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2025

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ANIMAL SCIENCE:
SUPPORTING LIVESTOCK'S ROLE
IN A GLOBAL SOCIETY

BSAS Annual Conference
8 - 10 April 2025
PROCEEDINGS

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british society of animal science

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The international journal for conference proceedings

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animal - science proceedings is part of the animal family of journals (animal, animal - open space).
The journal publishes high-quality conference, symposium and workshop proceedings on aspects of the life sciences with emphasis, on farmed, other managed animals, leisure and companion animals, aquaculture and the use of insects for animal feed and human food. These can be in the form of a book of abstracts or one to two-page summaries. The format will highlight the title of the meeting and organisations involved but the publications will have the added advantage of being gold open access and forming a series under animal - science proceedings. This gives conferences wide exposure and conference proceedings a wide circulation.
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animal Management Board

The Proceedings of the British Society of Animal Science
The Proceedings of the British Society of Animal Science constitutes abstracts of papers presented at the Society's Annual Conference, BSAS 82nd Annual Conference 2025 held at the Galway Bay Hotel, Galway, Ireland, 8-10 April 2025.

Guest Editors

All the research reported in this issue complied with the relevant country regulations on ethics and animal welfare.

Introduction

The British Society of Animal Science (BSAS), established in 1944, is a charity dedicated to supporting animal science in the UK and Ireland. BSAS has an interest in science related to all animals with the Society's core focus being livestock. We communicate, influence, and engage interest in how sustainable livestock production systems can contribute to socio-economic and ecosystem outcomes.

Our diverse membership comprises all career stages and includes leading academics, government, and industry professionals in all areas of animal science and related areas. This creates a dynamic and dedicated community committed to the betterment of the sector.

BSAS collaborative global networks aim to address climate change, global food security and one health challenges. BSAS provides an active platform that enables the dissemination of evidence-based research findings to show the role of livestock and other domesticated animals in high welfare and holistic sustainable agri-food systems.

BSAS aims to stimulate interest and discussion with focus on food security, addressing climate change and wider ecosystem challenges related to livestock production by providing a platform for individuals and organisations that are committed to the advancement of sustainable livestock systems.

And to then bring forward and disseminate evidence-based science and sector knowledge to champion and transform, through global collaboration, a trans- and multi-disciplinary one health approach. Using this evidence-based knowledge, we aim to inform government policy and related decision-making activities.

We want to work and collaborate with all sectors in, and related to, animal science to encourage and ensure sharing of factual information to improve all animal health, care and welfare.

BSAS encourages and supports the development of early career professionals in the field of animal science and associated areas helping them to develop, throughout their careers, into tomorrow's leaders.

We aim to be a society that is universally recognised and respected with an engaged membership encompassing academic, industry and public sectors within animal science and related fields.

Today, as ever, the Society is the natural connecting point for all of those with an interest in animal science and related sectors. Its membership is drawn from research, education, advisory work, commerce and practical animal keeping.

The 2025 annual conference addresses the topic of 'Animal Science: Supporting livestock's role in a global society'.

If you would like to join or receive further information about the British Society of Animal Science, please contact: E-mail: bsas@bsas.org.uk

Registered address: BSAS, 18 North Street, Glenrothes, Fife, KY7 5NA
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Leading Animal Scientific Journals Unite to Address Challenges in Science Publishing

Prominent peer-reviewed journals in animal science have collaborated on a groundbreaking article titled "Values Shared by Journals of Learned Societies, Associations, and Scientific Institutions in Animal Science". This joint effort emphasises the critical need to uphold scientific quality standards and integrity, ensure rigorous peer review, and embrace responsible publishing practices in an increasingly author-driven landscape.

The article will be simultaneously published by the following journals:
animal and animal – open space (Elsevier Limited on behalf of the animal Consortium)
Journal of Dairy Science and JDS Communications (Elsevier Limited on behalf of the American Dairy Science Association)
Poultry Science and Journal of Applied Poultry Research (Elsevier Limited on behalf of the Poultry Science Association)
Journal of Animal Science and Translational Animal Science (Oxford University Press on behalf of the American Society of Animal Science)

Scientific publishing has undergone a tremendous change in recent years. The move towards open access publishing has shifted the financial model in scientific publishing. Authors now bear the cost of publication, creating an author-driven market where income precedes the product. This shift presents challenges, including pressures on publishers to accept articles quickly and on researchers to publish frequently to advance their careers. Both dynamics can strain the peer-review process, potentially compromising its depth and thoroughness.

Challenges in Peer Review and the Role of Society-Owned Journals

One significant challenge highlighted in the article is the growing difficulty of securing reviewers. The peer-review system, widely regarded as a “mark of quality,” depends on the expertise and voluntary contributions of the scientific community. However, many scientists face time constraints that limit their ability to act as reviewers, leaving journals to grapple with maintaining the integrity of the process.

In this shifting landscape, journals owned by scientific societies and institutions play a critical role. As not-for-profit publications, they are driven by their mission to serve the scientific community rather than commercial gain. Their guiding principle—"For scientists, by scientists"—priorities the publication of sound and reliable research. By promoting and defending scientific integrity within their communities, society-owned journals ensure that rigorous standards are maintained even amidst market pressures.

Jaap van Milgen, Chairman from the animal Consortium commented:

"The role of society and institution-owned journals is more important than ever in an open-access world. We are committed to prioritising scientific quality and reliability above market trends, ensuring that the needs of science and society are at the forefront of publishing."

The article serves as a call to action for the scientific community to navigate these challenges collaboratively. It underscores the shared responsibility of researchers, reviewers, and publishers in safeguarding the credibility and utility of animal science research for the global community.

The full article is now available through participating journals' platforms - https://animal-journal.eu/

BSAS Proceedings

Welcome Note

Dear Delegates

It is a pleasure to welcome you to the BSAS 2025 Annual Conference here in Galway. This event is a highlight of the BSAS calendar, bringing together researchers, industry leaders, and policymakers to explore the challenges and innovations shaping the future of animal science.

Our theme this year, ‘Supporting Livestock’s Role in a Global Society,’ has attracted an exceptional lineup of keynote speakers. The President’s and Hammond sessions will tackle key issues, from the global role of livestock and its interaction with society to demand and supply trends for livestock-derived food products. Discussions will also focus on the nutritional value of meat and the importance of consumer trust and confidence in the industry.

This year’s programme reflects the diversity and impact of our sector, with sessions on climate and livestock, equine science, and the latest research showcased through our poster presentations and awards. We were also pleased to host the Early Career Symposium here in Galway yesterday, reaffirming BSAS’s commitment to supporting the next generation of animal scientists.

I encourage you to fully engage in the conference, take part in discussions, and make the most of the digital proceedings available in your conference pack. The BSAS 2025 Conference Proceedings will be available open access shortly after the event.

Thank you for joining us for BSAS 2025—I look forward to an insightful and inspiring few days ahead!

Best wishes

David Kenny
President, BSAS

Proceedings of the British Society of Animal Science Annual conference 2025

Tuesday 8th

Wednesday 9th

Thursday 10th

001

Opening Address - Getting the metrics right to define the critical role livestock will play in future sustainable circular food systems

M. R.F. Lee[1]
[1]Harper Adams University, Edgmond, Newport TF10 8NB, UK and LEAP Food and Agricultural Organisation of the United Nations

Presenting author
Michael Lee. E-mail: mrflee@harper-adams.ac.uk

In most farming systems, livestock's main function is food production, but they provide many other products and services such as fibre, hides, energy, fertilisers, feed, insulation, pharmaceuticals, nutraceuticals, biodiversity and landscape management (e.g. fire prevention), traction, contributions to culture, mental wellbeing as well as being an integral part of vibrant rural communities across the globe. In the context of climate change, measurement of the use of animal resources in all its components is a key question, and it is important to take all the products and services provided by livestock into account when assessing their contribution versus their environmental impact. The multiple products provided by livestock limit the value of comparisons with other foodstuffs using single factor assessments as often conducted in life cycle analysis exercises. All these non-food uses, in addition to food (considering its nutritional value), contribute to the closing of the biological cycle, and are key points to reduce waste and valorise the contribution of animal production to the circular bioeconomy. Some of them, like manure and drug production are alternatives to the use of fossil resources and contribute significantly to improving the climate balance of animal production. Animal by-products also contribute significantly to land use efficiency where alternative plant-based products would use land which could have otherwise been used to produce food directly (e.g. processed animal-protein versus soya as feed). Circular bioeconomy practices need to be designed and assessed regionally so that livestock production promote circularity in a manner that is economically viable and minimises the environmental footprint, while optimising the delivery of food and ecosystem services from agricultural systems which critically meet the needs of local communities and cultures. Defining the metrics and assessing these practices will generate the data needed to inform the development of livestock food systems through a sustainable circularity lens.

002

Efficacy of cattle slurry amendments to mitigate gaseous emissions during storage and land spreading

D. Krol[1], P. Morton[2], S. Connolly[1], M. Owusu-Twum[3], G. Gleasure[1], D. Kelleghan[4], K. Richards[1], G. Lanigan[1]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[2]*Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast BT9 5PX, UK*
[3]*School of Health and Science, Dundalk Institute of Technology, Dublin Road, Dundalk A91 K584, Ireland*
[4]*Environmental Protection Agency, Johnstown Castle, Wexford Y35 W821, Ireland*

Presenting author
Dominika Krol. E-mail: Dominika.krol@teagasc.ie

Application
Slurry amendments have the potential to reduce gaseous emissions from storage and landspreading of liquid manures.

Introduction
Slurry storage and landspreading can lead to large losses of gaseous emissions to the environment in the form of an air pollutant ammonia (NH_3) and greenhouse gases (GHG), mainly methane (CH_4; in storage) and nitrous oxide (N_2O; at land spreading). In Ireland, manure management is responsible for approximately 10% of agricultural GHGs and 80% of NH_3. Considering legally binding targets to reduce both types of emissions, there is an urgent need for mitigation. Slurry amendments or additives have long been of interest to the agricultural industry seeking ways to reduce gaseous emissions, improve nutrient use efficiency and overall sustainability. While slurry acidification is an established abatement technique, questions remain over many alternative additives such as waste products, by-products or commercial materials (Owusu-Twum et al., 2024). This work aimed to quantify NH_3 and GHG emissions from storage and landspreading of cattle slurry treated with sulphuric acid, gypsum and biochar compared with untreated slurry. Landspreading experiment also aimed to investigate efficacy of slurry treatments on two contrasting soil types and during varying environmental conditions at spreading.

Materials and methods
In this work, two experiments were carried out to assess efficacy of slurry amendments during slurry winter storage (Experiment 1) and land spreading (Experiment 2). Experiment 1 was a large-scale incubation simulating slurry storage under mild, temperate climatic conditions carried out during winter of 2022 at Teagasc Johnstown Castle, Co. Wexford. Twelve pilot-scale underground storage tanks were filled with 660L slurry and amended with either sulphuric acid, biochar or gypsum, or left untreated, resulting in three replicates of each treatment laid out in a randomized block design. Slurry was stored for 77 days post-treatment and NH_3 and GHGs were monitored regularly throughout this period using dynamic (acid trap method) and static chamber techniques for both types of gases, respectively.

Experiment 2 was a slurry land spreading trial carried out across three seasonal applications, over two years (2021-2022), at two grassland sites on contrasting soils in Ireland. The sites were: a) moderately to poorly drained soil at Teagasc Johnstown Castle, Co. Wexford and b) an imperfectly drained clay loam at the Agri-Food and Biosciences Institute (AFBI) in Loughgall, Co. Armagh. Slurry amended with the same treatments as during storage (sulphuric acid, biochar, gypsum, untreated slurry) was spread to 1.5 x 1.5 m plots laid out in a fully randomised block design, with five replicates for nitrous oxide measurements and three replicates for ammonia measurements. Ammonia was measured using dynamic chamber technique with an INNOVA 1412 photoacoustic gas analyser for a week following application. N_2O was measured using static chamber technique for approximately 2 months following application. Data was checked for normality prior to statistical analysis of variance (SAS version 9.4; SAS Institute Inc). Multiple comparisons between means were done using the Tukey test and differences among means were considered significant at a p-value below 0.05.

Results

During storage, slurry acidification with sulphuric acid showed a significant reduction (32%) in NH_3 emissions relative to the control, while CH_4 was reduced significantly in the sulphuric acid (46%), gypsum (39%) and biochar (15%) treatments relative to the control.

Upon landspreading, acidification with sulphuric acid significantly reduced NH_3 whereas the other slurry amendments (i.e., biochar and gypsum) showed variable results. N_2O emission factors were in the order; slurry + sulphuric acid (0.23%) > slurry + gypsum (0.17%) > untreated slurry (0.11%) > slurry + biochar (0.09%).

Conclusions

Slurry acidification proved a reliable method of reducing NH_3 and CH_4 in storage under mild winter conditions. Sulphuric acid was also effective at reducing NH_3 during land spreading, however it led to a small increase in N_2O emissions. However, other tested additives showed variable efficacy of mitigation in both phases of slurry management.

Acknowledgements

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003

Comparing ammonia emissions from low emission slurry spreading techniques using cattle slurry on cool temperate grasslands

P. Morton[1], G. Gleasure[2], S. Higgins[1], D. Kelleghan[3], G. Lanigan[2], J. McIlroy[1], R. Olave[1], R. Ramsey[1], K. Richards[2], D. Krol[2]
[1]Agri-Food and Biosciences Institute, Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast BT9 5PX, UK
[2]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[3]University College Dublin, Belfield, Dublin D04 V1W8, Ireland

Presenting author
Dominika Krol. E-mail: Dominika.krol@teagasc.ie

Application

Using low emission slurry spreading (LESS) equipment provides the simultaneous benefits of reducing the amount of ammonia emitted to the local atmosphere, which protects both human and sensitive habitat health, whilst increasing the amount of nitrogen available to the plants and thereby reducing additional fertiliser costs to the farmer.

Introduction

Gaseous ammonia (NH_3) negatively impacts both human and environmental health (Wyer et al., 2022). Agriculture is responsible for about 90% of NH_3 emissions on the island of Ireland, with over a third arising from the landspreading of cattle slurry (Bourdin et al., 2014). Whilst slurry is used as the main source of fertiliser, thus necessitating landspreading of slurry, the traditional spreading method involves a splashplate attachment to broadcast slurry, which encourages NH_3 volatilisation. It has been shown in several countries that LESS technologies reduce the NH_3 emissions from slurry spreading by depositing slurry closer to the ground and reducing the surface area of spread slurry, which minimises contact with ambient air (Nyameasem et al., 2022). However, the efficacy of different LESS technologies on NH_3 emission reduction is uncertain under Irish conditions. Therefore, the aim of this study was to investigate the ability of three different LESS techniques to reduce NH_3 emissions compared to splashplate on Irish soils.

Material and methods

Six paddock-scale plots (50 m x 50 m) were spread with cattle slurry at each of two permanent grassland sites, Loughgall in Northern Ireland and Johnstown Castle in Ireland. Loughgall has sandy clay loam soil and Johnstown Castle has loamy soil; the soil at these sites is representative of that found at a large proportion of grassland fields across the island of Ireland. Two of the plots at each site were spread by splashplate and two by trailing shoe. The other two plots at Loughgall were spread by trailing hose (dribble-bar) and the other two at Johnstown Castle were spread by open-slot injection. These trials were run for two years, with three applications at each site per year. The NH_3 emissions were monitored using integrated horizontal flux (IHF) shuttles for at least five days following each spreading event. Emission factors were calculated as the background-corrected cumulative NH_3 emissions for each trial divided by the total ammoniacal nitrogen (TAN) applied as slurry to each plot at the beginning of a trial. Linear mixed effects models were used to test for differences in TAN loss between slurry spreading techniques and spreading seasons.

Results

NH_3 emissions tended to peak on the day of spreading, with an average of 64% of NH_3 loss measured within 6 hours of spreading, and emissions then decreased exponentially to background emissions within five days. Mostly, the emissions were lower during spring (March/April) trials, when temperatures were cooler and plants were growing rapidly, and highest in late summer (August). However, splashplate-spread plots at Loughgall had the lowest emission factors in late summer in both years, when the ground was very dry. Due to the variability in NH_3 emissions from spreading techniques (Figure 1) and between years, the season slurry was spread did not significantly affect the NH_3 emissions ($p > 0.05$). At both sites, NH_3 emission factors were significantly higher from splashplate plots than from slurry spread by LESS techniques ($F_{2,28} = 7.87$; $p < 0.01$ for Johnstown Castle, $F_{2,24} = 20.4$; $p < 0.01$ for Loughgall; Figure 1). Despite reducing TAN loss by 37% on average compared to splashplate, there were no significant differences between the LESS techniques ($p > 0.05$; Figure 1).

Figure 1. Mean (± standard deviation) percentage of total ammoniacal nitrogen (TAN) lost over five days from the slurry applied by splashplate and by three forms of LESS equipment

Conclusions

On average, the LESS techniques do lower NH_3 emissions from Irish soils. However, contrary to that demonstrated in previous research in other countries, there is very little difference between LESS techniques and so there should be a focus on encouraging farmers and contractors on the island of Ireland to use any LESS equipment, rather than specifically pushing the more expensive form, as this may increase uptake.

Acknowledgements

This research was financially supported by the Irish Department of Agriculture, Food and Marine (grant number 2019R554) and the Northern Irish Department of Agriculture, Environment and Rural Affairs (grant number 19/4/16).

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004

Wind Tunnels as a Qualitative Tool for Measuring Ammonia Emissions from Manure: Enhancing Accuracy and Reliability

S. Connolly[1], D. Krol[1]
[1]Teagasc, Environment, Soils and Land Use Department, Johnstown Castle, Wexford Y35 Y521, Ireland

Presenting author
Shaun Connolly. E-mail: shaun.connolly@teagasc.ie

Application

This study contributes valuable insights into the optimization of wind tunnel methodologies for ammonia emission research. The findings provide practical recommendations to improve data reliability and quality for future applications.

Introduction

Ammonia is a significant atmospheric pollutant contributing to soil acidification, the indirect production of nitrous oxide, and the formation of particulate matter (PM2.5). In Ireland, over 99% of ammonia emissions originate from agriculture, with manure storage and spreading alone responsible for more than 75% of these emissions. Ammonia volatilization from animal manure depends on factors such as pH, surface area, temperature, and moisture. Numerous mitigation techniques, including acidification, covering manure during storage, and employing low-emission slurry spreading methods, have been developed to reduce emissions. Farmers are also encouraged to apply manure under cooler, slightly damp conditions rather than during hot, dry weather to minimize volatilization. Measuring and quantifying ammonia for emission inventories remains challenging. “Silver standard” micrometeorological methods, such as integrated horizontal flux and eddy covariance, provide accurate measurements but operate over large areas (40 m^2 to several hectares), complicating the comparison of treatment effects. For smaller-scale, qualitative measurements, wind tunnels are a more practical alternative. These systems typically cover a surface area of 0.5–3 m^2 and utilize either acid scrubbers or laser measurement techniques to capture ammonia emissions. Wind tunnels consist of two main components: a transparent canopy placed over the experimental area and a fan-enclosed metal body. These components are connected and sealed during experiments to minimize gas leakage. Ammonia measurements are taken from ambient air entering and exiting the tunnel.

Materials and Methods

For this study, wind tunnels were constructed based on Lockyer’s (1984) design. An ammonia recovery experiment was conducted at Teagasc Johnstown Castle in August 2024 over a 24-hour period. The study addressed three key questions:

1. Is a boundary required between the emission source and the ambient sampling port?
2. Does the calculation method for canopy volume (elliptical vs. integration) affect the recovery rate?

3. What is the effect of low vs. high airflow rates on ammonia recovery in wind tunnels?

Trays containing ammonium buffer solution were placed at three distances (0.02 m, 0.15 m, and 0.5 m) from the ambient sampling ports in separate wind tunnels (n=3). Canopy surface areas were measured, and the area of the irregular elliptical tunnel openings were calculated using either the elliptical area formula (Equation 1) or integration (Equation 2). Figure 1 illustrates the basic measurements of both methods. The ammonia recovery rate (ARR) was calculated using Equation 3. High airflow wind tunnels averaged 3.22 m s^{-1}, while low airflow tunnels averaged 1.21 m s^{-1} over the experimental period.

Equations:

- Equation 1 – $A = (\pi ab)/2$
  where A is the area, a is the length of the longest axis, b is the length of the shortest axis.

- Equation 2 - $A = (\int_0^{50} f(x)dx) * 2$
  where A is the area, integration upper and lower limits are 0 and 50, f(x) is the curve produced after measuring the out edge of the canopy to the centre line which varied among each canopy, dx denotes differential.

- Equation 3 – ARR = (AE/RA)*100
  where ARR is the ammonia recovery rate, AE is the ammonia emitted in 24 hours and RA is the recovered ammonia.

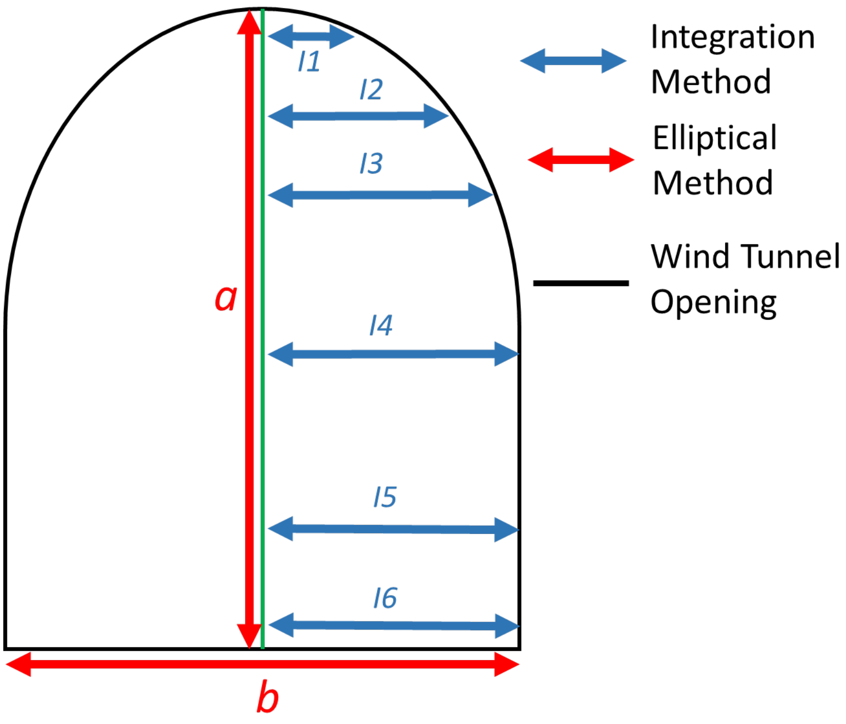

Figure 1. Illustration of the basic measurements needed to estimate wind tunnel opening area. Red lines demonstrate the elliptical method (Equation 1) while the blue lines demonstrate the integration method (Equation 2)

Statistical Analysis

Data were analyzed using GraphPad Prism (v9.1.0). NH_4^+ concentrations and recovery rates were compared using a two-tailed unpaired parametric t-test with Welch's correction to account for differing variances. Results with $P<0.05P < 0.05P<0.05$ were considered significant.

Results

- Sampling ports located within 15 cm of the emission source exhibited significantly higher ammonia concentrations than those placed further away at 0.15m and 0.5m. A minimum boundary of 0.15cm is therefore recommended to prevent contamination of ambient air samples during recovery experiments.
- There was approx. a 6.5% difference between volume estimates (Elliptical – 0.208 m^2; Integration – 0.223 m^2). The method of canopy volume calculation did not significantly influence emission estimates ($P>0.05$). The elliptical area method however is recommended due to its simplicity and practicality in field applications.
- Low airflow wind tunnels recovered 77% ± 2.17% of ammonium, while high airflow tunnels recovered 67% ± 0.79%, a statistically significant difference ($P<0.05$).

Conclusions

Wind tunnels provide a reliable method for measuring ammonia emissions across different treatments, and experimental designs should account for this capability. This study demonstrates that while both methods for calculating the tunnel opening are valid, the elliptical method is more practical and robust for field applications. For accurate results, future studies using wind tunnels should maintain a boundary layer of at least 0.15 m between the emission source and the ambient sampling port. Additionally, low-airflow wind tunnels recover more ammonia than high-airflow tunnels, making them a preferred option for precise measurements, though they may not fully reflect ambient conditions.

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005

Beyond Respiration Chambers: A Field-Deployable Device for Continuous Methane and Carbon dioxide Emission Measurement in Cattle

R. Bica[1], N. Coetzee[1]
[1]ZELP, Unit B, 449 Holloway Road, London N7 6LR, UK

Presenting author
Riccardo Bica. E-mail: riccardo.bica@zelp.co

Application
The zero emissions livestock project (ZELP) is advancing sustainable livestock management by introducing an innovative device designed to monitor methane (CH_4) and carbon dioxide (CO_2) emissions in cattle. This device allows for non-invasive, continuous measurement in natural settings, addressing the limitations of traditional respiration chambers and enabling more accurate data on greenhouse gas (GHG) emissions.

Introduction
Methane emissions from cattle contribute significantly to greenhouse gases, intensifying the need for effective monitoring to support environmental sustainability. Current gold-standard methods, such as respiration chambers, while precise, are resource-intensive, costly, and restrict natural behaviours due to confinement, potentially affecting emission data accuracy (Troy et al., 2016). By contrast, this novel device provides a field-deployable solution that collects emissions data in real-world conditions. The design overcomes respiration chamber limitations by enabling continuous and high-frequency monitoring across varied environments without disrupting animal behaviours or compromising welfare.

Materials and Methods
The device is equipped with an array of advanced sensors: Near Infrared optical and catalytic sensors for gas concentration, thermistors and thermocouples for temperature, and accelerometers and inertial measurement units (IMUs) for motion and position. These sensors are mounted on the animal’s head using a specially designed face sock and positioned near the nostrils to capture exhaled gases before significant dilution. The face sock, made from ventilated neoprene, has proven in lab and field tests to cause no abrasion and allows airflow to prevent sweat and dirt buildup. Data is recorded continuously or intermittently, allowing detailed respiratory profiles and gas concentrations to be captured. The device supports both onboard data storage for extended offline processing and wireless transmission for real-time or scheduled updates, depending on connectivity and power availability. To validate the device, a two-week trial was conducted in October 2024 at Hall Farm, Reading, England. Three Holstein Friesian heifers were involved, with two heifers tested each week and one heifer participating in both weeks with a one-week break between. Objectives included obtaining methane emissions data at a granular 15-minute interval, assessing device performance in comparison to respiration chamber readings, collecting high-quality thermistor data, and documenting prototype performance. The trial setup included two CCTV cameras per heifer for real-time behavioural observation, complemented by hourly

respiration rate checks via visual inspection. Data collected from the trial included respiratory rates, breath temperatures, gas concentrations, and heifer behaviours, with continuous monitoring by personnel to identify any welfare impacts. Statistical comparisons of methane emission data from the device and respiration chambers were performed, accounting for variables such as feed intake and activity level. Weight measurements were taken before and after the trials to monitor any adverse effects on animal health. Methane (CH_4) and carbon dioxide (CO_2) concentrations at the nostril were measured, and proprietary machine learning models were applied to estimate total emissions.

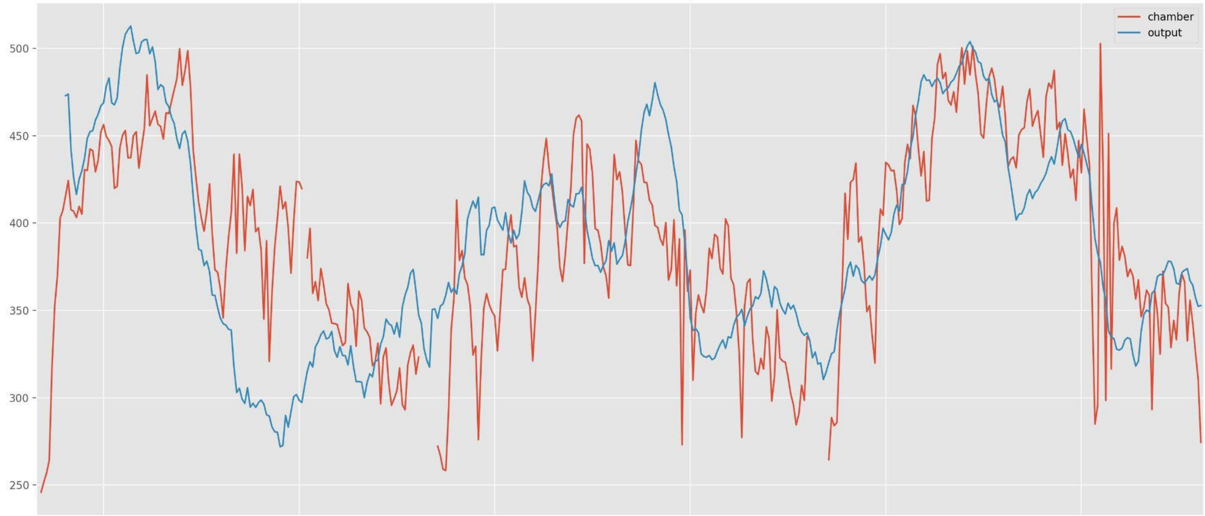

Figure 1. Comparison between CH_4 measured in respiration chambers (red line) and CH_4 measured by the device (blue line) over a 3-day period, with a measurement error of approximately 14%. The x-axis depicts time and the y-axis depicts methane concentration

Results

Preliminary results suggest that the device accurately mirrors respiration chamber data, providing comparable emission readings while capturing additional data in naturalistic environments. A moderate positive correlation ($r = 0.612$, $P < 0.05$) was found between the device's methane emission measurements and those obtained from the respiration chamber, indicating a moderate strength relationship between the two methods. This suggests that the device can reliably replicate the emission data in natural settings while maintaining consistency with the more controlled chamber environment. Respiration rates remained within normal limits of 26–50 breaths per minute, the behavioural assessment showed no signs of distress, confirming that natural behaviours were maintained. A minor weight loss in heifers was observed, consistent with restricted feeding patterns in respiration chambers (Llonch et al., 2018).

Conclusions

The device demonstrates significant potential as an alternative to traditional respiration chambers, enabling scalable, accurate, and behaviourally relevant emissions monitoring. This technology could facilitate more widespread methane management in livestock, supporting the sector's shift toward sustainable practices.

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006

Investigating methane emissions from nulliparous female sheep using GreenFeed technology

E. Dunne[1,2]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]University of Palermo, Piazza Marina, 61, 90133 Palermo PA, Italy

Presenting author
Eoin Dunne. E-mail: Eoin.Dunne@teagasc.ie

Application

Novel research using GreenFeed technology in a pasture-based sheep production system will generate data contributing to the national inventory, enabling more precise calculations of CH_4 emissions from sheep.

Introduction

The accurate measurement of methane (CH_4) emissions in grazing sheep is essential for improving the understanding of CH_4 production patterns, generating data for national inventory calculations and identifying dietary strategies to mitigate emissions. This study evaluates CH_4 emissions in sheep over a six-month period, from spring to autumn, using GreenFeed technology. The GreenFeed technology intermittently captures and quantifies eructated CH_4 emissions during individual animal visits to the feeding station at pasture. GreenFeed proved effective for longitudinal CH_4 monitoring, demonstrating its utility in capturing real-time, non-invasive emissions data in grazing systems, (Starsmore et al., 2024) however most of the work to date has focused on cattle or measuring animals indoors. The objective of this study was to profile CH_4 output on nulliparous hoggets across the grazing season and to identify the effect of seasonal forage and animal live-weight variation on CH_4 emissions in sheep.

Materials and Methods

This experiment was carried out over a six month period from March until August 2024. Forty nulliparous female sheep (20 Suffolk and 20 Texels), circa 12 months of age at commencement of the experiment, were monitored on a pasture-based diet. Daily CH4 emissions data were recorded during brief feed station visits, with individual measurements aggregated for analysis. Each animal was permitted a maximum of six visits per day to the GreenFeed system, with a minimum interval of two hours between consecutive visits to ensure temporal spacing and measurement independence. During each visit, animals received eight individual feed allocations, each consisting of 7.5 g of feed (dried grass pellet), dispensed at 30-second intervals to standardise feeding events. Additionally, weekly forage samples were collected from the grazing area throughout the study period. These samples were analysed for dry matter (DM) and nutritional composition. Animal live-weight was recorded monthly throughout the study. Two main CH4 output traits were investigated, namely CH4 expressed per day (g/day) and CH4 expressed per kg live-weight (g/kg LW). The effect of seasonal variation, forage quality parameters and breed on CH4 output were analysed using linear mixed models in PROC MIXED (SAS Inst. Inc., Cary, NC, USA).

Results

Across the six-month experimental period, the average CH4 output was 38.82 (SD = 7.46) g/day, with animals averaging 3.02 (SD = 1.16) visits to the machine per day. The repeatability of the animal's individual CH4 output (g/day) was 88% across the experimental period. Methane output expressed as g/day differed by month ($P<0.001$; Figure 1) with the lowest CH4 output associated with March (31.58 g/day) and the highest reported in the months of June to August, which did not differ from each other. Methane output, expressed per kg live-weight also differed by month ($P<0.001$; Figure 1) with the lowest values recorded for the months of March, April and June, which did not differ from each other. Additionally, breed-specific differences in CH4 output (g/day) were identified, with Suffolk hoggets producing 39.47 (0.30) g/day, compared to the Texel hoggets producing on average 37.63 (0.30) g/day ($P<0.001$). However when CH4 was expressed per kg live-weight the two breeds did not differ from each other ($P>0.05$) suggesting that differences in live-weight accounted for the differences in CH4 output expressed in g/day between both breeds. Grass quality analysis identified monthly differences OMD ($P<0.001$), with the highest OMD levels associated with March and April, 858.94 g/kg.

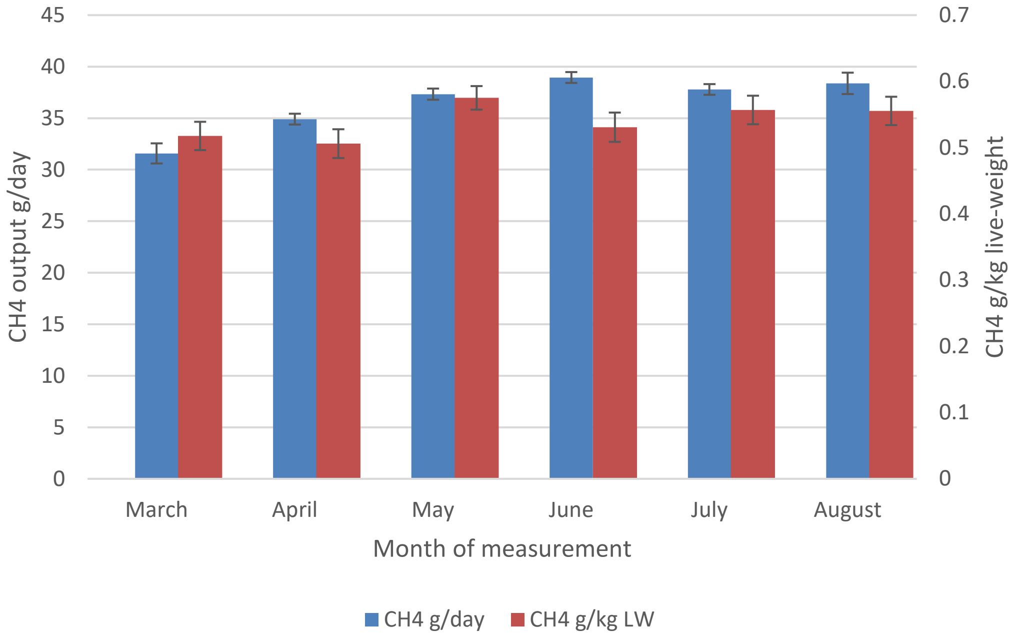

Figure 1. Average monthly methane output (error bars represent one standard error above and below the mean methane output) expressed as CH_4 g/day and CH_4 g/kg live-weight

Conclusions

Enhancing the understanding of enteric CH4 emissions from sheep in pasture-based systems is essential for refining Ireland's greenhouse gas inventories and supporting targeted mitigation strategies for small ruminants in extensive grazing systems. This study highlights the temporal and breed-specific variability in CH4 emissions among nulliparous female sheep. CH4 output, both in absolute terms (g/day) and relative to live-weight, was influenced by seasonal factors, animal bodyweight and a change in grass nutritional composition. Breed differences were evident in absolute CH4 output but not when expressed per kg of live-weight, emphasising the role of animal size in CH4 emissions. The high repeatability 88%, associated with individual animal CH4 measurements indicates the consistency among the individuals in terms of CH4 output across the study period. Furthermore, CH4 expressed as g/day increased over the trial period, correlating with an increase in animal live-weight and a variation in grass OMD. This demonstrates the potential in improving sward quality to reduce CH4 output. These findings provide valuable insights into the dynamics of CH4 emissions, with implications for breed selection and seasonal management strategies in livestock farming.

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007

The effect of rumen ORP modulation using calcium peroxide on feed intake and digestibility in fistulated beef cattle

A. Graham[1], C. Thorn[2], A. Bartle[1], M. Mcdonagh[1,2], A. C. Villa Montoya[1], S. Nolan[2], A. Hall[1], R. Friel[2], S. Waters[3,4], S. Kirwan[3], V. O'Flaherty[1,2]
[1]Microbial Ecology Laboratory, Microbiology, School of Biological and Chemical Sciences and Ryan Institute, University of Galway, University Road, Galway, Co. Galway H91 TK33, Ireland
[2]GlasPort Bio, Unit 204, Business Innovation Centre, Galway, Co. Galway H91 TK33, Ireland
[3]Animal Bioscience Research Centre, Grange, Dunsany, Co. Meath C15 PW93, Ireland
[4]School of Biological and Chemical Sciences, University of Galway, Co. Galway H91 CF50, Ireland

Presenting author
Alison Graham. E-mail: 2009alisongraham@gmail.com

Application

The development and *in-vivo* assessment of novel oxygen-releasing feed additives, to reduce enteric ruminant methane emissions, in both intensive and pasture-based production settings.

Introduction

As the reduction of enteric methane (CH_4) production commands global attention, coupled with emerging acceptance that current available mitigative solutions fall short, there is a growing demand for alternative feed additive approaches. The rumen oxidation reduction potential (ORP) parameter influences CH_4 production rates, as methanogens are typically active only at ORPs below -300 millivolts. Thus, a controlled elevation of rumen ORP offers a potentially benign means of CH_4 suppression, where extending the period of elevated rumen ORP would serve to inhibit the activity and growth of methanogens. Previous *in-vitro* work demonstrated successful CH_4 mitigation, with consistent CH_4 reductions of >50% observed when supplementing with various oxygen-releasing compounds, including calcium peroxide and magnesium peroxide (CaO_2, MgO_2). CaO_2 was proposed as a potentially viable rumen ORP modulator, pending further *in-vitro* and *in-vivo* assessment (Graham et al., 2024).

Materials and methods

This research evaluated the *in-vivo* effects of various doses of CaO_2 on animal performance for the first time in beef cattle. Nine cannulated Aberdeen Angus × Friesian steers (739 ± 67 kg BW) were used in an 84-day Latin Square design trial (n=9). Several CaO_2 inclusion rates were evaluated during once-a-day and twice-a-day feeding, providing valuable insights into animal response to varying CaO_2 concentrations. *In-vivo* ORP (continuous), static ORP, static pH, ammonia, volatile fatty acids (VFAs), *in-sacco* diet digestibility, feed intake, palatability, and rumen microbiome were all monitored, while a portable CH_4 analyser provided preliminary CH_4 emission indications.

Results

No significant negative impacts of inclusion rates of CaO_2 up to 1.7% of dry matter intake (DMI) were observed, while positive indications of reduced enteric CH_4 emissions were observed from animals receiving treatment vs. controls. Equivalent rumen ORP elevations were observed at both the initial higher and lower doses applied during once-a-day feeding, alongside similar levels of CH_4 suppression. Thus, to determine the mitigative efficacy of CaO_2 on enteric CH_4 production at lower inclusion rates, an additional lower dose of CaO_2 was introduced during the twice-a-day feeding phase. Consistently greater ORP values of >100 mV were observed in the rumen of treatment vs. control animals during twice-a-day feeding, which was again associated with reduced enteric emissions. These findings, together with the minimal palatability issues encountered at each CaO_2 inclusion rate and CaO_2's capacity to withstand both heat and pressure during incorporation into a feed pellet format, suggest that it may have potential as a tangible alternative to existing ruminant feed additives with a prospective contribution to supporting global methane mitigation goals.

Conclusions

The potential and merit of employing ORP as a tool for the direct manipulation and modulation of the rumen environment, using CaO_2, was demonstrated. The evaluation of CaO_2 in larger-scale animal trials using more robust emissions monitoring, is supported by this work. The long-term goal of this research is to establish a slower-release formulation, to allow more consistent, controlled, and sustained CH_4 suppression, effectively mitigating CH_4 in both intensive and pasture-based production systems. Controlled-release formats of CaO_2 would also serve to reduce the complexity of application on farm, allowing for continuous CH_4 suppression, while animals are grazing on pasture.

Acknowledgements

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008

Effect of genetic divergence for carcass fatness, slaughter age and body site on the hypertrophy of subcutaneous adipose tissue in beef steers

M. O'Rourke[1], A. K. Kelly[2], M. McGee[1], E. O'Riordan[1], D. Kenny[1]
[1]*Teagasc, Animal and Bioscience Research Department, Grange, Dunsany, Co. Meath C15 PW93, Ireland*
[2]*University College Dublin, Belfield, Dublin D04 V1W8, Ireland*

Presenting author
Marie O'Rourke. E-mail: MarieEKORourke@gmail.com

Application

Explain the effect of genetic divergence for carcass fatness, age at slaughter and body site on the hypertrophy of subcutaneous adipose tissue biopsies harvested at slaughter in beef steers

Introduction

Carcass fat cover is typically the most challenging market criterion to consistently achieve, particularly where pasture reared beef cattle are slaughtered at a young age (Regan et al., 2018). Whilst it is possible to produce late-maturing beef steers at 24 months from pasture, with an adequate carcass fat score (Doyle et al., 2021), late-maturing steers slaughtered at 19 months from pasture don't meet the minimum carcass fatness specification (Regan et al., 2018). In Ireland, carcass fat cover is measured at beef processing plants using video image analysis (VIA) and the trait is a key criterion governing the value of the carcass. The Irish cattle breeding federation (ICBF), harness these data to generate estimated breeding values (EBV) for carcass fatness. Previous research has shown that progeny performance, including carcass fat score, can be influenced by the genetic merit of sires (Clarke et al., 2009). At a laboratory scale, histological analysis of subcutaneous adipose tissue is an accurate approach in determining the extent of tissue adiposity including adipocyte hyperplasia (numbers) and hypertrophy (size), which is an objective measure of subcutaneous carcass development to assess subcutaneous fat development. Adipogenesis, and the development of fat, are influenced by genetics, age, diet and body depot location (Jin et al., 2012, Romao et al., 2014). Although it is well accepted that the hypertrophic expansion of adipocytes exists with increasing slaughter age (Hood & Allen, 1973, Du et al., 2013), there is a dearth of information regarding the effect of genetic divergence for carcass fatness, various slaughter ages, on the hypertrophy of subcutaneous adipocytes, in both the shoulder and the loin. Additionally, there is a paucity of information regarding the effect of body location, i.e. the forequarter (shoulder) vs. the hindquarter (loin) on the hypertrophy of subcutaneous adipocytes. Therefore, the objective of this study was to investigate hypertrophic differences of subcutaneous adipocytes in cattle genetically divergent for carcass fatness, with different slaughter ages and of contrasting body sites.

Materials and methods

As part of a larger experiment, seventy two weaned spring-born late-maturing crossbred (Limousin and Charolais sired) steers were assigned to the study (Burke et al., BSAS 2025) Cattle selected were genetically divergent for carcass fatness, 'fat' cattle had an EBV: 0.14 for carcass fatness, and 'lean' cattle had an EBV of -0.55. Cattle were finished at 19, 23 and 26 months, with a target carcass weight of 390kg for each production system. During their first winter, steers were offered silage *ad libitum* and 1.5kg/animal daily of a barley based concentrate, and subsequently turned out to pasture in the spring. The 19 month group were finished at the end of the second grazing season. The 23 and 26 month groups were offered ensiled forage, as well as 5kg and 1kg concentrates/animal daily, respectively. The 23 month group were finished at the end of the second winter, and the 26 month group were finished off pasture after grazing for approximately 2 months of the third grazing season. Prior to slaughter, ultrasonic fat depth was recorded at the rib, lumbar and rump. At slaughter, carcasses were weighed and graded for carcass fatness through VIA. A ~2g biopsy of subcutaneous adipose tissue was harvested from the shoulder and the loin, on the left side of the carcass. Samples were fixed in 10% neutral-buffered formalin for 48 hours, following storage in 70% ethanol <5days. Subcutaneous adipose tissue was then processed, and embedded in paraffin wax in accordance with standard procedures. Sections (5µm thick) were dehydrated in ascending grades of alcohol, followed by clearing with xylene and were stained using haematoxylin and eosin (H&E) stain. Nuclear counts of adipocytes on the depth of colour were conducted using an algorithm developed in Aperio ImageScope (v12.4.6.5003) to count and measure fat cells. Data were analysed using the Mixed Model procedures of SAS (9.4). Partial correlation coefficients of EBV for carcass fatness, live animal measurements, carcass fat scores and subcutaneous adipocyte histology data were derived.

Results

Results are displayed in Table 1 below. Briefly, 'Fat' animals had greater ultrasonic fat depth at the rib, lumbar and rump than 'Lean' animals. 'Fat' animals had greater carcass fat scores, than 'Lean' animals. Increasing slaughter age resulted in increased ultrasonic fat depth at the rib, lumbar and rump, in tandem with increased carcass fatness scores. This trend persisted at the subcutaneous adipocyte level, where 'Fat' cattle exhibited greater hypertrophic expansion of adipocytes by increased average adipocyte diameter and nuclei density, than 'Lean' cattle, at both the shoulder and the loin. Coincident with increasing slaughter age, increased average adipocyte diameter and increased nuclei density was observed at both sites. There were no differences ($P > 0.05$) in average adipocyte diameter or nuclei density, between the shoulder and the loin i.e. the forequarter and the hindquarter of the animal. Significant positive correlations were obtained for average adipocyte diameter and EBV (r=0.63). Significant positive correlation values were obtained for average adipocyte diameter and ultrasound fat depth at the rib (r=0.68), lumbar (r=0.66) and rump (r=0.54). Significant positive correlation values were obtained for average adipocyte diameter and VIA measured carcass fatness (r=0.864). Furthermore, the amount of total variation explained in VIA measured carcass fatness was lower for ultrasound fat depth at the rib (R^2=0.621) and higher in average adipocyte diameter (R^2=0.832). From these results, it can be observed that average adipocyte diameter, at both the shoulder and loin, exhibits the highest correlation values with VIA measured carcass fatness. Therefore, the use of VIA to measure carcass fatness is the greatest predictor of subcutaneous adipocyte diameter, in the shoulder and the loin, in this study.

Conclusion

The hypertrophy of subcutaneous adipocytes at both sites were greater in ‘Fat’ vs. ‘Lean’ animals, and with increasing slaughter age. There were no differences in hypertrophy of subcutaneous adipocytes between the loin or the shoulder regions. VIA measurements of carcass fatness explained a greater proportion of the variance in subcutaneous adipocyte diameter at both sites, compared to EBV and ultrasonic fat depth in this study.

Table 1. Effect of genetic divergence for carcass fatness, slaughter age and body site on ultrasound fat depth, carcass traits and hypertrophy of subcutaneous adipose tissue

	Genotype (G)			Slaughter age (S)				Body Site (B)			P-value		
	Fat	*Lean*	s.e.m.	*19m*	*23m*	*26m*	s.e.m.	*Shoulder*	*Loin*	s.e.m.	G	S	B
Ultrasound fat depth: rib (mm)	5.17	3.76	0.1825	3.52^{b}	4.90^{a}	5.10^{a}	0.2235	-	-	-	***	***	-
Ultrasound fat depth: lumbar (mm)	3.51	2.45	0.1201	2.46^{b}	3.28^{a}	3.28^{a}	0.1470	-	-	-	***	***	-
Ultrasound fat depth: rump (mm)	7.56	4.67	0.3642	4.12^{c}	8.11^{b}	6.37^{a}	0.4460	-	-	-	***	***	-
Final weight (kg)	680	668	4.584	616^{b}	702^{a}	704^{a}	5.614	-	-	-	NS	***	-
Carcass fat score (1-15)	8.4	6.6	0.132	6.3^{c}	7.6^{b}	8.6^{a}	0.162	-	-	-	***	***	-
Average adipocyte diameter (mm)	0.194	0.165	0.003	0.163^{c}	0.175^{b}	0.201^{a}	0.004	0.180	0.180	0.003	***	***	NS
Nuclei Density (mm)	0.030	0.022	0.001	0.021^{c}	0.024^{b}	0.032^{a}	0.001	0.026	0.026	0.001	***	***	NS

***P-value is significant at the 0.001 level, **P-value is significant at the 0.01 level, *P-value is significant at the 0.05 level, NS: Not significant (>0.05).

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009

Impact of Pre-slaughter Stress on the bovine Longissimus thoracis et lumborum and Semitendinosus muscle proteomes: A comparative study based on label-free proteomics data

S. Hazra[1,2], R. M. Hamill[1], J. P. Kerry[2], I.e Cassar-Malek[3], M. Gagaoua[4], Claudia Terlouw[3]
[1]Food Quality and Sensory Science Department, Teagasc Food Research Centre, Fermoy, Co. Cork P61 C996, Ireland
[2]School of Food and Nutritional Sciences, College of Science, Engineering and Food Science, University College Cork, Cork T12 K8AF, Ireland
[3]Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, 11 Boulevard Charles de Gaulle 63000 Cermont-Ferrand, France
[4]PEGASE, INRAE, Institut Agro, Pegase 16, le clos 35590 Saint-Gilles, France

Presenting author
Sudipta Hazra. E-mail sudipta.hazra@teagasc.ie

Application

The investigation of how molecular alterations in bovine muscle, under a stress challenge, are conserved or variable according to metabolic and structural properties, has the potential to enhance our understanding of the general stress response and its relationship with *post-mortem* muscle physiology to inform meat management systems.

Introduction

Previous studies have demonstrated a relationship between elevated pre-slaughter stress levels and reduced meat quality in farm animals (Xing et al., 2019). Currently, a comprehensive understanding of the biological mechanisms underlying the effects of stress on meat quality is lacking. However, minimising pre-slaughter stress would be beneficial for improving animal welfare and meat quality (Terlouw et al., 2021). The proteome is dynamic and responsive to *in vivo* and *post-mortem* environmental influences, making it a useful biological source for a better understanding of the biological mechanisms at interplay (Gagaoua et al., 2024; Wu et al., 2014). It can be further used to address the knowledge gap on the link between stress and meat quality (Sierra et al., 2021). This study sought to leverage a shotgun proteomic approach to examine the effects of pre-slaughter stress on the *post-mortem* physiological response in two bovine muscles with contrasting physiological properties.

Material and Methods

Thirty-two Normand cull cows (mean live weight 642 kg) at 54 ± 8.5 months of age were used in a 3×2 factorial design with three “feeding regimes” (FR) and two “pre-slaughter stress conditions” (PSC). During the finishing period, the cows received three different FR treatments: a diet of straw and concentrate (0.8 and 1.8 kg/day, respectively) supplemented with either a) lipid (40 g oil/kg diet DM), b) lipid + vitamin E (155 IU/kg diet DM), or c) lipid + vitamin E + plant extract (7 g/kg diet DM). The animals had ad libitum access to water. After the finishing period (100 days), the animals were slaughtered under either PSC: a) limited stress (n = 16) or b) physical and psychological stress (n = 16) (Bourguet et al., 2010). Within

15 min of slaughter, tissue samples were collected from two muscles: *M. longissimus thoracis et lumborum* (LT), a postural muscle essential for spinal support and stability, and superficial *M. semitendinosus* (ST), a key locomotor muscle involved in hind limb movement. The ST contains a greater proportion of fast oxido-glycolytic and fast glycolytic muscle fibres and a lower proportion of slow oxidative muscle fibres than the LT (Chriki et al., 2012). These samples were immediately flash-frozen in liquid nitrogen and stored at -80°C until proteomic analysis. After sample preparation (protein extraction and enzymatic digestion with trypsin), data were acquired using high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS). The ST and LT samples were analysed using a single procedure. The resulting data were then processed for protein identification using the Mascot search engine and relative label-free quantification (LFQ) using the Progenesis QI software. Data preprocessing (filtering, k-nearest neighbor imputation, log10 transformation, and Pareto scaling) and statistical analysis (two-way analysis of variance) were performed to identify Differentially Abundant Proteins (DAPs). The analysis was conducted using R packages, including tidyverse, UniprotR, MetaboAnalystR, car, ggplot2, and ggvenn. DAPs in the LT and ST muscles associated with PSC underwent additional filtering using fold change (FC). Subsequently, Metascape® (Zhou et al., 2019) was used for a comparative analysis of these DAPs in both muscle types to identify enriched biological processes within differentially abundant proteins.

Results

In total, 815 and 817 proteins were identified in the ST and LT muscles, respectively. FR influenced ($p<0.05$) the abundance of 160 and 66 proteins in ST and LT, respectively. PSC influenced ($p<0.05$) the abundance of 140 and 95 proteins in ST and LT, respectively. Eighteen and 42 of these proteins were influenced by an FR × PSC interaction in ST and LT, respectively (Fig 1 A & B). We present only the results of the subsequent analysis carried out on 122 (ST) and 53 (LT) proteins showing the main effect of PSC without interaction. Of these, 102 (ST) and 41 (LT) proteins showed FC> 1.2 or FC< -1.2 (Fig 1 C & D). Two proteins were affected by PSC in both LT and ST muscles, these being BLTP3B (Bridge-Like Lipid Transfer Protein Family Member 3B), and PAEP (progestagen associated endometrial protein), as shown in the Circos plot (Fig 2A), but the effect on BLTP3B was opposite in LT and ST. Metascape® revealed four common enrichment terms for PSC effect in LT and ST muscles; “GO:0019752: carboxylic acid metabolic process”, “R-HSA-71387: Metabolism of carbohydrates”, “GO:0006575: cellular modified amino acid metabolic process”, and “GO:0044283: small molecule biosynthetic process”. Several enrichment terms were identified specific to particular muscles. Twenty-four enrichment terms were only identified for ST muscles, whereas six enrichment terms were found to be specific to LT muscles (Fig 2B).

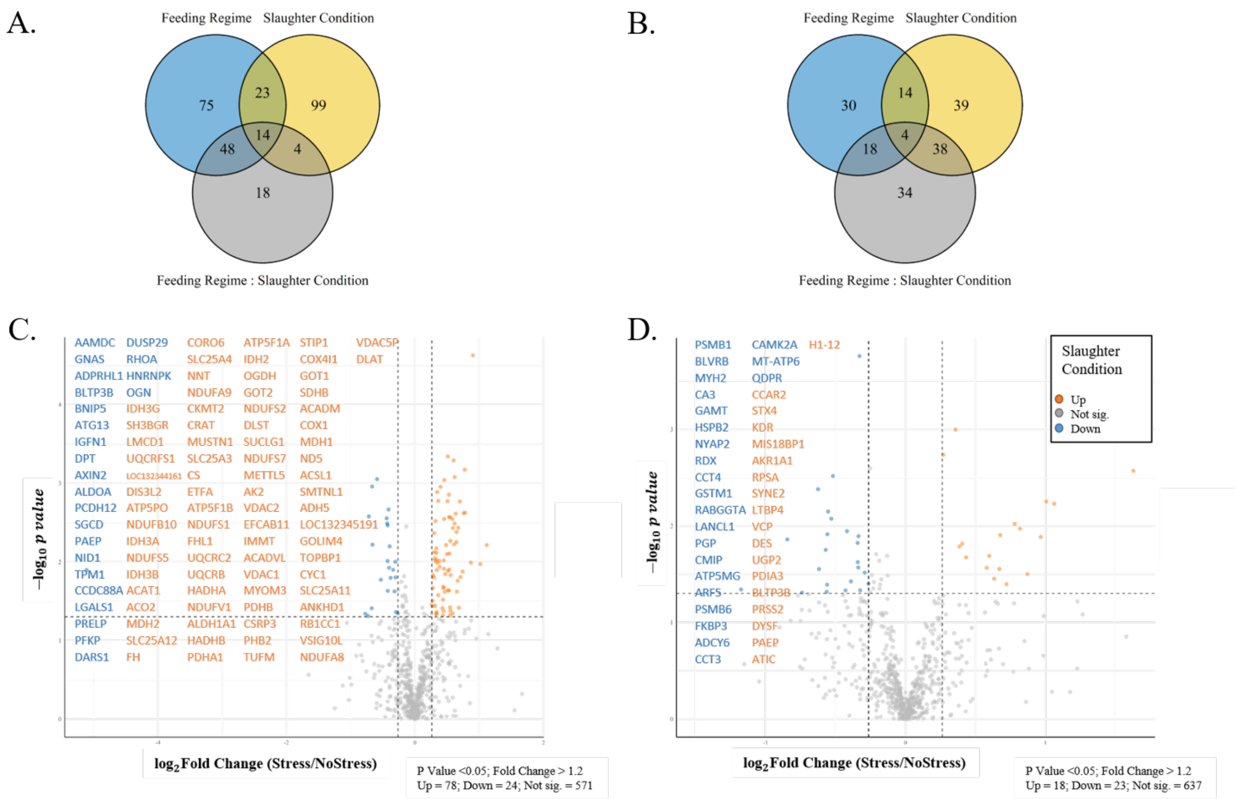

Figure 1. Venn diagrams illustrating the overlap in the number of differentially abundant proteins for the comparison of two treatments and their interaction in the A) ST and B) LT muscles; Volcano plots depicting log2 fold changes and p-values for the comparison of proteins in stressed *versus* non-stressed animals in C) ST and D) LT muscles

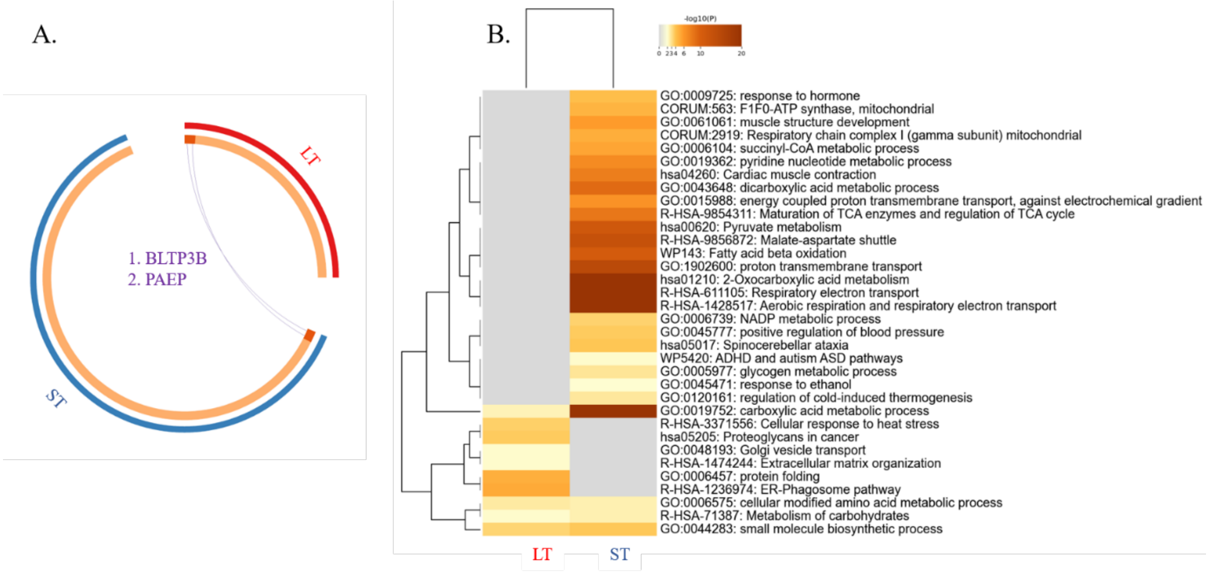

Figure 2. A) Circos plot illustrating overlap between proteins, and B) Heatmap representing all enriched terms across for LT *versus* ST muscles

Conclusions

PSC and FR affected a higher number of proteins in the ST than in the LT muscle (z-test for proportions: $p < 0.0001$), while the opposite trend was observed in transcriptomic data from the same experiment (Cassar-Malek et al., 2022). A number of biological processes were influenced by PSC in both muscles, particularly aerobic respiration, respiratory electron transport, and carboxylic acid metabolism. The ST muscle, however, exhibited a number of additional affected processes (24 vs 6 distinctive GO terms) relative to LT, suggesting a greater biological response to PSC in this muscle.

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010

Growth and carcass characteristics of late-maturing suckler steers genetically divergent for carcass fatness produced on grass-white clover or multi-species swards and finished at 19-, 23- or 26- months of age

S. Burke[1,2], E. O'Riordan[1], P. Crosson[1], A. K. Kelly[2], M. McGee[1]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland

Presenting author
Sarah Burke. E-mail: sarah.burke@teagasc.ie

Application

Identifying late-maturing suckler steers with greater genetic potential for carcass fat deposition can help achieve a market acceptable fat score at an earlier age when produced on grass-white clover or multi-species swards.

Introduction

Reducing cattle finishing age is a means of mitigating greenhouse gas emissions from beef production (Taylor et al., 2020); however, achieving an adequate carcass fat score (≥6.0, 15-point scale) on grass-based systems at a young age is challenging, particularly for late-maturing breed types, which predominate in Ireland (Regan et al., 2018). Exploiting within-breed genetic variance in carcass fatness may be a means of identifying more suitable genotypes within late-maturing breeds (Drennan and McGee, 2008). The inclusion of herbs (e.g. chicory/plantain) in grass-white clover swards may be a strategy to further improve animal growth (and thus fatness), herbage productivity, as well as mitigate environmental impacts from grass-based beef production (Sheridan et al., 2022). The objective of this study was to compare growth and carcass characteristics of late-maturing suckler steers genetically divergent for carcass fatness, produced on grass-white clover and multi-species sward systems and finished at 19-, 23- or 26-months of age.

Materials and methods

Over two weanling-to-beef production system cycles, weaned Charolais crossbred suckler steers genetically divergent in carcass fat score (Estimated Breeding Value; Lean-EBV, top 5% vs. fat-EBV, bottom 25%) were randomly assigned to one of two pasture types; 1). Perennial ryegrass-white clover (GC) and 2) multi-species (MS, perennial ryegrass, white and red clover, chicory and plantain) and finished at 19-, 23-, or 26-months of age (FA). The EBV for carcass fat score (scale 1-15) for the lean-EBV and fat-EBV steers were -0.55 and 0.14 in production cycle 1, and -0.58 and 0.17 in production cycle 2, respectively. During their first winter, steers were offered silage *ad libitum* and 1.5 kg/animal daily of a barley-based concentrate, following which they were turned-out to pasture in spring. The 19-FA were finished at the end of the second grazing season. For the second winter, steers were offered their respective silages *ad libitum* and the 23-FA and 26-FA groups received 5 kg and 1 kg concentrates/animal daily, respectively. The 23-FA were finished at the end of the second winter, while the 26-FA underwent a short (~ 2 months) third grazing season. Steer live weight was determined regularly and carcasses were weighed and graded for conformation and fat score. Data for each production cycle were analysed separately using linear models. The statistical model contained the fixed effects of pasture type, carcass fat-EBV, slaughter age and their interactions.

Results

For both production cycles, fat-EBV steers had a greater carcass fat score, lower kill-out proportion and similar carcass weight compared to lean-EBV steers (Table 1). There was no effect of pasture type on any of the carcass traits measured, expect for carcass fat score in production cycle 1 where MS had a lower fat score than GC (Table 1). For production cycle 1, 19-FA had a lower final and carcass weight compared to 23-FA and 26-FA, which did not differ (Table 1). Carcass fat score was lowest for 19-FA and greatest for 26-FA. For production cycle 2, 19-FA had the lightest final weight, 23-FA was heavier, followed by 26-FA, which was heaviest. Carcass weight was lighter for 19-FA compared to 23-FA and 26-FA, which did not differ. Kill-out proportion was greatest for 23-FA, lowest for 19-FA, with 26-FA being intermediate. Carcass fat score and conformation score was lower for 19-FA compared to 23-FA and 26-FA, which did not differ.

Table 1. Effect of EBV for carcass fatness, pasture type and finishing age on carcass traits at slaughter for production cycle one and two

	Carcass fat-EBV		Pasture type (PT)		Finishing age (mths) (FA)			SEM	P-value		
	Lean	Fat	GC	MS	19	23	26		EBV	PT	FA
Production cycle 1											
Final wt. (kg)	668	680	671	676	616^{b}	702^{a}	704^{a}	7.9	NS	NS	***
Carcass wt. (kg)	388	382	384	386	348^{b}	401^{a}	406^{a}	4.7	NS	NS	***
Kill-out proportion (g/kg)	582	561	572	571	566	571	577	3.4	***	NS	NS
Conformation score (1-15)*	9.3	8.6	8.9	8.9	8.7	9.2	8.9	0.22	**	NS	NS
Fat score (1-15)	6.6	8.4	8.0	6.9	6.3^{c}	7.6^{b}	8.6^{a}	0.22	***	***	***
Production cycle 2											
Final wt. (kg)	599	617	606	610	509^{c}	636^{b}	678^{a}	8.4	NS	NS	***
Carcass wt. (kg)	330	327	329	329	269^{b}	351^{a}	366^{a}	5.2	NS	NS	***
Kill-out proportion (g/kg)	551	529	542	538	528^{b}	551^{a}	541^{ab}	3.9	***	NS	***
Conformation score (1-15)	8.7	7.4	8.3	7.9	7.1^{b}	8.6^{a}	8.5^{a}	0.25	***	NS	***
Fat score (1-15)	4.0	6.2	4.9	5.3	2.8^{b}	6.0^{a}	6.5^{a}	0.26	***	NS	***

* Carcass fat-EBV × pasture type × FA interaction

Conclusion

Fat-EBV steers achieved greater carcass fat scores and a similar carcass weight compared to lean-EBV steers. Pasture type had little effect on carcass traits except for production cycle 1 whereby MS had a lower fat score than GC. Carcass weight, fat and conformation scores were lower for 19-FA than 23-FA and 26-FA.

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Advancing meat product quality prediction with spectral imaging of beef short-rib surfaces

J. Mishra[1,2], A. Ferragina[1,3], S. Hegarty[2], C. Esquerre[1], R. M. Hamill[1]
[1]*Food Quality and Sensory Science Department, Teagasc, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*Centre for Advanced Photonics and Process Analysis, Munster Technological University, Cork City T12 VN56, Ireland*
[3]*Department of Veterinary Science, University of Parma, Str. dell'Università, 12, 43121 Parma PR, Italy*

Presenting author
Jyoti Mishra. E-mail: jyoti.mishra@teagasc.ie

Application

Hyperspectral imaging has relevance to online prediction of rib product quality within meat management systems.

Introduction

The balance of fat and lean in the product is an important parameter of short rib product quality for certain markets. However, sortation for customer specification based on the fat content of rib products is normally deferred until the product is finally prepared, because EUROP grade is not considered informative on the variability of short rib product quality. In order to optimise carcass utilisation in the meat factory, a machine vision system to provide rapid non-invasive prediction of product quality would be advantageous. As compared with video image analysis, hyperspectral imaging (HSI) combines both spatial and spectral information, and has shown potential for meat quality prediction on the cut face of premium cuts (Hamill et al., 2024; Konda Naganathan et al., 2015). X-ray imaging technologies permits segmentation of the fat, muscle and bone through computed tomography (CT) scanning and has been used as reference data in carcass composition studies (Gardner et al., 2018). This research aims to evaluate the effectiveness of hyperspectral surface information in predicting CT-estimated fat % in short-rib products. The study includes development and comparison of several models trained with EUROP class, carcass weight and hyperspectral data or combinations.

Materials and Methods

Beef short-ribs (98) from bovine carcass (right side) sides at 24 hours post-mortem, representing a range of categories (young bulls, cows, steers and heifers), fat scores (2, 3, 4), conformation classes (E+, E=, U, R, O, P+), and side weights (122 - 297 kg) were collected. The short-ribs were scanned using a CT scanner (Aquilion Lightning, Canon Medical Systems Corporation) with a voxel resolution of 0.526 × 0.526 × 0.88 mm^3. Scan data were exported to 3D slicer in DICOM format for further analysis, including volume rendering, segmentation, and volume estimation for individual tissue components of interest (bone, lean, fat). The segmentation of voxels based on Hounsfield Unit (HU) barriers were established by analysis of CT scan of isolated fat, lean and bone tissues. The associated HU ranges were -227 to -47 for fat, 24.8 to131.8 for lean, >287 for bones, following modified approach of Gardner et al. (2018). Following exclusion of bone voxels, fat-associated % volume for each short-rib was estimated with respect to the combined volume of fat- and lean-associated voxels.

Hyperspectral images of each shortrib dorsal surface were recorded in reflectance mode (wavelength range: 900–1700 nm; spectral resolution: 5 nm) and the average spectrum was used for model calibration. The dataset was divided into training and test sets in an 80:20 ratio. Four Partial Least Squares (PLS) regression models were calibrated on the training set to model the fat percentage (Y variable) using different combinations of predictor variables (X variables): Model 1 included EUROP class; Model 2 included EUROP class and carcass weight; Model 3 was built on average spectra; and Model 4 included EUROP class, carcass weight, and spectra. The models were assessed on the test set by examining the coefficient of determination (R^2) and root mean square error of prediction (RMSEP).

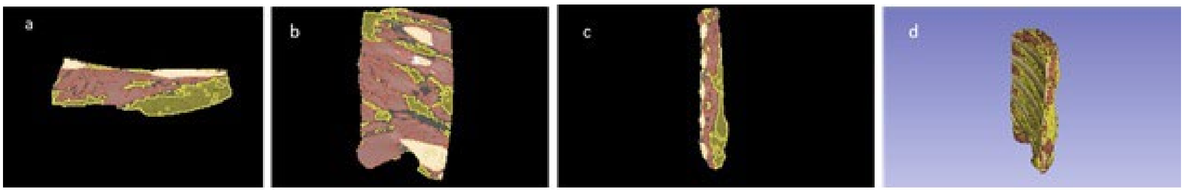

Figure 1. Segmentation of CT image for bone, fat and muscle a) axial b) coronal c) sagittal d) 3 dimensional view

Results

The CT-estimated fat percentage ranged from 11 to 51% with a mean of 30.4% and coefficient of variation of 27.3%, which implies that the short-ribs included in the model development show good variability.

Table 1. PLS fitting statistics of CT derived fat percentage prediction obtained for each model

PLS Models	Predictors	Prediction	R^2	RMSE
Model 1	EUROP class	CT fat %	0.24	7.50

Model 2	EUROP class + Carcass weight	CT fat %	0.21	7.68
Model 3	Average Spectra	CT fat %	0.86	3.23
Model 4	EUROP class + Carcass weight + Average Spectra	CT fat %	0.84	3.47

Models 1 and 2 developed using EUROP class and EUROP class combined with carcass weight showed relatively low prediction accuracy on the test set with R^2 below 0.3 and RMSEP greater than 7. Inclusion of the spectral data greatly improved the accuracy of the models (R^2 > 0.8, RMSEP reduced to <4), but it was noteworthy that inclusion of EUROP class and carcass weight did not improve the accuracy of the model achieved using the average spectral data alone.

Conclusions

Hyperspectral imaging of the short-rib surface provides an improved accuracy of prediction of rib product quality compared to EUROP class and carcass weight, suggesting the potential for improved meat sortation tools with machine vision approaches.

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O12

Developing chemometric models to classify eating quality of beef using algorithms produced by Rapid Evaporative Ionisation Mass Spectrometry (REIMS) and consumer sensory scores

L.Perkins[1], W. Jia[1], A. Neveu[2], T. Rowe[3], R. Polkinghorne[2], P. Nicholas-Davies[4], A. Koidis[1], N. Scollan[1]

[1]Institute for Global Food Security, Queen's University Belfast, 19 Chlorine Gardens, Belfast BT9 5DL, UK

[2]Birkenwood Pty Ltd, NSW, 2338 Australia

[3]Castell Howell Foods Ltd and Celtica Foods Ltd, Myrtle Hill, Pensarn, Carmarthen SA31 2NG, UK

[4]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Plas Gogerddan, Aberystwyth SY23 3EB, UK

Presenting author

Lynda Perkins. E-mail: l.perkins@qub.ac.uk

Application

REIMS output can successfully detect and categorise unique chemical fingerprints related to muscle, maturation, season and sensory quality of beef. Rapid evaluation of beef is valuable to industry as an objective method of classification. REIMS research highlights the potential for the implementation of an automated online method of assessing meat quality traits, the potential to forecast product demand and the opportunity for branding and marketing of products.

Introduction

Rapid Evaporative Ionisation Mass Spectrometry (REIMS) is a rapid ambient analytical technique without the need for sample preparation. Applications of REIMS include determination of biological tissue, food authenticity and more recently meat quality (Robson et al, 2022; Liu et al, 2024). Common traits used in evaluating sensory quality of red meat include tenderness, juiciness, flavour, and overall liking which are directly influenced by animal age, breed, sex and diet. Determination of beef muscle, maturation and season as well as discrimination of sensory eating quality using consumer taste panels and REIMS data are currently unknown. The aim of this study was to use REIMS and advanced chemometric modelling to uniquely connect REIMS data from four raw beef muscles, maturation, season and consumer sensory scores.

Materials and Methods

Welsh Beef samples (N=149) from four muscles; Tenderloin (TDR), Striploin (STR), Eye Round (EYE) and Feather blade (OYS), were collected from 31 carcasses across two seasons (summer and winter) after 7- or 21-day maturation post-slaughter. Consumer taste panels were conducted which evaluated tenderness, juiciness, flavour and overall liking. Beef samples were burned using a monopolar electrosurgical “iKnife” attached to the REIMS system (Waters Corporation, Wilmslow, UK) and a Xevo G2-XS QTof Mass spectrometer (Waters Corporation, Wilmslow, UK). Data were performed in sensitivity mode with continuum data acquisition in negative ionisation mode REIMS data was extracted using the Abstract Model Builder software and analysed using Simca 18 software. Muscle was classified into TDR, STR, EYE and OYS, while maturation and season was classified into 7- and 21-days and summer and winter. Consumer sensory scores ranged between 0-100 and classified meat quality into low (0-46), medium low (47-64), medium high (65-76) and high (78-100). MQ4 which represents the weighted combination of consumer scores of the four sensory attributes (tenderness, juiciness, flavour and overall satisfaction) was additionally classed into accepted (>40) and not accepted (<40). Models were calibrated, cross-validated and validated using an 80/20 calibration/validation training sample set.

Results

REIMS output and consumer sensory analysis successfully discriminated four beef muscles, in addition to 7- and 21-day maturation and season with an overall LDA model accuracy of 82, 100 and 96%, respectively. The most robust model for tenderness, flavour and juiciness were SVM where an overall model accuracy of 46, 39 and 43% was reported, respectively (Table 1). Similarly, the MQ4 SVM model classified for accepted or not accepted was reported at 85%. The most robust models for satisfaction, overall-liking and MQ4 (four rule classification) were LDA where an overall model accuracy of 45, 46 and 63%, respectively.

Table 1. Model accuracy for beef sensory traits according to class and model type using REIMS output

Trait	Model	Class	Accuracy (%)	Overall accuracy (%)	Accuracy (%)	Overall accuracy (%)	Accuracy (%)	Overall model accuracy (%)
			Calibration		Cross-validation		Validation	
Tenderness	SVM	L	100	100	48	48	72	46
	SVM	M-L	100		48		70	
	SVM	M-H	100		48		72	
	SVM	H	100		48		78	
Flavour	SVM	L	100	100	76	76	72	39
	SVM	M-L	100		76		66	
	SVM	M-H	100		76		68	
	SVM	H	100		76		72	
Juiciness	SVM	L	100	100	45	45	78	43
	SVM	M-L	100		45		66	
	SVM	M-H	100		45		67	
	SVM	H	100		45		76	
Satisfaction	LDA	L	100	100	39	39	75	45
	LDA	M-L	100		39		64	
	LDA	M-H	100		39		72	
	LDA	H	100		39		78	
Overall liking	LDA	L	100	100	34	34	76	46
	LDA	M-L	100		34		71	
	LDA	M-H	100		34		71	
	LDA	H	100		34		74	
MQ4	LDA	L	100	100	48	48	73	63
	LDA	M-L	100		48		96	
	LDA	M-H	100		48		75	
	LDA	H	100		48		81	
MQ4	SVM	A	100	100	90	90	85	85
	SVM	NA	100		90		85	

L - low
M-L – medium low
M-H – medium high
H – high
A – accepted
NA – not accepted

Conclusions

REIMS can detect and categorise chemical information related to muscle, maturation, season and sensory meat quality. Model optimisation to reach a minimum of 70% overall accuracy is required. This study has identified the potential of REIMS as a complementary tool for evaluating meat quality, offering a fast, accurate, objective, and cost-effective method for the meat industry to ensure product safety, authenticity and quality.

Acknowledgements

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013

Sperm head morphology and fertility: insights from high-throughput nuclear analysis of boar semen

J. Perry[1], A. Mandawala[2], B.Skinner[1], G. Walling[3], S. Harvey[4], K. Harvey[5]
[1]University Of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK
[2]Canterbury Christ Church University, N Holmes Rd, Canterbury CT1 1QU, UK
[3]Topigs Norsvin Research Center, Meerendonkweg 25, 5216 TZ 's-Hertogenbosch, Netherlands
[4]University of Greenwich (Faculty of Engineering and Science), Medway Campus, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK
[5]The Open University (School of Life, Health and Chemical Sciences), Walton Hall, Kents Hill, Milton Keynes MK7 6AA, UK

Presenting author

Joseph Perry. E-mail: jp19193@essex.ac.uk

Application

Artificial insemination is commonplace in commercial pig breeding, and as such, ensuring sperm sample quality is important to avoid reduced farrowing rates and litter sizes. Semen sample quality control often includes tests of sperm motility and sperm morphology using computer assisted semen analysis (CASA) systems that calculate semen parameters from microscopy data. While motility is well studied, it is harder to detect variation in morphology; most analysis considers overall sperm width and height, area, and presence of

vacuoles, acrosomes or tail abnormalities. Here, we demonstrate the use of high-throughput nuclear morphometric analysis of boar sperm to identify subtle sperm shape phenotypes, demonstrating progressive abnormalities. The specific phenotypes identified suggest aspects of spermiogenesis that may be disrupted in the production of these sperm and indicate future avenues to investigate the functional impacts of genetics and environment on pig sperm quality.

Introduction

Sperm shape is known to play a role in male fertility, affecting fertilisation rates (e.g Coetzee et al. 1998). However, since sperm are asymmetric cells, detailed shape analysis requires correct orientation and identification of key landmarks, which has limited the scale of most studies. Studies in pigs have been limited, finding some association between sperm morphology and litter size ((Barquero et al., 2021), but more detailed studies are needed. We previously developed high-throughput image analysis methods for mouse sperm (Skinner, 2022; Skinner et al., 2019), enabling automated recognition and morphological analysis of asymmetric cell nuclei within an image. Since the majority of a sperm head is taken up by the nucleus, we can examine nuclear shape as a proxy for overall sperm head shape using nuclear stains to generate images amenable to automated image analysis. Here, we investigate the use of this method for characterising boar semen samples.

Materials and Methods

94 boar sperm samples were imaged in this study. Fresh ejaculates were collected by JSR Genetics Ltd, fixed with 3:1 methanol:acetic acid, and nuclei stained with DAPI. Images were captured on an Olympus IX83 fluorescence microscope at 100x magnification with at least 200 nuclei per sample. Images were analysed in Nuclear Morphology Analysis (NMA) v2.2.1 (Skinner 2022) and data was further processed in R (v4.4.0). The analysis converts the outline of an object into linear profiles of internal angle, diameter and radius, allowing consistent detection of landmarks. Nuclei were oriented by the tail attachment region at the base, characterised by a ‘dimple’ in the nucleus. (Figure 1A, reference point 1). Cells with abnormal phenotypes were detected by identification of outlier cells in either of the angle, radius or diameter profiles.

Results

We analysed 21002 nuclei in total. We aggregated outlier nuclei with similar shape profiles to identify continuous phenotypes, iteratively grouping nuclei with the greatest differences to normal profiles. This amalgamation of outlier shapes yielded a normal phenotype group (85% of nuclei), intermediate phenotype group (13%) and extreme phenotype group (2%). Example images showing the progressive phenotypes from normal to extreme are shown in Figure 1. We found two main paths of abnormality: firstly, basal compression and failure to elongate (Figure 1A; 7.75%); secondly, basal compression and abaxial tail attachment (Figure 1C; 6.25%). We also saw within the intermediate phenotypes minor tapering and formation of spikes in the basal region (Figure 1B 1%). We looked at the frequency of abnormal sperm per sample via ordinal logistic regression, and saw no association with breed ($p>0.05$); however, some collection dates had higher frequencies of abnormal sperm ($p<0.001$).

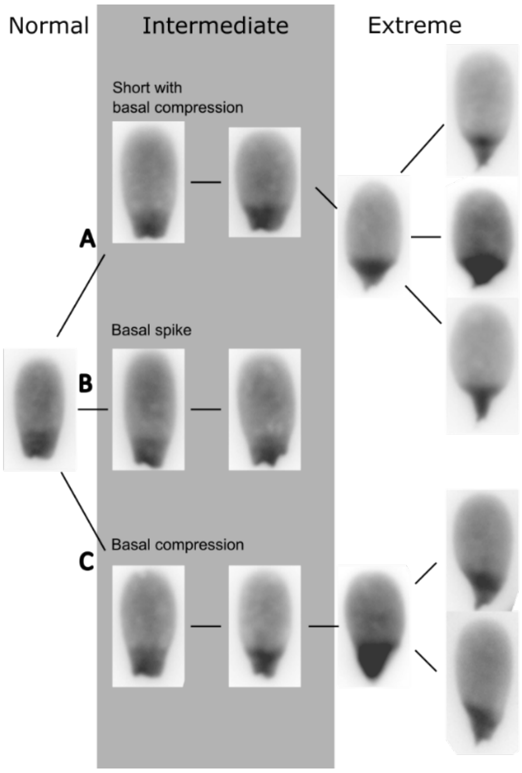

Figure 1. Example DAPI stained images of progressively abnormal sperm phenotypes. A) short progressing to compression around the base of the nucleus and a pyriform phenotype; B) extrusion of nuclear DNA forming a spike near the base of the nucleus with some tapering; C) abaxial compression around the base of the nucleus, leading to a pyriform phenotype

Conclusions

We demonstrate that high-throughput morphometric analysis of pig sperm nuclei can reveal morphological abnormalities that are not detected by conventional semen analysis. Our initial findings suggest breed may be less important than environment in ejaculate quality. Further study is needed to understand how these phenotypes develop, and the functional consequences for boar fertility.

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014

Assessing the surface temperature and thermal windows of newborn piglets undergoing routine procedures (teeth clipping, tail docking, and ear tagging) using infrared thermography

C. Mulvenna[1], A. Domínguez-Oliva[1,2], D. Mota-Rojas[2], I. Hernández-Ávalos[3], A. Jones[1], R. Muns[1]
[1]AFBI, 18a Newforge Lane, Belfast, Co Antrim BT9 5PX, UK
[2]Universidad Autónoma Metropolitana, Prolongación Canal de Miramontes 3855, México
[3]Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, CP 04510, México

Presenting author
Ramon Muns. E-mail: ramon.muns@afbini.gov.uk

Application

Infrared thermography can be used as a non-invasive tool to detect surface temperature changes of piglets

Introduction

Procedures such as Teeth clipping (TEE), tail docking (TAI), and ear tagging (TAG) are conducted routinely in commercial pig production units during the first days of a piglet's life. However, as these procedures are conducted without anesthetics, there is controversy surrounding these procedures due to the pain/stress they may cause. The assessment of vasomotor activity using non-invasive infrared thermography (IRT) is increasingly being promoted as a tool to detect acute stress (Ghezzi et al. 2024). Within this study, IRT was used to assess the surface temperature of new-born piglets before and after the aforementioned routine procedures and various thermal windows were compared.

Materials and methods

A total of 116 crossbred piglets (Duroc Danish x Landrace x Large white) from multiparous sows were randomly assigned to one of ten cross-over experimental groups of procedure combinations; 1: TEE, 2:(TEE+) TAI, 3:(TEE+TAI+) TAG, 4:TAI, 5:(TAI+) TEE, 6: (TAI+TEE+) TAG, 7:TAG, 8: (TAG+) TEE, 9: (TAG+TEE+) TAI, 10: All At Once (AAO), further descriptions of the groups an procedures are presented in Table 1.. Between 09:00 and 16:00 h, thermal images of four thermal windows (ocular (OCU), nose (NOSE), ear (EAR) and upper lip (LIP)) were collected using a high-resolution handheld infrared camera FLIR® model T650sc (FLIR Systems, Wilsonville, OR, USA) at an uniform distance of 1 m on the left side of the face. Images were taken before and after each individual procedure. GraphPad Prism 10.0.2 (San Diego, CA, USA) statistical software was used to analyze the obtained data. A linear mixed model for repeated measures was performed and Multiple comparisons were calculated using a post-hoc Tukey test.

Results

Results from the analysis comparing surface temperature before and after each procedure combination group and comparisons between procedure combination groups for each of the thermal windows are presented in Table 2. There was no significant difference in the ‘before’ surface temperature between any of the procedure combinations for any of the thermal windows assessed (OCU, EAR, NOSE, or LIP; P=0.99, respectively). This was also the case when the ‘after’ procedure surface temperatures of each of the four thermal windows were compared between procedure combination groups (OCU, EAR, NOSE, or LIP; P=0.99, respectively). When comparing temporal changes in surface temperatures within each procedure combination group, significant differences were observed in OCU and NOSE temperatures (Table 1). Specifically surface temperature of the OCU ‘before’ was significantly greater than ‘after’ in 2: (TEE+) TAI (0.57°C reduction, P<0.001), 6: (TAI+TEE+) TAG (0.65 °C reduction, P<0.001), 7:TAG (0.58°C reduction, P<0.001), 9: (TAG+TEE+) TAI (0.50°C reduction, P<0.001) and 10:AAO (0.75°C, P<0.001). For NOSE, significant reductions in the surface temperature before and after 2:(TEE+) TAI (0.77°C reduction, P<0.001), 7:TAG (1.07°C reduction, P<0.05) and 10:AAO (2.08°C reduction, P<0.001) were observed.

Table 1. Description of each of the experimental procedure groups.

Experimental group number	Procedure(s)	Description
1	**TEE**	Teeth clipping first procedure performed on the piglet
2	**(TEE+) TAI**	After a period of recovery* following teeth clipping, piglet tail docked
3	**(TEE+TAI+) TAG**	After a period of recovery following teeth clipping & tail docking, piglet ear tagged
4	**TAI**	Tail docking first procedure performed on the piglet
5	**(TAI+) TEE**	After a period of recovery following tail docking, piglet teeth clipped
6	**(TAI+TEE+) TAG**	After a period of recovery following tail docking & teeth clipping, piglet ear tagged
7	**TAG**	Ear tagging first procedure performed on the piglet
8	**(TAG+) TEE**	After a period of recovery following ear tagging, piglet teeth clipped
9	**(TAG+TEE+) TAI**	After a period of recovery following ear tagging & teeth clipping, piglet tail docked
10	**AAO**	TEE, TAI, and TAG were performed consecutively without any recovery time allowed in between procedures.

*A recovery period (12±2.5 min) was allowed between procedures

Table 2. Results of comparisons between the mean (±SD) surface temperatures of each thermal window before and after each procedure combination group

Thermal window	Treatment	Before	After	P-value
OCU	TEE	34.45 ± 0.55	34.27 ± 0.45	0.99
	(TEE+) TAI	35.48 ± 0.18	34.91 ± 0.16	0.0005
	(TEE+TAI+) TAG	34.15 ± 0.59	34.44 ± 0.16	0.99
	TAI	34.98 ± 0.16	34.74 ± 0.15	0.99
	(TAI+) TEE	34.63 ± 0.41	34.91 ± 0.13	0.99
	(TAI+TEE+) TAG	35.34 ± 0.14	34.69 ± 0.12	0.0003
	TAG	35.02 ± 0.18	34.44 ± 0.14	0.01
	(TAG+) TEE	35.39 ± 0.19	34.96 ± 0.11	0.99
	(TAG+TEE+) TAI	35.07 ± 0.19	34.77 ± 0.10	0.002
	AAO	34.93 ± 0.12	34.18 ± 0.11	0.0001
	P-value	0.99	0.99	
EAR	TEE	35.93 ± 0.62	35.26 ± 0.75	0.99
	(TEE+) TAI	36.61 ± 0.23	36.47 ± 0.25	0.99
	(TEE+TAI+) TAG	35.54 ± 0.15	35.57 ± 0.16	0.99

	TAI	36.28 ± 0.21	35.97 ± 0.22	0.99
	(TAI+) TEE	35.77 ± 0.19	36.16 ± 0.24	0.99
	(TAI+TEE+) TAG	35.68 ± 0.25	35.75 ± 0.16	0.99
	TAG	35.71 ± 0.26	35.78 ± 0.18	0.99
	(TAG+) TEE	36.52 ± 0.17	36.41 ± 0.15	0.99
	(TAG+TEE+) TAI	35.50 ± 0.25	36.32 ± 0.18	0.99
	AAO	36.38 ± 0.15	35.93 ± 0.16	0.99
	P-value	0.99	0.99	
NOSE	TEE	28.88 ± 0.58	28.61 ± 0.53	0.99
	(TEE+) TAI	31.11 ± 0.73	30.34 ± 0.66	0.0001
	(TEE+TAI+) TAG	29.62 ± 0.61	28.15 ± 0.68	0.99
	TAI	28.85 ± 0.55	27.71 ± 0.52	0.99
	(TAI+) TEE	28.56 ± 0.59	27.40 ± 0.45	0.99
	(TAI+TEE+) TAG	30.19 ± 0.59	29.45 ± 0.53	0.99
	TAG	28.49 ± 0.75	27.42 ± 0.62	0.006
	(TAG+) TEE	29.59 ± 0.79	29.45 ± 0.73	0.99
	(TAG+TEE+) TAI	29.15 ± 0.80	28.42 ± 0.64	0.99
	AAO	29.91 ± 0.47	27.83 ± 0.32	0.0005
	P-value	0.99	0.99	
LIP	TEE	32.83 ± 0.31	31.43 ± 0.66	0.99
	(TEE+) TAI	31.88 ± 1.35	33.11 ± 0.46	0.99
	(TEE+TAI+) TAG	32.55 ± 0.53	32.11 ± 0.51	0.99
	TAI	32.52 ± 0.34	32.39 ± 0.37	0.99
	(TAI+) TEE	32.18 ± 0.33	32.23 ± 0.33	0.99
	(TAI+TEE+) TAG	32.80 ± 0.41	32.61 ± 0.36	0.99
	TAG	31.57 ± 0.61	31.57 ± 0.38	0.99
	(TAG+) TEE	33.33 ± 0.45	32.82 ± 0.38	0.99
	(TAG+TEE+) TAI	31.92 ± 0.48	31.85 ± 0.36	0.99
	AAO	32.91 ± 0.24	31.64 ± 0.28	0.99
	P-value	0.99	0.99	

Discussion

From the thermal windows assessed, OCU and NOSE showed potential in their use to assess surface temperature changes in newborn piglets. According to the rapid drop in temperature observed in OCU and NOSE windows it could be suggested that TAG and TAI appear to trigger a more marked thermal change in piglets than TEE. This temperature change may be a result of a higher sympathetic nervous system response to a stressor, as cutaneous capillary blood flow shifts due to transient peripheral vasoconstriction. However, this would merit further investigation. This temperature change observed is further magnified when procedures are performed all at once (AAO) which suggests a period of rest between procedures to allow piglets to recover or adjust may be beneficial. However, to be sure if these changes in temperature are a result of an acute stress response, further comparison with simultaneous, direct physiological stress measures (e.g. cortisol) is required.

Conclusions

Infrared thermography shows potential as a tool to assess the reaction of newborn piglets to harmful stimuli, potentially linked to a stress response, via detection of surface temperature change. Allowing a period of rest between procedures may be beneficial in terms of reducing distress experienced by newborn piglets, however, confirmation with simultaneous, direct measures of stress is required to validate or confirm this.

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015

Optimum post-weaning dietary regime for weaner pigs was unaffected by level of pre-weaning creep consumption

S. Icely[1], A. Mackenzie[1], S. Mansbridge[1], A. Stewart[1]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*

Presenting author
Sarah Icely. E-mail: sicely@harper-adams.ac.uk

Application

The financial cost of post-weaning piglet diets could be reduced by feeding lower specification and lower quality diets, in healthy pigs. Lower FCR immediately post-weaning of pigs with high pre-weaning creep consumption demonstrates the importance of maximising creep intake.

Introduction

High-quality diets are commonly fed to pigs during the initial period post-weaning to ease the transition from sows' milk to solid feed, but they are expensive (Muns and Magowan, 2018). They contain a high proportion of highly digestible protein from animal sources, such as fishmeal and milk powder, and lower proportion of soya, which may have inflammatory effects on the intestine. Due to the zinc oxide ban, reduction in crude protein level is utilised as a strategy to reduce the risk of post-weaning diarrhoea resulting from microbial fermentation of excess undigested protein in the small intestine (Batson *et al.,* 2021), but risks under-supplying protein. The aim of this study was to determine whether level of pre-weaning creep consumption affected post-weaning performance when pigs were fed diets with differing lysine levels (specification) and diet quality.

Materials and Methods

Piglets from 56 litters equally spread between two batches farrowing six week apart were utilised. Piglets had *ad libitum* access to standard commercial creep from 14 days prior to weaning (wn-14), until weaning. Creep was provided through a single-space hopper, and consumption was quantified using the PigTrack® system (Asserva, France). The electronic ID (EID) tag of the piglet and duration of visit was recorded by an antenna each time it visited the creep feeder. Piglets were individually weighed on wn-7 and wn-1, and a creep score calculated as duration/ADG, designed to differentiate the contribution of creep to the individual piglet's nutrient intake. Piglets were ranked by creep score into low (first quartile; CL) and high (third quartile; CH), then allocated a post-weaning two-stage dietary regime of low or high specification (SL, SH) and low or high quality (QL, QH). Specification was differentiated by lysine level, with SL conforming to minimum requirements as recommended by Whittemore *et al.* (2003) and SH had 1.5 g/kg higher lysine for each stage. Amino acid balance relative to lysine was maintained across diets. Low quality diets had a lower proportion of milk protein, cooked cereals and fishmeal, but higher proportion of raw cereals and soya. The SLQH regime was considered as equivalent to an industry-standard commercial regime. After day 22, a common grower diet was fed to all pigs to day 54 (nursery exit). At weaning, pens of six pigs were established, with variation in average pig weight minimised between pens, but difference in weight due to pre-weaning creep consumption was maintained. Pigs were weighed and pen feed intake recorded on day 3, 6, 9, 12, 15, 22, 36 and 54. Pen performance from weaning to day 54 post-weaning was analysed by ANOVA (Genstat 23rd edition, UK). Where a repeated measures model was utilised, deviance and Akaike Information Criterion were utilised to select the most appropriate model. Significant differences between treatments were determined by *post-hoc* Bonferroni test.

Results

Weaning weight of pigs classified as CH (8.97 kg) was lower than CL (9.27 kg, $P <0.001$, s.e.m =0.011), but there was no difference in weight at nursery exit on day 54 (43.3 kg, $P =0.899$, s.e.m =0.38). For CH pigs, ADFI and ADG were higher from weaning until day 12 post-weaning, and FCR was lower from weaning until day 9 post-weaning ($P <0.05$), than CL pigs. There was no three-way interaction on any performance parameters measured ($P >0.05$) to day 22 (end of experimental diets) or 54 post-weaning. Pigs fed SLQH were lightest at day 22 ($P =0.003$; Figure 1), and day 54 ($P =0.025$).

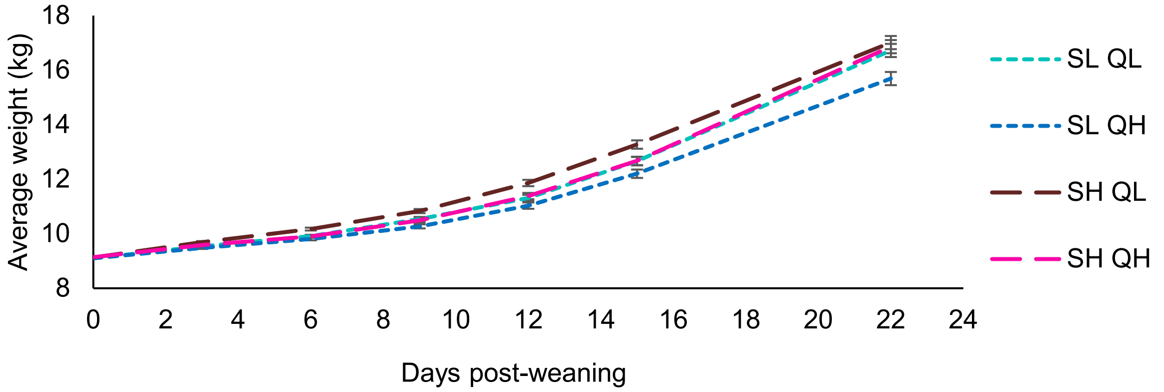

Figure 1. Effect of low (L) or high (H) diet specification (S)*diet quality (Q) on pig weight from weaning-day 22 post-weaning. Error bars denote pooled s.e.m, antedependence order 1 with additional uniform correlations within subject

Pigs fed SLQH had highest FCR and lowest ADG from weaning-day 9 and day 9-22 (Table 1).

Table 1. Effect of low (L) or high (H) diet specification (S) and diet quality (Q) on post-weaning performance of pigs

	SLQL	SLQH	SHQL	SHQH	s.e.m	*P*-value
ADFI wean-d9 (kg/d)	0.22	0.21	0.24	0.22	0.009	0.149
ADFI d9-22 (kg/d)	0.60	0.56	0.59	0.58	0.012	0.205
ADG wean-d9 (g/d)	160^{ab}	129^{a}	189^{b}	153^{ab}	12.1	0.011
ADG d9-22 (g/d)	476^{a}	420^{b}	485^{a}	475^{a}	16.9	0.037
FCR wean-d9	1.50^{ab}	1.81^{a}	1.30^{b}	1.60^{ab}	0.111	0.017
FCR d9-22	1.27^{ab}	1.37^{a}	1.23^{b}	1.24^{ab}	0.036	0.036

Superscripts denote significance at $P <0.05$.

The optimum regime in terms of cost of feed/kg pig post-weaning weight gain was SLQL.

Conclusions

Piglets classified as CH were probably lighter at weaning due to lower sow milk consumption than CL. Post-weaning dietary cost could be reduced by feeding lower quality diets with no adverse effects on nursery performance. There was no performance benefit to feeding diets with higher lysine levels.

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016

Effect of weaner pen hygiene, floor space allowance and feeder space allowance on post-weaning pig growth

J. Glynn[1,2], P. G. Lawlor[1], G. E. Gardiner[2]
[1]*Teagasc, Pig Development Department, Animal & Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*Eco Innovation Research Centre, Department of Science, South East, Technological University, Kilkenny Road, Carlow, Co. Carlow R93 V960, Ireland*

Presenting author
Joe Glynn. E-mail: joe.glynn@teagasc.ie

Application

Similar growth performance is achieved at both low and high floor space allowances in pens with optimal hygiene; however, increasing floor space allowance will improve growth and feed efficiency in pens with sub-standard hygiene. Providing an additional feeder space will improve feed efficiency of pigs when both low and high floor space allowance is provided.

Introduction

Management factors that can influence post-weaning (PW) pig growth include pen hygiene (Halpin *et al.*, 2024) and floor space and feeder space allowance (Wolter *et al.*, 2003). However, it is likely that the growth responses to some of these factors will be affected by the others. The hypothesis was that increasing pen hygiene (sub-standard to optimal), floor space allowance (0.35m^2/pig to 0.70m^2/pig) and feeder space allowance (single to double) will each increase post-weaning pig growth to day (D)45 PW and that there will be interactions in effect between each. This hypothesis will be tested in a 2x2x2 factorial arrangement.

Materials and methods

The experiment was a 2x2x2 factorial arrangement with factors being weaner pen hygiene (sub-standard vs optimal), floor space allowance (0.35m^2/pig or 0.70m^2/pig) and feeder space allowance (single or double). The optimal hygiene protocol was applied to vacated weaner pens as follows: 1. 1% carboxylic acid-based detergent (Blast Off) application allowing a contact time of ~40 minutes, 2. Washing with cold water, 3. Drying for 48h, 4. Chlorocresol-based disinfectant (Interkokask) applied at a rate of 3%, 5. Further drying for 12 days before introduction of weaned pigs. The sub-standard hygiene protocol was applied to vacated weaner pens as follows: 1. Washing with cold water, 2. Overnight drying (<18h) before entry of pigs. Prior to entry of pigs, pens from both hygiene treatments (N=24 sub-standard and N=20 optimal) were swabbed at three locations (piglet lying area, dunging area and feeder) using adenosine triphosphate (ATP) bioluminescence swabs. Single and double feeder space allowance was achieved by providing a single-spaced (width = 225mm) or a double-spaced (width = 400mm) wet-dry feeder per pen, respectively. A floor space allowance of 0.35m^2/pig or 0.70m^2/pig was achieved by stocking pens (2500 x 2000mm) with 14 and 7 pigs/pen, respectively. At weaning (28 ±1.2 days of age), pigs were formed into same-sex (entire male or female) pen groups of even weight. Pen groups were blocked on sex and weaning weight (8.7±1.5 kg) and randomly assigned to treatment (N=12 pen replicates per treatment). Pigs were fed a common sequence of diets until transfer at D45 PW (32.8±5.0kg). Pig weight and feed disappearance were determined at D6, 13, 20, 27 and 45 PW to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Data were analysed using repeated measures analysis in PROC MIXED (v9.4, SAS Institute Inc.). The model included pen hygiene, floor space allowance, feeder space allowance and their associated interactions as fixed effects with block included as a random effect and weaning weight included as a co-variate when significant.

Results

At entry of pigs to the weaner accommodation, ATP concentrations (log$_{10}$ [Relative Light Units]/cm^2) were 1.85 and 0.44, ±0.11 for the piglet lying area (*P*<0.001); 1.93 and 1.21, ±0.10 for the dunging area (*P*<0.001); and 1.04 and 0.63, ±0.09 for the feeders (*P*<0.001), for the sub-standard and optimal hygiene protocols, respectively. There was no pen hygiene x floor space allowance x feeder space allowance interaction (*P*>0.05) for any parameter of interest; therefore, only 2-way interactions were considered. There was a pen hygiene x floor space allowance interaction for body weight (BW), ADFI and ADG. Increasing floor space (0.70m^2/pig) with sub-standard hygiene increased BW at D45PW (*P*<0.001); ADFI from D27-45PW (*P*<0.05); ADG from D20-27 (*P*<0.05) and D27-45PW (*P*<0.05); and reduced FCR from D20-27PW (*P*<0.05) but not with optimal hygiene (*P*>0.05). There was a pen hygiene x feeder space interaction for FCR from D27-45PW. Increasing feeder space allowance under both sub-standard and optimal hygiene conditions reduced FCR (*P*<0.001) with increased feeder space giving better FCR with optimal than with sub-standard hygiene (*P*<0.05). There was an overall floor space allowance x feeder space allowance interaction for BW and ADG (Table 1). Increasing floor space allowance in pens with single-spaced feeders increased BW (*P*<0.001) and ADG (*P*=0.02) but this was not the case when pigs were fed from double-spaced feeders (*P*<0.05). Providing additional feeder space at both low or high floor space allowances reduced FCR from D27-45PW (*P*<0.02; Table 1).

Table 1. Effect of floor space and feeder space allowance on post-weaning pig growth performance.

Floor Space Allowance	0.35m²/pig		0.70m²/pig			P-value	
Feeder space allowance	Single	Double	Single	Double	SEM	Floor	Feeders
No: Pens	24	24	24	24			
BW[1] (kg)							
Overall (D0-45PW)[5]	16.9^{b}	17.4^{ab}	17.8^{a}	17.5^{ab}	0.21	0.001	0.47
ADFI[2] (g/d)							
Day 27-45PW	1156^{b}	1149^{b}	1258^{a}	1192^{ab}	23.5	0.001	0.10
ADG[3] (g/d)							
Overall (D0-45PW)	475^{b}	504^{a}	527^{a}	521^{a}	9.8	<0.001	0.11
FCR[4] g/g							
Day 20-27 PW	1.41^{a}	1.30^{b}	1.28^{b}	1.22^{b}	0.044	0.01	0.05
Day 27-45 PW	1.50^{a}	1.42^{b}	1.50^{a}	1.40^{b}	0.034	0.50	0.001

[1]BW= Body Weight [2]ADFI= Average Daily Feed Intake [3]ADG= Average Daily Gain [4]FCR= Feed Conversion Ratio [5]= PW= Post-weaning

Conclusion

Providing a double-spaced feeder reduced FCR from D27-45 PW in both sub-standard and optimal hygiene environments, with the greatest effect observed with optimal hygiene. Increasing floor space allowance improved growth and feed efficiency from D27-45 PW in pens of sub-standard hygiene but not in pens of optimal hygiene. Therefore, it is possible to achieve the same growth and feed efficiency at a floor space of 0.35m^2/pig or 0.70m^2/pig when pen hygiene is optimal. Providing an additional feeder space will reduce feed efficiency of pigs when both low and high floor space allowance is provided.

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017

Relationships between oral fluid biomarkers and ear necrosis in weaned pigs

L. Markland[1,2], K. O'Driscoll[1], F. Leonard[2], L. Boyle[1]
[1]Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]School of Veterinary Medicine, University College of Dublin, Belfield D04 C1P1, Dublin 4, Ireland

Presenting author
Lucy Markland. E-mail: lucy.markland@teagasc.ie

Application

Ear necrosis is a major welfare concern in Irish pigs. However, little is known about physiological alterations associated with the disease. This study investigated the use of oral fluid biomarkers to determine systemic alterations in markers of inflammation, stress and immune function in pigs with ear necrosis.

Introduction

Ear necrosis presents as scabbed and bleeding sections of the ear tip that vary in severity but which can progress to the stage that the ear structure is degraded (Richardson et al., 1984). Affected pigs clearly suffer from a health and welfare point of view but there is limited information on the associated physiological alterations and implications for pig performance. Some pigs may suffer from a failure to thrive and lameness (Papatsiros., 2011) and there is an association between ear necrosis in growing pigs and pericarditis at slaughter (Pessoa et al., 2021). This could be due to pathogens entering the lesions on the ear and causing secondary disease (Boyle et al., 2022). However, the mechanisms behind these relationships are unknown and it is not fully established if ear necrosis affects the pig systemically. Oral fluids can be used as a non-invasive method to measure internal biomarkers and assess the health and welfare of animals. This study investigated the differences in oral fluid biomarkers for stress, inflammation, and immune activation between pigs with and without ear necrosis. It was hypothesized that pigs with ear necrosis would have increased biomarker levels than pigs without ear necrosis.

Materials and Methods

Oral fluid samples were collected from weaner pigs (N = 61) across two farrowing batches at 9 weeks of age. Sampled pigs were healthy with no visual impairments other than the presence (n = 31) or absence (n = 30) of ear necrosis, and were balanced by sex. Pigs were further categorised based on ear necrosis severity: mild (n = 8), moderate (n = 8), and severe (n = 15). Samples were analysed for the following biomarkers: cortisol, alpha-amylase, and butyrylcholinesterase as indicators of stress, haptoglobin as an indicator of inflammation, adenosine deaminase and ferritin as indicators of immune activation, and creatine kinase as an indicator of general tissue damage. Ear necrosis category (none, mild, moderate, severe), and sex effects were investigated using separate linear models for each of the biomarkers. Pairwise comparisons between categories were determined using Tukey’s method.

Results

Levels of all biomarkers were similar between sexes (*P* > .05). The only biomarker that was statistically different between EN categories was haptoglobin. Since there were no differences between 'none' and 'mild' categories (*P* > .05), and 'moderate' and 'severe' categories (*P* > .05), the final model combined these categories. Haptoglobin was higher in both moderate and severe (6353.4 ± 5234.93 ng/mL) pigs compared to pigs with mild and no EN (2193.7 ± 2322.69; $F_{1,60}$ = 26.29, *P* < .0001). The other biomarkers showed no differences (*P* > .05).

Conclusions

These results indicate that both moderate and severe ear necrosis is associated with an inflammatory response. Further research on whether these inflammatory alterations can lead to secondary health and welfare impairments is warranted. A longitudinal study would also be of interest to determine the development and duration of the inflammatory response. There was a large variation between pigs, thus further research using a larger sample size and additional/alternative biomarkers to more precisely identify systemic responses to ear necrosis is warranted.

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018

A comparative study on the effect of feeding DevAmine technology products and traditional protein sources on the concentration of Myo-inositol in weaned piglets

M. Quinn[1], G. Brychkova[2], M. Scott[1], M. O'Connell[1]
[1]*Devenish Nutrition, 96 Duncrue Street, Belfast BT3 9AR, UK*
[2]*University of Galway, Galway H91 TK33, Ireland*

Presenting author
Michaela Quinn. E-Mail: michaela.quinn@devenish.com

Application

A nutritionally enhanced protein source (DevAmine) influences the synthesis of myo-inositol, a conditionally essential nutrient for optimal growth performance of weaned pigs.

Introduction

Myo-inositol is a naturally occurring sugar alcohol enzymatically synthesised from glucose (Su et al., 2023). In mammals, myo-inositol plays an essential role in many physiological processes, such as cell signaling, glucose homeostasis and osmolarity regulation (Kiani et al., 2021). Recent studies have shown that increasing concentrations of myo-inositol in pigs can improve growth performance, feed efficiency and regulate the integrity of the intestinal barrier (Moran et al., 2019, Ogunribido et al., 2022). DevAmine is manufactured by processing a mixture of proteins and amino acids with sugars under conditions of temperature and pressure to ensure all amino acids and sugars are released at a rate commensurate with efficient absorption and ultilisation. The sugar released from DevAmine could act as a precursor for myo-inositol synthesis. The objective of this study is to evaluate if diets containing different protein sources balanced for dietary sugar content affect the plasma concentration of myo-inositol in weaned pigs. The study focuses on weaned pigs as myo- inositol may be a conditionally essential nutrient in weaned pigs due to greater metabolic effects observed in this age group. The study balanced dietary sugar levels to ensure that any effect demonstrated on myo-inositol levels was due to the inclusion and release mechanism of DevAmine, rather than the addition of pure sugar sources.

Materials and Methods

Ninety-six piglets (14.5kg ± 0.35 s.e.m.) were fed a diet containing either vegetable protein (VP), synthetic amino acids (SYN), nutritionally enhanced protein product (DevAmine), or a combination of sources (COM) (Table 1).

Table 1. Proximate analysis of diets containing vegetable protein, synthetic amino acids, DevAmine or a combination of all three sources.

Nutrient	*Combination*	*Intact Protein*	*Synthetic amino acids*	*DevAmine*

Energy, DE MJ/Kg	15.4	15.7	15.4	15.4
Protein	19.6	24.8	19.6	19.6
SID Lysine, %	1.3	1.3	1.3	1.3
Sugar, %	10.4	10.2	10.4	10.6

Pigs were assigned to the dietary treatments based on weight, sex and dam. Blood samples were collected post-feeding and analysed using GC-MS with d27-myristic acid as an internal standard (Palazoglu, 2009). The myo-inositol concentration was normalised to the internal standard and reported as the mean normalised value. Each individual pig was considered a replicate, and results were analysed by one-way ANOVA, with statistical significance determined by P<0.05. The Tukey-Karmer test was used for post-hoc analysis to assess the significance between treatment groups.

Results

Plasma concentrations of myo-inositol were significantly increased when pigs received the DevAmine protein source compared to all other treatments (Table 2). The results indicate that a DevAmine-based diet will result in a 139-fold increase in myo-inositol compared to typical commercial diets.

Table 2. Normalised plasma Myo-inositol levels in weaned piglets fed diets containing either a combination or one of 3 protein sources (Intact protein, synthetic amino acids or DevAmine) at 360 minutes post-feeding.

	Combination	Intact Protein	Synthetic amino acids	DevAmine	S.E.M	P-Value
Myo-inositol, normalised	0.54[a]	0.06[a]	0.16[a]	22.24[b]	0.82	<0.05

[a,b] superscript of different letters between columns indicates statistical significance P<0.05

Previous myo-inositol studies in pigs focused on direct supplementation of the diet or from the supplementation of phytase; catabolism of phytase produces myo-inositol as a breakdown product (Moran et al., 2019, Kiani et al., 2021). This study, in comparison, had no supplemented myo-inositol or phytase, so the increases observed are due to increased synthesis from glucose. Due to the relationship between myo-inositol and improved animal performance and biological processes, the results indicate that DevAmine-based diets could improve pig growth, particularly in weaned piglets, by increasing the supply of a conditionally essential nutrient.

Conclusions

Inclusion of DevAmine into weaned piglet diets results in a significant increase in myo-inositol, a conditionally essential nutrient for optimal growth performance at weaning.

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019

The positive and negative impact of grazing on biodiversity of grasslands

M. Fraser[1]
[1]Aberystwyth University, Aberystwyth, Ceredigion SY23 3FL, UK

Presenting author
Mariecia Fraser. E-mail: mdf@aber.ac.uk

Grasslands support multiple ecosystem services and functions, including biodiversity. Managed grazing for the production of animals for meat, milk and other animal products is one of the most extensive forms of land use on the planet and covers around a quarter of the global land surface. Grazing impacts are recognised as being strongly influenced by factors such as grassland type, species of animal grazing, and stocking rate. Both overgrazing and undergrazing can have enormously damaging effects on botanical composition and related habitat value; so how do we get the balance right? Livestock Units (LUs) are a commonly used means by which the impact potential of different grazers is quantified, and LUs commonly underpin grazing prescriptions imposed as part of agri-environment schemes and similar initiatives seeking to improve and/or preserve biodiversity. However, for the most part these have been calculated based on crude estimates of the comparative energy requirements of very broad categories of livestock (e.g. dairy cow, beef cow, sheep) and do not take into account key factors such as the type or quality of grassland being grazed, within-breed variations in body size or hardiness, physiological status (e.g. pregnant, lactating, growing), or environmental challenges. It is therefore perhaps unsurprising that the majority of grazed ecosystems in the UK and beyond remain in poor condition. Major improvements to LUs and related prediction models are urgently required if grassland-based livestock production is to effectively support achieving national and international targets of restoration of 30% of degraded ecosystems by 2030.

020

Multispecies Swards Outperform Perennial Ryegrass Monocultures Under Long-Term Drought

R. Valencia[1], J. Hammond[1], C. Reynolds[1]
[1]*University Of Reading, Whiteknights, Reading, Berkshire RG6 6AH, UK*

Presenting author
Chris Reynolds. E-mail: c.k.reynolds@reading.ac.uk

Application

Multispecies swards containing grasses, legumes, and herbs provide farmers with a more drought-resilient, sustainable, and bio-diverse pasture requiring less N fertilizer compared to perennial ryegrass monocultures.

Introduction

The use of multispecies swards has many purported ecological and agronomic benefits, particularly in sustainable livestock systems. By integrating legumes, these swards reduce reliance on nitrogen fertilizers, while the complementary traits among mixed species enhance forage yield (Lüscher et al., 2014). Additionally, the inclusion of herbs has shown potential in lowering greenhouse gas emissions from ruminants (Khan et al., 2023). However, despite these benefits, the impact of climate change on pasture systems—an essential component of sustainable livestock—is less well understood. This knowledge gap underscores the importance of investigating adaptive strategies for climate resilience. Finn et al. (2018) reported that multispecies swards in intensively managed grasslands can mitigate climate stressors, such as drought, by promoting yield resilience and aiding grassland recovery post-drought. In this context, our objective was to assess the impact of drought stress on forage yield of multispecies swards compared to a perennial ryegrass monoculture.

Materials and Methods

A two-year field experiment was established at the University of Reading with sowing in April 2023 followed by a clearing cut in Autumn. The study employed a 2 x 2 factorial design to investigate the effects of species richness and water availability on forage yield across 20 plots (2 m x 12.5 m each), each with polytunnels (3 m x 12 m, height 2.5 m) to simulate drought conditions erected in April 2024. Treatments included two species richness levels (10 plots each): monoculture perennial ryegrass (PRG; *Lolium perenne*) control and a six-species mixture (2 grasses: *L. perenne*, *Phleum pratense*; 2 legumes: *Trifolium repens*, *T. pratense*; and 2 herbs: *Cichorium intybus*, *Plantago lanceolata*) and two levels of moisture, with five plots for each species richness level irrigated with tap water (11 mm applied once per week) and five plots without additional water. Moisture treatments were maintained from May 1 to September 19, 2024, for a total of 142 days. Nitrogen fertilization was applied at 200 kg N/ha in monoculture plots and 100 kg N/ha in six-species mixture plots. Between March and July 2024, PRG control plots received three applications (75 kg N with 40 kg SO_3 per hectare on March 22, 75 kg N on May 21, and 50 kg N on July 12), while six-species mixture plots received two applications (50 kg N with 40 kg SO_3 per hectare on

March 22, and 50 kg N on May 21). Above-ground biomass was harvested three times throughout the duration of the moisture treatment using three randomly placed quadrats (50 × 50 cm) per plot, cut to a residual height of 4 cm with shears. Harvests were conducted approximately two weeks, ten weeks, and twenty weeks after the initiation of the moisture treatment, following the removal of the polytunnels. Fresh forage mass from quadrat cuts was recorded, and a 1 kg subsample was oven-dried at 60°C to determine dry matter (DM) yield. The cumulative yield from the three harvests for each plot was analysed using Mixed models to determine the fixed effects of mixture, moisture, and their interaction and random effects of the plot. Least squares means were compared using Tukey's adjustment at $P < 0.05$.

Results

The six-species mixture had a higher ($P < 0.0001$) total DM yield (12,742 ± 437 kg/ha) compared to the PRG control (8,896 ± 437 kg/ha). Drought had a negative impact on forage production ($P < 0.0001$), with well-watered plots yielding 12,976 ± 437 kg DM/ha compared to drought conditions (8,662 ± 437 kg/ha). The interaction between species richness and moisture availability was not significant ($P = 0.9278$; Figure 1), indicating the six-species mixture and PRG monoculture responded similarly to the drought stress. However, due to the yield benefit of species richness, the yield of the drought-stressed six-species mixture receiving 100 kg N/ha (10,557 ± 618 kg/ha) was not significantly different from the yield of well-watered PRG monoculture receiving 200 kg N/ha (11,025 ± 618 kg/ha). These results highlight the resilience of diverse mixtures, providing a yield advantage over PRG monoculture despite similar impacts from drought.

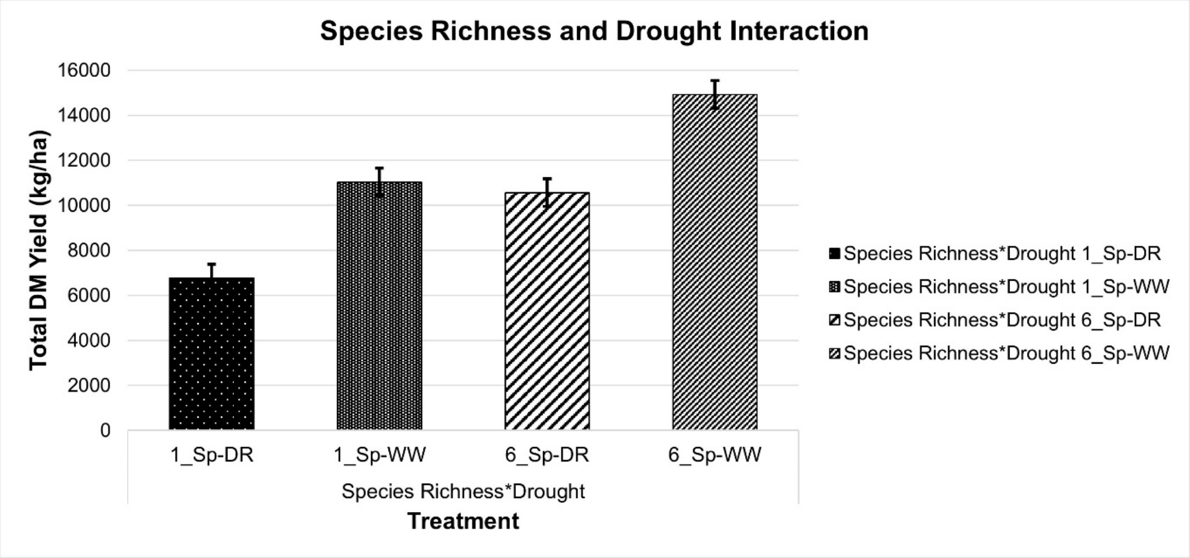

Figure 1. Interaction effects of species richness (perennial ryegrass, 1_Sp; 6 species mixture, 6_Sp) and moisture treatment (DR: drought; WW: well-watered) on total forage dry matter (DM) yield. Data are presented as mean values and SEM per treatment combination (n=5).

Conclusion

Overall, the findings demonstrate that species richness, including grasses, legumes, and herbs, enhances yield compared to a PRG monoculture receiving greater N fertilizer. Whilst the response to drought was similar in magnitude for two levels of species richness, the drought-affected six-species mixture achieved yields comparable to well-watered PRG monoculture. These results underscore the advantages provided by species richness by counteracting drought effects and reducing reliance on N fertilizer.

Acknowledgments

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021

How does white clover inclusion in perennial ryegrass swards within intensive grazing dairy systems impact pasture and animal performances on a wetland soil type?

H. Walsh[1,2], D. Patton[1], J. Collins[1], K. M Pierce[2], B. Horan[1]
[1]Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]University College of Dublin, Belfield D04 C1P1, Dublin 4, Ireland

Presenting author

Helena Walsh. E-mail: helena.walsh@teagasc.ie

Application

How does white clover inclusion in perennial ryegrass swards within intensive grazing dairy systems impact pasture and animal performances on a wetland soil type?

Introduction

The volatility of both milk prices and farm input costs within modern dairy production systems emphasises the necessity for the development of more resilient, low-input, pasture-based systems (Horan and Roche, 2020). This necessity has been reinforced by intensifying pressures to reduce the impacts of agriculture on the natural environment and meet environmental policy targets within the European Union, including a 20% reduction in Nitrogen (N) fertiliser use by 2030. These pressures have prompted a shift towards legume incorporation, particularly white clover (WC; *Trifolium repens* L.), within grazed pastures to fix atmospheric N and reduce requirements for chemical N fertiliser. The incorporation of WC in grazing swards can fix up to 220 kg N/ha/year displacing the requirement for chemical N applications (Schils et al., 2005). Although the effects of WC inclusion on milk production performance have proven inconsistent, recent evidence suggests that pasture DM intake is increased within swards containing WC (McClearn et al., 2021) thereby supporting higher production levels per cow and similar levels per hectare compared to grass only swards. Outstanding concerns remain as to the suitability of WC within swards on wetland soil types.

Material and methods

This study was conducted on 144 cows, divided into one of two sward system treatments during 2023 and 2024. In 2021, 2022 and 2023, both perennial ryegrass only (PR) and PR white clover (PRWC) sward systems underwent intensive sward renewal through reseeding and oversowing, which resulted in the entire PRWC farmlet area with some clover establishment by the end of 2023. Following establishment, PR only swards received a maximum of 220 kg N/ha of chemical N per year, whereas chemical N application was reduced to a maximum of 125 kg N/ha for PRWC swards, with significant reductions in N fertilisation from May each year based on prevailing sward clover contents. Grazing management was similar for all treatments in terms of pre- and post-grazing sward height, pre-grazing herbage mass and residency time. Total net herbage production, botanical composition and sward chemical composition were measured for the duration of the study and analysed for sward, season and year effects using mixed models (Proc Mixed, SAS Institute, 2006).

Results

Grazing characteristics were similar for both sward systems. Average pre- and post-grazing heights and pre-grazing masses were 113.8 and 47.1 mm and 1,584 kg DM/ha, respectively. Annual average WC contents were similar for both years during the study (190 and 180 g/kg for 2023 and 2024, respectively), however a slight increase in WC content was observed from August 2024 (Figure 1). There were no differences in total pasture production between swards (11,930 and 11,770 kg DM/ha for PR and PRWC, respectively). Nonetheless, established PRWC swards resulted in a significant reduction in chemical N fertiliser application from 189 kg for PR to 128 kg for PRWC. Sward nutritive value parameters (Ash, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF)) were significantly ($P<0.001$) improved with PRWC swards during the 2 year study period. In particular, increased ash and CP content were observed in PRWC compared to PR only swards (101.2 and 187.0 versus 98.8 and 181.1 g/kg DM, respectively). In contrast, PR only swards had higher NDF and ADF contents (406.8 and 224.2 compared to 390.1 and 220.8 g/kg DM, respectively). The increase in sward quality resulted in increased ($P<0.05$) milk (4,464 and 4,668 kg/cow for PR and PRWC, respectively) and fat plus protein yield (414 and 428 kg/cow for PR and PRWC, respectively) during the study with no significant differences in milk composition (54.6, 37.2 and 47.7 g/kg for milk fat, protein and lactose, respectively) between sward types. There were also no significant differences in individual animal body weight or body condition score observed during the 2 year study period.

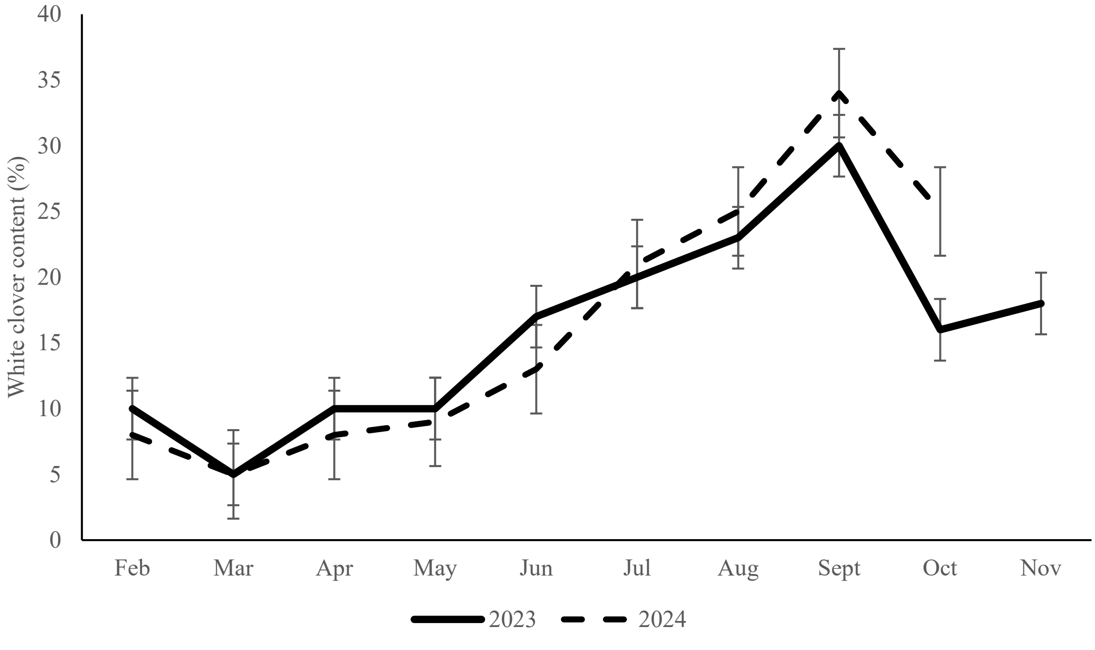

Figure 1. Mean monthly sward white clover (WC) content for the grazing season (February to November) during the experimental period 2023-2024.

Conclusion

The results of this study demonstrate that WC incorporation in swards can significantly reduce the requirement for chemical N fertiliser applications, while maintaining pasture performance and increasing animal productivity within a wetland intensive grazing dairy system.

Acknowledgements

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022

Grassland Management as a Socio-Cultural Practice: farmers' values, decisions and behaviours regarding use of paddock grazing and clover in cattle pastures

Á. Macken-Walsh[1]

[1]Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author

Áine Macken-Walsh. E-mail: aine.mackenwalsh@teagasc.ie

Application

Grassland management is explained as a socio-cultural practice, and a practice-ready extension approach, developed using socio-cultural insights, is described.

Introduction

Farmers' decisions, particularly those of family farmers, are informed by a range of information sources, which often include forms of knowledge accumulated within territories and communities over generations. New grassland management practices are introduced to farmers often by agricultural advisors, who formulate advice on the basis of science. The process of translation from science to advisors to farmers is a socio-cultural practice of communication, deliberation and, often, negotiation between the cohorts involved. This paper explicates farmers' decisions and behaviours regarding grassland management as a socio-cultural practice. Taking a focus on the Agricultural Knowledge and Information Systems

(AKIS) and efforts and influences of scientists, advisors, farmers and others within it, the paper outlines a socio-cultural approach that accommodates multidirectional flows within the AKIS, resulting in interventions that directly support more sustainable grassland use.

Materials and Methods

A qualitative research approach was taken using sociological frameworks and in-depth interviews and focus groups. Interviews were undertaken with Irish dairy and beef farmers over a ten-year period, involving two separate studies. Interviews elicited farmers' experiences of grassland management and the values, perspectives, knowledges and cultural scripts generated during those experiences that resulted in particular decisions and behaviours. Analysis of interviews was conducted thematically and similar experiences were reported by farmers pertaining to two grassland management practices: paddock grazing and use of clover. On the basis of findings of the analysis of interview data, a multi-actor co-design process was implemented with agricultural advisors, scientists and farmers to devise an extension approach to address the challenges and opportunities identified.

Results

Grassland management practices (whether paddock grazing or use of clover) are likely to be rejected by farmers if the practices don't correspond to their existing values, experiences, knowledge etc. When practices are translated from science in generic form from the lab' to the farm, they are less likely to be understood and accepted by farmers than when advisors take active roles in translating and adapting practices to have specific relevance to a specific farmer and farm. Knowledge flows within AKISs are multi-directional and different actors (scientists, advisors and farmers) have various and intersecting influences in processes of interrogation, translation and adaptation of new knowledge. An approach for agricultural extension that foresees, accommodates and enhances the socio-cultural nature of these processes is likely to be more successful than traditional 'top down' approaches.

Conclusions

As new practices emerge and are promoted to farmers, particularly in the context of climate change, it is not necessarily the case that entirely new extension challenges arise. Regarding the two technologies/practices under focus in this paper - paddock grazing and clover use - the same socio-cultural processes emerge and similar types of interventions can successfully address challenges and opportunities. The results of this paper suggest that AKIS-oriented extension approaches based on a socio-cultural understanding of grassland management are potentially adaptable and transferable to a wider range of practices. This is important, particularly in the context of climate change and the need to amplify the impacts of science at farm-level.

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023

Effect of perennial ryegrass and white clover or perennial ryegrass, white clover, and plantain swards on the milk mineral and fatty acid concentrations of grazing dairy cows

C. Minogue[1], T. Boland[1], I. Etxeberria[2], N. Walsh[2], M. Dineen[3], Z. McKay[1]
[1]School of Agriculture and Food Science, University College Dublin, Belfield D04W656, Dublin 4, Ireland
[2]University College Dublin Lyons Farm, Lyons Estate, Celbridge, Naas, Co. Kildare W23ENY2, Ireland [3]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author
Cian Minogue. E-mail: cian.minogue@ucdconnect.ie

Application

Dairy cows grazing pasture containing plantain produce milk with a greater concentration of human-beneficial unsaturated fatty acids.

Introduction

Including plantain (*Plantago lanceolata* L.) in pastures offers the opportunity to reduce N loss to the environment from dairy cows, by reducing the concentration and excretion of N in cows' urine, while improving milk production (Minnée et al., 2020). However, only a few studies have reported the effect of plantain inclusion on milk quality parameters, such as fatty acids (Mangwe et al., 2020), and no study has reported the effect on milk mineral concentrations. Milk from cows grazing pasture is an important source of unsaturated fatty acids and minerals for human consumption (Cimmino et al., 2023). Thus, there is a requirement to quantify the concentrations of individual fatty acids and minerals in milk from cows grazing pasture containing plantain. It was hypothesised that plantains' inclusion in grazed pasture for dairy cows would result in milk being produced with greater concentrations of poly-unsaturated fatty acids, with no difference in the milk mineral profile.

Materials and methods

This study was conducted at UCD Lyons Farm. A randomized complete block design was implemented using 26 Holstein × Friesian spring-calving dairy cows (*Bos taurus*). Cows were blocked based on parity and randomly assigned to one of two treatments (n = 13). The pasture treatments offered were 1) perennial ryegrass and white clover (**GC**) and 2) perennial ryegrass, white clover, and plantain (**GCP**). Cows grazed their pasture treatment from day four post-partum. The experiment consisted of two measurement days conducted during early lactation on the 19th of April and late lactation on the 20th of September 2023. Cows were on average 61 ± 12 (early lactation) and 214 ± 16 (late lactation) days in milk on each measurement day.

All cows were offered on average 16.3 ± 2.59 kg DM pasture plus 5.75 kg DM concentrate feed per day in early lactation and 17.6 ± 2.03 kg DM pasture plus 2.66 kg DM concentrate feed per day in late lactation, respectively. Pasture dry matter intake was determined through herbage disappearance (n = 6) (Nguyen et al., 2024). Cows were milked at 0800 and 1530 h and offered half of their daily concentrate feed allocation at each milking. Individual milk samples (100 ml) were obtained from successive a.m. and p.m. milkings and pooled in proportion to the respective yields. The concentrations of fatty acids were determined using gas chromatography with Flame Ionisation Detection. The concentrations of calcium, magnesium, phosphorous, potassium, and sodium were determined through inductively coupled plasma atomic emission spectroscopy.

Data was analyzed for each stage of lactation using the PROC MIXED procedure of SAS® Studio (version 3.81). The model included the fixed effect of treatment, block, and their interaction. The cow was included as a random effect.

Results

In early lactation, perennial ryegrass and plantain comprised 46 and 47 % (DM basis) of GCP pasture, whereas perennial ryegrass dominated (93%) the GC pasture. In late lactation, perennial ryegrass (44%) and plantain (37%) dominated the GCP pasture and perennial ryegrass was still present in the greatest proportion in the GC pasture (72%). The nutritive value of the pastures is presented in Table 1. Cows grazing GCP had a greater concentration of C18:3 cis-9, 12, 15 in milk on both measurement days (Table 2). The calcium, magnesium, and phosphorous concentrations were lower in milk from cows grazing GCP in late lactation.

Table 1

Mineral and fatty acid concentrations of the experimental pastures and concentrate on each measurement day.

	Period							
	Early lactation				**Late lactation**			
Treatment[1]	**GC**	**GCP**	SEM	*P*-value	**GC**	**GCP**	SEM	*P*-value
Dry matter intake (kg/day)								
Pasture	13.6	15.0	0.68	0.04	14.6	16.9	0.63	<0.01
Concentrate	5.75	5.75	-	-	2.66	2.66	-	-
Total	19.3	20.8	0.68	0.04	17.2	19.5	0.63	<0.01
Milk yield (kg/day)	31.6	31.8	0.98	0.86	18.7	21.0	0.62	0.02
Milk fat (%)	4.05	3.73	0.108	<0.05	5.23	4.68	0.121	<0.01
Milk fat (kg/day)	1.27	1.19	0.049	0.28	0.97	0.98	0.036	0.81
Milk minerals (mg/kg)								
Calcium	1174	1192	32.9	0.70	1274	1149	27.0	<0.01
Magnesium	100	101	3.6	0.84	125	116	2.3	0.01
Phosphorous	1048	997	23.7	0.14	1043	982	21.7	0.06
Potassium	1567	1564	37.2	0.95	1475	1445	31.8	0.52
Sodium	294	299	8.6	0.68	341	336	14.3	0.81
Milk fatty acids (g/100g)								
Saturated	2.39	2.34	0.161	0.84	2.49	2.69	0.146	0.36
Mono-unsaturated	0.78	0.76	0.069	0.82	0.81	0.81	0.041	0.98
Poly-unsaturated	0.12	0.15	0.009	0.03	0.13	0.13	0.008	0.80
Trans	0.19	0.17	0.015	0.20	0.16	0.18	0.012	0.32
Milk fatty acids (g/100g fat)								
C18:1 trans-FA	4.18	4.12	0.362	0.90	3.49	4.15	0.226	<0.05
C18:2 cis - 9, 12	1.70	1.98	0.235	0.41	1.75	1.79	0.131	0.85
C18:3 cis - 9, 12, 15	0.82	1.14	0.107	<0.05	0.80	1.18	0.085	<0.01
ΣOmega-3	0.86	1.18	0.117	0.06	0.80	1.18	0.085	<0.01
ΣOmega-6	1.74	2.03	0.236	0.39	1.75	1.79	0.131	0.85
Omega-6:Omega-3	2.01	1.85	0.110	0.31	1.99	1.52	0.101	<0.01

1 Treatment, GC = Perennial ryegrass and white clover pasture; GCP = Perennial ryegrass, white clover, and plantain pasture.

Table 2. The effect of treatment on milk yield, milk mineral, and milk fatty acid concentrations on each measurement day.

	Period					
	Early lactation			**Late lactation**		
Treatment[1]	**GC**	**GCP**	**Concentrate**	**GC**	**GCP**	**Concentrate**
Minerals (g/kg DM)						
Calcium	8.03	17.7	6.78	7.55	15.8	10.8
Magnesium	1.72	1.54	5.21	0.98	1.04	11.3
Phosphorous	5.42	5.69	5.38	3.03	3.52	6.16
Potassium	30.5	27.2	9.02	23.7	22.4	8.51
Sodium	0.85	1.77	5.92	1.14	1.75	8.95
Fatty acids (g/kg DM)						
Saturated	6.9	5.0	2.4	4.1	3.3	3.6
Mono-unsaturated	1.2	2.1	2.3	-	-	3.3
Poly-unsaturated	15.6	11.5	1.5	13.0	10.7	2.9
Omega-3	11.9	8.5	-	10.9	8.6	-
Omega-6	3.7	3.0	1.4	2.0	2.0	2.7
Fatty acids (g/100 g fat)						
C18:2 cis-9, 12	14.8	15.2	22.0	11.0	13.3	26.3
C18:3 cis-9, 12, 15	47.8	43.5	1.25	58.9	57.2	1.18
ΣOmega-3	47.8	43.5	1.76	58.9	57.2	1.73
ΣOmega-6	14.8	15.2	22.4	11.0	13.3	26.7
Omega-6:Omega-3	0.31	0.35	12.7	0.19	0.23	15.4

1 Treatment, GC = Perennial ryegrass and white clover pasture; GCP = Perennial ryegrass, white clover, and plantain pasture.

Conclusion

Including plantain in perennial ryegrass and white clover pasture for spring-calving dairy cows results in a greater concentration of human-beneficial poly-unsaturated fatty acids, namely C18:3 cis-9, 12, 15, in milk fat, through a greater transfer efficiency of poly-unsaturated fatty acids from feed to milk. No negative effect on the individual milk mineral profile was recorded from plantain inclusion in pasture.

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024

An investigation to the effect of a multispecies sward on herbage availability and lamb performance on a commercial farm

N. Williams[1], M. Williams[1], N. Meades[2]
[1]*Mentera - Farming Connect, 82 Lon Tanyrallt, Alltwen, Pontardawe SA82 3AS, UK*
[2]*Aberystwyth University, Penglais, Aberystwyth SY23 3FL, UK*

Presenting author
Non Williams. E-mail: non.williams@mentera.cymru

Application

Bridging the gap between agricultural research and practice is becoming increasingly important as new information emerges. This knowledge transfer project provided an opportunity for Welsh farmers to trial a multispecies ley on farm and compare it with a conventional ley of perennial ryegrass, timothy and clover. Preliminary results from one farm indicated the multispecies ley to have a tendency to produce greater volumes of herbage biomass over the grazing season in comparison to the conventional ley, however no differences were observed between the daily liveweight gains of select weaned lambs grazing each ley.

Introduction

The inclusion of multispecies swards, often referred to as herbal leys into a farm's grazing rotation is increasing in popularity, with a wealth of benefits seen to livestock, forage, and soils, when managed appropriately. Existing research has indicated that multispecies leys may result in improved lamb performance and reduced faecal egg counts (FEC) in comparison with perennial ryegrass-clover swards (Cooledge et al., 2024, Grace et al., 2019). Whilst current studies have largely focused on assessing animal and forage performance from multispecies leys (where there are more than two species included in the ley) in comparison with conventional leys (e.g. perennial ryegrass-based), there are still key research gaps, in particular relating to the success of multispecies leys on various sites on multiple soil types.

The aim of this ongoing project was to compare herbage availability and the performance of lambs grazing either a multispecies ley reseed or a conventional perennial ryegrass, timothy and clover reseed on several commercial farms in Wales. The project also offers an opportunity to investigate the impact of varying site conditions (i.e. differing farms on a range of soil types) on the leys' success. This project was set up as part of a wider knowledge exchange programme, where knowledge exchange in agriculture is important for transferring scientific knowledge and communicating scientific research to farmers. Due to the project still ongoing, here we present the preliminary findings from one farm.

Materials and Methods

The project consisted of a 2-hectare field split into two equal sizes, ploughed and reseeded with either a perennial ryegrass, timothy and clover seed mix or a 15-species multispecies ley (grass = 4, legumes = 6, herbs = 5) at a rate of 34.6kg/hectare. Each treatment was further split into three equal sized paddocks with electric fencing to allow for the rotational grazing of plots by weaned lambs. In August 2024, 100 weaned lambs were selected and allocated into one of two groups balanced for gender and liveweight, to graze each treatment. Ten lambs from each group were further selected, balanced for live weight and gender (32.3 kg ± 0.43 kg) and monitored throughout the grazing season. Lamb numbers were reduced gradually as grass growth decreased, leaving 10 lambs to graze each treatment (a total of the 20 monitor lambs on the field) by December 2024 at which point a final sward height of approximately 4-5 cm was achieved, after which lambs were removed to allow the leys to rest for the subsequent grazing season. Lambs were weighed monthly during the grazing period (August – December) to monitor daily liveweight gain (DLWG) and faecal samples were collected monthly from a representative proportion of the lambs (n = 10) to monitor worm burden by faecal egg count (FEC).

Herbage biomass availability was measured monthly from August - November by placing three 50 m x 50 m exclusion cages in each treatment ley and cutting herbage present within a 50 cm x 50 cm quadrat to a height of 5 cm. Herbage recovered was weighed to determine fresh weight before drying in a microwave until a constant weight was achieved. Results were upscaled to determine the total dry matter herbage biomass over the grazing period. Botanical species composition was assessed on the farm on ungrazed plots before grazing. Lamb average daily live weight gain data were analysed using a repeated measures analysis of variance and the total herbage biomass over the grazing season were analysed using a two-tailed t-test.

Results

The preliminary results from this farm demonstrated that the multispecies ley had a tendency to produce increased herbage biomass over the duration of the grazing season in comparison to the conventional ley (Figure 1).

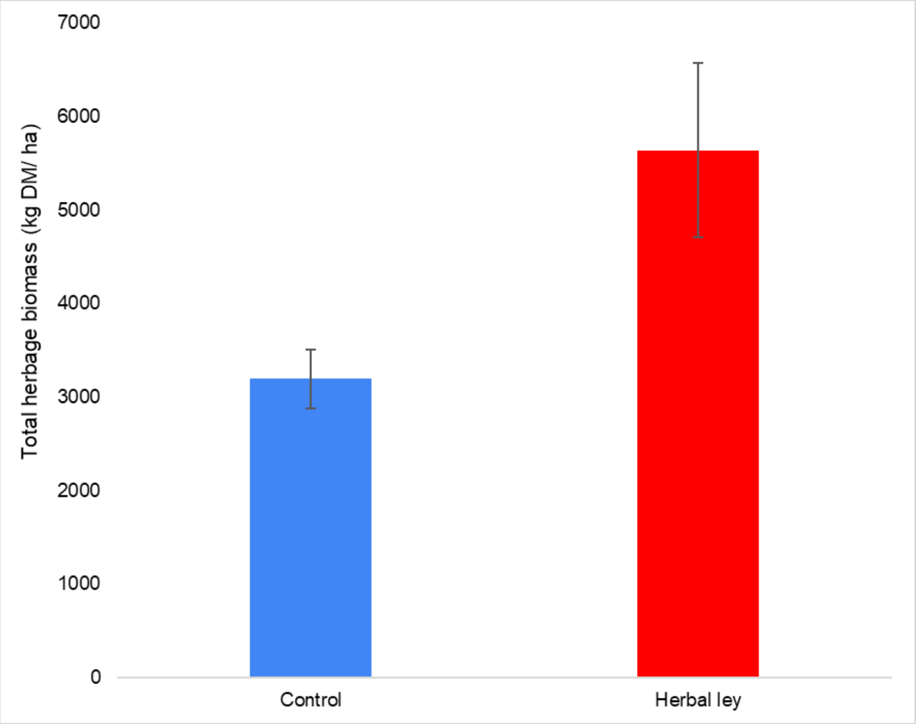

Figure 1. Total herbage biomass of both treatments over the grazing period (August - November 2024).

Dry matter content was higher on average in the conventional ley than the multispecies ley throughout the grazing season. There was no significant difference in lamb liveweight gain between the lambs grazing the multispecies ley compared to the conventional ley ($P > 0.05$).

Conclusions

Preliminary results from the project's establishment year indicate no significant differences in lamb performance or total herbage biomass from either sward during the grazing season. However, as only limited measurements were available for analysis, further measurements taken in 2025 is required to determine the impact of the multispecies leys on these measured parameters.

Acknowledgments

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Enhancing the Climate Resilience of UK Dairy Systems: Case Studies and Next Steps for the Sector

H. McFadzean[1]
[1]*Promar International, London Rd, Nantwich CW5 7JW, UK*

Presenting author
Hattie McFadzean. E-mail: hattie.mcfadzean@genusplc.com

Application

The application of this research is pertinent to economic, social, and sustainability objectives within the agricultural, and specifically dairy, sector. By exploring the relationship between climate trends and the on-farm implications, and how to effectively utilise adaptation practices to mitigate negative financial and environmental consequences, the sector may become better prepared to face unprecedented change.

Introduction

For the UK, climate projections indicate a potential average of warmer and drier summers, and wetter winters, with increased intensity in rainfall events (Met Office, 2022). For dairy enterprises, heat stress is likely to be a growing concern, rising from 1-2 days per annum in 2014, to over 20 by 2100, holding consequences for milk yield and animal welfare (Dunn et al., 2014). The impacts on forage/crop yields are more complex, whilst expected to increase due to warmer temperatures, changes in pest and disease prevalence, grazing periods, and water availability add complexity (Cho et al., 2012; Skendžić et al., 2021; Wreford & Topp, 2020).

For dairy farms, the interdependencies of forage production, animal welfare, milk production, cost of production and farmers wellbeing prompts the need to find solutions that are not single focused but consider a whole farm approach.

The research presented builds from the study conducted by Wreford and Topp (2020), which presented a list of adaptation methods for UK livestock farmers, and reviewed the financial, capital, operational, productivity, and opportunity costs of each practice.

By utilising a case study approach, an opportunity is presented to compare published climate trends, impacts, and adaptation practices, with adopted resilient initiatives, practical barriers and solutions, under a whole-farm approach.

Materials and Methods

This research utilised a mixed-method approach, initiated with a review of secondary literature to build an understanding of main adaptation practices for UK dairy farmers, and the level of adoption throughout the industry.

A series of case studies were conducted globally through semi structured interviews with farmers, academics, and industry stakeholders across UK, Brazil, US, Mexico, Ireland, and India. Case studies were collected from dairy and other livestock and arable farmers in order to encourage cross-sector learnings. Interviews were conducted with representatives of key organisations such as Teagasc, Met Éireann, Cornell University, University of Georgia and Universidad Autónoma de Baja California.

The interviews centred around a series of themes, including but not limited to:

- Enterprise type and objectives
- Climate trends, past, present, and future
- Impacts of the climate
- Key adaptation practices employed and results
- Barriers to adaptation
- Accessibility of data for decision making

Transcripts were analyzed, with key findings were grouped into themes on climate risk, adaptation feasibility, and research needs.

Results

The case studies highlighted the demand for a support tool to aid climate resilient dairy farming. This was suggested to include climate trend data, localised environmental data, such as soil erosion risks, and recommended adaptation practices. Whilst similar tools or platforms existed, they were not widely adopted, such as US Climate Resilience Toolkit, or the Georgia Climate Project (GCP). The GCP consisted of a consortium of academics who aim to enhance the transparency of climate trend and impact data, and present risk mitigation. The direction of the platform was steered by the public, and could be utilised as a concept for the UK dairy sector.

Many case studies highlighted similarities between resilient and regenerative principles, especially regarding soil health protection, mixed livestock integration, multi species lays, and rotational/cell grazing. Rancho AgroEcologica, for example, was situated in Yucatan Mexico, and suffered from extremely degraded soil due to a long-term monocrop of cactus cultivation, heighted by drought risks. The 120-hectare ranch has seen stark improvements in soil health, biodiversity, drought resilience, and productivity, now supporting dairy, goat, sheep, poultry and fruit enterprises. Initiatives included silvopasture, mixed grazing, multi species grasses, and minimum tillage practices. Whilst the objectives of the initiatives were resilience, the initiatives fell under the umbrella of regenerative principles, and subsequently enhanced soil, biodiversity, and environmental indicators.

Lastly, the interplay of key adaptation practices in response more longer-term or fixed variables such as access to capital and land availability is an important result. Utilising genetics, such as high-yielding breeds crossed with heat tolerant native breeds, in comparison to infrastructure investments, such as high sheds with fans and misters presented as a common example. Whilst both adaptation practices contribute towards mitigating the impact of heat stress, investment into modern infrastructure and equipment is often capital intensive, whilst utilising native breeds, such as Brahman and Holstein crosses, are typically less capital intensive, but will impact milk yield significantly. Whilst the trade-offs between these two initiatives were intensified in more extreme climates, such as in Brazil and Mexico, however similar case studies, although muted, were found in more temperate climates such as Ireland and the UK. The trade-offs between adaptation recommendations must therefore be reflective of the enterprise constraints and management objectives of the farm leadership and sector objectives.

Conclusions

Through these case studies, a call for a climate resilience network has been established, to utilise climate data with sector resilience knowledge and support the decision making of UK dairy farmers. Whilst many resilient practices could be considered regenerative, these practices were broader than regenerative principles and need to remain flexible to suit the inherent capabilities of the enterprise.

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You can’t mitigate what you don’t measure - quantifying baseline greenhouse gas emissions and key performance indicators from UK beef and sheep production via the ABP PRISM2030 programme

J. Capper[1], K. Perham[1], K. Behrendt[1], E. Harris[1], M. Lee[1], J. McCaughern[1], S. Morgan[1], M. Haverty[2], K. Thorley[3]
[1]Harper Adams University, Edgmond, Newport TF10 8NB, UK
[2]Andersons Farm Consultants, Melton Mowbray LE13 0PB, UK
[3]ABP UK, Vienna House, Starly Way, Birmingham B37 7GN, UK

Presenting author

Jude Capper. E-mail: JCapper@harper-adams.ac.uk

Application

Variation in greenhouse gas emissions (GHGe) across UK beef and sheep production systems may highlight opportunities for cutting carbon footprints of ruminant livestock.

Introduction

The sustainability of ruminant livestock systems is under considerable scrutiny given the contributions of methane and other greenhouse gases to climate change. Although data relating to GHGe from beef and sheep systems are widely reported, these data are either based on global averages that are not specific to or characteristic of UK systems; or are dated, both in terms of farm performance and GHGe calculation methodology. Reducing GHGe from UK beef and sheep production therefore relies on a trifurcate approach: 1) establish the baseline, 2) quantify the impacts of specific management practices and systems, and 3) evoke behavioural change such that producers adopt new practices and/or improve existing operations. On-farm practices account for up to 90% of the GHGe from beef and lamb production from farm to fork, therefore meat processors have an obvious interest in encouraging mitigation measures to reduce their Scope III emissions. As the largest beef and lamb processor in the UK, ABP Foods therefore initiated the PRISM2030 programme to enhance the sustainability (GHGe plus wider environmental, economic and social metrics) of their suppliers.

The objective of this study was to quantify a baseline for GHGe from UK beef and sheep operations (as represented by 352 farms nationwide) and to identify the range of key performance indicators (KPI) associated with these operations.

Materials and Methods

A total of 352 beef and/or sheep farms were identified and recruited into the PRISM2030 programme. All farms supplied beef cattle, lambs or both into ABP's British (300 farms) or Northern Irish (52 farms) red meat supply chain and were recruited through recommendations from livestock buyers, posts on social media and in email newsletters, or word of mouth from farmers in the programme. Farms were assessed against the demographics of the ABP red meat supply chain to ensure that there was a wide spread of flock or herd sizes, enterprise types, locations and elevations across the sample population. Farm consultants visited each farm, collecting data with which to populate the Agrecalc (https://www.agrecalc.com/) farm carbon calculator to quantify on-farm GHGe. Farms that had an additional beef or sheep enterprise on farm that did not supply ABP, or that had insufficient sales to calculate accurate GHGe were excluded from the analysis. The GHGe data from the resulting sample populations (332 beef and 200 sheep enterprises) were used to calculate overall and sector-specific baselines within which to compare data. Statistical analyses on the GHGe data were executed using a single-factor ANOVA followed by f-tests and t-tests to determine differences between variance and means.

Results

Data relating to KPI are summarised in Table 1. Lowland suckler cattle herds within the population were bigger, had heavier cows and a younger age at first calving, although calving percentages were similar between lowland and upland/hill enterprises. Suckler breeder-finisher cattle were slaughtered earlier, but with lower slaughter weights and DLWG compared to cattle reared on finishing units. Crossbred and early-lambing ewe flocks had increased lambing % and slaughter weights but lower flock sizes compared to late lambing or hill flocks.

The overall mean GHGe intensities from UK beef and sheep production were 26.7 kg CO_2e/kg dwt and 23.47 kg CO_2e/kg dwt, respectively. However, mean GHGe intensity varied considerably between beef enterprise types. Suckler enterprises had significantly higher GHGe intensities compared to finisher enterprises ($P<0.001$), ranging from, 22.6 kg CO_2e/kg dwt for dairy-bred finishers to 32.9 kg CO_2e/kg dwt for upland and hill suckler-finishers (Table 2). Similar trends were exhibited by ewe flocks compared to store lamb enterprises, with store lamb enterprises having a significantly lower ($P<0.01$) GHGe intensity than crossbred, hill or late-lambing flocks; but a similar GHGe intensity to early-lambing ewe flocks (Table 2).

Table 1. Key performance indicators from 332 beef and 200 sheep enterprises across the UK[1]

Suckler Cattle	**Cow liveweight (kg)**	**Age at first calving (mo)**	**Calving %**
Lowland[2]	667 (61.2)	27.0 (3.97)	95.4 (6.49)
Upland and hill[3]	636 (57.3)	28.9 (4.43)	95.4 (6.75)

Finishers	**DWLG (kg/d)**	**Age at slaughter (mo)**	**Slaughter weight (kg)**
Lowland[2]	1.10 (0.23)	20.1 (4.11)	621 (62.4)
Upland and hill[3]	1.01 (0.22)	20.5 (4.76)	624 (80.2)
Beef-bred	1.19 (0.30)	22.2 (3.12)	659 (54.3)
Dairy-bred	1.05 (0.27)	22.2 (3.88)	608 (65.0)

Sheep	**Ewe numbers (head)**	**Lambing %**	**Slaughter weight (kg)**
Crossbred flock	453 (495)	169 (21.7)	66.9 (11.0)
Hill flock	554 (444)	146 (19.8)	56.3 (8.60)
Early flock	410 (319)	162 (22.7)	67.4 (10.6)
Late flock	573 (503)	147 (24.3)	62.3 (13.9)
Store lambs	-	-	58.3 (14.8)

[1] Means followed by standard deviation in brackets

[2] Lowland suckler-finisher enterprises

[3] Upland suckler-finisher enterprises

Table 2. Greenhouse gas emission intensities from UK beef and sheep enterprises[1]

Beef enterprise	**Lowland Suckler**	**Upland/hill suckler**	**Beef-bred finisher**	**Dairy-bred finisher**
GHGe (kg CO_2e/kg dwt)	30.5^{a} (10.0)	32.9^{a} (13.9)	24.0^{b} (13.9)	22.6^{b} (8.03)

Sheep enterprise	**Crossbred flock**	**Hill flock**	**Early-lambing**	**Late-lambing**	**Store lambs**
GHGe (kg CO_2e/kg dwt)	27.1^{c} (12.6)	$24.3^{c,d}$ (12.6)	20.4^{d} (7.08)	24.1^{c} (10.1)	19.6^{d} (10.6)

[1] Means followed by standard deviation in brackets

[a,b] Means with different super-scripts are significantly different ($P<0.001$)

[c,d] Means with different super-scripts are significantly different ($P<0.01$)

Conclusions

The increased GHGe intensities from suckler-finisher enterprises compared to beef- or dairy-bred finishers; and for store lamb enterprises compared to most types of ewe flock were not unexpected given that both suckler-finishers and ewe flocks maintain a breeding population in addition to finishing cattle. However, further research is required to elucidate which beef and sheep KPI have the greatest influence upon and therefore mitigation potential for GHGe intensities.

027

Chance of nil returns: a novel indicator to measure the changing impact of climate volatility on grassland productivity and support on-farm decisions

T. Takahashi[1]
[1]*Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Taro Takahashi. E-mail: taro.takahashi@afbini.gov.uk

Application
The message that our climate is becoming more volatile is often communicated to farmers in a confusing and inactionable manner. To support proactive climate adaptation, this study proposes a novel indicator of pasture growth volatility that is clear, transparent and directly usable for on-farm decision making.

Introduction
As with many temperate grassland regions in the world, ruminant production in Northern Ireland is predominantly pasture-based, wherein 92% of the farmed land area is currently classified as grassland. Increasing occurrences of extreme weather events and erratic weather patterns is therefore likely to have a substantial impact on grassland productivity and its consistency, with farmers repeatedly advised to 'prepare' for climate change. However, the majority of these communications remain ambiguous about what the 'preparation' actually entails, creating a sense of anxiety and mistrust within the farming community. The present study was designed as a measure to overcome this issue.

Materials and methods
We employed AFBI GrazeGro pasture growth model (Barrett et al., 2005) to predict the daily grass growth rates for the whole of Northern Ireland over a 200-year period (1900-2100) under UK Met Office UKCP18 (RCP8.5/HadGEM3) climate change projections (Met Office, 2019). The model was pre-calibrated with 20-year controlled plot trial data, originating from AFBI Hillsborough (Co. Down) and CAFRE Greenmount (Co. Antrim), for a reseeded pasture dominated by perennial ryegrass (*Lolium perenne*) on a moderately drained soil. Mirroring the typical grazing management on commercial farms in the study region, the baseline model was run under a 10-way split nitrogen fertilisation, totalling to 270 kg N ha^{-1} $year^{-1}$, each following a simulated 21-day rotational grazing cycle. Following the initial run, a similar task was repeated for 10 more iterations, but each time removing one of the 10 fertilisation events to quantify the marginal grass yield (kg DM ha^{-1}) attributable to that particular event. Once all outputs have been collated, fertilisation events associated with < 200 kg DM ha^{-1} of marginal grass yield were marked as 'nil return events', on the basis that the investment was unlikely to have been fully rewarded. Finally, the entire procedure was repeated for all 15 sets of UKCP18 projections (i.e. 3000 annual weather patterns), to account for uncertainty with respect to both long-term trend and short-term volatility.

Results

The baseline (fully fertilised) model predicted a substantial (~2 t DM ha^{-1} year^{-1}) increase in the expected annual yield between 2024 and 2100; however, the yield volatility also increased as indicated by the wider range of the 95% confidence interval **(Figure 1)**. Within each season, grass production in early spring and late autumn (where the current limiting factor is primarily the low temperature) demonstrated a clear pattern of higher growth rates into the future, whereas that in mid-summer was found to become more erratic and often lower than the current average (figure not shown). The analysis of marginal yield suggested that the occurrences of nil returns will become substantially more common as a result of climate change, with the probability increasing more than threefold (5.3% to 16.7%) between 2024 and 2100 **(Table 1)**. Cost-effective climate adaptation strategies, such as model-assisted adaptive fertilisation programmes and greater incorporation of legumes and other non-grass species (Hopkins & Del Prado, 2017), are therefore likely to be the key to minimise the economically unfavourable use of fertilisers.

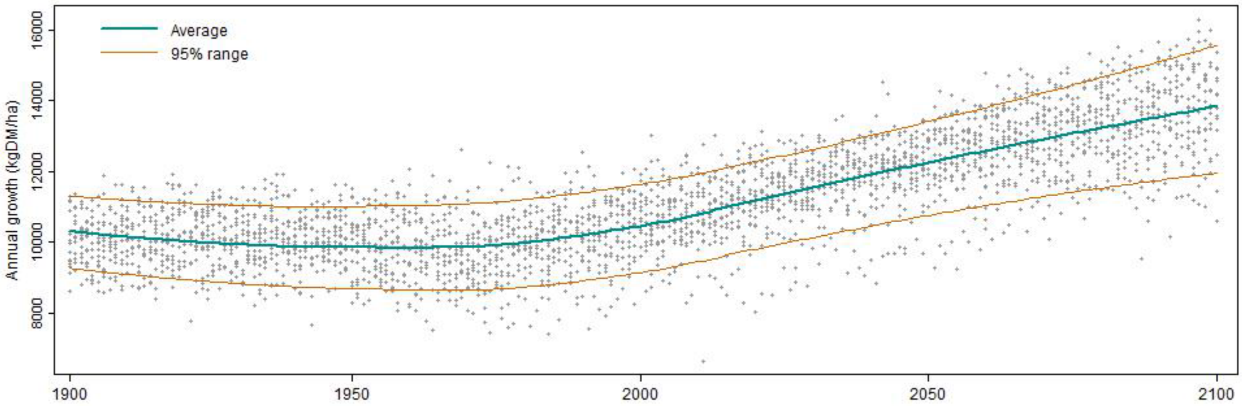

Figure 1. Annual yield prediction (1900–2100) for Northern Ireland

Table 1. Probability of ‘nil returns’ to fertilisation events

Marginal yield	2024	2100
< 100 kg DM ha^{-1}	No case	2.7%
< 200 kg DM ha^{-1}	5.3%	16.7%
< 300 kg DM ha^{-1}	22.0%	30.7%

Conclusions

In order to encourage actions and perseverance towards climate adaptation by farmers, the clarity of messages matters. It is hoped that the concept developed herein can be utilised as an effective communication tool to make climate science feel more relevant to the farming community.

Acknowledgements

Funding sources are withheld to ensure the blind review.

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028

Exploring the trade-offs between ammonia mitigation and greenhouse gas emissions on dairy farms

L. McNicol[1], J. Newbold[1], C. Duthie[1], R. Ramsey[1,2], G. Miller[1]
[1]*Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK*
[2]*Agrecalc, West Mains Road, Edinburgh EH9 3JG, UK*

Presenting author
Louise McNicol. E-mail: louise.mcnicol@sruc.ac.uk

Application

This study examines how strategies to reduce ammonia emissions on dairy farms may inadvertently impact greenhouse gas (GHG) emissions. By modeling various ammonia mitigation measures on real farms, this research offers insights into balancing ammonia reduction with GHG emissions, helping dairy farmers meet two important policy targets.

Introduction

Under the Gothenburg Protocol and the National Emissions Ceilings Regulations (2018), the UK is required to reduce ammonia emissions by 16% by 2030, relative to 2005 levels. Agriculture is responsible for approximately 88% of ammonia emissions in the UK, with cattle alone contributing 44% of the total (DEFRA, 2024). The UK has also committed to achieving net zero greenhouse gas (GHG) emissions by 2050. To meet this target, the UK Climate Change Committee has recommended a 64% reduction in GHG emissions from the agriculture and land use sector. While reducing both ammonia and greenhouse gas (GHG) emissions reduction are key policy objectives, little research has examined the trade-offs between these two goals. The aim of this work was therefore to investigate the effects of various ammonia mitigation measures on both ammonia and GHG emissions.

Materials and Methods

Nine Scottish dairy farms were selected from a large dataset provided by Agrecalc. Farms were chosen to represent high, average and low emitters (based on emissions per kg fat- and protein-corrected milk (FPCM)) and included three different systems: all year-round calving (AYR) 9500 litre herd, AYR 8000 litres herd and traditional 6500 litre herd. Farms were categorised using Agrecalc's benchmarking function, which determines the average, top 25%, and bottom 25% of emissions among all farms assessed within each system. The selected farms ranged in baseline product emissions from 1.00 - 1.35 kg CO_2e/kg FPCM. Baseline GHG emission estimates were calculated for each farm using the carbon calculator Agrecalc and ammonia emissions were calculated using a bespoke whole-farm ammonia footprinting tool. A range of ammonia mitigation measures were then modelled, including urease inhibitors, low-protein diets, slurry injection, slurry crusts and slurry covers. Assumptions were based on the UK Ammonia Inventory (Misselbrook et al., 2023), IPCC (2019) guidelines and a comprehensive literature review.

Results

All ammonia mitigation measures, with the exception of slurry crusts, were predicted to reduce both whole-farm ammonia and GHG emissions on average. Urease inhibitors proved the most effective, reducing ammonia emissions by 29% and GHG emissions by 1% (Table 1). Both slurry injection and floating covers were assumed to have no impact on GHG emissions. Only slurry crusts slightly increased GHG emissions on average across all farms (0.03%) due to the higher direct N_2O emissions associated with manure management.

Table 1. Average baseline ammonia and greenhouse gas emissions for nine farms presented alongside ammonia mitigation scenarios. Percentages in brackets represent the change from baseline.

	Ammonia emissions (kg NH_3)	**GHG emissions (kg CO_2e)**
Baseline	27,288	3,663,024
Urease Inhibitors	19,388 (-28.65%)	3,628,047 (-1.26%)
Low protein diet	25,142 (-10.80%)	3,611,579 (-1.41%)
Slurry Injection	25,142 (-8.89%)	3,663,024 (0.00%)
Slurry Crust	26,680 (-2.51%)	3,664,039 (+0.03%)
Floating Cover	26,376 (-3.01%)	3,663,024 (0.00%)
Tight Cover	26,559 (-3.46%)	3,662,687 (-0.10%)

Conclusions

Overall, based on baseline emission estimates from Agrecalc, this study has demonstrated that ammonia mitigation strategies for dairy farms can be implemented without significant trade-offs for GHG emissions. These finding suggest that dairy farms can adopt measures to reduce ammonia emissions while continuing to make progress towards broader climate goals.

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029

Modelling enteric methane emissions from a suckler beef production system

D. Meehan[1], P. Crosson[1]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*

Presenting author
Denis Meehan. E-mail- Denis.Meehan@teagasc.ie

Application

The diversity in direct methane emissions (kg/year) shown here highlights the complexity in modelling enteric fermentation across all stages of the animals lifetime and may lead to greater emphasis on the use of multi-factor enteric methane prediction equations in whole farm beef production systems.

Introduction

Ruminant production systems constitute a significant source of anthropogenic greenhouse gas (GHG) emissions, the principal cause of global warming and climate change. GHG emissions are typically assessed using modelling approaches such as life cycle assessment (LCA) and these models incorporate equations to predict methane (the principal GHG) emissions derived from enteric fermentation. Dry matter intake is recognised as a key driver of enteric methane emissions and a range of equations have been developed to predict enteric methane (Ellis *et al.* 2007). The aim of this present study was to compare enteric methane emissions (kg/year) in a modelled beef herd for a number of published enteric methane prediction equations. The herd level analysis was conducted using the Grange Beef Systems Model (GBSM; Crosson *et al.* 2006).

Materials and Methods

The GBSM was populated as a spring-calving suckler calf-to-beef farm system using cross-bred cows mated to late-maturing beef sires with all cow replacements bred on farm. The farm system incorporated a farmed area of 40 ha, two silage cuts (29th May and 24th July) and a mean calving date of 6th March with inorganic fertiliser application of 131 kg N / ha. Diets fed comprised a mix of grass, grass silage and concentrate with feeding activities quantified monthly to account for seasonal variation in diet composition._ Calves were single-suckled at grass and weaned in October before a 112-day indoor winter feeding period on a grass silage + concentrate diet. Following this indoor feeding period, animals were turned out to pasture in March of their second year. Heifers not required for replacing cows culled from the breeding herd were finished at pasture at 19.5 months of age (330 kg carcass weight). Steers were housed for a second winter, fed a diet of grass silage + concentrates and finished at 22.5 months of age (389 kg carcass weight). Direct methane emissions derived from enteric fermentation were modelled using seven prediction equations (Table 1) to reflect a range of diet and stock types.

Results

Direct methane outputs across methodologies varied in both total emissions and within stock types (Table 1). Relative to Yan *et al.* (2009; the baseline prediction equation used in GBSM), total direct emissions ranged from 90% (Galyean & Hales, 2022) to 127% (Axelsson 1949) with Ellis *et al.* (2007) 2, Ellis *et al.* (2007) 4, Ellis *et al.* (2007) 1 and Kriss (1930) being intermediate at 95%, 101%, 115% and 122% respectively. Similarly, within stock types, emissions across equations relative to those of Yan *et al.* (2009) ranged from -13% (Galyean & Hales 2022) to +27% (Axelsson 1949) for steer yearlings, -8% (Ellis *et al.* 2007, 2) to +34% (Axelsson 1949) for heifer yearlings and -16% (Ellis *et al.* 2007, 2) to +18% (Kriss 1930) for suckler cows. The largest deviations within stock type were seen for heifer calves (+74%) and bull calves (+67%, both Ellis *et al.* 2007, 1) which may reflect differences in dietary composition and animal class compared to those in the baseline model (Yan *et al.* 2009).

Table 1. Direct methane emissions (kg/year) in GBSM and other prediction equations in a suckler beef system

Stock type	Yan *et al.* (2009)*	Ellis *et al.* (2007) 1	Ellis *et al.* (2007) 2	Ellis *et al.* (2007) 4	Galyean & Hales (2022)	Kriss (1930)	Axelsson (1949)
Suckler cows	3671	3753	3069	3378	3287	4340	4280
Heifer calves	535	930	794	779	487	729	766
Bull calves	604	1006	853	857	594	859	947
Heifer yearlings	1234	1393	1133	1205	1134	1516	1651
Steer yearlings	1342	1433	1176	1273	1166	1561	1711
Total	7386	8515	7025	7492	6668	9005	9355
Total *vs.* * %		115	95	101	90	122	127
Parameters	ME, GE, DE LW, FP	MEI	DMI	DMI, FP	DMI	DMI	DMI

ME = Metabolisable Energy (MJ); GE = Gross Energy (MJ); DE = Digestible Energy (MJ); LW = Liveweight (kg); FP = Forage Proportion; MEI = Metabolisable Energy Intake (MJ/day); DMI = Dry Matter Intake (kg/day).

Conclusions

Differences exist in direct methane emissions from enteric fermentation using either single (DMI) or multi-factor (DMI and FP) prediction equations in whole farm beef systems on high forage diets. Emissions (kg/year) are positively correlated to live weight (thus DMI capacity) and duration of animal stay on farm.

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030

Readily available measures of greenhouse gas mitigation are more than sufficient for UK livestock industry to stop contributing to further climate change if fully implemented

T. Takahashi[1]

[1]Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author

Taro Takahashi. E-mail: taro.takahashi@afbini.gov.uk

Application

In order to encourage the uptake of greenhouse gas (GHG) mitigation measures by livestock farmers, the narrative behind the encouragement matters. This study proposes a new and more positive framing of the industry's potential to transform itself into a low emissions sector.

Introduction

For the UK to achieve the legislated target of becoming a net zero economy by 2050, the livestock industry must accelerate effort to reduce its contribution to the national GHG inventory. A recent study encompassing the industry's six most important sectors (dairy, beef, sheep, pigs, broilers and layers) indicates that adopting an economically realistic set of currently available GHG mitigation measures would result in a 23% reduction in inventory-relevant emissions (Magowan et al., 2022). This estimate, however, was based on the 100-year time horizon global warming potentials impact assessment method (GWP_{100}), which is known to overstate the effect of constant methane emissions on global surface temperature while understating the effect of new methane sources (McAuliffe et al., 2023). As such, the exact level of mitigation required for the livestock industry to stop contributing to further temperature increases remains unquantified to date.

Materials and methods

We used the 2022 edition of the UK devolved administration GHG inventory (NAEI, 2024), which separately reports emissions that occurred in England, Scotland, Wales and Northern Ireland. In addition to agricultural source categories of 3A (enteric fermentation), 3B (manure management) and 3D/ 3G/3H (soil emissions), a subset of sources within source categories 4B (Cropland) and 4C (Grassland) were extracted to account for the background (landscape-originated) emissions associated with current agricultural land use. The gas-by-gas emission data thus compiled were then integrated using the GWP* impact assessment method (Cain et al., 2019) to estimate the approximated short-term (< 20 years) relative temperature effect. Following a baseline simulation, methane emissions by each nation were iteratively reduced to identify an equilibrium point where the global cooling effect of methane mitigation (Δ stock) equates to the global warming effect of carbon dioxide/nitrous oxide emissions (flow). In order to eliminate the effect of transborder emissions displacement, livestock numbers (and therefore food supply) were assumed to be constant. The background emissions from organic soil (peatland) were excluded from the main analysis due to their uncertainty but considered in the form of a sensitivity analysis.

Results

The level of methane mitigation required to achieve a short-term equilibrium in each nation was found to be 22% (England), 27% (Scotland), 15% (Wales) and 12% (Northern Ireland), respectively **(Figure 1)**. The inter-regional variability in the threshold was primarily driven by the relative importance of ruminant agriculture, which, in turn, contributed to a greater proportion of methane within the GHG inventory. Without any mitigation in place, this share was found to be 61% (England), 68% (Scotland), 79% (Wales) and 78% (Northern Ireland). Inclusion of peatland emissions slightly increased the level of methane mitigation required, to 29% (England), 33% (Scotland), 15% (Wales) and 16% (Northern Ireland), primarily due to a low water table to facilitate greater carbon dioxide emissions under these conditions. To contextualise these values, Magowan et al. (2022) estimate that existing mitigation technologies alone could lead to a ~28% reduction in methane from UK livestock farming if fully implemented, whereas Newbold et al. (2022) predict that near-market-ready feed additives would further reduce enteric methane by 13–56%. Combined together, our results indicate that readily available mitigation measures are more than sufficient for the UK livestock industry to stop contributing to further climate change if fully implemented. It should be noted, however, that the derived equilibrium will only last for ~ 20 years (during which the methane stock in the atmosphere continues to decrease), meaning that a new set of mitigation technologies will be required to maintain the zero temperature effect beyond then.

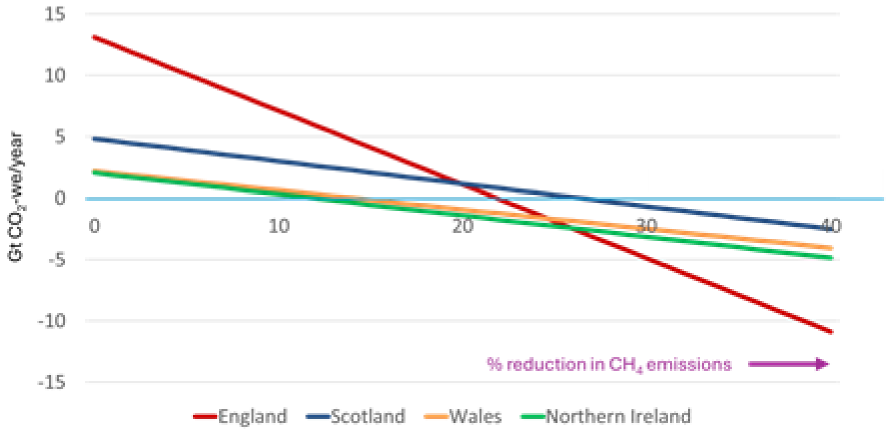

Figure 1. Impact of methane reduction on temperature effect

Conclusions

The global cooling effect considered herein is primarily underpinned by the livestock industry's own past methane emissions and, therefore, from the legal and societal justice points of view, the absence of the incremental temperature effect does not immediately mean that the industry bears no further responsibility in climate change. Notwithstanding, this study demonstrates that a seemingly small reduction in GHG emissions can reverse the contemporaneous temperature effect — thereby likely the public perception — of livestock farming, reiterating the importance of actions and perseverance towards mitigation efforts.

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031

Identifying the biogenic and thermogenic carbon sources at farm level to support the transition to carbon net zero

A. Rogers[1], E. Harris[1], G. Milburn[1], E. Siqueiros[1], M. J. Lewis[1], M. Lee[1]
[1]Harper Adams University, Edgmond, Newport TF10 8NB, UK

Presenting author

Michael Lee. E-mail: mrflee@harper-adams.ac.uk

Application

Understanding the biogenic and thermogenic carbon sources on a farm will assist in the transition towards net zero farming. It will help identify the areas of a farm that are reliant on the use of fossil fuels (decarbonisation), whilst recognising the natural carbon sinks (net zero carbon emissions).

Introduction

Climate change has a dramatic impact on many human activities, including agri-food, resulting in an urgent need to act. In a recent update of the planetary boundaries (Rockström *et al.* 2023), it was identified that due to anthropogenic activity the planet's natural homeostatic capacity has surpassed six of the nine defined boundaries. The release of anthropogenic greenhouse gas (GHG) emissions has increased the levels of the three main GHG's in the atmosphere: carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O); identifying a need to significantly reduce these emissions by 2050 to pre-industrial levels. The three GHG's can be identified as either biogenic sources (part of a natural cycle of capture and release) or thermogenic sources (fossil fuel derived). For environmental assessment the GHG's are converted to CO_2e based on their associated global warming potential (GWP) over a defined period e.g., 100 years for GWP-100 (IPCC. 2021); $CO_2 = 1$ (thermogenic); $CH_4 = 27$ (biogenic), 29.8 (thermogenic); $N_2O = 298$ (biogenic and thermogenic). It is important to understand these sources due to the nature of agriculture utilising biogenic cycles, however, there is currently no advice on disaggregating thermogenic sources that could be decarbonised from biogenic sources, that can be balanced with their sink to achieve carbon net-zero. Here, we demonstrate biogenic and thermogenic GHG disaggregation at a farm level to identify a pathway towards decarbonisation and carbon net-zero farming.

Materials and Methods

The emissions of the three main GHG's for Future Farm at Harper Adams University were calculated using a market-leading carbon calculator. The result output from the calculation evaluated the total GHG emissions for the beef, dairy, sheep, pig, and oilseed rape enterprises which were then disaggregated into individual gases and then further between biogenic and thermogenic sources. Future Farm is variable and unique, there is a mixed enterprise of beef, dairy, sheep, pigs, and arable contributing to our carbon footprint. Our intensive dairy beef production enterprise finishes approximately 70 British-Blue cross steers and heifers from an all-year round calving Holstein-Friesian herd, within 12 months, on a TMR ration. The dairy enterprise is a high performance indoor dairy system formed of a main unit and "smart unit" featuring elements of automation. The main unit accommodates up to 400 cows milked three times a day with a target annual yield of 10,000 litres per cow. The smart unit houses 45 cows and these are milked using a robotic milking system. The sheep enterprise is a grass-based outdoor system formed of a pedigree Lleyn flock, with 600 breeding ewes that rear over 1,000 lambs every year, and most ewes are housed indoors for the lambing season. The pig enterprise is a closed herd consisting of 230-sow farrow-finish in an indoor unit running a 3-week batch operation and finishing over 6,500 pigs annually. The oilseed rape enterprise uses a small proportion of our total land use, and its sole purpose is for commercial production and revenue. As a commercial farm, Future Farm also manages grass leys, produces forage crops (e.g., silage and maize) and other combinable crops (e.g., wheat and barley), which are fed to the farm livestock enterprises and are embedded in the CO_2, CH_4, and N_2O emission totals for these enterprises.

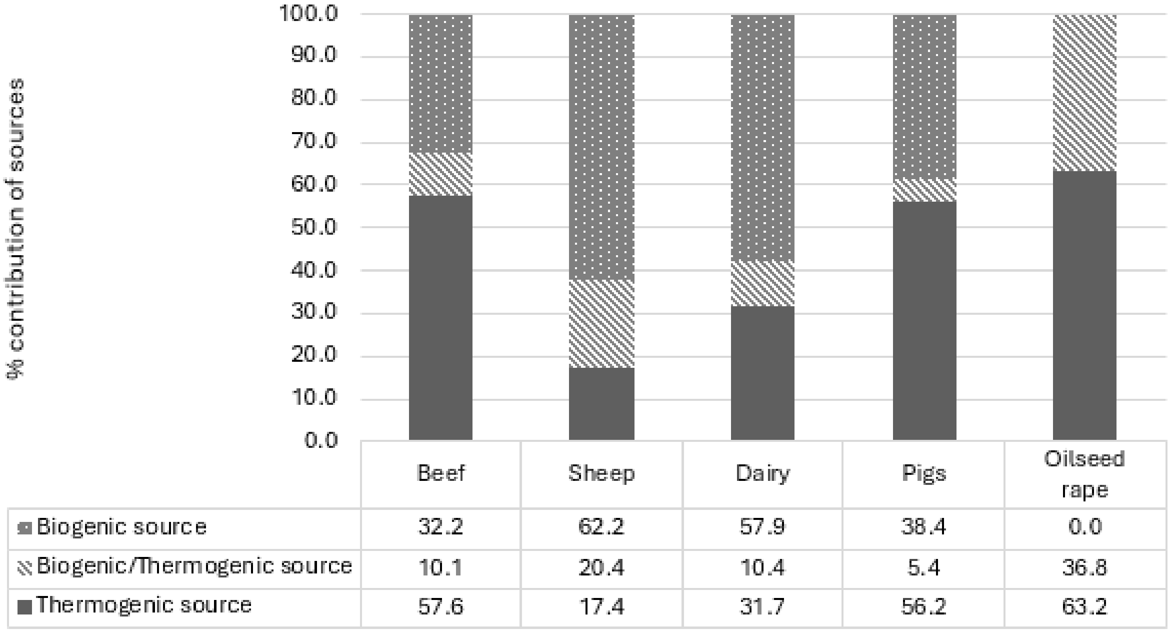
Results

We categorised biogenic source as CH_4 and the thermogenic source as CO_2 (Table 1; direct and indirect). When determining the source of N_2O, it was not possible to differentiate when N_2O emissions became an act of biogenic or thermogenic activity due to the nature of the N inputs in the Harber-Bosch process (focus of future work). Therefore, N_2O was defined as both biogenic and thermogenic (Figure 1).

Table 1. Emissions from the farm by enterprise

Emissions	Enterprise				
	Beef	Sheep	Dairy	Pigs	Oilseed rape
Total direct CO_2 (kg CO_2e)	37,967	42,934	220,831	85,717	18,216
Total indirect CO_2 (kg CO_2e)	84,122	39,411	1,262,368	1,329,279	28,160
Total CH_4 (kg CO_2e)	68,266	294,695	2,707,546	966,256	0
Total N_2O (kg CO_2e)	21,433	96,457	488,174	136,526	26,954

Total CO_2e emissions from farming	**211,787**	**473,596**	**4,679,919**	**2,517,778**	**73,330**



	Beef	Sheep	Dairy	Pigs	Oilseed rape
▒ Biogenic source	32.2	62.2	57.9	38.4	0.0
▨ Biogenic/Thermogenic source	10.1	20.4	10.4	5.4	36.8
■ Thermogenic source	57.6	17.4	31.7	56.2	63.2

Figure 1. Percentage contribution of biogenic and thermogenic sources per enterprise

Conclusions

The balance between total biogenic and thermogenic sources differed with each enterprise within Future Farm, as did the proportion of emissions where biogenic and thermogenic sources were indistinguishable. As part of a net-zero transition, the thermogenic sources derived from fossil fuels would be the focus of decarbonisation and the biogenic sources would be balanced by sinks in concert with decarbonisation. The ability to disaggregate the sources within Agri-food is crucial to inform management decisions regarding natural sinks and those that need to be removed through decarbonisation, and to assist the development and implementation of a net-zero carbon strategy at farm level.

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032

Identification of the core microbiota of bovine colostrum collected fresh from the dam

S. Scully[1,2], B. Earley[1], P. Smith[1], F. Buckley[3], C.McAloon[2], D. Kenny[1], S. Waters[4]
[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*
[3]*School of Biological, Earth and Environmental Sciences, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*
[4]*School of Biological and Chemical Sciences, University of Galway, University Road, Galway H91 TK33, Ireland*

Presenting author
Sabine Scully. E-mail: sabine.scully@teagasc.ie

Application
The microbial, physiological, and immunological development of the calf in the first few hours of life will be influenced by the colostral microbiome, as evidenced by the diversity of community members and their possible functions. Colostrum quality and its timely intake are key to preventing diseases and ensuring optimum calf health.

Introduction
Once believed to be sterile, there is increasing evidence of a naturally occurring colostral microbiome. Recent work in other mammals provides evidence that an entero-mammary pathway that mobilizes gut microbes and transfers them to the mammary during colostrogenesis does exist (Derakhshani et al., 2018). The microbes present in colostrum are ingested by the calf, and are integral to calf gut microbiome and immune development (Messman and Lemley, 2023). During development, commensal gut microbiota and their metabolites function in conjunction with the host to improve mucosal barrier and immune function (Chase and Kaushik et al., 2019). The origins of the pioneering microbes that colonize the calf gut are still debated, with microbes being sourced maternally and environmentally (Messman and Lemley, 2023). One potential maternally driven source would be that of a colostral microbiome, which may be an evolutionary adaptation designed to aid in gut microbiome colonization and development in the calf. To better understand the potential implications of a colostral microbiome on calf health and development, it is important to identify the microbes present in fresh, unprocessed colostrum as this is what the calf would be consuming naturally. Thus, the study objective was to examine the core bacteria identified in fresh colostrum collected from a single, spring-calving herd of primi and multiparous Holstein-Friesian (n=17) and Jersey (n=10) cows.

Materials and Methods
Colostrum samples (10mL × 3) were collected from all four mammary gland quarters within 2h of parturition (n=27) and were immediately snap frozen and stored at -80°C. Prior to microbial DNA extraction 600µL EDTA per 10mL colostrum was added and centrifuged to generate a microbial pellet (Siebert et al., 2021). Microbial DNA was extracted via repeated

bead beating and column purification using a Qiagen DNeasy® PowerSoil® Pro Kit and extracted DNA was sent to Macrogen for Illumina amplicon sequencing of the V4 region of the 16S rRNA gene. Sequencing data underwent PERMANOVA, α- and β-diversity, and core analysis using R packages DADA2, Phyloseq, and Microbiome. Core analysis was performed once sequencing data had been filtered for a relative abundance (RA) of >0.05%. Core bacteria was defined as those bacteria, which were present in abundance of greater than 0.05% in all samples analysed. Colostrum immunoglobulin (Ig) concentration was determined using single radial immunodiffusion.

Results

There were no effects of breed or parity on colostrum IgG concentrations. Colostrum was determined to be of excellent quality (mean IgG 150.59 (SE 6.60) mg/ml). Based on PERMANOVA analysis, breed and parity had no effect on α-diversity, nor an effect on microbial composition. Fresh colostrum was observed to harbour a diverse microbial community with homogenous microbial composition across samples. Twenty-six bacterial genera were observed to contribute to the core microbiota. Bacillota (39.05%; 17 core), Pseudomonadota (21.56%; 5 core), Actinomycetota (6.88%; 2 core) and Bacteroidota (2.52%; 2 core) were the major phyla observed to contribute to the colostral core bacterial community. Of these, Christensenellaceae R-7, Lachnospiraceae NK3A20, and Oscillospiraceae UCG-005 are regularly identified as bovine gut commensals. The archaeal genera with the greatest relative abundance (RA) were Methanobrevibacter (85.5%) and Methanosphaera (13.5%). Both of these have previously been reported to be the dominate archaea present in the neonatal calf hindgut, indicating that archaea present in colostrum may contribute to the pioneering archaeal community in the calf gut.

Conclusions

The core colostral bacterial community observed in this study was highly diverse, in not only genera but also potential function, including acetate, propionate, and butyrate producers. These short chain fatty acids are critical to the development of the mucosal immune system of the calf in early life. The presence of strict anaerobes like archaea and commensal gut bacteria lend support to the theory of an endogenous entero-mammary pathway in cattle and may be reflective of a naturally occurring colostral microbiota. Mechanisms behind the formation of a colostral microbiome in ruminants are still unclear. To fully comprehend how the colostral microbiome influences the calf's intestinal microbiome, more research is necessary.

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033

The effects of an Aspergillus niger product and yeast cell wall mannan-rich fraction on dairy calf performance and rumen fermentation

S. Yerby[1], J. Huntington[2], N. Jonsson[1], H. Warren[3]
[1]University Of Glasgow, West of Scotland Science Park, Maryhill Rd, Glasgow G20 0SP, UK
[2]Harper Adams University, Edgmond, Newport TF10 8NB, UK
[3]Alltech, Ryhall Rd, Stamford PE9 1TZ, UK

Presenting author
Stefan Yerby. E-mail: s.yerby.1@research.gla.ac.uk

Application

Inclusion in pre-weaned dairy calf diets of commercially available feed materials derived from the solid-state fermentation of a fungus (Synergen®) and a mannan-rich fraction of yeast cell wall (MRF) promotes solid feed intake and a more efficient rumen fermentation in calves. Increased solid feed intake is positively associated with rumen development, which can ultimately promote calf performance. Inclusion of Synergen® also improved rumen fermentation post-weaning. More data are needed to demonstrate effects on calf performance.

Introduction

Aspergillus niger fermentation products can contain a suite of bioactive compounds including enzymes, alkaloids and plant growth like factors, which can enhance rumen fermentation and productivity in mature cattle (Meale et al., 2014). There is a considerable body of research indicating that yeast cell wall fractions are beneficial for calf health (Broadway et al., 2015). However, there is limited research examining the effects of *Aspergillus* products in calves, and to our knowledge, there is no published literature examining the effects of an *A. niger* fermentation product in combination with the mannan-rich fraction of *Saccharomyces cerevisiae* cell walls (MRF). This study measured the effects of an *Aspergillus niger* fermentation product (Synergen®) alone, or in combination with MRF, on dairy calf performance and rumen fermentation.

Materials and Methods

Thirty male, Holstein-Friesian calves (age 19 ± 5 days) were obtained from a single farm and enrolled into a 70-d study, including 7 d adaption to environment and treatments. Calves were blocked by weight and randomly assigned to three balanced groups: CTRL, SYN, MRF. CTRL calves received no treatment, SYN calves received 3 g of Synergen®/d, MRF calves received 3 g of Synergen® and 2 g of MRF/d. Calves were individually housed and treatments were combined with milk replacer (6 l/d) for the first 27 d, after which they were abruptly weaned, and received treatments in starter concentrates. Calves had *ad libitum* access to straw, water and concentrates; concentrate refusals were measured daily. Calves were weighed on two consecutive days every week in the pre-weaned period, and bi-weekly post weaning. Digestive health of calves was measured weekly following the University of Wisconsin faecal scoring protocol (Jaureguiberry et al., 2023). Immediately prior to weaning, 15 calves were culled and duplicate samples of rumen digesta were collected for volatile fatty acid (VFA) analysis. The remaining calves were culled for rumen sampling at week 10. VFA analysis was performed using gas chromatography according to the protocol of Erwin et al. (1961). Statistical analysis was performed in R (R Core Team, 2024). Differences in feed intake, FCE, ADG and faecal score were calculated using repeated measures ANOVA, differences in VFA concentrations and proportions were calculated using one-way ANOVA.

Results

Table 1 shows the FCE and starter intake of calves during the trial. SYN calves had increased starter intake in both the pre-weaned and weaned periods, however, neither treatment consistently improved FCE during the pre-weaned or weaned periods. MRF calves had higher FCE compared to CTRL calves in weeks 1, 8 and 9. The FCE of SYN calves was lower than CTRL calves in week 3 but increased in week 6 and 7. ADG was unaffected by treatment, as was faecal score; all calves remained healthy throughout the trial. At weaning, MRF calves had an acetate: propionate ratio of 2.18 in rumen fluid, lower than the CTRL ratio of 2.7 (Pooled SE = 0.09; *P* = 0.047). At week 10, SYN calves had an acetate: propionate ratio of 1.6 compared to the CTRL ratio of 2.27 (Pooled SE = 0.103; *P* = 0.009), and a higher proportion of propionate in rumen fluid, 32 % compared to 25 % (Pooled SE = 0.951, *P* = 0.004). SYN and MRF calves had higher proportions of valerate, 4.52 % and 4.54 % respectively, compared to CTRL of 3.28 % (Pooled SE = 0.17; *P* < 0.001), as well as lower proportions of isovalerate and isobutyrate (*P* < 0.001).

Conclusions

Neither treatment consistently improved FCE or ADG in dairy calves. Synergen® increased feed intake in pre-weaned and weaned dairy calves and shifted rumen fermentation towards propionate post-weaning. The propionate pathway is more efficient than acetate, suggesting enhanced starch hydrolysis and rumen function, however, this effect did not translate into consistent improvements in feed efficiency. The VFA profile of MRF calves pre-weaning indicates that the combination of treatments could improve the capacity of calves to digest solid feed prior to milk withdrawal. These results show that both Synergen® and Synergen® + MRF can positively influence rumen fermentation but at different stages of calf development. Commercial trials using a larger number of calves, in more challenged environments, would reveal if effects of treatments on calf performance are more pronounced than those realised in this study.

Table 1. Mean feed conversion efficiency, average daily gain and starter intake of thirty Holstein Friesian bull calves treated with an *Aspergillus niger* product alone (SYN), or in combination with a yeast cell wall fraction (MRF).

FCE		Treatment					
Week	CTRL	SYN	MRF	Pooled SE	*P*-value Treatment	*P-value* Time	*P*-value Treatment × Time
1	0.64^{a}	0.64^{a}	0.69^{b}	0.008	0.0171	0.3743	0.9342
2	0.62	0.62	0.63	0.009	0.9387	0.7772	0.9361
3	0.63^{a}	0.59^{b}	$0.60^{a,b}$	0.007	0.03	0.0205	0.321

Pre-weaned period	0.63	0.62	0.64	0.006	0.2115	< 0.0001	0.0239
4 & 5	0.56	0.55	0.61	0.025	0.447	< 0.0001	0.7038
6 & 7	0.34^{a}	0.38^{b}	0.34^{a}	0.007	0.0016	< 0.0001	0.1589
8 & 9	0.30^{a}	$0.32^{a,b}$	0.33^{b}	0.003	0.0026	< 0.0001	0.2
Weaned period	0.40	0.42	0.43	0.001	0.4404	< 0.0001	0.5845
Starter intake (g)							
Pre-weaned	360^{a}	417^{b}	328^{a}	11.52	0.0008	< 0.0001	0.9695
Weaned	3155^{a}	3398^{b}	3203^{a}	50.17	0.0021	< 0.0001	0.0513
ADG (g)							
Pre-weaned	773	817	778	7.9	0.6312	0.0002	0.767
Weaned	1110	1236	1184	12.05	0.4728	0.0016	0.7276

Abbreviations: FCE, feed conversion efficiency; ADG, average daily gain; CTRL, control calves; SYN, calves fed 3g/d Synergen®; MRF, calves fed 3g/d Synergen® + 2g/d Actigen®; SE, standard error.
Values not sharing a superscript are significantly different ($P < 0.05$)

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034

The impact of pre-weaning nutrition and a methane-suppressing feed additive on Holstein heifer development

S. O'Callaghan[1,2], G. Arnott[1], O. Cristobal Carballo[2], D. Seymour[3,4], S. Stergiadis[5], G. Scoley[2]
[1]Queen's University Belfast, University Rd, Belfast BT7 1NN, UK
[2]Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK
[3]Trouw Nutrition R&D, Stationsstraat 77, 3811 MH Amersfoort, The Netherlands
[4]Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, 50 Stone Road East, Guelph N1G 2W1, Canada
[5]School of Agriculture, Policy and Development, University of Reading, Whiteknights House, Reading RG6 6UR, UK

Presenting author
Sinéad O'Callaghan. E-mail: socallaghan08@qub.ac.uk

Application
Feeding an elevated level of milk will result in a sustained growth advantage in the pre-wean period. Calcium peroxide supplementation at 2.25% of DM lowered concentrate intake and live weight in pre- and post-weaning periods. Elevated milk replacer allowance increased calves' average daily gain in the pre-weaning period.

Introduction
Feeding calves higher milk replacer (MR) levels in the pre-weaning period could improve growth in early life. Elevated MR can boost long-term production performance regarding average daily gains and greater milk yield in the first lactation. Reduction in methane production of cattle can increase performance such as average daily gain (ADG) which could result in a lowered age at first calving. Higher milk replacer intake and reduced methane emissions can increase calves' growth. A feed additive, calcium peroxide, has been proven to reduce methane emissions in adult cattle by 16-28% (Roskam et al., 2024). However, no research has been reported using calcium peroxide as a methane-suppressing additive for calves and its effects on performance. This study aims to assess the effects of milk replacer level and calcium peroxide supplementation during the first 12 weeks of life on methane production and calf development. Our hypotheses are i) calves fed higher milk replacer levels will have higher body weights and lower concentrate dry matter intake from birth to week 12, and ii) calcium peroxide supplementation at 2.25% will reduce methane production (g CH_4/day).

Materials and Methods
Holstein heifer calves (n = 53) were allocated to treatment in a 2 MR feeding level (Conventional vs. Elevated) x 2 Concentrate type (Additive vs. Control) design and balanced for birth weight, dam parity and colostrum quality. Calves were individually housed from birth, and experimental diets were offered on day 4. MR was offered at conventional (4 L/day) or elevated (8 L/day) feeding levels until weaning at day 63. Ad libitum additive (2.25% calcium peroxide) and Control (2.25% limestone) were offered from day 4 to week 12. Concentrate and MR intake were measured daily, and calves had free access to drinking

water and chopped straw. Live weight was recorded weekly from birth to week 18. Methane emissions were recorded using metabolic chambers at week 2 and 9. From week 12, all treatments ceased, and calves were moved from individual pens to group housing. Once in group housing, calves were fed ad libitum grass silage alongside a standard heifer developer concentrate (2kg/day) using a GreenFeed unit (C-Lock Inc., Rapid City, SD.), with this being used to record their methane emissions. Daily concentrate intake and weekly live weight were fitted to a repeated measures REML model (GenStat 21st ed., VSNI Ltd) where milk replacer level, concentrate treatment, day and their interactions were fitted as fixed effects and birth weight was included as a covariate. Daily live weight gain was fitted to a linear mixed model with fixed effects of milk level and concentrate treatment and birth weight as a covariate. Pen was a random effect in all models.

Results

As expected from day 4 – 83 calves fed the conventional MR level consumed more concentrate (Table 1; P = 0.021). Average daily concentrate intake in Control calves was higher than that of calves offered Additive concentrate – during the first 12 weeks of life (Table 1; *P* <0.001). In the pre-wean period (d4-63), calves offered conventional levels of MR consumed ~ 134 g DM/day more concentrate than those offered elevated levels of MR (*P* = 0.002). In the same period, calves fed control concentrate consumed ~109 g DM/day more than those offered the Additive concentrate (*P* = 0.008). The difference in intake between concentrate treatments continued in the post-wean period (d64-84), whereby Control calves consumed ~ 403 g DM/day more than Additive calves (*P* <0.001).

Table 1. Daily concentrate intake (g DM/day) in the first 12 weeks of life

	MR Treatment			Concentrate Treatment			P-Value		
	Conventional	Elevated	SED	Additive	Control	SED	MR	Conc	MR xConc
Day 4 – 83 (gDM/day)	888.2	766.4	49.20	736.2	918.3	48.54	0.021	<0.001	0.928
Day 4– 63 (gDM/day)	430.8	296.7	40.41	309.5	418.1	39.86	0.002	0.008	0.578
Day 64-84 (gDM/day)	2260	2175	59.45	2016	2419	58.69	0.233	<0.001	0.420

In line with expectations, elevated levels of MR resulted in improved growth from week 1 to week 18 (P = 0.016). Within the first 18 weeks of life, calves fed the control concentrate had increased live weights (P = 0.036; Figure 1). Between birth and weaning (day 63), calves fed control concentrate had a 70 g/day increased gain (additive ADG = 0.54kg/day vs. Control ADG = 0.61kg/day; P = 0.026). Within the post-weaning period (days 63 – 84), calves fed the control concentrate had a 140 g/day increased ADG over calves fed the additive concentrate (additive ADG = 0.91kg/day vs Control ADG = 1.05kg/day; P = 0.031). Analysis of the methane emissions of these calves is currently underway.

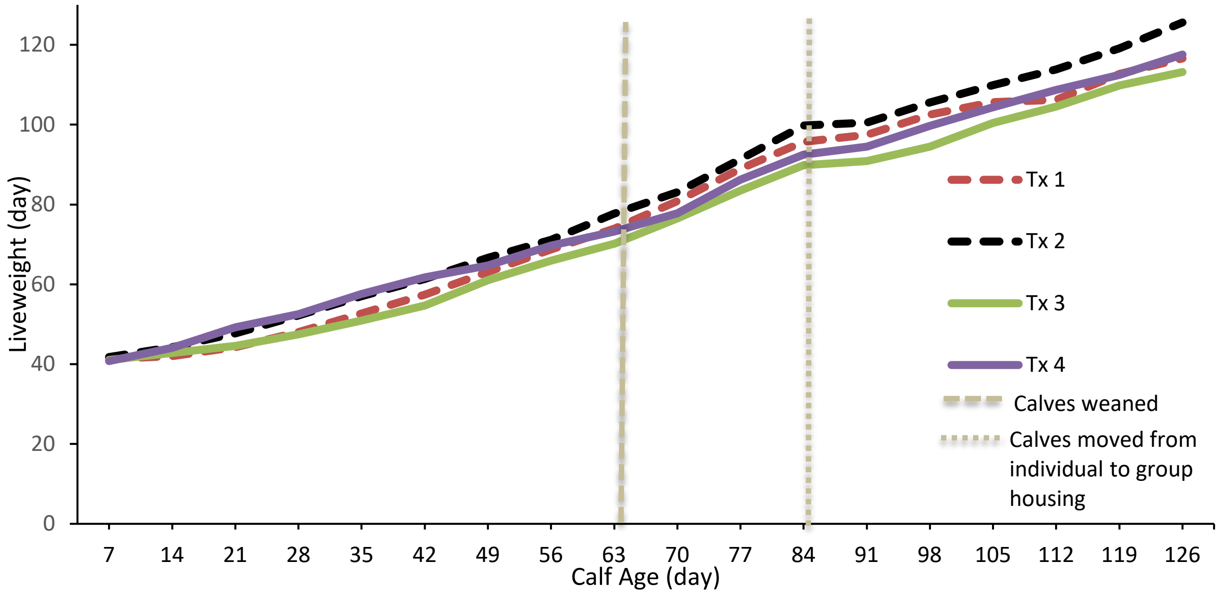

Figure 1. Live weight (kg) of calves fed each treatment (Tx) from day 7 to day 126. The treatments are as follows Tx 1 = Conventional MR, Control concentrate; Tx 2 = Elevated MR, Control concentrate; Tx3 = Conventional MR, Additive concentrate; Tx4 = Elevated MR, Additive concentrate.

Conclusions

The study shows that feeding calves elevated levels of MR during the pre-weaning period enhances growth as evidenced by increased average daily gains. The supplementation of calcium peroxide at 2.25% DM reduced concentrate intake in the first 12 weeks of life and live weight from day 7 to day 84. Liveweight was not significantly different between additive and control treatments from week 12 to week 18. More examinations are needed on completion of methane emissions analysis to investigate the effectiveness of calcium peroxide at suppressing methane, specifically in the post treatment period.

Acknowledgements

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035

Exploring farmers' perceptions of the value and management of dairy-bred calves in block calving dairy systems in England

C. Green[1,2], A. Mackenzie[2], D. Gilbert[2], P. A. Robinson[1,2,3]
[1]Harper & Keele Veterinary School, Edgmond, Newport TF10 8NB, UK
[2]Harper Adams University, Edgmond, Newport TF10 8NB, UK
[3]Keele University, Keele, Newcastle ST5 5BG, UK

Presenting author
Chloe Green. E-mail: cgreen@live.harper.ac.uk

Application

This research highlights that the lower perceived value of dairy-beef and bull calves from block calving systems has led to the differing management of many of these calves compared to replacement heifers.

Introduction

With the rise of plant-based milk alternatives and studies indicating the awareness of consumers towards dairy calf welfare (Perttu et al., 2021; Sirovica et al., 2022), the UK dairy industry has seen the introduction of more calf welfare-focused policies. For example, the GB dairy calf strategy (NFU and AHDB, 2021) sets several key targets including the standardisation of treatment of dairy-bred beef and bull calves with that of dairy heifers along with a milk purchaser commitment to prevent the slaughter of healthy calves. Similarly, a milk processor operates a calf policy within their milk contract terms that encompasses these aims, stating that no healthy calf may be slaughtered on farm before eight weeks of age (Arla, 2019). In the 2023 season, over 1.9million dairy-bred calves were sent to slaughter from four days of age (Ministry for Primary Industries, 2024). Policies such as those operated by Arla remove the option for the early culling of calves. With the majority of block calving systems aiming to calve the herd within a 12-week period, calves for the production cycle are reared within a small proportion of the year. With the increased management pressure on these systems and the potential for other milk buyers to implement such policies in the future, the objective of the study was therefore to investigate the perceived value of surplus and replacement calves, and the effect this has on management and welfare.

Materials and Methods

Participants were recruited through purposive and snowball sampling (Cohen, Manion and Morrison, 2007), starting with existing contact networks followed by farmers suggested by participants, usually through farmer discussion groups.

In total, 32 face-to-face semi-structured interviews were conducted with spring and autumn block calving farmers (16 spring, 16 autumn) in the West Midlands and South-East of England.

The topic guide used to inform the interviews contained a mix of question types to allow for the collection of demographic data as well as to explore opinions and experiences relating to system management, the farmer’s experience in dairying, and the care of calves.

Interviews were audio recorded and manually transcribed with transcripts thematically coded in NVivo for Windows (QSR International, Version 14).

Results

Spring calving farms in the sample had greater herd sizes, yet lower average yields per animal than autumn herds ((mean ± SD) 563±332 cows producing 5359±682 l/lactation compared to 416±134 cows producing 7232±969l/lactation). While Friesian and Jersey were the two most common cow breeds (pure- or crossbred) in both systems, Jersey was more commonly seen in spring calving herds (62.5%). A third of the farms in the sample were under Arla milk contracts.

While both spring and autumn calving herds produced dairy-bred beef calves from Angus and Hereford sires, the use of continental beef sires was only reported in the autumn cohort (43.7% of herds). Spring calving farms were almost twice as likely to provide beef and dairy bull calves with the same quality colostrum as replacement heifers. However, while the majority (87.5% of spring and 92.9% of autumn) of farms fed replacements on milk replacer, spring calving farms were more likely to feed beef and dairy bull calves on waste or whole milk (62.5% compared to 50% of autumn farms).

Many participants viewed beef and bull calves as having a lower value than heifer replacements, with beef calves perceived as 'second class citizens' (F6) while replacement calves were 'the future of what we're doing' (F12).

For the majority of participants, the treatment of calves was generally driven by one of three factors:

(i) Value to the system: 'We're going to have only heifers… and then we're going to have all Belgian blue calves so they're all really valuable lives…'(F27), 'then you've got your tail end calves and you know the fate of them anyway (slaughter) so it's a matter of fact of keeping them alive for ten days' (F1).

(ii) Welfare: 'Welfare is massive here and we've worked really hard the past two or three seasons… we're very lucky that we've got a really very passionate team when it comes to cow health and calf health' (F2).

(iii) Simplicity: 'You can only do it (the same) across all the calves, you can't pick and choose. We're not a laboratory or anything' (F11).

Conclusions

Dairy-beef and bull calves were generally viewed as being of lower or little value in comparison to replacement heifers, with the majority of farms managing the two cohorts differently, usually with regards to colostrum and/or milk feeding. Despite this, many participants described striving to meet a minimum standard of care for all calves, driven by a range of social and economic factors, but particularly the requirements of their milk supply contracts.

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036

Modelling the effect of commingling events on bovine coronavirus shedding in beef-on-dairy calves

J. Urbaniec[1], A. Anagnostopoulos[1], S.Gould[2], J. Ellis[3], F. Hoffman[4], F. Meyer[4], P. Morley[5], M. Scott[5], A. Wollums[6], N. Noyes[7], P. Davies[1], J. Neary[1]

[1]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool L69 7ZX, UK

[2]Department of Clinical Sciences, Liverpool School of Tropical Medicine and Tropical and Infectious Diseases Unit, Royal Liverpool University Hospitals Foundation Trust, Liverpool L69 7ZX, UK

[3]Western College of Veterinary Medicine, University of Saskatchewan, 105 Administration Pl, Saskatoon SK S7N 5A2, Canada

[4]Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology, Mississippi State University, Hood Drive MS 39762, USA

[5]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, 400 Bizzell St, College Station TX 77840, USA

[6]College of Veterinary Medicine, Mississippi State University, Hood Drive MS 39762, USA

[7]Department of Veterinary Population Medicine, University of Minnesota, Minneapolis, MN 55455, USA

Presenting author

Joanna Urbaniec. E-mail: j.urbaniec@liverpool.ac.uk

Application

Modelling the effect of length and intensity of commingling events on development of pneumonia and shedding of pneumonia-associated pathogens could aidin respiratory disease management and prevention.

Introduction

Bovine respiratory disease (BRD) is a leading cause of calf morbidity and mortality, costing the UK cattle industry approximately £50 million a year (Agriculture and Horticulture Development Board, 2024). Commingling events, such as mixing animals from different sources at auction markets or feedlots, are well-known to increase risk of disease transmission, and are a major risk factor for the development of BRD. In this study we aimed to model the relationship between commingling length and intensity, development of BRD, and shedding of bovine coronavirus (BCV).

Materials and methods

The study was conducted on weaned Angus x Holstein calves: 40 per cohort were obtained from five source farms (SF). In total, 80 calves (two cohorts) from seven SF were enrolled in the study, as due to practical constraints two SFs differed between cohorts. Animals were transported to the University of Liverpool research facility and quarantined for 12-14 days. Following quarantine calves were randomised within the strata of farm and sex, into one of four commingling groups: none (single-source pen), low-level (pen with 2 calves from one SF and 8 calves from a second SF), moderate (pen with 5 calves from one SF and 5 calves from a second SF), and intensive (pen with 2 calves from each of the 5 SFs). There was at least 3m air gap between pen groups. Serum, nasopharyngeal swabs and faecal samples were collected on source farms on the day of calf collection (arrival), the day of commingling (day 0), and then 3, 7 and 21-days post commingling. Swabs and faecal samples were subjected to quantitative PCR for BCV, targeting the conserved region of the membrane (M) protein. Serum samples were subjected to anti-BCV IgG ELISA at arrival, and then commingling days 0, 7 and 21. Furthermore, calves were scored daily for signs of clinical BRD using a modified Wisconsin system, and for subclinical BRD by thoracic ultrasonography on arrival and commingling days 0 and 21.

To assess the potential of airborne BCV transmission Sartorius MD8 Airport and SKC button air samplers were fitted in two selected pens on commingling days 7 and 21. Aerosol samples were collected from the button samplers by continuous filtration over gelatine membranes on sampling days (~ 8h) and from MD8 Airport samplers by 30-minute filtration both prior and during sampling events. RNA extracted from aerosol samples was subjected to quantitative PCR for BCV. Furthermore, biological material extracted from aerosol samples was subjected to serial passage in VERO cells as previously described (Gould *et al.* 2022).

Relationship between commingling length and intensity, development of BRD, and BCV shedding was modelled with linear mixed-effects models in R studio release 2024.09.0+375.

Results

Based on preliminary results from the first cohort, crude prevalence of nasopharyngeal BCV on arrival and on the day of commingling was 10 and 5%, respectively. After commingling, nasal BCV prevalence remained relatively low (<10%) until day 21, when 53% of samples were positive. Highest prevalence (100% on day 21) was observed in the pen with animals from a single source farm, with prevalence decreasing with commingling intensity: 12.5% in low and moderate commingling pens, and 7.5% in the intensive-commingling pen. Intensity of nasal BCV shedding was also relatively low until day 21, rising from an average of 10^{4-5} to 10^7 viral copy number/µL in positive animals. Additionally, air swabs collected in the pens were positive for BCV RNA at low copy number ($< 10^4$ viral copy number/µL).

Crude prevalence of faecal BCV was 15 and 10% on arrival and commingling day respectively. Following a similar pattern to nasal BCV, faecal prevalence remained at <10% until 21 days post-commingling when it rose to 43%. There was a positive correlation between nasal and faecal BCV shedding, with the odds of calves shedding faecal BCV being 3.8 times greater if they were also shedding BCV from the nares at any experimental timepoint.

All calves were seropositive for BCV antibodies on arrival and their antibody levels remained high throughout the study. Both commingling time and intensity influenced BCV shedding, with commingling time of 21 days modelled to increase BCV shedding by 3.1 log viral copies/µL relative to the day of commingling (p=2.11e-10) and assignment to high-commingling pen to decrease it by 1.27 log viral copies/µL relative to the single source pen (p=0.0062). Neither clinical pneumonia signs, nor presence of lung lesions were found to be significantly associated with BCV shedding.

Conclusions

Nasal BCV shedding was significantly impacted by commingling length and intensity; however, we found no association between BCV shedding and development of clinical or subclinical pneumonia. At the time of submission, we have completed sample collection of two cohorts and data analysis of the first cohort. Data analysis of the second cohort, including additional environmental sampling, is ongoing. Results from these analyses should help to further disentangle the relationship between BCV and BRD.

Acknowledgements

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037

Effects of age and diet on the physiology of unweaned calves during ferry and road transport

S. Siegmann[1,2], L. van Dijk[1,3], N. L. Field[1], K. Sugrue[1], G. Sayers[3], K. van Reenen[2], E. A. M. Bokkers[2], M. Conneely[1]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]Wageningen University & Research, Droevendaalsesteeg 2, 6708 PB Wageningen, Netherlands
[3]Munster Technological University, 46 Grand Parade, Cork City T12 VN56, Ireland

Presenting author
Susanne Siegmann. E-mail: susanne.siegmann@teagasc.ie

Application

This study emphasizes the importance of feeding unweaned calves adequate amounts of milk replacer before transport to reduce dehydration, but shows that long-distance transport compromises calf physiology regardless of age or pre-transport diet, highlighting the need for more comprehensive strategies to improve welfare during transportation.

Introduction

Long-distance transport poses many challenges to calf welfare and physiology, including extended fasting intervals, which may lead to depleted energy levels, dehydration, and elevated stress. Older calves with more body reserves may be able to better cope with long fasting periods, and feeding calves higher volumes of milk replacer before departure may aid in maintaining a positive energy balance and hydration status throughout transport. The aim of this study was to investigate how calf age and pre-transport diet affect the physiological status of unweaned calves during and after long-distance transport via ferry and road.

Materials and Methods

We followed a commercial shipment of 138 male calves (118 Holstein-Friesian, 20 Holstein-Friesian crossbreds) from an assembly centre (AC) in Ireland via road and ferry to a lairage (LA) in France (24.5h + 13h rest stop) and then via road to a veal farm (VF) in the Netherlands (13.5h). The study had a 2 x 2 factorial design with factor 1) calf age: 2-3 weeks (“younger”) or 4-5 weeks (“older”), and factor 2) pre-transport diet: calves were fed 2L or 4L of milk replacer the morning of transport. Blood samples were collected at the AC and LA, upon arrival at the VF and on Day 8 after arrival. Body weight (BW) was recorded at the same time points and on Day 22 after arrival. Blood samples were analysed for variables indicating energy balance (glucose, β-hydroxybutyrate, non-esterified fatty acids), hydration status (urea, total protein, albumin, sodium, potassium, chloride, haematocrit), muscle fatigue (creatine kinase, lactate), and stress (cortisol). Linear mixed models with age, diet, time points and their interactions as fixed effects and animals and farms of origin as random effects were used to analyse all variables.

Results

Younger calves had higher glucose levels than older calves at the AC (4.51 ± 0.09 vs 4.16 ± 0.09 mmol/L, $P < 0.01$), arrival on the VF (4.52 ± 0.09 vs 4.13 ± 0.09 mmol/L, $P < 0.01$), and Day 8 (5.59 ± 0.09 vs 5.28 ± 0.09 mmol/L, $P = 0.01$). Calves fed 2L had higher urea levels at the LA than calves fed 4L (4.01 ± 0.17 vs 3.10 ± 0.13 mmol/L, $P < 0.001$). Younger calves had lower sodium levels at arrival on the VF (139.9 ± 0.3 vs 140.8 ± 0.3 mmol/L, $P = 0.03$), and lower chloride levels at the AC (97.41 ± 0.29 vs 99.01 ± 0.29 mmol/L, $P < 0.001$) and LA (96.60 ± 0.28 vs 97.50 ± 0.29 mmol/L, $P < 0.01$). Indicators of muscle fatigue and stress were not affected by age or diet. Age had an overall effect on BW, with younger animals weighing less than older animals over the entire study period (49.8 ± 0.9 vs 53.0 ± 0.9 kg, $P < 0.01$), but neither age nor diet affected changes in BW between time points. Mean values for all treatments were outside reference limits for healthy calves for 7/13 blood variables at AC (and glucose for older calves), 9/13 at LA (and urea for 2L calves), 7/13 at arrival (and glucose for older calves, potassium for 2L calves), and 2/13 on Day 8 after arrival (and creatine kinase for older calves).

Conclusions

Calves were notably challenged by long-distance transport, with more than half of all blood variables outside reference limits during transport regardless of treatment, but normal calf physiology was mostly restored by Day 8 after transport. Calf age affected energy balance, hydration status and BW: younger calves had higher glucose levels and showed fewer signs of dehydration (lower levels of electrolytes) during transport but weighed less throughout the study period. Feeding calves 4L of milk replacer instead of 2L before transport had limited positive effects on their hydration status: urea levels were higher and outside reference limits for calves only fed 2L after ferry transport, but none of the other hydration variables were affected by diet.

038

BSAS Undergraduate Thesis of the Year 2024 Winner | An investigation into the effect of probiotic supplementation on the prevalence and severity of Cryptosporidium infections in young calves

M. Allan[1,2]
[1]SRUC,
[2]The University of Glasgow

Presenting author
May Allan. s30075417@student.sruc.ac.uk

Introduction
Cryptosporidiosis causes substantial health and economic issues for the livestock industry, particularly affecting newborn calves. This study investigates the effectiveness of probiotic supplementation in reducing the prevalence and severity of *Cryptosporidium* infections in young calves in a commercial setting. Probiotics, which are known for their benefits on gastrointestinal health, have been suggested to offer therapeutic therapy against *Cryptosporidium* due to their impact on gut microbiota and immune response.

Materials and methods
The study, which was conducted over 6 weeks on a commercial dairy farm, involved 50 calves systematically separated into treatment and control groups. Probiotic supplementation was administered to the treatment group following manufacturer guidelines. Data collecting methodologies included faecal sample testing, used to confirm the presence of *Cryptosporidium*, as well as to confirm that *Cryptosporidium* was the causative agent of diarrhoea amongst the calves. Scour scoring, body weight measurements, and body temperature were also monitored and recorded to compare the severity of symptoms amongst groups.

Results
Results indicated a significant decrease in *Cryptosporidium* prevalence in the treatment group, as well as significant impacts on milk consumption, temperature regulation and weight gain ($P < 0.05$). Probiotic supplementation resulted in greater morning milk consumption (Estimate = 0.327 litres, SE = 0.046 litres, t-value = 7.145, $P < 0.0001$) and evening milk consumption (Estimate = 0.1847 litres, SE = 0.0446 litres, t-value = 4.139, $P < 0.0001$). These findings demonstrate the positive impact of probiotic supplementation on feeding behaviour.

Temperature regulation in treated calves was notably improved, as evidenced by lower temperatures compared to the control group (Estimate = -0.36°C, SE = 0.09°C, $P = 0.0052$). Additionally, the analysis of scour scores revealed a significant effect of the treatment on reducing scour severity (Estimate = -1.83, z = -8.25, $P < 0.001$). Despite variations in breed, sex, and pen, the probiotic supplementation consistently resulted in lower scour scores, indicating improved gastrointestinal health and reduced disease severity. Treatment effects varied with age, suggesting dynamic responses over the study period. Post-hoc comparisons identified significant temperature changes at ages 7, 8, 9, 10, 11, and 12 days old ($P < 0.05$), indicating the course of infection, and how symptoms vary over time.

Conclusion

This research highlights the potential of probiotic supplementation as a viable option for reducing *Cryptosporidium* infections in young calves, opening up new possibilities for improved livestock health and economic sustainability in the agricultural industry. Further research into probiotic mechanisms and long-term effects is needed to optimise their use in cattle management methods.

039

Economic, environmental and food security contribution of mixed farming crop-livestock rotational-based grazing beef systems

F. Pereryra-Goday[1], J. Rivero[2]
[1]*Instituto Nacional de Investigación Agropecuaria (INIA), Rincón del Colorado, Canelones, Uruguay*
[2]*Rothamsted Research North Wyke, Okehampton EX20 2SB, UK*

Presenting author
Jordana Rivero. E-mail: jordana.rivero@genusplc.com

Introduction

This work addresses the sustainable intensification of production systems through the analysis of four systems that combine agriculture and livestock with varying levels of soil use intensification. We evaluated their economic outcomes, environmental impact (i.e., emission profiles, contribution of each process, and partial carbon footprint), and contributions to food security using data from a long-term experiment.

Material and Methods

The study evaluated four crop-livestock systems in Uruguay, with temporal and spatial combinations of land use (Table 1). The continuous cropping system (CC, 12 ha) involves reseeding two crops per year without rotating with pastures but includes an external 6 ha area of permanent pasture with tall fescue, birdsfoot trefoil, and white clover, reseeded every five years for continuous growth. The short rotation system (SR, 24 ha) alternates two years of crops, similar to the CC system, with two years of grass-legume pastures, including Yorkshire fog and Italian ryegrass with red clover. The long rotation system (LR, 36 ha) alternates two years of crops, as in the CC and SR systems, with four years of pastures composed of tall fescue, birdsfoot trefoil, and white clover. The forage rotation system (FR, 24 ha) is reseeded with tall fescue and does not include crop rotations. Each pasture-crop rotation (CC, SR, and LR) is divided into two areas: one for grain production, planted with oats, wheat, soybean, and sorghum; and one for grazing, where Italian ryegrass and oats are planted in winter, and sorghum and moha are grown in summer.

In the CC, SR, and LR systems, animals are introduced to their paddocks from April to May and remain for one year (for rearing) or until reaching slaughter weight (for finishing). In the FR system, animals are introduced from November to December annually. In CC, the objective was to rear male calves for one year. In SR, the objective was to rear heifers, complemented by finishing culled cows during May and September. In LR, the objective was to rear male calves and finish steers over 18 months. FR begins at the end of spring (Nov-Dec) with yearling steers instead of weaned calves. The objective of the livestock strategy in FR was to produce a finished steer ready for slaughter in 12-15 months. British early-maturing beef cattle were used in the four systems (Hereford, Aberdeen Angus, and Hereford-Angus cross).

Table 1. Cropping and pasture sequences of the four pasture-crop rotations at the ‘Palo a Pique’ long term experiment.

Rotation[1]	**Purpose of the crop phase**	**Years of the rotation**					
		Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**
Cntinuous cropping (CC)	Crop	Oat/Sorghum	Black Oat/ Soybe	Wheat/Sorghum			
	Grazing	Oat/Sorghum	Ryegrass/Moha	Oat/Sorghum			
Short rotation (SR)	Crop	Idem CC	Idem CC	Wheat + P1	P2		
	Grazing	Idem CC	Idem CC	P1	P2		
Long rotation (LR)	Crop	Idem CC and SR	Idem CC and SR	Wheat + P1	P2	P3	P4
	Grazing	Idem CC and SR	Idem CC and SR	P1	P2	P3	P4
Forage rotation	Grazing	Fescue	Fescue	Fescue	Fescue	Fescue	Fescue

Note: Yellow and green areas represent crop and pasture phases, respectively. P: pasture, followed by pasture age (i.e.: P2: second-year pasture). All pastures, including those following the grain/hay crop phase, were available for grazing.

Production data (grain and livestock) was obtained from Pereyra-Goday et al. (2022). Carbon footprints were calculated following Life Cycle Assessment (LCA) methodologies, as reported in Pereyra-Goday et al. (2024). Nitrogen use efficiency (NUE) was calculated as nitrogen in food outputs relative to total nitrogen inputs, as reported in Pereyra-Goday (2024). Economic data was collected on input costs and revenues from grain and livestock sales based on the physical parameters for tree cycles (2019-2022), as reported by Pereyra-Goday et al. (2022). We evaluated the contribution to food security through the production of human-edible protein (HEP) and human-edible energy (HEE), using the methodology proposed by Mosnier et al. (2021). Lastly, soil erosion was estimated using the Universal Soil Loss Equation (USLE-RUSLE) model (software Erosión 6.0, MGAP, Uruguay).

Results and Discussion

A summary of the main sustainability indicators calculated are shown in Table 2. The grey cells indicate that no statistical analysis was permitted due to the lack of replicates, green cells mean high/desirable values, red cells mean low/non-desired values, and yellow cells mean intermediate values. Systems with integrated crop-livestock components, especially those with longer pasture phases (LR), provide greater environmental benefits by reducing soil erosion and carbon emissions. These systems also enhance NUE due to biological nitrogen fixation from legumes. However, the CC system, with its intensive cropping strategy, delivers higher short-term economic returns and food production but poses long-term sustainability risks due to higher soil losses and emissions. Pasture-based systems (FR and LR) offer valuable ecosystem services, including nutrient recycling, carbon sequestration, and biodiversity conservation. Although they yield lower human-edible protein and energy, they avoid direct competition with human food supply and contribute to a more sustainable agricultural model. The systems combining pasture and cropping (SR and LR) yielded the highest economic returns due to the diversification of income sources. The CC system showed high production costs but delivered higher returns in favourable years. Systems with a greater focus on pasture, such as FR, exhibited lower production variability but also lower overall productivity.

Table 2

Main indicators calculated for the four systems ("*" there was not harvest, "-" does not apply).

Parameter	Unit	CC				SR				LR				FR			
Liveweight production[1]	kg LW/ha	426				418				369				310			
		Soybean	Wheat	Oats	Sorghum	Soybean	Wheat	Oats	Sorghum	Soybean	Wheat	Oats	Sorghum	Soybean	Wheat	Oats	Sorghum
Crop production[1]	kg/ha	2.40	2.21	*	4.97	2.48	2.68	1.81	5.65	2.76	2.59	*	5.29	-	-	-	-
Forage production[1]	kg DM/ha	5206				5763				5399				6867			
GHG emissions intensity[2]	kg CO_2 eq/kg LW	11.3				11.8				11.8				16.4			
GHG emissions intensity[2]	kg CO_2 eq/ha	2795				2734				2727				2607			
GHG emissions intensity[2]	kg CO_2 eq/kg crop	1.36	0.61	*	-	1.24	0.54	0.57	-	1.01	0.43	0.47	-	-	-	-	-
Nitrogen use efficiency (crop)[3]	%	62.5				83.8				77.5				-			
Nitrogen use efficiency (livestock)[3]	%	24.4				9.9				14.3				5.5			
Gross margin[4]	US$/ha	181				200				285				197			
Emissions per US dollar[4]	kg CO_2 eq/US$	15.4				13.7				9.6				13.2			
HEP and HEE[4]	kg/ha	High				Medium				Medium				Low			
Soil losses[4]	tonnes/ha	7.16				4.11				3.17				2.49			

[1]Pereyra-Goday et al (2022); [2]Pereyra-Goday et al (2024); [3]Pereyra-Goday et al (2025);[4]This study

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040

The impact of incorporating ribwort plantain into perennial ryegrass swards on the performance of high-yielding dairy cows

N. Rutherford[1], L. Chesney[1], C. Holohan[1], D. Patterson[1]
[1]*Agri-food And Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Naomi Rutherford. E-mail: naomi.rutherford@afbini.gov.uk

Application

Similar levels of cow performance can be achieved when grazing plantain-ryegrass swards and perennial ryegrass swards.

Introduction

In an endeavour to reach net zero targets and reduce the environmental impacts of our dairy production systems, there has been growing interest in alternative sward types. Ribwort plantain (*Plantago lanceolata L.*) is one such forage, that could be incorporated into perennial ryegrass swards. Research from New Zealand has shown reductions in nitrogen (N) losses and nitrate leaching (Navarrete et al., 2022) and lower nitrous oxide emissions (Vi et al., 2023) in plantain swards compared to ryegrass-based swards. Recent research conducted at AFBI (unpublished) has produced similar findings. However, the impact of alternative sward types on cow performance, must also be assessed, as this will be a large determinant of the adoption of plantain swards within the dairy industry. The aim of this study was to investigate the impact of plantain inclusion in a perennial ryegrass (PRG) sward on cow performance.

Materials and methods

This experiment was conducted at the Agri-Food & Bioscience Institute in Hillsborough, Northern Ireland. A total of 44 spring-calving Holstein-Friesian cows were assigned to one of two sward type treatments in a randomized complete block design, with two replicate groups of 11 cows per treatment. Each replicate was balanced for parity, days in milk, body weight, BCS, milk quality and milk yield. The two sward types were a PRG and a plantain + perennial ryegrass sward (PLN). Swards were initially established in 2021 with additional plantain being stitched into the PLN swards in spring 2024. Cows were paddock grazed (night & day) from May to October 2024 at a stocking rate of 3.8 cows/ha. Cows were turned out in May due to breeding programme commitments and poor weather in March and April. Mean pre-grazing herbage mass was 3203 and 3318 kgDM/ha for PRG and PLN, respectively, likewise mean post-grazing herbage mass was 2010 and 1947 kgDM/ha. Cows received concentrates through the milking parlour at a rate of 4 kgDM/cow/day. Throughout the study, milk yields were monitored twice daily. Milk samples were taken weekly from one successive evening and morning milking for the determination of milk fat, protein, urea and lactose concentrations. Live weight (LW) was recorded daily and body condition score (BCS) was recorded monthly. Pre- and post-grazing herbage mass was determined using a rising platemeter (Jenquip, Fielding, New Zealand), while daily herbage samples were taken for the determination of nutrient composition. Daily sward botanical compositions were completed on both sward types. Statistical analyses were conducted using Genstat (21st Edition) by REML repeated measures.

Results

Mean botanical composition over the course of the study showed that plantain content of the PLN sward was 27.9%. The proportion of unsown species within the swards were 2.4% and 6.0%, for PRG and PLN, respectively (*P*<0.001). The remaining proportion in each sward was PRG (*P*<0.001). The results in Table 1 show that there was no difference in cow performance between a PRG sward and a 27.9% PLN sward. These findings are in agreement with that of Nguyen et al. (2024).

Table 1.
The effect of sward type on cow performance

	PRG	**PLN**	**SED**	**P-value**
Milk yield (L/cow/day)	23.3	23.0	0.80	ns
Milk solids (kg/cow/day)	1.97	2.02	0.048	ns
Milk fat (%)	4.98	5.15	0.172	ns
Milk protein (%)	3.59	3.59	0.085	ns
Milk urea	275	278	8.4	ns
BCS	2.55	2.58	0.059	ns

Conclusion

Results from this study show that similar levels of cow performance can be achieved from both PRG swards and PLN swards with a mean 27.9% plantain inclusion rate over the season.

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041

Cell grazing enhances soil carbon storage in intensively managed sheep pastures

T. T. Akpensuen[1,2], C. Morten[1], S. Demirkaya[1], M. S. A. Blackwell[1], J. Rivero[1]
[1]*Rothamsted Research, West Common Harpenden AL5 2JQ, UK*
[2]*Faculty of Agriculture University of Jos, Plateau State, Nigeria*

Presenting author
Tersur Theophilus Akpensuen. E-mail: theo.akpensuen@rothamsted.ac.uk

Application

Cell grazing and undrained pasture plots had greater total soil carbon concentration and stock than continuous stocking after five years, which could enhance overall total soil health.

Introduction

Grasslands are vital for supplying food, goods, and services that support the livelihoods and economies of people globally. They are a key resource for livestock, offering grazing opportunities and conserved feeds. There are distinct benefits and drawbacks associated with livestock grazing systems. Therefore, developing effective grazing management strategies that prioritize the benefits is essential. Grazing management that benefits the soil's physical and chemical properties should be encouraged. Effective management of grassland ecosystems involves carefully balancing priorities like food production, economic sustainability, and the preservation of ecosystem services (Boval and Dixon, 2012). Drainage is believed to cause a significant decrease in total carbon (TC) storage in soil (Harris et al., 2018). The relationship between grazing method (GM) and drainage system (DS) is crucial for managing soil moisture and enhancing pasture productivity, adding complexity to soil chemistry. This study aimed to measure the impact of two GM (cell grazed, CG; and continuous stocking, CS) and DS (drained and undrained) on the TC concentration and stock in intensively managed grassland soils.

Materials and Methods

The study was carried out at Rowden Moor near North Wyke, Rothamsted Research, southwest England. The soil is derived from the underlying Carboniferous Crackington Formation. There were 12 experimental plots of 1-ha each. The plots consist of a series of hydrologically isolated fields (lysimeters). Six plots have surface drainage only (undrained) and six plots have surface drainage plus 85-cm-depth subsurface drainage (drained). Half of the plots of each drainage type were managed under CG (in 20-day rotation cycles) and the other 6 under CS. The CG plots were divided into two lanes of 0.5 ha and each lane was subdivided into 30 cells of 167 m^2 using bespoke electric fencing and water supply equipment (KiwiTech Int). All plots were grazed with 10 - 15 ewes and 20-25 lambs from 2018 to 2022 during the grazing season (April to October). Ten soil cores were sampled from each of the 1-ha plots evenly distanced along a '**W**' shape to capture field heterogeneity to a depth of 30 cm in August 2022. At each sampling point, the core was sub-divided into two cores (0-10 cm and 10-30 cm), making 240 soil samples in total. The soil samples were processed and analysed for TC concentration and stock. *Lolium perenne*, *Agrostis stolonifera* and *Holcus lanatus* were the dominant grass species. Data analysis was performed using the linear mixed model of JMP statistical software with GM, DS, and their interaction as fixed effects while plot, and cores were used as random effects.

Results

Significant effects of GM and DS on soil TC were found at 0 – 10 cm soil depth. CG had 0.66 greater percentage points (pp- difference between percentages) carbon concentration than CS (Figure 1a). Undrained plots were 0.71 pp greater than the drained plots. However, the main effects and their interaction were not significant at 0 – 30 cm (p = 0.6158, 0.6349, and 0.3097 for GM, DS, and GM x DS, respectively). Significant interaction existed between GM x DS on TC stock (*F* = 8.1890, *P* = 0.0050) at 0 – 10 cm depth with the highest stocks observed in the undrained CG plots (Figure 1b). At 10 – 30 cm, there were no significant effects from the main factors or their interaction effect (*P* = 0.7123, 0.3178, and 0.8149, for GM, DS, and GM x DS, respectively). CG pastures are typically reported to have more plant litter and standing herbage that reduces evaporation losses by moderating extremes in soil surface temperatures, protecting the soil from drying winds, and conserving moisture better than CS pastures (Teutscherova et al., 2021), which could promote organic matter turnover compared to CS systems.

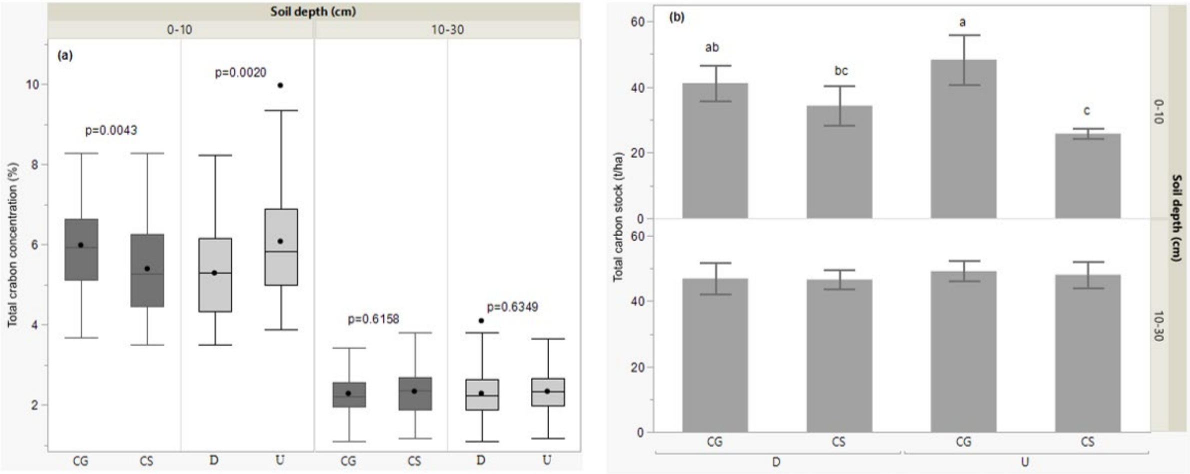

Figure 1. Box plots showing the main effect grazing method (GM; cell grazed, CG; and continuous stocking, CS) and drainage system (DS; drained, D; and undrained, U) for total carbon concentration (a) and the bar graph with GM x DS (b) at 0 -10 cm and 10 – 30 cm soil depth. Error bars are constructed from a 95% confidence interval.

Conclusions

Greater TC concentrations and stocks occurred in the upper soil layer (0-10cm) in CG plots compared to CS plots after five years of different management.

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042

Effect of silage species and silage feeding rate on the milk production of late lactation, grazing dairy cows

M. Chaize[1,3], M. Egan[1], R. Fitzgerald[1], R. van Emmenis[1], A. Y. Ikoyi[2], N. Byrne[2], T. F. O'Callaghan[3], M. Dineen[1]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[3]School of Food and Nutritional Sciences, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland

Presenting author
Mathilde Chaize. E-mail: mathilde.chaize@teagasc.ie

Application

Red clover has the ability to produce high herbage yields at reduced chemical nitrogen application, and to maintain dairy cow performance.

Introduction

The European Green deal set a target to reduce greenhouse gas emissions by 55% and to reach a 20% reduction in inorganic nitrogen (N) fertiliser use by 2030. Perennial ryegrass (Lolium perenne L.; PRG) monocultures are highly dependent on inorganic N to maintain high herbage production. The inclusion of red clover (Trifolium pratense L.) into PRG silage swards without inorganic N application maintained herbage production, similar to PRG monocultures receiving 412 kg of inorganic N/ha/year (Clavin et al., 2016). In grass-based systems, increasing the proportion of grazed pasture in the diet of dairy cows was shown to improve farm profitability (Dillon et al., 2005). During restricted grazing conditions or low pasture supply, cows typically graze by day and are supplemented with silage at night. In indoor feeding systems, dry matter (DM) intake and milk production were improved when red clover silage was fed to dairy cows, when compared to PRG silage (Steinshamn, 2010). The objective of this study was to investigate the effect of silage species and silage feeding rate on the milk production of late lactation dairy cows, within a pasture-based system.

Materials and Methods

This study was conducted from mid-September to the end of November 2023. Eighty Holstein-Friesian and Jersey x Holstein-Friesian crossbred dairy cows in late lactation were blocked based on pre-experimental milk production, parity and breed. Cows were then randomly assigned to one of four dietary treatments in a 2×2 factorial design including two silage species (PRG silage (GS) vs. PRG-red clover silage (GRCS)) and two silage feeding rates (restricted to 8 kg of DM/cow (L) vs. ad libitum (H; targeting 5% refusals)). All groups grazed separately by day and received silage at night along with 2.68 kg of DM of a concentrate comprising 166 g of crude protein/kg of DM. The experiment consisted of a 2-wk covariate period, followed by eight weeks of data collection. Individual milk yields were recorded daily, and milk composition weekly. Milk samples from two consecutive evening and morning milkings were collected and analysed using a Milkoscan 7. Weekly milk solids yields (fat yield + protein yield) were then calculated. Data were analysed using a mixed model in R. The fixed effects included in the model were silage species, silage feeding rate, week, their interaction, parity and breed. Cow was included in the model as a random effect and a covariate adjustment was applied for each cow. The repeated effect of the model was based on week. Statistical significance was considered at $P \leq 0.05$ and statistical trends at $0.05 < P \leq 0.10$.

Results

Cows fed GRCS tended to have higher milk yield than cows fed GS ($P = 0.07$; Table 1). Cows fed GS had higher milk protein and fat concentrations when compared with cows fed GRCS ($P < 0.01$), leading to similar milk solids yield among treatments ($P = 0.57$). Cows fed H silage rates had higher milk protein concentration, when compared to cows fed L silage rates ($P = 0.03$). There was no effect of silage feeding rate on milk yield, milk fat concentration and milk solids yield ($P = 0.48$, $P = 0.30$ and $P = 0.89$, respectively). There was no interaction between silage species and silage feeding rate.

Table 1 Effect of silage species and silage feeding rate on the milk production and milk composition of grazing dairy cows in late lactation

	Diet[1]					*P*-value		
Item	H-GS	L-GS	H-GRCS	L-GRCS	SEM[2]	Silage	Rate	Silage*Rate
Milk yield, kg/d	13.5	13.7	13.9	14.2	0.26	0.07	0.48	0.94
Protein, g/kg	44.9	44.4	43.8	43.2	0.27	< 0.01	0.03	0.84
Fat, g/kg	62.3	60.8	59.7	59.9	0.70	< 0.01	0.30	0.19
Protein yield, kg/d	0.60	0.60	0.60	0.61	0.01	0.67	0.98	0.88
Fat yield, kg/d	0.83	0.81	0.82	0.84	0.02	0.53	0.88	0.35
Milk solids yield, kg/d	1.43	1.42	1.42	1.45	0.03	0.57	0.89	0.54

[1]H = *ad libitum* silage; L = 8 kg DM of silage/cow; GS = grass silage; GRCS = grass-red clover silage;
[2]SEM = standard error of the mean.

Conclusions

Feeding grass-red clover silage maintained animal performance, irrespective of the feeding rate. Future research should investigate the environmental impact of feeding red clover silage within a pasture-based system, along with a more in depth study of the physiological mechanisms occurring to explore the reduction in milk protein and fat concentrations from cows fed PRG-red clover silage.

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043

Effect of grass-only and grass-clover based swards on the growth performance of suckler bred heifers and steers finished at 19.5 months of age

P. Bennett[1,2], M. McGee[1], A. K. Kelly[2], M. O'Donovan[3], P. Doyle[1]
[1]*Teagasc Animal and Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath C15 PW93, Ireland*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*
[3]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*

Presenting author
Peter Bennett. E-mail: peter.bennett@teagasc.ie

Application

Incorporating red and white clover into grass swards can reduce the requirement for chemical nitrogen fertiliser and increase animal live weight gain within a suckler calf-to-beef system.

Introduction

Grazed grass-white clover is recognized as a cost-efficient feed source for beef cattle production systems (Doyle et al., 2024). The inclusion of clover species in pastures, particularly white and red clover, provides the dual benefit of biologically fixing nitrogen (N) and reducing dependency on chemical fertilisers. Although white clover is optimized for grazing systems, red clover is more suited for silage production. Research has shown that cattle grazing grass-white clover swards achieve approximately 0.1 kg greater live weight gain during the second grazing season compared to grass-only swards (Doyle et al., 2024; Fitzpatrick et al., 2024). Despite these advantages, there is limited research on the long-term performance of suckler beef cattle raised on grass-only versus grass-clover systems from birth-to-finish. The present study evaluates the growth performance and carcass traits of suckler cattle produced on perennial ryegrass-only (GO) or perennial ryegrass-clover (GC) systems, incorporating grazed white clover and red clover silage.

Materials and methods

This research was conducted at Teagasc Grange Beef Research Centre between February 2023 and October 2024. Eighty spring-born Aberdeen Angus- and Charolais-sired calves, from Limousin × Holstein-Friesian dams were assigned to two different pasture farm-treatments; GC or GO. Calf mean date of birth was 26 February 2023, and mean birth weight was 45 kg. Treatments were balanced for sire breed, calf sex, calf weight, date of birth, and dam parity and live weight. All cows and calves were turned out to pasture on 3 April 2023. The GO progeny grazed perennial ryegrass-only swards with their dam up to ca. 7.4 months of age, following which they were weaned, and four weeks later were housed and offered grass-only silage *ad libitum* + 1.25 kg dry matter (DM) concentrate/head for 155 days. At the end of the indoor winter they were turned out to pasture (12 April 2024) and grazed perennial ryegrass-only swards for their ‘second’ grazing season; heifers were grazed separately to steers.

Management of the GC progeny was the same except that they grazed perennial ryegrass-white clover swards and were offered grass-red clover silage during the indoor winter. All animals were finished at pasture (10 October 2024) at a mean age of 19.5 months. On average, the GO and GC land areas received 145 and 75 kg of chemical N fertiliser per hectare, respectively. Male calves were castrated at ca. 6 months of age. Pre-grazing herbage mass, sward pre- and post-grazing height, and clover composition were determined as described previously (Fitzpatrick *et al.,* 2024). Animal live weight was measured at birth, at the start and end of the grazing seasons and every three weeks in between. Post-slaughter, carcasses were weighed and conformation and fat score were determined. Data were analysed using the GLM procedure in SAS; the model had fixed effects for pasture treatment, sex, sire breed, dam parity and their interactions.

Results

Mean white clover content in the grazing swards for GC cattle was 11% and 22% during the first and second grazing season, respectively. Red clover content was 14% and 59% for silage harvests 1 and 2, respectively.

There was no interaction between pasture treatment and sex ($P > 0.05$). The GC cattle tended to be heavier than GO cattle at housing post-weaning (+12 kg; $P = 0.06$), and were heavier at turn-out to pasture in spring (+27 kg; $P < 0.01$), and at finishing (+27 kg; $P < 0.01$), leading to an 18 kg heavier carcass weight ($P < 0.001$) (Table 1). Average daily live weight gain to 200-days of age (1.20 vs. 1.26 kg) and during the first winter (0.54 vs. 0.62 kg) was lower ($P < 0.05$) for GO than GC cattle, but live weight gain did not differ during the second grazing season (1.03 vs. 1.02 kg). There was a positive live weight gain response to grass-white clover during the first grazing season, and to grass-red clover silage during the first indoor winter, but no response to grass-white clover during the second grazing season.

Table 1. Effect of grass-only or grass-clover production system on growth and carcass traits of heifers and steers.

Sex	**Heifers**		**Steers**		**SEM**	**P-value**	
Pasture treatment (PT)	**Grass-only**	**Grass-clover**	**Grass-only**	**Grass-clover**		**PT**	**Sex**
Live weight (kg)							
Birth	41.4	41.5	46.6	45.7	0.96	0.758	0.002
Housing	312	315	322	344	5.0	0.064	0.005
Turn-out to pasture	393	411	406	442	6.2	0.001	0.009
Final	571	592	598	630	6.9	0.004	0.001
Carcass traits							
Carcass weight (kg)	299	308	313	340	3.9	0.001	0.001
Conformation score (1-15)	7.76	8.14	6.81	7.61	0.137	0.001	0.001
Fat score (1-15)	8.35	8.48	6.27	6.80	0.174	0.153	0.001

Conclusion

Incorporating red and white clover into a perennial ryegrass sward in a suckler calf-to-beef system increased beef cattle live weight gain and carcass weight, while reducing chemical N fertiliser requirement.

Acknowledgements

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044

Mapping Antimicrobial Resistance Across Dairy Cows, Human, and Environmental Interfaces in Food-Producing Regions of Brazil: A One Health Perspective

L. Oyama[1], S. Huws[1], H. Mantovani[2], N. Scollan [1], S. McCloughlin[1]
[1]Queens University Belfast, Belfast BT7 1NN, UK
[2]University of Wisconsin-Madison, 500 Lincoln Dr, Madison WI 53706, USA

Presenting author
Linda Oyama. E-mail: l.oyama@qub.ac.uk

Application

Antimicrobial resistance (AMR) poses a global health and economic challenge, with predictions of up to 10 million annual deaths by 2050 (O'Neill, 2014). It complicates disease management, increases healthcare costs, and threatens sustainable agricultural practices. This study focuses on AMR risks associated with livestock farming in Brazil, a key agricultural producer among BRICS nations. A One Health approach was applied to investigate the presence of AMR genes across dairy cow microbiomes, human nasal and hand microbiomes, and farm environments. Findings contribute to scientific understanding and provide actionable insights to inform policy changes, enhance antimicrobial stewardship, and support sustainable agricultural practices to mitigate resistance risks and preserve public health.

Introduction

AMR emerges when bacteria, viruses, or fungi no longer respond to antimicrobials, making infections harder to treat (WHO, 2024). In livestock farming, unregulated antimicrobial use and/or difficulties in implementing regulations exacerbate resistance development. Within the UK, sales patterns for veterinary antibiotics have remained stable over the last few years (UK-VARSS report, 2024). Nonetheless, prescription of antimicrobials for livestock animals in the BRICS countries (Brazil, Russia, India, China, and South Africa) is increasing, and set to double between 2010 and 2030 as a consequence of increased demand for animal protein (O'Neill, 2014). The scale of antibiotic use in agriculture and the large numbers of livestock involved has provided a significant route for dissemination of AMR bacteria to carcasses, to the environment, and subsequently to the human population (Van Boeckel et al., 2015). Brazil's prominence in global livestock and food production and antimicrobial practices make it an important focus for investigating AMR development and dissemination. This study aimed to map AMR trends across human, animal, and environmental interfaces on Brazilian farms and assess the relationship between farming practices and AMR burden in major food-producing regions.

Materials and Methods

A total of 186 samples, including ruminant faeces (36), soil (35), udder swabs (32), and human nasal swabs (20), were collected from farms across four major food-producing states: Minas Gerais, Paraná, Santa Catarina, and São Paulo, with varying practices. Illumina sequencing generated 40 GB of metagenomic data, with an average of 2.37 million reads per sample. Quality filtering and assembly using MetaSPADES resulted in 554 million high-quality reads with read counts per sample ranging from 37,008 to 4,066,265 and a mean read count of 1,491,834 reads per sample. The reads were assembled into contigs using MetaSPADES, resulting in contigs with a median N50 value of 250 bp. Contigs with a length of less than 500 bp were filtered from the dataset for downstream analysis.

Microbiome profiling was conducted with MetaPhlan4, and antimicrobial resistance genes (ARGs) were identified using KMA against the PanRes database (Martiny et al., 2023). Community composition and resistance distribution were analyzed using Principal Coordinate Analysis (PCoA) and Permanova tests. OH relevance was categorized into two levels: Level 1 (OH-L1) if ARGs were detected in at least one sample type across all three OH groups (Animal: Faeces and Udder; Environment: Soil and Bedding; and Human: Hand, Nasal, and Saliva), and Level 2 (OH-L2) if ARGs were present in at least one sample type across two OH groups.

Results

Across all samples in our dataset, 886 genera and 1,786 prokaryotic species were detected. The relative abundance of dominant taxa associated with human hand samples was observed to vary greatly between states. For instance, at the genus level, hand samples from Minas Gerais were dominated by *Kocuria*, *Corynebacterium*, and *Acinetobacter*. Hand samples from Paraná and Santa Catarina were overwhelmingly dominated by *Rothia* and *Cutibacterium*, respectively, while in São Paulo, hand samples were dominated by *Ornithinimicobium* and *Deinococcus*. Human nasal samples were dominated, by varying amounts across states, by *Cutibacterium*, *Corynebacterium*, and *Staphylococcus*. Human saliva samples were largely dominated by *Staphylococcus*, *Prevotella*, and *Actinomyces*.

In relation to animal samples, the abundance of *Bifidobacterium* and an unclassified genus (GGB25583) dominated the microbiota of faecal samples. In addition, *Methanobrevibacter* was found to largely dominate faecal samples from São Paulo. Udder samples were largely dominated by *Corynebacterium* and an unclassified genus (GGB44037). Environmental samples revealed that *Corynebacterium* and GGB44037 were prevalent in samples from Paraná and Santa Catarina, while *Brachybacterium*, *Corynebacterium*, and *Dietzia* dominated bedding samples from São Paulo.

Across all samples, 1,686 ARGs conferring resistance to 25 antibiotic classes were identified, with dominant classes including glycopeptides (284 genes), beta-lactams (279), and aminoglycosides (158). Resistance genes were abundant in human samples, ruminant faeces, udder swabs, and soil samples. Human hand samples were dominated by Tetracyclines and Aminoglycosides (34-55% across samples), human saliva samples by Glycopeptides, Antifolates, and Elfamycins (43-64%), and human nasal samples by Multi-compound, MLS, and Elfamycins (39-57%). Faecal resistomes were dominated by ARGs conferring resistance to Tetracycline, MLS, and Elfamycin (55-74%), while udder samples were dominated by ARGs classified as conferring resistance to Elfamycins, Tetracycline, and MLS (43-59%). Soil ARGs predominantly conferred resistance to Elfamycins, Glycopeptides, and Rifamycin (53-66%), while bedding samples were dominated by Elfamycin, Aminoglycoside, and Rifamycin resistance (41-63%).

PCoA revealed clustering by sample type, with significant differences in microbial and resistome compositions influenced by farming practices and state ($P < 0.001$). Although self-reported antimicrobial usage did not correspond to observed AMR patterns, AMR genes associated with mobile genetic elements (MGEs) were identified and shared across human, animal, and environmental interfaces. Resistance genes for vancomycin, colistin, and quinolones—last-resort antibiotics—were detected, highlighting the critical need for regulation. Cross-interface analysis indicated the presence of ARGs relevant to One Health (OH), with 434 genes detected across human, animal, and environmental samples (OH-L1 level).

Conclusions.

This study underscores the widespread dissemination of AMR genes in Brazilian livestock farming systems, which may be driven by unregulated antimicrobial use. Findings highlight the urgent need for regulatory interventions to limit antimicrobial use in livestock farming to reduce resistance risks, protect critical antimicrobials, and ensure sustainable agricultural production. Insights from this work provide a foundation for evidence-based policy changes to inform AMR strategies in Brazil and other BRICS nations.

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045

Bacteriocin-Producing Lactic Acid Bacteria as Silage Inoculants to Reduce Methane Emissions in Dairy Cattle

K. Linehan[1]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*

Presenting author
Kevin Linehan. E-mail: kevin.linehan@teagasc.ie

Application

Methane emissions from dairy cattle are a significant contributor to agricultural greenhouse gases (GHG), with implications for climate change and sustainability. This study evaluates bacteriocin-producing lactic acid bacteria (LAB) as silage inoculants for methane reduction, offering a practical and scalable solution for dairy farmers. The LAB inoculants reduced methane emissions by 5.7% on average, with a significant 7.74% reduction observed in week 5, while maintaining milk yield, dry matter intake (DMI), and animal health. This approach aligns with national and international climate targets, providing environmental and economic benefits for the dairy industry.

Introduction

Methane, a potent GHG, contributes significantly to climate change, with dairy cattle responsible for a large share of emissions due to enteric fermentation (Wójcik-Gront, 2020). Methane also represents a loss of feed energy, reducing animal efficiency. Addressing these emissions is critical for achieving climate targets, particularly in countries like Ireland, where enteric fermentation accounts for 63% of agricultural methane emissions (Kamyab et al., 2024). This study aimed to evaluate the potential of LAB-based silage inoculants containing *Lactococcus lactis* subsp. *lactis* SL242 (*L. lactis* SL242) and *Lactiplantibacillus plantarum* LP58 (*L. plantarum* LP58) to reduce methane emissions and assess their impact on dairy cow productivity and health.

Materials and Methods

Thirty late-lactation Holstein dairy cows were randomly assigned to treatment (LAB-inoculated silage) and control (non-inoculated silage) groups (n = 15/group). Silage was treated with LAB inoculants at a dose of ~10^{11} colony-forming units (CFU)/cow/day and fed ad libitum over a seven-week period, including a two-week adaptation phase. Methane emissions were measured weekly using the GreenFeed system, while DMI, milk yield, and body condition score (BCS) were monitored. Microbial composition of rumen samples was analyzed using 16S rRNA gene sequencing. Statistical analyses, including mixed models and Wilcoxon tests, were performed, and results were considered significant at $p < 0.05$.

Results

Methane emissions were reduced by an average of 5.7% in the LAB-treated group from weeks 5 to 7, with the greatest reduction observed in week 5 (7.74%, $P < 0.001$). On average, treatment cows produced ~20 g less methane per day than controls (~360 g/day vs. ~380 g/day). DMI, milk yield, and BCS did not differ significantly between groups. Microbial analysis revealed no significant differences in alpha diversity (Shannon and Chao1 indices), but beta diversity analysis identified significant clustering between groups at weeks 4 (*P* = 0.045) and 7 (*P* = 0.042). Taxonomic shifts were observed in bacterial families linked to fiber degradation, while archaeal communities dominated by *Methanobrevibacter* remained stable.

Conclusions

The results demonstrate that LAB-based silage inoculants can reduce methane emissions in dairy cattle without compromising productivity or animal health. The observed 5.7% reduction aligns with climate targets, providing a cost-effective, practical strategy for mitigating GHG emissions in dairy systems. Further research should explore the long-term effects of LAB inoculants on rumen microbiota and methane production under varying conditions to optimize their use for methane mitigation.

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046

Temporal establishment of the colon microbiota in Angus calves from birth to post-weaning

M. Stafford[1,2], P. Smith[1], S. Waters[3], F. Buckley[2], S. McLoughlin[4], S. Kirwan[1], E. O'Hara[5,6], D. Kenny[1]

[1] *Teagasc Animal and Bioscience Research Department, Teagasc Grange, Meath, C15 PW93, Ireland*

[2] *School of Biological, Earth and Environmental Sciences, University College Cork, Cork, T23 N73K, Ireland*

[3]*School of Biological and Chemical Sciences, University of Galway, University Rd, Galway, H91 TK33, Ireland*

[4]*School of Biological Sciences, Queens University Belfast, Belfast, BT7 1NN, UK*

[5]*Department of Agricultural, Food, & Nutritional Sciences, University of Alberta, Edmonton, AB T6G 2P5, Canada*

[6]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, 5403 1st Ave S, Lethbridge, AB, T1J 4B1, Canada.*

Presenting author

Michelle Stafford. E-mail: michelle.stafford@teagasc.ie

Application

The colon plays a pivotal role in early life nutrition, performance and health of calves, particularly during the pseudo-monogastric phase. Optimising the early establishment of a resilient microbiome helps limit the infiltration of pathogens and reduces disease incidence, while enabling better growth and performance in neonatal calves.

Introduction

The colonisation of the gastrointestinal microbiome begins shortly after parturition and is central to modulating host defence mechanisms and contributes to the regulation of inflammatory response. Early microbial colonisation of the lower GIT is influenced by numerous factors including maternal microbiota, age, diet, weaning and environmental factors (Amin and Seifert, 2021). This early microbial colonisation supports the production of various pre-immune B cells and the expression of tight junction proteins, which are protective mucosal barriers (Malmuthuge et al., 2013). The neonatal lower GIT microbial community is a diverse and volatile environment. Therefore, the aim of this study was to characterise the temporal establishment and dynamics of the bacterial community of the colon digesta of Aberdeen Angus calves using DNA amplicon sequencing of the 16S rRNA gene from birth to post-weaning.

Materials and Methods

Animal management protocols were previously described by Surlis et al. (2017) and O'Hara et al. (2020). Heifers were subject to AI from a single Aberdeen Angus Bull sire, 42 heifers utilised for the experiment were housed in Teagasc Mellows Campus, Athenry, Co. Galway, Ireland (Farm 1; F1). During the third trimester, a subset of heifers (n = 18) were transported to the Department of Agriculture, Food and Marine (DAFM) Longtown Research Facility in

Clane, Co. Kildare, Ireland (Farm 2; F2) for calving. Both facilities adhered to the same housing and feeding practices. All calves were delivered transvaginally. The calves assigned to the D0 cohort had no contact with the cow or their external environment following birth. Calves not in the D0 cohort, nursed from their dam for 48 hours before being housed in individual pens. Calves were euthanised via lethal intravenous injections of pentobarbital sodium, according to their allocated groupings. Death was determined by the absence of cardiac activity and the lack of a corneal reflex. The colon digesta samples were collected from calves at day 0 (D0, n=7), D7 (n=7), D14 (n=5), D21 (n=7), D28 (n=5), and D96 (n=7) of life. Samples were snap frozen in liquid nitrogen and stored at -80°C. The DNA was extracted from approximately 250 mg of ground frozen sample, using a modified version of repeated bead beating and column purification method (Yu and Morrison, 2004) and as previously described (Smith et al., 2022). Sequencing was carried out on the Illumina MiSeq platform.

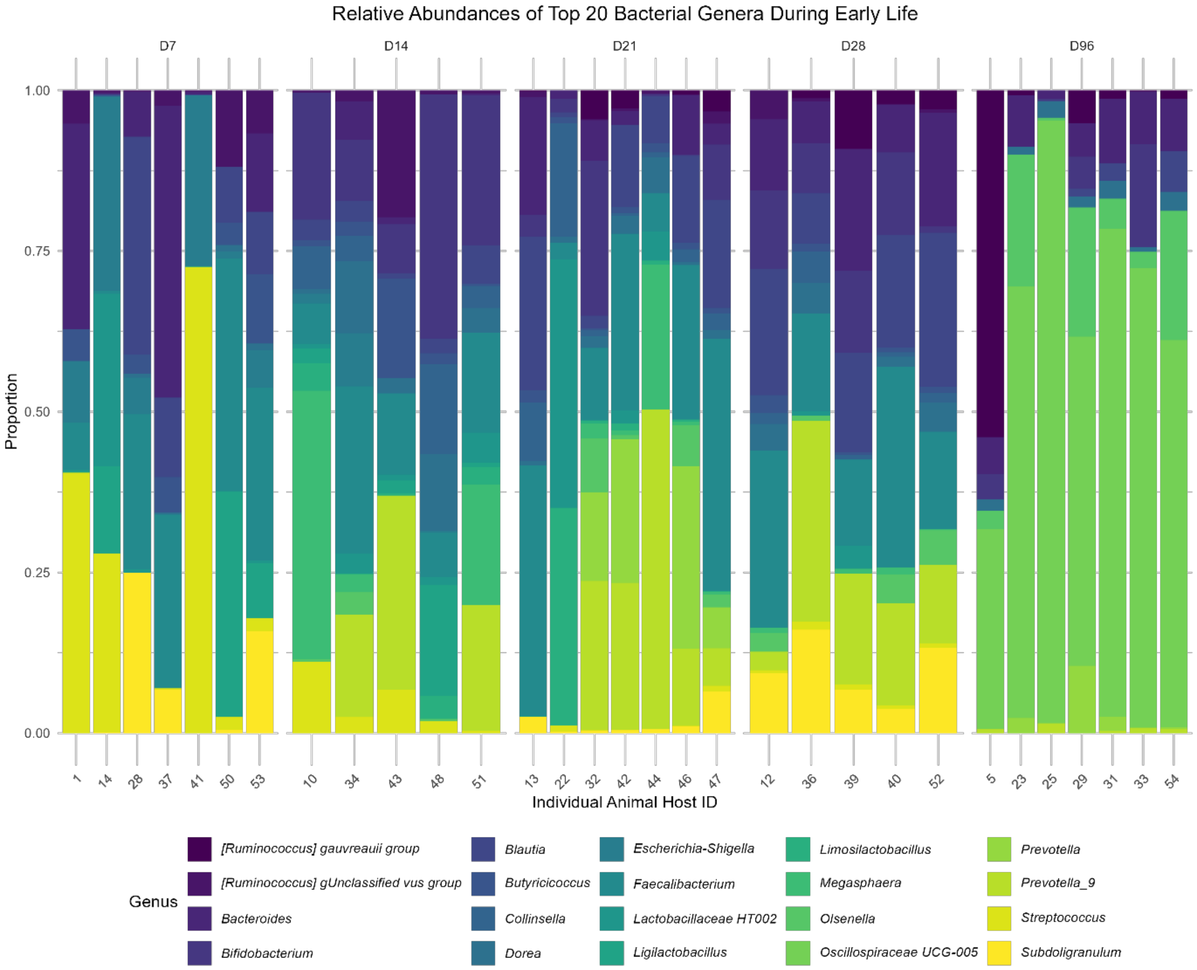

Figure 1. Bacterial genus abundance of the colon digesta at different time points during early life. Each bar represents an individual animal host and the x-axis shows the host ID. The y-axis depicts the proportion of the sum of the 20 most abundant bacterial genera.

Results

Alpha and beta diversity were assessed using GLM (SAS 9.4) and PERMANOVA, with age and farm as fixed effects. Bacterial α-diversity increased significantly with age, showing marked differences between D7 and later stages, including D21, D28, and D96 (each $p < 0.0001$), indicating substantial shifts in microbial diversity as the calves mature. Microbial colonization began to stabilize between D14 and D21 ($P = 0.22$), suggesting an early-stage equilibrium in microbial composition. Weaning further altered the microbiota, with significant diversity differences between D28 and D96 ($p < 0.001$). PERMANOVA analysis shows that both age ($P < 0.001$) and farm ($I < 0.001$) affect bacterial community composition in calves, with age explaining the largest proportion of variance.

Conclusions

The neonatal GIT microbiome is a highly complex environment that plays a pivotal role in immune development, nutrient absorption and disease resistance. This study demonstrates the considerable changes the colon digesta microbiota undergoes in response to various factors such as the environment and dietary changes particularly as the calf transitions from a milk-based to solid-feed diet.

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047

The potential selection response of microbiome-driven breeding to mitigate methane emissions from beef cattle considering correlated production traits

T. Nguyen[1], M. Martinez-Alvaro[2], J. Lima[1], M. Cleveland[3], R. Roehe[1]
[1]Scotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3JG, UK
[2] Polytechnic University of Valencia, Camí de Vera, Algirós, 46022 València, Spain
[3]Genus plc, Matrix House, Basing View, Basingstoke, Hampshire RG21 4DZ, UK

Presenting author
Tuan Nguyen. E-mail: tuan.nguyen@sruc.ac.uk

Application

Microbiome-driven breeding, as a cost-effective strategy to mitigate methane (CH_4) emissions, is recommended to be used in a multiple trait model with correlated production traits, as it substantially increased the accuracy of estimation of breeding values (EBVs) and thus enhances selection response.

Introduction

Roehe et al. (2016) found that rumen microbial gene abundances are closely linked to CH_4 emissions and highlighted these as a highly informative proxy for breeding low CH_4 emitting cattle. Later, Martinez-Alvaro et al. (2022) demonstrated the effectiveness of using microbial genes in microbiome-driven breeding to reduce CH_4 yield (expressed as g of CH_4/kg of dry matter intake). In this study, we applied microbiome-driven breeding for mitigating daily CH_4 emissions (g of CH_4 /day), and incorporated information from key performance traits genetically associated with CH_4 emissions, such as daily feed intake (DFI), average daily gain (ADG) and carcass weight (CCW).

Materials and Methods

The experiment was conducted following the UK Animals Act 1986 and was approved by the Animal Experiment Committee of SRUC. Three hundred sixty-three steers raised under the same housing conditions on the same research farm were used in this project. The animals were balanced for different breeds (Aberdeen Angus, Limousin, Charolais crosses and purebred Luing) and basal diets (two diets of 520:480 and 920:80 forage:concentrate ratios). Blood and rumen fluid samples were collected at slaughter. Microbial DNA sequence reads from rumen fluid samples were aligned to the Kyoto Encyclopedia of Genes and Genomes database, resulting in the identification of 3362 microbial genes. To account for the compositionality of microbiome data, microbial gene abundance data were transformed using the additive log-ratio method. CH_4 production was measured individually for 285 of the 363 animals over a 48-hour period using six respiration chambers and expressed as CH_4 emissions per day (CH_4p).

Firstly, we conducted multiple bivariate genomic (37K SNPs) analyses to obtain genetic variances and covariances between CH_4p and microbial genes. Secondly, we identified the most informative microbial genes that yielded the largest correlated response in CH_4p. Thirdly, we conducted genomic bivariate analyses between the identified microbial genes and the performance traits DFI, ADG, CCW to obtain the genomic (co)variances. Lastly, we used these genomic (co)variances for different breeding strategies to reduce CH_4 production: 1) univariate analyses, using measured CH_4p only (CH_4p measured), 2) multivariate analysis using only the most informative microbial gene abundances genetically correlated with CH_4p, i.e., microbiome-driven breeding (MDB.43), 3) multivariate analysis, including DFI, ADG, CCW, and measured CH_4p (Four traits measures), and 4) multivariate analysis, including DFI, ADG, CCW, and predicted CH_4p using microbiome-driven breeding (Three traits & MDB.43). Three selection intensities (1.159, 1.400, and 1.755) were considered for each strategy.

Results

We identified 43 informative microbial genes, of which 17 were positively genetically correlated with CH_4p (r_{gCH4p}, ranging from 0.45 to 0.80) and 26 microbial genes were negatively correlated (r_{gCH4p} ranged from -0.32 to -0.75) with CH_4p. All correlations had more than 80% probability of being greater or lower than zero (Pr0). The heritability of these microbial genes ranged from 0.19 to 0.50.

Of all performance traits, DFI showed strong positive genetic correlations (ranging from 0.84 to 0.93, Pr0 = 100%) with ADG, CCW, and CH_4p. CCW had a marginally higher genetic correlation with CH_4p (0.61, Pr0 = 96%) than ADG (0.58, Pr0 = 96%).

Selection using microbiome-driven breeding (MDB.43) resulted in similar selection responses to those based on measured CH_4p using respiration chambers (Figure 1, -17.76 ± 2.30% vs -16.76 ± 2.26% at highest selection intensity). Including measured CH_4p in the multiple-trait model with ADG, DFI and CCW increased the accuracy of the EBVs from 0.63 ± 0.15 to 0.81 ± 0.06 and the selection response to -20.59 ± 2.13%). Replacing measured CH_4p by microbiome-driven breeding resulted in a further increase in response at -23.12 ± 2.88%.

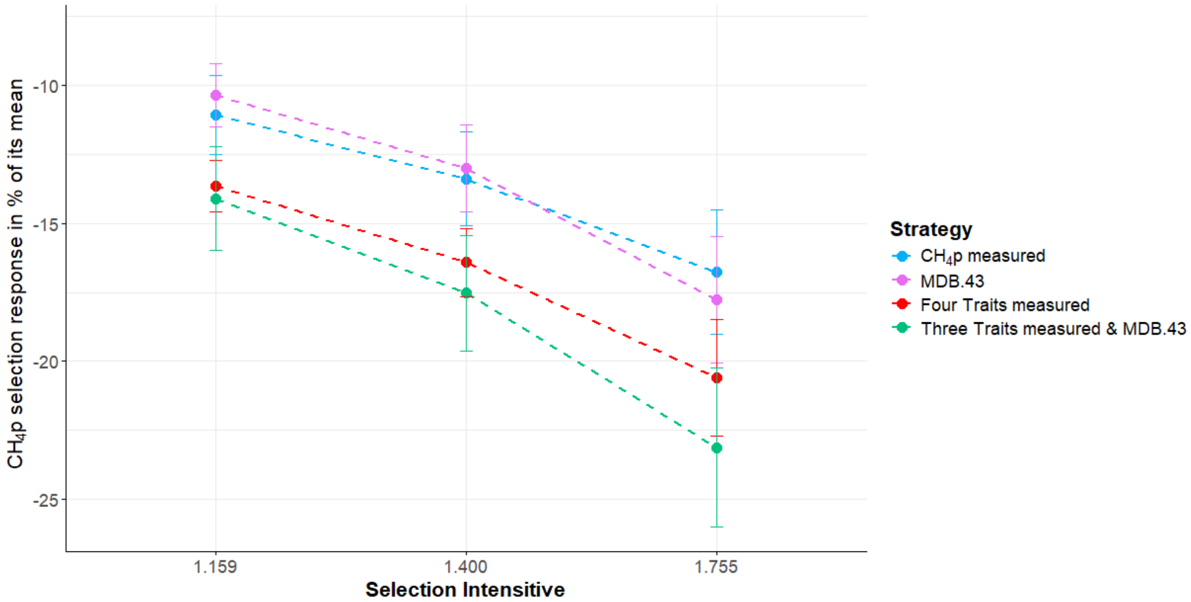

Figure 1. Methane mitigation using different selection strategies, considering three selection intensities (1.159, 1.400, 1.755, equivalent to selection of the best 30%, 20% and 10% of the population, respectively)

Conclusions

Microbiome-driven breeding for reduced CH_4p was successfully integrated into a multiple-trait model with production traits by considering all genetic and residual covariances between microbial gene abundances and those traits. Since microbiome-driven breeding is substantially more cost-effective than using measured CH_4 emissions and provide at least similar selection response to that obtained using the gold standard method of respiration chambers, this methodology provides large potential to effectively reduce this highly potent GHG gas in beef populations.

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048

Characterisation of the bacterial microbiota of nasal swab and pharyngeal tonsil samples from dairy calves following experimental challenge with bovine herpesvirus 1 (BoHV-1)

S. O'Donoghue[1,2], B. Earley[1], M. S. McCabe[1], D. Johnston[1], S. L. Cosby[3], K. Lemon[3], J. Kim[4], J. F. Taylor[4], D. Morris[2], S. Waters[2]
[1]Teagasc, Animal and Bioscience Research Department, Grange, Meath C15 PW93, Ireland
[2]School of Biological and Chemical Sciences, National University of Ireland, Galway, Ireland
[3]Virology, Veterinary Sciences Division, Agri-Food and Biosciences Institute, Stormont, Belfast, UK
[4]Division of Animal Sciences, University of Missouri, Columbia, MO, USA

Presenting author
Bernie Earley. E-mail: bernadette.earley@teagasc.ie

Application
Understanding the dynamics of respiratory microbiota across various sites in the respiratory tract during viral challenge with BoHV-1 offers significant insights into the pathogenesis of bovine respiratory disease (BRD).

Introduction
BRD is multifactorial and causes morbidity and mortality in cattle of all ages. Viral and bacterial infectious agents, in addition to psychological stress caused by management and environmental factors contribute to disease onset. The composition of the bovine respiratory tract microbiome has been shown to play a role in respiratory health. Studies have utilized 16S rRNA gene sequencing to characterize the cattle respiratory microbiome (Johnston et al., 2017; McDaneld et al., 2018; Centeno-Martinez et al., 2022). Despite current research, there is a lack of data concerning the perturbations to the respiratory microbiota as a result of viral infection. Bovine herpesvirus 1 (BoHV-1), a double stranded DNA virus, is a key pathogen associated with BRD. Therefore, the objective of this study was to characterise the bacterial microbiota of the nasal cavity and pharyngeal tonsil of dairy calves following experimental infection with a specific BRD-associated virus, BoHV-1.

Materials and Methods
Holstein-Friesian bull calves (mean age (SD) = 149.2 (23.8) days; mean weight (SD) = 174.6 (21.3) kg) were either administered BoHV-1 inoculate (1 × 107/mL × 8.5 mL) (n = 12) (challenged) or were mock challenged with sterile phosphate buffered saline (control) (n = 6). Clinical signs were monitored and scored daily, and nasal swabs were collected from each animal until euthanasia at day (d) 6 post-challenge. Pharyngeal tonsil tissue (PGT) samples were collected from all animals at post-mortem. Based on clinical scores and quantification of BoHV-1 virus by qPCR, nasal samples collected on d0, d4 and d6 post-challenge were selected for bacterial 16S gene amplicon analysis. Total DNA was extracted from nasal and pharyngeal tonsil samples and full-length bacterial 16S gene amplicon sequencing was conducted on the Oxford Nanopore Technologies MinION Mk1C sequencing device. Fastq files were generated during the sequencing run using GUPPY base-caller algorithm within the

Minknow software that was installed on the device. Clinical score data were analysed using SAS (9.4). Clinical score data were tested for normality using PROC REG and analysed using the PROC MIXED procedure of SAS (9.4), with time-point as the replicate measures. Fastq files were uploaded to the EPI2ME platform and analysed using the Fastq 16s workflow (v.2022.01.07). Results from the EPI2ME analysis were input into R and alpha diversity analysis was performed using the phyloseq (v1.40.0) package.

Results

There was a significant treatment by time interaction (*P* < 0.0001) for clinical score and rectal temperature between BoHV-1 challenged and control calves. Clinical scores were greater for BoHV-1 challenged calves on d 3, 4, 5, and 6 compared to d -1 (*P* < 0.001), and were greater for BoHV-1 challenged than control calves on d 4, 5, and 6 (*P* < 0.05) (Figure 1). No significant difference (*P* > 0.05) was detected in the relative abundance or diversity of bacterial genera between the control and challenged nasal swab samples. A total of 470, 571, and 569 bacterial genera were detected in the nasal swab samples collected on d 0, d 4, and d 6 respectively. The most prevalent genera identified in challenged calves across these days were *Moraxella* (mean (SD)) (25.1 (10.0) %), *Pasteurella* (51.6 (17.0) %), and *Pasteurella* spp. (64.7 (18.4) %), respectively. In controls the most prevalent bacterial genera were *Moraxella* (37.2 (10.2) %), Pasteurella (38.1 (16.0) %), and *Pasteurella* spp. (30.4 (12.5) %), respectively. In the PGT, 1479 genera were identified across all samples. No significant difference (P > 0.05) was detected in the relative abundance of bacterial genera between challenge and control PGT samples. The most common bacterial genera identified in challenged animals were *Streptococcus* (12.4 (4.5) %), *Mycoplasmopsis* (11.8 (5.3) %), *Mesomycoplasma* (7.9 (5.1) %), and *Pasteurella* spp. (7.3 (2.4) %). In control samples, the most prevalent genera identified were *Pasteurella* (30.3 (15.5%)), *Chitinophaga* (16.5 (14.8%)), *Moraxella* (11.4 (10.3%)), *Streptococcus* (7.2 (3.9%)) and *Escherichia* spp (6.6 (5.4%)).

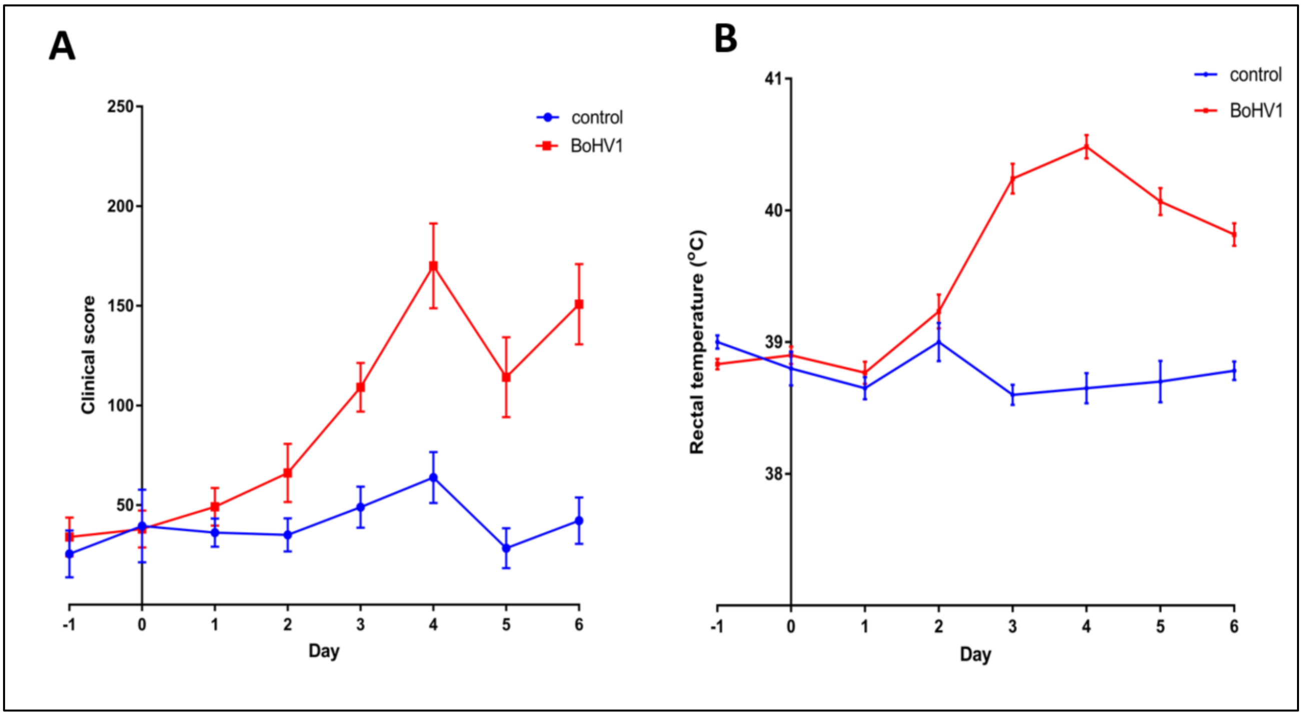

Figure 1. Clinical scores (**A**) and rectal temperatures (**B**) from the BoHV-1 challenged (n = 12) and the control (n = 6) calves during the BoHV-1 challenge study (means and their standard

errors are presented). The day on which the challenge as administered is represented as day 0.

Conclusions

BoHV-1 induced clinical signs of BRD and allowed for the characterization of the composition of the bacterial microbiome across upper and lower respiratory tract sites. Furthermore, the use of replicate nasal swab samples across multiple time-points allowed for the examination of these bacterial populations over the course of an active viral infection.

Acknowledgements

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The use of ruminal metabolic information to select microbial genes for microbiome-driven breeding to mitigate methane emissions from beef cattle

R. Roehe[1], T. Nguyen[1], J. Lima[1], M. Martinez-Alvaro[2]
[1] Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK
[2] Universitat Politècnica de Valéncia, Camí de Vera, Algirós, 46022 València, Valencia, Spain

Presenting author
Rainer Roehe. E-mail: Rainer.Roehe@sruc.ac.uk

Application

The identified animal genetic effects on ruminal metabolites concentrations and on the abundances of microbial genes together with their genetic correlations with methane emissions are expected to improve the accuracy of microbiome-driven breeding to reduce this highly potent greenhouse gas efficiently and cost-effectively by reliable selection of low emitting cattle.

Introduction

Volatile fatty acids (VFAs) in the rumen are the primary energy source for cattle and are known to be phenotypically related to CH_4 emissions. Our research aimed to investigate how these ruminal metabolites are animal genomically influenced and genetically correlated with CH_4 emissions. Additionally, we were interested in identifying microbial genes that are closely genetically correlated with both metabolites and CH_4 emissions. The identification of the most informative biomarkers (VFAs, microbial genes) is essential for the microbiome-driven breeding strategy (Roehe et al., 2016; Martinez-Alvaro et al., 2022) and for improving our understanding of the functional regulation of the ruminal metabolite metabolism and CH_4 production.

Materials and Methods

The animal trials were conducted following the UK Animals Act 1986 and were approved by the Animal Experiment Committee of SRUC. The data comprised of 363 steers that were deeply phenotyped (including CH_4 emissions measured using respiration chambers) and genotyped using a 50k SNP chip. The animals were tested at SRUC's Beef Research Centre across five trials and represented four breed types, with two basal diets (480:520 and 920:80 forage:concentrate ratios). In two of the trials, the feed additives nitrate and rapeseed oil were investigated. In addition, whole metagenome sequencing data of microbial DNA from rumen fluid samples taken at slaughter were available. Aligning the ruminal metagenomic sequence reads to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database resulted in the identification of 3362 microbial genes. For a subset of these animals (n = 137), VFA concentrations in the rumen fluid, collected at slaughter, were determined using HPLC. Bayesian genomic analyses were applied to estimate the heritabilities of the ruminal metabolites and their genetic correlations with CH_4 emissions as well as with functional microbial KEGG genes. The genomic model included fixed environmental effects and the animal's random genomic effects, considering 36780 SNPs. Fixed effects in the model for metabolites included trial, breed, basal diet, feed additives and as a covariable age at slaughter, whereas for CH_4 emissions and microbial KEGG genes, fixed effects were the combined trial-breed-diet effects with additional consideration of a covariable, either the age entering the respiration chambers or age at slaughter, respectively.

Results

Estimated heritabilities of molar proportion of (iso)butyrate were at high magnitude, whereas those of acetate, propionate and (iso)valerate were at moderate level, indicating a host genomic influence on the ruminal microbial metabolism of VFAs (Table 1). The genetic correlations of the main VFAs with daily CH_4 emissions (CH_4d) were moderate in magnitude and associated with probabilities (P_0) of more than 80% to be different from zero. The direction of the correlations indicates that higher molar proportions of acetate and butyrate in the rumen were genetically associated with increased CH_4d. In contrast, higher proportions of propionate and valerate were genetically correlated with decreased CH_4d. The genetic correlations of isobutyrate and isovalerate were close to zero, indicating that they did not relate to CH_4 metabolism. The magnitude of the genetic correlations between VFAs and CH_4 emissions per kg dry matter intake (CH_4y) were similar to those emissions obtained on a daily basis.

One interesting microbial gene group that was moderately to highly genetically correlated with the concentration of the main ruminal metabolites was anaerobic sulphite reductase (*asr*) subunits. The abundance of the microbial KEGG gene *asrC* showed genetic correlations with acetate, propionate, butyrate, and valerate of -0.74, 0.87, -0.67 and 0.77, respectively, which were associated with P_0 in the range of 0.95 to 0.98. Genetic correlations of equal direction and similar magnitude were also found for *asr* subunits A and B. The abundance of the *asr g*enes were negatively genetically correlated with CH_4d between -0.23 to -0.41 with P_0 ranging from 0.77 to 0.90. These results indicate that selection for increased abundances of the *asr* genes will decrease CH_4 emissions by favouring ruminal propionate and valerate metabolism compared to acetate and butyrate production.

Table 1.

Heritabilities (h^2) of ruminal metabolites and their genetic correlations (r_g) with daily CH_4 emissions and CH_4 yield

Trait	h^2	SD[1]	r_g with CH_4d[2]	P_0 r_g with CH_4d	r_g with CH_4y[3]	P_0 r_g with CH_4y[4]
Acetate	0.22	0.18	0.51	0.84	0.37	0.75
Propionate	0.34	0.24	-0.57	0.88	-0.62	0.89
Butyrate	0.51	0.26	0.43	0.82	0.52	0.87
Isobutyrate	0.46	0.27	-0.09	0.58	0.24	0.67
Valerate	0.36	0.26	-0.50	0.83	-0.41	0.77
Isovalerate	0.32	0.24	-0.01	0.50	0.26	0.68

[1]Standard deviation of the posterior distribution of h^2 (SD); [2]daily methane emissions (g/d) (CH_4d); [3]methane yield (g/kg dry matter intake) (CH_4y); [4]probability that the genetic correlation is different from zero (P_0). Heritabilities of CH_4d and CH_4y were 0.46 (±0.19) and 0.43 (±0.20), respectively.

Conclusions

The molar proportions of ruminal VFAs were found to be heritable and genetically correlated with CH_4 emissions. These VFAs could be combined with the microbial gene-based microbiome-driven breeding strategy to improve its accuracy to estimate breeding values for CH_4 emissions. In addition, key microbial genes (*asr* subunits), genetically correlated with both VFAs and CH_4 emissions were identified, which are of high value to be directly included into microbiome driven breeding to mitigate CH_4 emissions. The *asr* genes are involved in the reduction of sulphite to sulphide and might compete with methanogenic archaea for molecular hydrogen (H_2).

Acknowledgments

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050

Effect of protein source and a pasture-based diet on beef performance and methane emissions

C. Christodoulou[1], K. Kliem[1], M. D. Auffret[2], D. J. Humphries[1], P. Kirton[1], H. Jalal[3], J. Newbold[4], L. G. Smith[1,5], S. Stergiadis[1]
[1]*University Of Reading, School of Agriculture, Policy and Development, Earley gate, RG6 6EU Reading, United Kingdom*
[2]*Agrifirm, Booiebos 5, B-9031 Gent (Drongen), Belgium*
[3]*University of Teramo, Piano d'Accio, Teramo, 64100, Italy*
[4]*Scotland's Rural College, Dairy Research & Innovation Centre, Barony Campus, Parkgate, DG1 3NE Dumfries, United Kingdom*
[5]*Swedish University of Agricultural Sciences, Biosystem & teknologi, SE-234 22 Lomma, Sweden*

Presenting author

Christos Christodoulou. E-mail: christos.christodoulou@reading.ac.uk

Application

This study evaluated local brewers' spent grains and field beans as alternative proteins to soyabean meal, comparing concentrate-based and a pasture-based diets. While the pasture diet lowered methane emissions per kg intake, it reduced growth rates, raising environmental and cost concerns.

Introduction

Using sustainable feed proteins in livestock diets can enhance performance and nutrient use while reducing reliance on high-carbon-footprint proteins like soyabean (Pexas et al., 2023). Local proteins in the UK, such as brewers' spent grains and field beans, can reduce costs and minimise carbon footprint of transport. Furthermore, pasture-based beef production can reduce feed costs, support local economies, and preserve biodiversity (Boval and Dixon, 2012; Fraser et al., 2022). These systems are favoured by consumers because of the perceived improved animal welfare and nutritional composition of animal-derived foods (Klopatek et al., 2022). The present study examined the impact of substituting soyabean meal with brewers' spent grains or field beans on beef performance and enteric methane (**CH_4**) emissions; also comparing the concentrate-based diets with a fresh-cut ryegrass diet without concentrate supplementation.

Materials and Methods

Thirty-two Aberdeen Angus×Holstein beef cattle (16 steers, 16 heifers), were grouped and randomly assigned to one of four experimental diets, balanced for age and weight. Three groups received total mixed rations with grass and maize silages, but with soya-based (**SB**), brewers' spent grains-based (**BSG**), or field beans-based (**BNS**) concentrates; while a fourth group was fed fresh-cut ryegrass only (**GRA**). Weekly feed intake was calculated from daily feed offered and refusals. Measurements of body weights (**BW**), BW change (**BWc**), and feed efficiency were recorded, along with preserved feed samples for proximate analysis. Furthermore, CH_4 emissions were recorded daily by automated head chamber system (GreenFeed; C-Lock, Inc., Rapid City, SD). While on GreenFeed, animals had access to concentrate feed as bait, which made up 7.9–8.7 % of total dry matter (**DM**) across the different diets. Data were analysed using general linear models (SPSS 29.0; Armonk, NY, USA) with treatment, week, sex, and their interactions as fixed factors; animal ID nested within treatment as random factor; with week being the repeated effect. Where necessary, Fisher's Least Significant Difference test ($P < 0.05$) was used for pairwise mean comparisons.

Results

DM intake (**DMI**; kg/day) was higher for SB (9.78; $P = 0.002$) compared with BSG (8.50) and GRA (8.60), and organic matter intake (**OMI**; kg/day) was higher ($P < 0.001$) for SB (9.44) and BNS (9.58) compared with GRA (7.91). GRA had higher ($P < 0.001$) NDF (5.25 vs 4.16, 4.04, and 3.94 kg/day for GRA vs SB, BSG, and BNS, respectively) and ADF intakes (3.33 vs 2.60, 2.36, and 2.37 for GRA vs SB, BSG, and BNS, respectively). Crude fat intake (kg/day) was higher ($P < 0.001$) for BSG (0.35) compared with SB (0.24), BNS (0.23), and GRA (0.18). Starch intake (kg/day) was higher ($P < 0.001$) for BNS (2.58) compared with SB (2.07) and BSG (1.72), and water-soluble carbohydrate intake (kg/day) for GRA (0.61; $P < 0.001$) compared with SB (0.28), BSG (0.16), and BNS (0.24). BWc was higher ($P < 0.001$) for the concentrate-fed animals compared with GRA (1.42, 1.36, 1.58, and 0.72 kg/day for SB, BSG, BNS, and GRA, respectively). GRA had lower CH_4 yield (19.6 g/kg DMI and 22.3 g/kg OMI; $P < 0.001$ and $P = 0.006$, respectively) compared with SB (24.1 and 25.4 g/kg DMI and OMI, respectively), BSG (26.4 and 27.5 g/kg DMI and OMI, respectively), and BNS (22.5 and 23.4 g/kg DMI and OMI, respectively). CH_4/BW (g/kg) was higher ($P = 0.011$) for SB (0.50) and BNS (0.46) compared with GRA (0.39). Steers had higher (*P* < 0.001) nutrient intakes and BWc (1.72 vs 0.81 kg/day; *P* < 0.001), and lower CH_4/DMI (21.9 vs 24.4 g/kg; *P* = 0.019) and CH_4/OMI (22.9 vs 26.3 g/kg; *P* = 0.002) than heifers.

Conclusions

The study shows that high-quality protein diets with less fibre and more starch, like soya and field beans, enhance growth and nutrient use, while more fibrous diets like ryegrass reduce growth rates but also CH_4 emissions. The lower CH_4 yield could be attributed to the reduced availability of fermentable carbohydrates in fresh-cut ryegrass. The significant sex effect complies with Owens and Gardner (2000) who found that steers typically have higher feed intakes and growth than heifers. Brewers' spent grains and field beans can replace an equivalent amount of soyabeans in the diet without impacting productivity or CH_4 emissions. High-fibre pasture-based diets can be more cost-effective and reduce CH_4 yields but there is an environmental and economic trade-off as they also reduce growth rates.

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051

Manipulating composition and nutritional value in edible insects through agri-food co-products based diets

C. Alvarez
[1]Ashtown Teagasc Food Research Centre, Scribbletown, Dublin 15, Ireland

Presenting author
Carlos Alvarez. E-mail: carlos.alvarez@teagasc.ie

Application
Insects serve as bio-factories, converting food waste and by-products into highly nutritious proteins and fatty acids suitable for human consumption. This research explores how specific agri-food by-products affect the nutritional composition of edible insects, aiming to create a sustainable protein source.

Introduction
Insects are regarded as a more sustainable source of nutrients, especially protein, compared to traditional livestock. Insects intended for human consumption can be fed on a wide variety of substrates, provided they meet minimum nutritional requirements for protein, energy, minerals, and vitamins. The substrate used can also affect the nutritional composition of the final product, whether whole insects or insect flour. In this study, we fed two edible insect species (*Tenebrio molitor* and *Acheta domesticus*) with food by-products like chicken feather meal, red blood cells, horticulture foliage, potato cuttings, chicory roots, or vegetable mix, with the goal of reintroducing these materials into the food chain. Substrates were selected based on their availability, protein content, and format (wet or dry). Our hypothesis was that altering the diet's composition could potentially manipulate the protein, fat, and mineral content in the resulting insect biomass while minimising food waste and lossess.

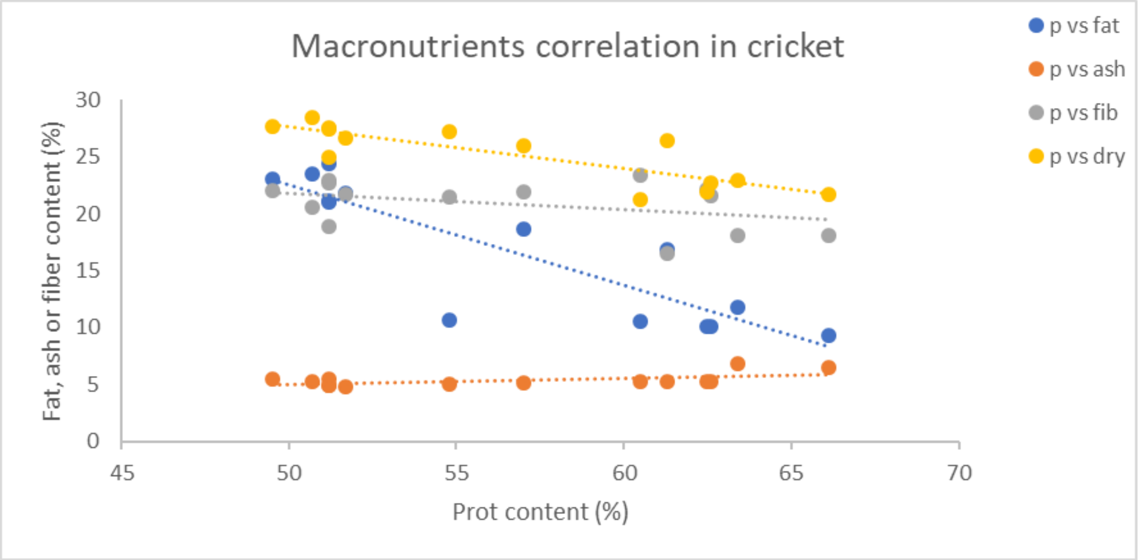
Material and methods
A wheat bran control diet was used alongside experimental diets. Dry diets were supplemented with protein-rich by-products (e.g., feather meal and dried blood cells up to 20% and 25% inclusion, respectivelly). Wet diets utilized potato peels, fermented chicory roots, vegetable mix, and horticultural foliage, with particles reduced to under 2 mm for mealworms and 0.5 mm for crickets. Each diet was replicated in triplicate per insect species. Harvested insects were dried and processed into flour, then analyzed for protein, fat, ash, fiber, and moisture. Amino acid, fatty acid, and mineral profiles were also assessed.

Results
The dietary impact on insect composition varied between Acheta domesticus (crickets) and Tenebrio molitor (mealworms). For crickets, higher dietary protein led to increased body protein and reduced fat content, without affecting dry matter levels. Mealworms showed an increase in dry matter with higher protein diets, but their fat and protein content remained relatively stable, indicating species-specific metabolic responses to protein-rich diets.
Wet diets also influenced composition distinctly. Crickets reared on fermented chicory roots accumulated more fat, while those fed potato cuttings had higher protein content. In

mealworms, potato cuttings led to increased lipid levels at the expense of protein, showing an inverse relationship between protein and fat accumulation. Overall, both species displayed a negative correlation between protein content and fat and dry weight, suggesting the possibility of tailoring insect nutrition by substrate selection.

Table 1. Proximate analysis of crickets and mealworms reared on dry protein-rich side streams. Mean ± Standard Deviation (n=3 independent batches). Different letter indicates significant differences $P<0.05$

Substrate	**Ether extract (g/100g DM)**	**Crude protein (g/100g DM)**	**Crude ash (g/100g DM**	**Chitin (g/100g DM)**	**NFCH (g/100g DM)**
Crickets (*Acheta domesticus*)					
Control Feather Meal	21,8^{a}±2,3	51,7^{d}±2,8	4,8^{a}±0,1	7,2 ^{b}±0,6	14,5 ^{d}±2,4
Feather Meal 5%	21,0^{a}±0,9	51,2^{d}±2,5	4,9 ^{a} ±0,2	6,9 ^{b}±0,7	16,0 ^{c}±2,1
Feather Meal 10%	21,1^{a}±0,9	51,2^{d}±2,5	4,9 ^{a}±0,2	6,6 ^{b}±0,7	16,1 ^{c}±2,0
Feather Meal 15%	18,7^{b}±3,6	57,0^{c}±6,7	5,1 ^{a}±0,4	7,8 ^{a}±1,0	14,1 ^{d}±3,4
Feather Meal 20%	10,7^{c}±0,9	54,8^{c}±2,6	5,0 ^{a}±0,1	7,0 ^{b}±0,5	14,5 ^{d}±2,0
Control Red Cells	10,6^{c}±0,4	60,5±^{b}0,7	5,3 ^{a}±0,1	ND	23,4 ^{a}±0,4
Red Cells 12.5%	10,1^{c}±1,8	62,6±^{a}1,7	5,2 ^{a}±0,2	ND	21,6 ^{b}±0,7
Red Cells 25%	10,1^{c}±1,1	62,5±^{a}1,4	5,2 ^{a}±0,3	ND	22,1 ^{b}±1,3
Mealworms (*Tenebrio molitor*)					
Control Feather Meal	20,3 ^{a}±1,1	51,9^{a}±0,2	5,0^{b}±0,5	7,2^{a}±0,1	15,7^{e}±1,5
Feather Meal 5%	18,0 ^{c}±0,8	53,0^{a}±1,1	4,8^{b}±0,1	7,2^{a}±0,4	17,1^{d}±1,0
Feather Meal 10%	18,7^{c}±1,1	51,3^{a}±1,4	4,6^{bc}±0,2	7,0^{a}±0,2	18,4^{c}±1,8
Feather Meal 15%	20,2 ^{b}±0,3	52,3^{a}±0,7	4,3^{c}±0,2	7,1^{a}±0,5	16,2^{de}±0,9
Feather Meal 20%	18,7^{c}±0,3	52,4^{a}±0,6	4,8^{b}±0,4	7,2^{a}±0,2	16,9^{d}±1,4
Control Red Blood Cells	12,9^{d}±1,4	53,4^{a}±0,8	5,6^{a}±0,3	ND	28,2^{a}±0,4
Red Blood Cells 5%	17,7^{c}±1,7	50,6^{a}±0,6	4,5^{c}±0,1	ND	27,3^{a}±1,6
Red Blood Cells 10%	17,7^{c}±5,1	50,2^{ab}±3,4	4,2^{c}±0,2	ND	27,9^{a}±2,0
Red Blood Cells 15%	20,0 ^{b}±0,9	49,7^{b}±,05	3,9^{d}±0,1	ND	26,4^{ab}±1,3
Red Blood Cells 20%	22,1^{a}±1,7	50,0^{ab}±0,5	3,5^{e}±0,1	ND	24,4^{b}±1,1



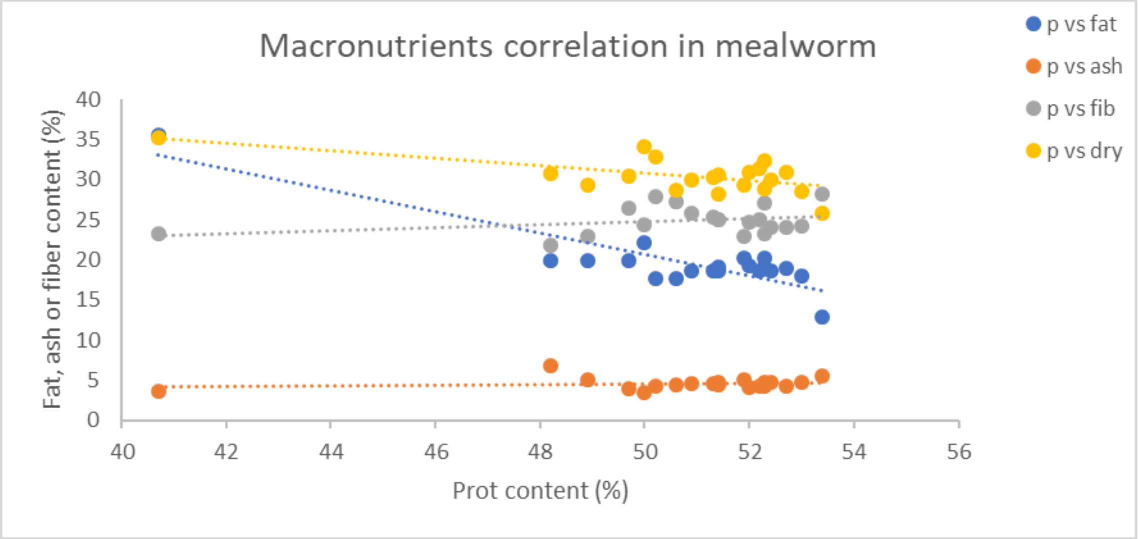


Figure 1. correlation between protein content and dry matter (yellow), fat (blue), ash (orange) and fibre (grey) in crickets (left) and mealworms (right)

From a nutritional point of view, Higher-protein diets also modified the fatty acid profile, particularly in mealworms, where increased C18:1 (oleic acid) and decreased C18:2 (linoleic acid) were observed. Crickets did not show a consistent trend in fatty acids across diets. Amino acid profiles remained stable across all diets and substrates, indicating that amino acid composition was less influenced by dietary changes. Finally, it was very interesting to see how both mealworms and crickets were able to accumulate some minerals such as iron, calcium or phosphorus when provided by the diet, i.e. red cells and fermented chicory roots.

Conclusions

Food losses and by-products are a suitable source of substrates for insect rearing, with the capacity of manipulate the proximate and the nutritional value of the insect derived products. By using these streams we have the potential of reducing food waste and transform them into valuable nutrients for human consumption.

Acknowledgements

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052

The effect of performing an economic allocation to partition total animal emissions of Aberdeen Angus Friesian steers finished on pasture or indoors

A. Mc Namee[1], P. Crosson[2] D. Mc Crudden[1], E. O Riordan[2], M. McGee[2]
[1]Atlantic Technological University, Port Rd, Gortlee, Letterkenny, Co. Donegal, Ireland
[2]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author
Andrew Mc Namee. E-mail: Andrew.mcnamee@atu.ie

Application

Presently, the beef carcass carries the entire environmental loading with all non-carcass ~offal components avoiding the allocation process**.** This disadvantages the 'carcass' as it unfairly carries the entire emission burden.

Introduction

Global demand for beef and animal-derived protein continues to rise. In addition to the dressed carcass, animal non-carcass components or co-products are also produced, which includes edible co-product meats that have a nutritional value comparable to lean meat (Soladoye et al., 2022). Most life cycle analysis studies, to date, have not considered the valuable contribution of animal co-products when calculating greenhouse gas (GHG) emissions or the additional protein generated in the co-product components and subsequently allocated all emissions directly to the final beef carcass (Dominguez Aldama et al., 2023). Using data generated from a dairy-beef cattle experiment conducted at Teagasc Grange Research Centre, the objectives of this study were to augment the existing Grange Dairy Beef System Model (GDBSM) and subsequently partition total animal GHG emissions into carcass and non-carcass using the economic allocation approach.

Materials and Methods

Sixty-six male spring-born Aberdeen Angus × Holstein-Friesian calves were sourced from commercial farms and reared in a standard calf-rearing system at Grange Research Centre. Animals were rotationally grazed on predominantly perennial ryegrass swards as one group. After 86 days at pasture, steers were weighed and blocked on weight and date of birth and assigned to one of three grazing treatments for 112 days: (i) grazed grass only (GG-0), (ii) GG plus 1.5 kg concentrate per animal daily (GG-1.5), and (iii) GG plus 3.0 kg concentrate (GG-3.0). At the end of this period, half of steers from each grazing treatment were slaughtered (mean age, 18 months - Early-Finish: EF), and the remainder were accommodated indoors and offered *ad libitum* concentrates for 89 days following which they were slaughtered (mean age, 22 months Late-Finish: LF-0, LF-1.5 and LF-3.0). Post-slaughter, individual animal hide, fore and hind feet, liver, head, lungs, kidneys, heart, empty reticulo-rumen and intestines, as well as perirenal and retroperitoneal fat were weighed. Overall, these organs accounted for 61% of the total differential between the mean pre-slaughter weight and the mean carcass weight. The weight of blood, tail, tongue, spleen, gall bladder and 'full' reticulo-rumen (25%)

and miscellaneous non-carcass components (i.e. tonsils, brain, spinal cord, scrotal sack, pizzle, pharynx, water, esophagus and 'other'; 14%) was estimated based on previous research (e.g. McGee et al., 2008; Campion et al., 2009). These miscellaneous non-carcass components were classed as non-edible and excluded from the allocation process. The data collected was incorporated into the Grange Dairy Beef System Model (GDBSM; Kearney et al., 2022) to include an emission allocation sub-model, incorporating the contribution of non-carcass components. The economic value of the edible non-carcass co-product components was then determined using international market price data published by the United States Department of Agriculture for 2022 (USDA, 2023). These prices, denominated in US dollars, were converted to euros (€) using published exchange rates, and the price per kg for each co-product was determined. These prices were multiplied by the measured or estimated weights of each co-product component to provide a total value per component and a combined carcass value. This exercise then allowed an economic allocation to be performed to assign the proportions of GHG emissions, in line with ISO (2006) guidelines, to the co-products. The allocation was obtained by expressing the monetary value of the non-carcass components, and expressing it relative to the total monetary value (carcass plus non-carcass) as determined by the price obtained upon slaughter within the model.

Results

The mean value of non-carcass components for the EF animals was €162 and for the older, heavier LF animals was €198 (Table 1). The total GHG emissions allocation on a per animal, carcass and non-carcass (co-products) basis are presented in Figure 1. As a proportion of total GHG emissions per finished animal carcass to non-carcass components showed a similar mean allocation ratio of 85:15 across the six treatments. In other words, approximately 15% of the animal emission allocations were attributable to the non-carcass components. This process does not increase nor reduce emissions per animal but merely reflects a fairer distribution for the valued non-carcass components.

Table 1.

The effect of performing an economic allocation of carcass and non-carcass components for Angus steers offered supplementary concentrates at pasture and finished at pasture or indoors.

	Pasture supplementation			Finishing strategy	
	GG-0	GG-1.5	GG-3.0	Early	Late
Economic allocation					
Beef price[1] (€/kg)	4.73	4.72	4.78	4.43	5.07
Carcass value (€)	1201	1225	1255	1025	1429
Non-carcass value (€)	176	180	182	162	198
Combined total (€)	1377	1405	1437	1187	1627

[1]Beef price varied according to carcass grading (conformation and fat) and seasonality (date of slaughter)

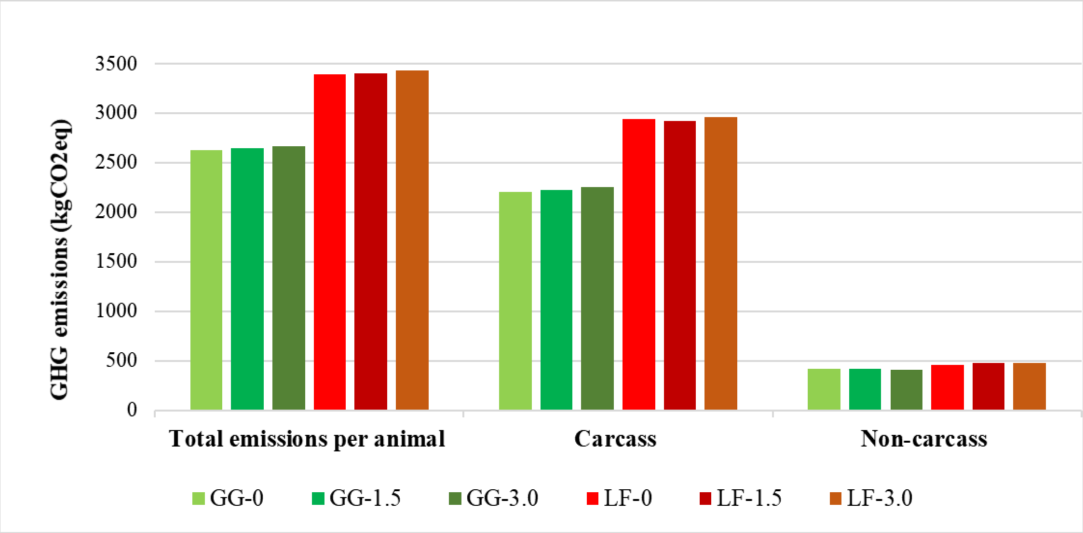

Figure 1. Emission distribution for carcass and non-carcass components expressed relative to total emissions per animal

Conclusion

In the current study, the economic allocation distributed emissions on a mean 85:15 ratio across the six individual treatment groups. The non-carcass component is a substantial share of the total animal emissions. To enhance beef sustainability in a bio-circular economy, policymakers should consider the novel practice of allocating emissions to co-products as non-carcass co-products have avoided this process. Doing so would offer a much fairer distribution pattern.

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053

Financial assessment of integrating cattle farming with anaerobic digestion for biomethane production

S. Tisocco[1,2], J. Lenehan[1], X. Zhan[2], P. Crosson[1]
[1]*Teagasc Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*Civil Engineering, School of Engineering, University of Galway, University Road, Galway H91 TK33, Ireland*

Presenting author
Sofia Tisocco. E-mail: sofia.tisocco@teagasc.ie

Application

This research could drive important progress in renewable energy and sustainable agriculture by demonstrating the economic potential of anaerobic digestion (AD) of livestock slurry and grass silage. Insights from this study may guide policy and funding strategies to encourage sustainable farming, reduce greenhouse gas (GHG) emissions and support rural economies by creating new revenue streams for grassland farmers.

Introduction

Anaerobic digestion of livestock slurry is an effective approach to reduce GHG emissions from slurry management, while generating valuable products such as energy and organic fertilizer, through biomethane and digestate, respectively. Available AD feedstocks in Ireland, where pasture-based livestock systems predominate, include cattle slurry and grass silage. However, there is little knowledge about the economic implications of integrating AD and livestock farms and the financial support required to make biomethane from agricultural feedstocks profitable. This study aimed to (1) assess the economic impact of integrating AD and livestock farms and determine the AD silage price needed to make this activity competitive with beef production, (2) carry out a cost-benefit analysis of a 40 GWh biomethane plant, and (3) determine the adequate support schemes needed to ensure the economic viability of producing biomethane from the co-digestion of cattle slurry and grass silage.

Materials and Methods

Farm scale

The farm-level model implemented was the Grange Dairy Beef System Model (GDBSM), which allows the evaluation of biophysical, economic and GHG outputs from pasture-based dairy-beef production systems (Kearney et al., 2022). This model was augmented with an AD submodel (Tisocco et al., 2024) to simulate the biomethane production using cattle slurry and grass-based feedstocks supplied from dairy-beef farms. The grass-based dairy-beef system modelled assumed a 50 ha farm stocked with February-born early-maturing progeny of dairy cows which were slaughtered at 24 months of age (Kearney et al., 2022). To provide the feedstock demand for a 40 GWh AD plant (43019 tonnes (t) of grass silage and 50042 m^3 of cattle slurry annually), 130 farms were required with 85% of each farm (42.5 ha) allocated for growing grass for livestock use, and the remaining 15% (7.5 ha) allocated to produce AD silage (Tisocco et al., 2024). The AD silage crop comprised a mixture of perennial ryegrass and red clover with a total annual yield of 14.2 t dry matter (DM)/ha and 22% losses. The gross margin of the livestock area was determined by considering revenue from animal sales and direct expenses such as concentrate feeds, milk replacer and fertilizers. Silage production costs were obtained from the Grange Feed Cost Model (Finneran et al., 2010). The total price of AD silage included production costs and an additional amount to offset the partial displacement (15%) of livestock, calculated based on the revenue generated from an equivalent area of a dairy-beef system.

Supply chain scale

The capital and operational expenditures of a 40 GWh biomethane plant were calculated, and a 20-year net present value (NPV) analysis was conducted to assess profitability. Two scenarios, reflecting current financial support structures, were evaluated: (1) a baseline scenario, which included the biomethane certificate price (€0.098/kWh) in addition to the prevailing market price for gas (€0.04/kWh), and (2) a grants scenario, which also incorporated AD plant grants (covering 20% of capital costs and 30% of gas grid connection costs) along with an AD silage establishment grant (€60/ha/year). Additionally, the maximum price that the AD plant could afford for AD silage in order to achieve a payback period of 8 years was determined for each scenario. The required AD farm subsidy was determined by the price gap between the farm's AD silage price and the maximum price the AD plant could afford.

Results

Results indicated that dairy-beef farms providing feedstock for AD reduced direct costs by 4% compared to conventional dairy-beef farms due to lower chemical fertilizers used, as the digestate (616.5 m^3/farm) provided more nutrients and had a higher volume than the slurry produced (454.2 m^3/farm). Consequently, the gross margin per hectare for dairy-beef farms integrated with AD was 5% higher than conventional dairy-beef farms. However, livestock gross margin per farm (50 ha) was 11% lower due to the allocation of 7.5 ha for AD silage cultivation. A total AD silage price of €245/t DM was required to cover the production cost and to compensate for the reduced gross margin per farm. For the baseline scenario, the 20-year NPV was -€2.4 10^6, indicating that a biomethane certificate price of €0.098/kWh was insufficient for financial competitiveness. Incorporating grants and the AD silage establishment grant in the analysis resulted in a 20-year NPV of €645013 (Figure 1a). For the baseline and grants scenarios, the AD plant could afford a maximum AD silage price of €134 and €169/t DM, respectively, to achieve an 8-year payback period. Consequently, price gaps of €111/t DM and €76/t DM would demand AD farm subsidies of €1229/ha and €840/ha, respectively (Figure 1b).

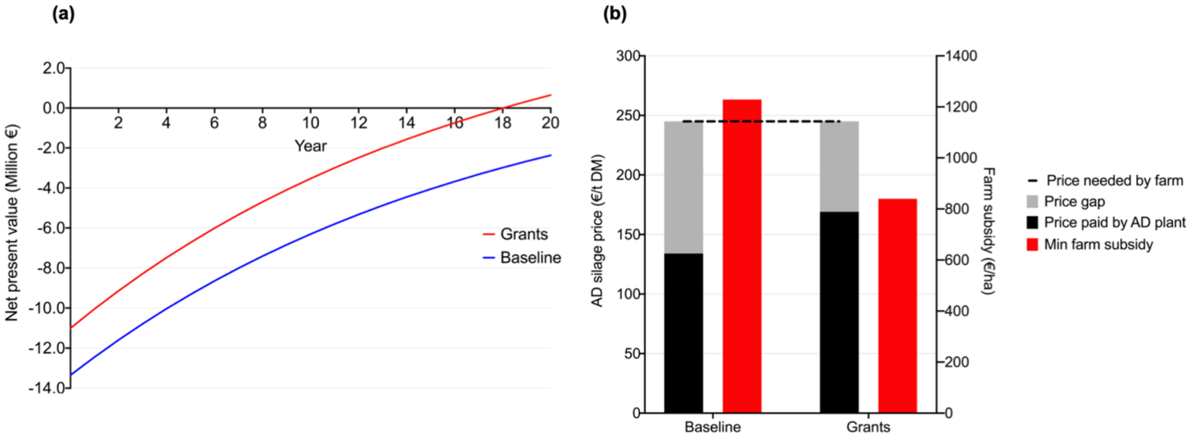

Figure 1. (a) Net present value analysis over 20 years for the baseline and grants scenarios; (b) Maximum AD silage price paid by the AD plant to achieve an 8-year payback period and minimum farm subsidy required to bridge the price gap.

Conclusions

Results indicated that a price of €245/t DM is needed to attain gross margin comparable to beef production. For the AD plant to fully cover this cost and remain profitable, a minimum biomethane price of €0.12/kWh is required, along with grants covering 20% of capital costs and 30% of gas grid connection costs. At current biomethane prices and grants, the plant could only afford a silage price of €169/t DM, necessitating AD farm subsidies of €840/ha. These findings highlight key economic thresholds for Irish farmers, policymakers, and industries to ensure the viability of AD systems, emphasizing the need for targeted subsidies and grants. The results also provide a transferable framework for bioenergy development in similar agricultural contexts globally.

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054

Optimisation of full-scale anaerobic digestion plant: Impact of organic loading rate, renewable energy criteria and biomethane price

A. Braga, C. Beausang, P. Crosson
Teagasc Animal and Bioscience Research Department, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author
Adriana Braga. E-mail: adriana.braga@teagasc.ie

Application

Identify the importance of operational parameters for optimising biogas production from an anaerobic digestion (AD) plant using silage and slurry as feedstock. Additionally, assess the potential profit from biomethane considering different biomethane prices and the emissions savings criteria from the Renewable Energy Directive.

Introduction

AD plants using agricultural feedstock offer an opportunity to increase renewable energy production, diversify the energetic matrix and land use, and secure the local energy supply. The co-digestion of crops and residues improves the robustness of the process and biogas production while improving waste management and mitigation of greenhouse gas (GHG) emissions. AD process has been continuously studied since 1980, with exponential growth in publications from the 2000s (Ampese et al., 2022) and several models have been developed to predict the behaviour of AD systems. Nevertheless, more recently statistical methodologies such as principal component analysis (PCA) are gaining attention as they can summarise complex data sets and indicate the crucial parameters to help operators (Kim et al., 2022). However, the optimisation of operational parameters on the efficiency of biogas production in full-scale AD plants using agricultural feedstock, considering also economic aspects and sustainability criteria from the Renewable Energy Directive (RED), is still incipient.

Materials and Methods

The analysis of operational parameters was performed on 6 months of data from an AD full-scale plant fed with grass silage and cattle slurry. The AD system was composed of two continuously stirred tank reactors (CSTR) working in series, C1 and C2, with working volumes (WV) of 2112 ± 37 m^3 and 2611 ± 20 m^3, respectively. The raw data provided are: daily feeding with silage (t/day) and slurry (m3/day), silage fraction in the feed (xSilage – dimensionless), feedstock flow rate (Q – m^3/day), feedstock organic loading (OL – kg-VS/day) in terms of volatile solids (VS), biogas production rate from both reactors combined (BPR – m^3/day), and percentage of methane (%CH_4) in the biogas. The following operational parameters and dependent variables from the system were calculated considering the combined WV of the system ($WV_{CS} = WV_{C1} + WV_{C2}$):

Hydraulic Retention Time (HRT – day): $HRT = \frac{WV_{CS}}{Q}$

Organic Loading Rate (OLR – kg-VS/m^3 per day): $OLR = \frac{OL}{WV_{CS}}$

Methane Production Rate (MPR – m^3-CH_4/day): $MPR = BPR \times \%CH_4$

Methane Yield (MY – m^3-CH_4/t-VS): $MY = \frac{MPR}{OL/1000}$

The analysis was performed as follows: i) descriptive analysis of data, ii) removal of outliers based on quartiles criteria, iii) standardisation of data, iv) PCA analysis, v) desirability function for optimisation of both profit and emissions savings for heat and only profit. The profit calculation considered a biomethane facility, with a digester of 4723 m^3 of working volume, producing biogas with 55% methane from silage with 25% of VS in the wet weight. The price of the silage was fixed at €35 per t-wet. Data for capex and opex and emissions savings were obtained from Beausang et al. (2024). The statistical analysis was performed in RStudio 2023.12.1 Build 402.

Results

The PCA analysis indicated a higher correlation for OLR, OL and MY in the PC1. The absolute value of the correlation was similar for all variables, but OLR and OL were inversely correlated to MY. HRT had the highest correlation for PC2, followed by xSilage and $\%CH_4$. The PC3 was strongly correlated to MPR and followed by $\%CH_4$ and xSilage. Investigating the correlation among the variables identified for each PC, it was found that MY can be estimated by linear regression of OLR, with the MY increasing with a decrease of the OLR. The desirability function results were plotted (Fig.1) against the price of biomethane and the OLR for different fractions of silage. The contour plots considering profit and emissions savings (Fig.1A) shows the desirability increases as the silage fraction and OLR decrease. As demonstrated by Beausang et al. (unpublished results) the maximum silage in terms of VS in the feeding mix should be 70% to achieve emissions savings required for heating. Emissions savings lower than 80% resulted in desirability equals to 0. The minimum price of biomethane for a breakeven profit is €0.10 per kWh when 60% or 70% of silage in VS is used, as long as the OLR is lower than 2.12 kg-VS/m^3 per day. For a gross profit margin of 33%, a biomethane price of €0.14 per kWh with 60% and OLR lower than 1.96 kg-VS/m^3 per day. However, the MY using a low percentage of silage might be overestimated due to the lack of data in the data set with silage lower than 80% after the removal of outliers.

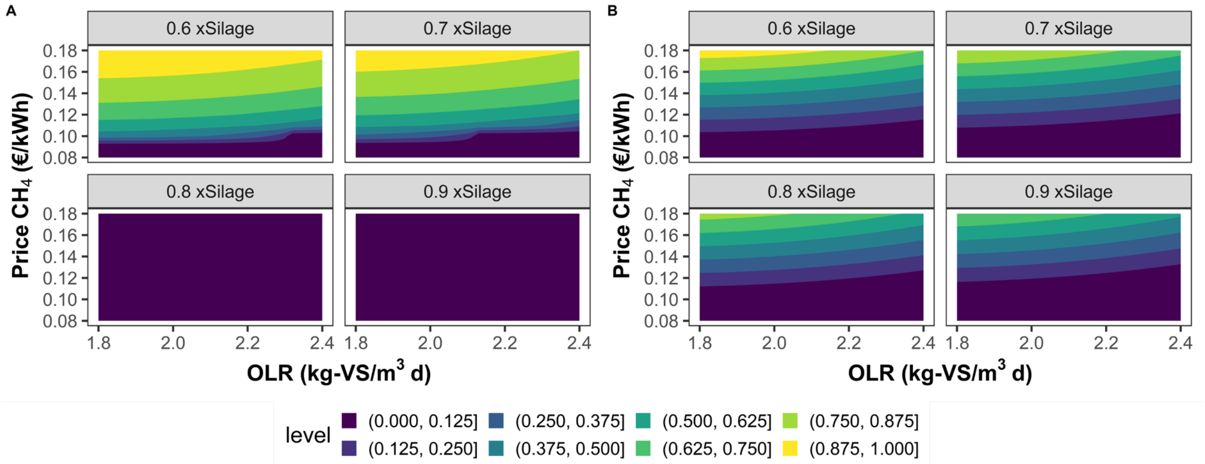

Figure 1. Contour plots of desirability function values obtained by maximising profit and emissions saving for heat (A) and only profit (B) for different prices of biomethane (Price CH_4), organic loading rates (OLR) and fractions of silage (xSilage) in the feeding mix.

When the objective is only optimising the profit (Fig.1B), the silage fractions of 80 and 90% can also be considered. The minimum price for breakeven would be €0.11 per kWh for 90%

of silage fraction and OLR lower than 2.17 kg-VS/m^3 per day. However, a minimum price of €0.16 per kWh would be required to ensure a 33% gross profit margin.

Conclusions

The data analysis demonstrated that higher methane yield (MY) and profits from the AD of grass silage and slurry can be improved using organic loading rate (OLR) below 2.0 kg-VS/m^3. The desirability function indicated that the minimum biomethane price of €0.16 per kWh is required to achieve a minimum 33% gross profit margin for all silage fractions. However, 70% of silage should be used to optimise profits and emissions savings. These findings can help the biomethane industry to optimise the operation of AD digesters to increase the profit while attending the renewable energy criteria. Besides, it highlights the opportunities for farmers to improve the slurry management and diversify activities by providing grass as feedstock to AD plants.

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Does space allowance and enrichment material influence the occurrence of tail biting outbreaks in pigs?

M. Cupido[1,2], S. Mullan[2], L. Boyle[1], K. O'Driscoll[1]

[1]*Pig Development Department, Animal & Grassland Research & Innovation Centre, Teagasc Moorepark, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*

[2]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*

Presenting author

Melissa Cupido. E-mail: melissa.cupido@teagasc.ie

Application

Understanding how type of enrichment and space allowance influence tail biting can help pig farmers implement effective preventative measures, and reduce the need for tail docking.

Introduction

Current EU legislation on space allowances for pigs were determined by considering production of pigs with docked tails, despite routine tail docking being banned. In addition, legislation mandates that pigs be provided with access to a sufficient quantity of material to enable proper investigation and manipulation activities, and the European Food Safety

Authority (EFSA) recommends that this material stimulates the exploratory behaviour of a pig in a way similar to straw. However, less than 5% of Irish producers provide pigs with such material. Concerns include the potential for loose material to block the slurry system, and the lack of availability of straw in Ireland. This study compared the effectiveness of three alternative manipulable materials to Straw (*n* = 12 pens); Haylage (*n* = 12), Hay (*n* = 12) and Grass (*n* = 11) in controlling tail biting in pigs. Materials were placed in mesh racks hung on the front wall of the pen (to limit potential slurry blockages) with the hypothesis that responses of the pigs to the various materials would not differ. Because space allowance is related to the risk of tail biting, we applied these treatments to pigs in equally dimensioned pens containing either 8 (*n* = 16), 10 (*n* = 16) or 12 (*n* = 15) pigs (weaner: 0.62, 0.49, 0.41m^2/pig; finisher: 1.24, 1.00, 0.83 m^2/pig, respectively), and hypothesised that there would be fewer incidences of tail biting in pigs in lower stocked pens.

Material and methods

Forty-seven litters containing healthy, undocked piglets, balanced by weaning weight and sex, were assigned at weaning to Enrichment and Space Allowance treatments in a 4x3 factorial design. Enrichment was provided *ad libitum* and was monitored twice daily. Pigs' tails were scored in detail weekly for lesions (0 = No damage, 1 = Swollen and/or bite marks, 2 = Open wound, 3 = Swollen and open wound). Each pig's tail was checked twice a day for the presence of blood. If an outbreak of tail biting occurred (at least two pigs had an open wound on their tail), supplementary enrichment (manila rope or hessian cloth) was added to the pen. If the outbreak persisted until the next inspection after the intervention, the biter or victim pigs were removed for 72hrs. If the outbreak persisted when removed pigs were re-introduced, biter pigs were removed permanently. All measurements were taken from weaning until slaughter (approx. 22 or 23 weeks of age). Data were analysed using SAS v9.4 accounting for repeated measurements, and considering the pen as the experimental unit.

Results

A total of 48 outbreaks occurred during this study (Table 1).

Table 1. Number of tail biting outbreaks and number of pigs removed across treatments

Treatment	Tail biting outbreaks	% of outbreaks	Pigs temporarily removed	% of temporarily removed pigs
Grass	5	10.42%	0	0.00%
Hay	18	37.50%	9	52.94%
Haylage	20	41.67%	7	41.18%
Straw	5	10.42%	1	5.88%
8	2	4.17%	2	11.76%
10	10	20.83%	4	23.53%
12	36	75.00%	11	64.71%
Grand Total	**48**		**17**	

Only four pigs were permanently removed (two biters and two victims) due to continuous tail biting, all from one pen (12 pigs, Hay). Type of enrichment had no significant impact on tail damage, but space allowance did (P = 0.05), with those in pens of 8 and 10 having a greater proportion of undamaged tails than those in pens of 12. At final inspection, 24.2% of pigs had tail amputations due to biting. Amputation rates were higher in pens of 12 (41.0%) compared to pens of 10 (14.2%) and 8 (12.6%). Enrichment type had similar amputation percentages observed across treatments; Haylage (27.2%), Grass (23.6%), Straw (23.5%), and Hay (22.3%).

Table 2. The amount (kg) and other characteristics of enrichment provided in 4 treatments on average for the duration of the trial.

		Average given/day (kg)		
	Average given total (kg)	Incl. moisture (kg)	Excl. moisture (kg)	DM%
Grass	155.39	1.13	0.26	0.23
Hay	6.93	0.05	0.04	0.88
Haylage	66.43	0.48	0.28	0.59
Straw	12.64	0.09	0.08	0.91

Conclusion

Tail biting outbreaks were more prevalent and harder to control in pens with Hay and Haylage compared to pens with Grass and Straw. The highest stocking density (i.e. largest group size) resulted in the highest number of outbreaks and proportion of tail damage and amputations. The findings suggest that both type of enrichment and stocking density influence the occurrence and management of tail biting in pigs with undocked tails.

Acknowledgements

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One Welfare as a framework to develop fair and resilient livestock production systems for the future: example from outdoor pig farming

L. Boyle[1], O. Menant[1], K. O'Driscoll[1], F. Butler[2], N. Senanayake[1,2]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[2]*University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*

Presenting author
Laura Boyle. E-mail: laura.boyle@teagasc.ie

Application

Recently, the focus on sustainability of livestock production shifted almost exclusively to the environment which may take precedence over practices associated with good animal welfare (AW) (Boyle and Stevenson, 2024). One Welfare recognises the interconnectedness of humans, animals and the environment they share (Pinillos et al., 2016). Hence, it provides a useful framework to ensure the development of fair and resilient livestock production systems in which animals do not fall further out of consideration in the future.

Introduction

There is increasing societal concern about AW in intensive systems, growing interest in pigmeat produced from alternative systems and awareness of their value in terms of ecosystem services. However, while outdoor farming is perceived as 'AW friendly' as it allows pigs to perform natural behaviours, they can adversely affect the environment, particularly soil health. Furthermore, there are potentially greater challenges to humans due to lack of policy supports and insecure income. The aim of this study was to use the One Welfare framework to evaluate outdoor pig farming.

Materials and Methods

Animal, environment and farmer data were collected on 17 outdoor farms between July and October 2023. Pigs (n=223) in 29 paddocks were observed for body condition, lameness, lesions, tear staining and the presence of external parasites. Paddocks were divided into foraging and defecation areas. Soil shear strength was measured three times/area and averaged and a control measurement was taken outside the paddock. The shear strength of each area was subtracted from the control value to obtain a delta value. All plant species were counted in the same areas and the delta value obtained in the same way as for shear strength. AW and environmental measures were averaged across paddocks. Farmers completed a Quality of Life (QoL) questionnaire (WHO, 2012) producing scores based on four sub-sections (0-100) and an overall health and QoL score (0 – 5). Principal Component Analysis (PCA) was conducted using Rstudio software (R version 4.3.1, R core team, 2022) using the FActMineR and missMDA packages. Data were scaled before PCA analysis. Biplot was obtained and clusters were created to explain the analysis. Means of each variable was calculated for each cluster and an ANOVA was performed between each cluster. A Tukey's test was performed post hoc.

Results

Bi-plots captured 13.7% from dimension 1 and 34.5% from dimension 2 revealing three distinctive clusters which were named according to characteristics shown by each of the mean scores in the cluster (Table 1). Cluster 1 represented paddocks where sows were kept (sows were only on 3 farms) and with the highest (i.e. worst) average scores for all AW measurements. This cluster also had the highest number of plants in both areas in the paddock. The majority of QoL sub-section (>80%) and overall scores were higher than in the other 2 clusters. Cluster 2 represented 5 paddocks in three farms which had the lowest (i.e. best) scores for AW and for soil compaction. However, this cluster also had the lowest QoL scores in the sub-sections (av. 43.75%) and overall. Cluster 3 contained 20 paddocks and it generally fell between Cluster 1 and 2 for AW. The farmers also had generally higher average QoL scores than Cluster 2 but lower than Cluster 1 in every sub-section.

There were differences ($P < 0.05$) between the three clusters in lameness, skin lesion counts, ear lesion and tear scores. The soil shear strength differed across the three clusters in the foraging area and plant species diversity differed across the clusters in the defecation area. All of the QoL measurements differed significantly ($P < 0.001$) across all the clusters.

Table 1. Mean values for animal, environment and human Quality of Life (QoL) measurements in three clusters

	Cluster			
	Low AW	Low QoL	Average	P- value
Animal welfare				
Body condition score	3.2	3.16	3.3	NS
Lameness	0.1	0	0	<0.01
Ecto – parasites	0.2	0.18	0.13	NS
Skin lesion count	3	0.3	1.1	<0.01
Tail lesion score	0.3	0	0.1	NS
Ear lesion score	0.9	0.3	0.5	<0.01
Tear stain score	2.4	1.3	1.7	0.039
Environment				
<u>Soil shear strength</u>				
Foraging area	-17.7	-82	-46.3	0.03
Defecation area	29.6	-37.2	-11.6	NS
<u>Plant species diversity</u>				
Foraging area	-0.3	-2.86	-2.3	0.05
Defecation area	1.2	-1.6	-1.1	0.04
QoL				
Physical	87.3	45.8	90.1	<0.001
Psychological	94	52.6	83.8	<0.001
Social relationships	95.8	36.6	83.0	<0.001
Environmental	81.3	40.0	81.0	<0.001
Overall health	4.6	2.6	4.6	<0.001
Overall QoL	5	4	4.5	<0.01

Conclusions

Using One Welfare measures (animal, environmental and human) within one analysis, we identified three clusters of paddock 'type'. When comparing clusters, most differences were between the AW and human QoL measures. The environmental parameters were less specific, possibly because there were only two measurements. Poor welfare of sows in Cluster 1 could be related to their age compared to the fattening pigs but may also reflect poorer levels of care directed towards lower value animals. The lower QoL scores in Cluster 2 could be because these three farmers were certified organic or providing GMO free feed which is difficult to source and expensive (Brajon et al., 2024). The generally better AW in this cluster could reflect stricter standards for organic farms. In general this exercise reflects the value of a more holistic approach to the evaluation of farming systems.

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057

Outdoor pig rearing on the Island of Ireland – does agroforestry improve pig health?

N. Mitchell[1], R. Muns[1], R. Olave[2], C. McFarland[3], C. Brown[3], S. Anna Francina Buijs[1]
[1]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[2]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[3]*School of Biological Sciences, Queen's University Belfast, Institute for Global Food Security, 19 Chlorine Gardens, Belfast BT9 5DL, UK*

Presenting author
Nicola Mitchell. E-mail: nicola.mitchell@afbini.gov.uk

Application

Good health can be achieved when rearing fattening pigs outdoors on the Island of Ireland. Doing so in an agroforestry system, rather than a grassland system, reduces sunburn and accelerates growth. The latter means that slaughter weight can be reached in a shorter period, potentially resulting in labour savings. The mechanism(s) behind the slightly higher gait scores and levels of skin damage in the agroforestry system require further attention.

Introduction

Outdoor pig rearing is often perceived as an animal welfare friendly alternative to indoor rearing, mainly because of increased opportunities for rooting and exploration (Studnitz et al. 2007). However, outdoor paddocks cannot be separated from the outside world or cleaned and disinfected in the same way that modern indoor pens can (Bonde and Sørensen 2004). Thus, there is potential for increased exposure to pathogens and adverse climatic conditions. Unless restricted to concrete runs, outdoor rearing is often carried out on grassland. Grassland offers little protection from adverse climatic conditions and is susceptible to poaching during rain, with potential knock-on effects on pig health. The use of wooded areas can be expected to limit such issues, as trees provide shelter and help evaporate water (Manevski et al. 2019). This study aimed to compare the health of pigs kept in grassland and agroforestry systems.

Materials and Methods

We used two batches of 48 undocked boars each (batch 1: May-August 2023, batch 2: September-December 2023). Pigs ((Landrace x Large white) x Hampshire)) were born indoors, received anthelmintic treatment, and had limited outdoor access during their last two weeks indoors (to acquire experience with electrical fencing). At 8 weeks of age, the pigs were brought to the outdoor plots site in Loughgall, Northern Ireland. The outdoor site contained four grassland plots (GRASS, ryegrass dominated) and four agroforestry plots (AF, ±354 22-year-old oaks/ha). Each ±1000 m2 plot was equipped with a 6 m2 insulated shelter and a 2-space feeder and housed 6 pigs simultaneously. Plots used for batch 1 were reused for batch 2. Attempting to minimize terrain degradation, plots were further divided into 3 strips, and pigs, shelters and feeders were moved to a new strip after 4-5 weeks. Pigs were fed commercial diets ad libitum. During a period of unforeseen heat (within batch 1), temporary wallows were dug for all plots, whereas tarpaulins were used to create shade on the grassland plots only. Pigs remained on site until 21-22 weeks, but since batch 1 breached the fences when 19 weeks old no data from this point onwards is described here, except for batch 2 data on endoparasites and performance.

Tail damage, ear damage, body lesions, pig cleanliness, swellings, ectoparasites, nasal discharge, gait scores, sunburn, and body condition scores were determined after arrival and at 13 and 18 weeks of age (all visual scores, taken from within 2 m). Faecal consistency, melena and hematochezia were determined visually weekly. Individual faecal samples were collected at 10, 15 and 19 weeks of age (batch 1 only) and 20 weeks of age (batch 2 only), pooled per plot and analysed for Ascaris, Trichuris and Strongyle eggs using mini-FLOTAC. Feed disappearance and weight gain were calculated over the entire experimental period (batch 1: 76 days, batch 2: 91 days).

Data were analysed using (generalized) linear mixed models. Plot type was included as a fixed factor and pig age, age×plot type and baseline values were added where appropriate. Batch was added as a random variable in all models, as was group (except for pen-level data, i.e., performance, weekly faecal scores).

Results

Ectoparasites, nasal discharge, melena, hematochezia and reduced cleanliness were completely absent, whereas tail damage, swellings and endoparasite eggs were only observed once each. Ear damage (never worse than superficial scratches) tended to occur more in AF than GRASS (odds ratio: 2.3 [CI95%:0.9-5.8], P=0.09). The number of body lesions was relatively low, although slightly higher in AF than GRASS, with greater differences at 18 weeks (mean lesions/side at 13 weeks: 1.2±0.3 vs. 0.5±0.2, at 18 weeks: 1.4±0.4 vs. 0.3±0.1, P=0.02). Similarly, gait scores were good overall but slightly worse for AF than GRASS (23 vs. 19±5 SEM on a scale of 0-150, P=0.02). Sunburn (never worse than reddened skin without blisters) occurred significantly less in AF than GRASS (odds ratio: 0.07 [CI95%:0.03-0.21], P<0.01). Weight gain and disappearance of grower and finisher feed (in kg/pig/day) were all significantly greater for AF than GRASS (1.10 vs. 1.03±0.02 SEM, P<0.01; 1.55 vs. 1.43±0.03 SEM, P=0.02; and 2.77 vs. 2.66±0.27 SEM, P=0.04, respectively). Body condition, faecal consistency and feed conversion were not significantly affected by plot type (P>0.10).

Conclusions

Although health was generally good in both outdoor rearing systems, rearing fattening pigs on agroforestry plots rather than grassland plots reduced sunburn, which occurred frequently even under local weather conditions. Furthermore, agroforestry resulted in more rapid growth which should allow slightly shorter rearing periods, potentially with beneficial effects on labour requirements. Effects on growth were clearest in the summer batch and may be due to the greater availability of shade in the agroforestry system. Slightly more skin damage and worse gait scores occurred in the agroforestry plots than in the grassland plots. The causal mechanism for this may require further study, even though scores were never severe.

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058

Reducing Crude Protein contents with amino acid supplementation in finishing pigs: an economic assessment in male versus female heavy Duroc X (Landrace X Large White) crosses

A. De Almeida[1], M. Latorre[2], J. Alvarez-Rodriguez[3]
[1]*LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, 1649-004 Lisboa, Portugal*
[2]*Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, 50009 Zaragoza, Spain*
[3]*Departamento de Producción Animal y Ciencia de los Alimentos, Escuela Politécnica Superior, Universidad de Zaragoza, 50009 Zaragoza, Spain*

Presenting author
André De Almeida. E-mail: aalmeida@isa.ulisboa.pt

Application

In male and female heavy Duroc X (Landrace X Large White) finishing pigs, reduced Crude Protein (CP) with amino acid (AA) supplementation feeding strategies are economically viable under high or low feed and carcass prices scenarios.

Introduction

Nutrition represents 70% of pig production costs, particularly protein feedstuffs. Reducing feeding costs is of major relevance, particularly in heavy pigs finished until 120kg. Decreasing CP contents below recommended supplemented with AA is effective in decreasing costs without affecting production performances and profitability under particular scenarios (Almeida et al., 2024a). The strategy has been conducted in lean genotypes in post-weaning piglets and grower stages, with few studies concerning heavy male and female finishers. Herein, we conduct an economic assessment in heavy (Duroc X (Landrace X Large White)) male and female finishing pigs under a 2% CP reduction with AA supplementation under four scenarios (low feed and carcass prices and high feed and carcass prices). We hypothesize that for both sexes and scenarios the CP reduction coupled to AA supplementation is economically viable.

Materials and Methods

A total of 60 males and 60 females heavy (Duroc X (Landrace X Large White) finishers were used. At 9 weeks, pigs were distributed in split-sex pens (6 pigs/pen) with ad libitum access to water and feed. At 15 weeks, two isoenergetic diets with different CP levels were provided (145 and 125 g CP/kg, control and low-CP) during 42 days when all animals were slaughtered. Low-CP pigs were supplemented higher levels of crystalline AAs to meet equal ideal protein balance. Males were 7.25% heavier and had 16% higher average daily gains than females in both treatments with no differences recorded for feed intake or feed conversion ratio (Almeida et al., 2024b; 2024c). Feed costs and carcass incomes were calculated for best and least favourable scenarios in weekly prices over the last three years. Feed price of the control diet matched mean values of finishing feed in Spain for that period. Feed costs were calculated by multiplying feed prices (€/kg of feed) by the amount of feed consumed during the experiment (in kg/pig) plus a fixed partial fixed feed cost of 80 €/pig from 6 to 70 kg of body weight (start of the experiment). Carcass income (€/pig) was obtained by multiplying carcass prices (€/kg of carcass) by carcass weight (kg of carcass). Margin-over-feed-cost (€/pig) was calculated as the difference between carcass income and feed costs. Data was analyzed using the simple least squares models that included dietary treatment and sex and their interaction as fixed effects with the pen (n=20) as the experimental unit. The University of Lleida Ethics Committee (CEEA 01/23) approved the protocol.

Results

Results are detailed in Table 1. In the first two scenarios (concomitant low feed costs and carcass incomes, as well as high feed costs and carcass incomes), decreased CP contents with AA supplementation lead to analogous feed costs for all experimental groups. Results are different regarding carcass incomes and margin-over-feed costs, as for both remaining scenarios (contrasting high and low feed costs with low and high carcass incomes), the males had higher margins over feed costs than females for both diets. Concerning the low feed and high carcass prices scenario, control males had higher margins than low-CP females, whereas low-CP males and control females showed intermediate values.

Table 1. Economic assessment of the effect of low CP diet with amino acid supplementation on entire male and female pigs

	Diet-Sex					**P-value**		
	Low-CP Female	**Low-CP Male**	**Control-CP Female**	**Control-CP Male**	**SEM**	**Diet**	**Sex**	**Diet x Sex**
Low feed and carcass prices scenario (2020)[1]								
Feed cost (€/pig)	112.05	112.69	112.52	113.07	0.788	0.599	0.461	0.956
Carcass income (€/pig)	142.04^{c}	150.00^{ab}	143.99^{bc}	152.64^{a}	1.647	0.182	<0.001	0.833
Margin-over-feed-cost (€/pig)	29.99^{b}	37.30^{a}	31.47^{b}	39.57^{a}	1.249	0.153	<0.001	0.754
High feed and carcass prices scenario (2022)[2]								
Feed cost (€/pig)	133.17	134.23	135.92	136.87	1.325	0.059	0.459	0.967
Carcass income (€/pig)	177.09^{b}	184.70^{ab}	179.39^{ab}	188.10^{a}	3.281	0.397	0.024	0.869
Margin-over-feed-cost (€/pig)	43.91^{b}	50.46^{a}	43.47^{b}	51.23^{a}	3.309	0.962	0.046	0.857
High feed and low carcass prices								
Margin-over-feed-cost (€/pig)	8.87^{b}	15.76^{a}	8.06^{b}	15.77^{a}	1.223	0.749	<0.001	0.742
Low feed and high carcass prices								

Margin-over-feed-cost (€/pig)	65.03^{b}	72.00^{ab}	66.87^{ab}	75.03^{a}	3.230	0.463	0.032	0.856

[a,b] Lines with different superscripts indicate significant differences ($P<0.05$); [1]Feed prices for the most favorable scenario were 0.258 €/kg and 0.264 €/kg (+6 €/t) in the control and low-CP feeds, respectively. Prices of the control diet was set according to mean values of finishing feed in Spain during 2020; [2]Feed prices for the least favorable scenario were 0.428 €/kg and 0.454 €/kg (+26 €/t) in control and the low-CP feeds, respectively. The feed price of the control diet was set according to mean values of finishing feed in Spain in 2022

Conclusions

Reduced CP feeding strategy with AA supplementation is economically viable under the scenarios studied. Regardless of dietary CP level, sex was the major determinant of the economic margin-over-feed-cost, as a result of marked differences in carcass incomes.

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059

Variation and factors influencing ammonia concentrations levels in finishing pig facilities, Ireland

I. Martin Aroh[1], L. Clarke[1], G. McCutcheon[1], T. Curran[2]
[1]Pig Development Department, Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]School of Biosystem and Food Engineering, University College Dublin Belfield, Dublin D04 V1W8, Ireland

Presenting author

Izuchukwu Martin Aroh. E-mail: Izuchukwumartin.aroh@teagasc.ie

Application

This work is vital for addressing gaps in monitoring and understanding ammonia concentration levels and its influencing factors in Irish pig farms.

Introduction

Ammonia (NH_3) and greenhouse gas emissions, including methane (CH_4), and nitrous oxide (N_2O) are receiving increased attention due to environmental and health concerns (Kriz et al., 2021). NH_3 is not only a significant air pollutant due to its strong odour and toxicity at high levels (Roney & Llados, 2004), but in Europe, animal production alone accounts for over 75% of NH_3 emissions. In Ireland, the agricultural sector contributes 98% of the total national NH_3 emissions, with pigs and poultry responsible for 7% (Kelleghan et al., 2021). Past studies have largely concentrated on NH_3 levels, their spatial distribution, emissions, and seasonal and diurnal variations, particularly in poultry and livestock farms across Europe (Wyer et al., 2022). However, there is limited scientific knowledge regarding ammonia concentration monitoring in pig facilities in Ireland. This research builds on existing work by monitoring NH_3 concentrations within a pig research facility in Ireland. Using Off-axis Integrated Cavity Output Spectroscopy, the study examined NH_3 concentrations levels in a finishing pig building, evaluated NH_3 concentrations patterns and its influencing factors.

Keywords: ammonia, pigs, ventilation, temperature

Materials and methods

NH_3 concentrations were monitored continuously for 76 days in three finishing pig rooms (Rooms A – 117 pigs, Room B – 112 pigs, and Room C – 102 pigs) measured using a Los Gatos Research (LGR) gas analyser. Indoor temperature and ventilation were continuously monitored using the Big Dutchman system. All measurements were conducted from 9th August – 23rd October 2024. The pigs were fed a 16% crude protein diet. NH_3 concentrations were sampled for one hour at the exhaust fan according to the VERA protocol (Losada et al., 2014). The LGR provided 115 readings per hour at each sampling location, with a 31-32 second set interval. Data manipulation and visualisation was done using MS excel. Daily averages were used.

Results and discussion

The study observed that ammonia concentration levels and other measured parameters followed a consistent pattern across the three rooms during the measurement period, with Room B exhibiting the lowest ventilation requirement, indoor temperature, and highest ammonia concentrations levels **(Figs. 1, 2 and 3)** respectively. During the early phase of the finishing pig period, NH_3 concentration levels increased, gradually declined during the mid-growth phase, and increased again in the final finishing period **(Fig. 3)**. **Figures 1, 2, and 3** illustrated that an increase in NH_3 concentration levels was associated with a decrease in ventilation requirements and indoor temperatures across all rooms, and vice versa. This relationship was particularly evident in Room B, which recorded the highest ammonia concentration levels but the lowest ventilation requirements among the three rooms. These findings suggest a significant interaction between NH_3 concentration levels, ventilation requirements, and indoor temperature, with each factor influencing the other. Also, variation in temperature was greatly influenced by ventilation requirement of the rooms. Moreover, although Room A had the highest stocking density, other environmental factors beyond the number of animals played a more critical role in determining NH_3 concentration levels. For instance, Room B, with fewer animals, exhibited higher NH_3 concentration levels, indicating that factors such as ventilation requirement and manure management had a greater impact on NH_3 accumulation in those rooms.

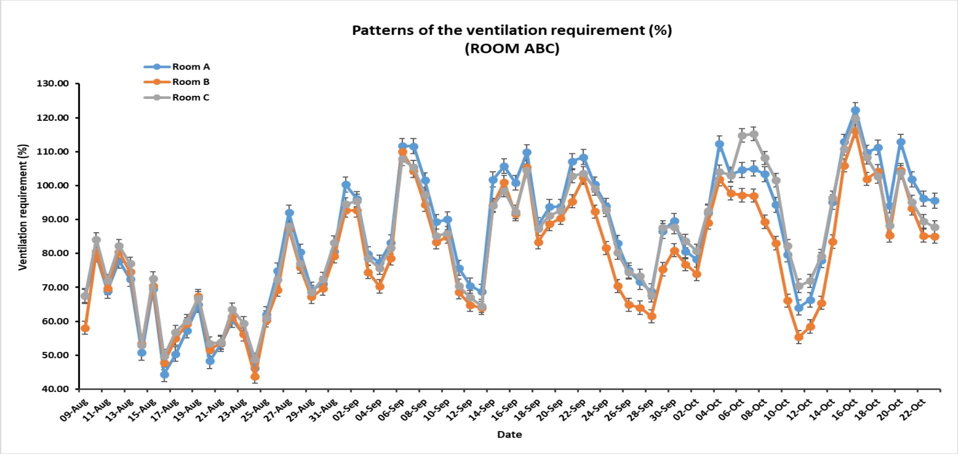

Figure 1. Patterns of ventilation requirement

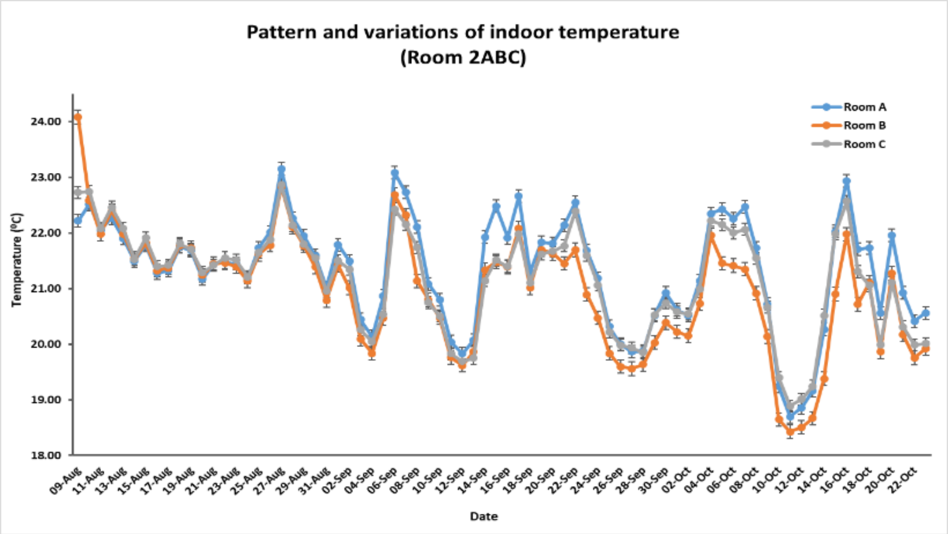

Figure 2. Patterns and variations of indoor temperature

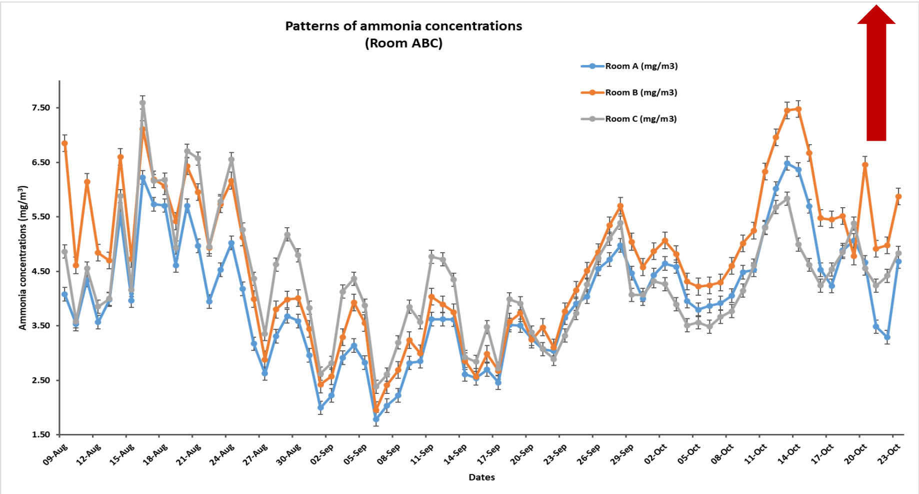

Figure 3. Patterns of ammonia concentrations

Conclusion

The NH_3 concentration levels measured in all three rooms stayed within the 10 ppm limit (7.16 mg/m^3) proposed in the Best Available Techniques guidelines for indoor pig environments. Also, facilities with higher stocking densities can effectively control ammonia concentration levels through good manure management practices and proper maintenance of indoor environmental conditions. Factors such as ventilation and indoor temperature played a key role in influencing/determining NH_3 concentration levels in the rooms. This study suggests that improving air quality in a finishing pig house can be achieved by examining several factors to monitor and reduce ammonia levels.

Acknowledgements

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060

Red meat: Balancing nutritional benefits with health and sustainability

M. Elmes[1]
[1]*AHDB, Siskin Parkway East, Middlemarch Business Park, Coventry CV3 4PE, UK*

Presenting author
Matthew Elmes. E-mail: matthew.elmes@ahdb.org.uk

In his presentation, **"Red meat: Balancing nutritional benefits with health and sustainability"** Dr Matthew Elmes highlights the nutritional benefits of red meat while addressing the concerns surrounding its consumption, particularly in relation to non-communicable diseases and environmental impacts. He explores the role of red meat in providing essential nutrients such as protein, iron, zinc, and vitamin B12, and discusses how it contributes to overall health, especially in vulnerable population groups. Dr Elmes also reviews current dietary guidelines and consumption trends, emphasizing the need for a balanced approach to red meat in diets. While acknowledging health risks like cardiovascular disease and colorectal cancer, he calls for a better understanding of red meat's role in nutrition, urging for clearer, evidence-based public health messaging that considers both individual and societal health needs.

061

Milking your milk contract

A. Sutcliffe[1]
[1]AB Agri, 64 Innovation Way, Lynch Wood, Peterborough Business Park, Peterborough PE2 6FL, UK

Presenting author
Anna Sutcliffe. E-mail: anna.sutcliffe@abagri.com

This session explores the structure of current milk contracts, focusing on maximising returns by achieving optimal benchmarks such as somatic cell count, milk fat, and protein content, all of which directly influence financial outcomes. Actionable strategies to optimise these metrics will be outlined, with particular emphasis on tailored nutritional approaches that improve gut health and integrity.
It also examines the evolving landscape of milk contracts, considering the impact of carbon-related pressures and sustainability metrics on the future of dairy farming.

062

Impact of Early-Life Nutrition on Lifetime Performance and Carcass Composition in Dairy-Bred Bulls

J. Abbott[1,2], A. K. Kelly[2], D. A. Kenny[1]
[1]Animal & Grassland Research and Innovation Centre, Teagasc Grange, Dunsany, Co. Meath C15 PW93, Ireland
[2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland

Presenting author
Jennifer Abbott. E-mail: jennifer.abbott@teagasc.ie

Application

Increasing the plane of nutrition offered to bull calves during either the pre-weaning or early post-weaning phases of development resulted in higher weight gain respectively, with post-weaning differentials retained to slaughter at 16 months of age, affecting carcass weight, a key economic trait.

Introduction

Only 38% of Irish dairy-bred bull beef carcasses meet the desired slaughter specifications, resulting in both economic and environmental inefficiencies (Kenny et al., 2020). Early-life nutrition can play a central role in determining lifetime growth potential and carcass composition. Strategic nutritional intervention during key developmental windows can positively influence carcass gain, adiposity, and meat quality. However, limited data exist on the immediate and long-term effects of such interventions on animal performance, carcass traits, and the production and environmental efficiency of pasture-based dairy bull-beef production systems. This study aimed to assess the impact of both pre- and post-weaning nutrition and their interaction, on performance and carcass composition in dairy-bred male calves slaughtered at 16 months of age.

Materials and Methods

Spring-born dairy-bred Angus and Holstein Friesian bull calves (n=56), were blocked by breed, birth date and birth weight, and assigned within block to either a moderate (M PRE) or high (H PRE) pre-weaning plane of nutrition and allocated across four pens. Calves (7 days of age) assigned to M PRE were offered a typical rearing diet of 6L/day containing 750g milk replacer (MR; 26% protein and 16% fat) for 50 days followed by a 2-week weaning period. H PRE were offered 8L/day containing 1200g MR for 14 days, 10L/day containing 1500g MR for 24 days followed by a 3-week step-down weaning period. Both pre-weaning treatments had *ad libitum* access to concentrate feed. Post-weaning, from within their respective pre-weaning treatments, calves were allocated to either a moderate (M POST), 1.5kg/day concentrate, or high (H POST), *ad libitum* concentrate, post-weaning diet for four weeks. All calves were individually offered their respective dietary allocation of MR and pelleted concentrate (18% CP) via an electronic calf feeding system (Foster-Technik Vario; Engen, Germany). Subsequently, all animals were turned out to pasture and managed under a standard 16-month-old pasture based dairy bull beef system blueprint with bodyweight and

other anatomical measurements collected throughout and carcass characteristics data collected at slaughter.
All data were analysed in SAS (v9.4) using ANOVA (MIXED procedure the model included fixed effects PRE and POST plane of nutrition, their interaction along with random effects of block and pen.

Results

No interactions were observed between pre- and post-weaning diets for animal performance and carcass variables. An interaction between week and both pre and post-weaning dietary treatment was observed for both dry matter (DMI) and energy intake as per design. H PRE calves had greater ADG than M PRE pre-weaning resulting in higher bodyweight at weaning (+8.4kg, *P* = 0.0002), turnout (5.7kg, *P* = 0.024), housing (+11.3kg, *P* = 0.05), however, no effect (*P* >0.05) of pre-weaning diet was observed on bodyweight or carcass characteristics at slaughter (Table 1). As expected H POST had higher ADG post-weaning than M POST resulting in a bodyweight advantage at turnout (+19.3kg, *P* <0.0001), housing (+20.0kg, *P* = 0.001) and slaughter (+29.0kg, *P* = 0.001) with a 16kg difference in carcass cold weight (*P* = 0.001; Table 1). Weight gain during the grazing and finishing periods were similar across both dietary groups thus preserving the weight differentials established as a consequence of the early life nutritional treatments.

Table 1. Effect of pre- and post-weaning plane of nutrition (M or H) on performance of dairy-bred bull calves slaughtered at 16 months of age

Plane of nutrition	**Pre-weaning**		**Post-weaning**			**Significance***	
	M PRE	**H PRE**	**M POST**	**H POST**	**SEM**	**Pre-weaning**	**Post-weaning**
Bodyweight (kg)							
Birthweight	35.3	36.9	36.4	35.9	1.07	NS	-
Arrival (3 Weeks)	46.8	52.0	49.9	48.9	1.20	**	-
Weaning (11 Weeks)	83.7	92.0	87.9	87.8	1.70	**	-
Turnout (15 Weeks)	108.8	114.5	102.0	121.3	2.00	*	***
Housing (9 months)	188.2	199.5	183.8	203.8	4.72	*	**
Bodyweight at slaughter (16 months)	522.0	531.3	512.0	541.2	9.27	NS	**
Carcass weight	270.1	273.8	263.8	280.1	5.40	NS	**
Conformation	5.4	4.9	5.0	5.4	0.32	NS	NS
Fat score	7.2	6.6	7.1	6.7	0.45	NS	NS
Average daily gain (kg)							
Pre-weaning period (11 weeks)	0.71	0.81	-	-	0.024	*	-
Post-weaning period (4 weeks)	0.91	0.84	0.55	1.20	0.040	NS	***
Grazing period (5 months)	0.57	0.61	0.59	0.59	0.030	NS	NS
Housing to Slaughter (7 months)	1.34	1.32	1.32	1.35	0.035	NS	NS

*Non-significant (P>0.05) pre-weaning x post-weaning diet interaction was observed. *, ** and *** indicate P < 0.05, < 0.01 and < 0.001, respectively

Conclusions

Pre-weaning plane of nutrition had impacts on bodyweight only to 9 months, with no impact on carcass traits. However, **o**ffering dairy-bred male calves a high plane of nutrition for four weeks post-weaning, irrespective of pre-weaning plane of nutrition, led to enhanced bodyweight gain which was subsequently retained to slaughter at 16 months of age. This also translated to higher carcass weight at slaughter in these animals. There was no evidence of compensatory gain in animals offered a moderate pre-weaning plane of nutrition, followed by a high plane of nutrition post-weaning. These findings highlight the vital role of optimising post-weaning nutrition to enhance overall lifetime growth and feed efficiency. Research is needed to further refine post-weaning nutritional strategies and evaluate their impact on the economic and environmental efficiency of sustainable pasture-based dairy calf-to-beef production systems.

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063

Rumen degradability and intestinal digestibility of toasted faba beans

J. Houdijk[1], M. Hussein[1], D. Davies[2], L. Smith[3], K. Hertogs[4], L. Vandaele[5], S. Pauwelyn[4], D. Van Wesemael[5], W. McArthur[3]
[1]*Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK*
[2]*Silage Solutions Ltd, Llanbadarn Road, Aberystwyth, Ceredigion SY23 1EY, UK*
[3]*McArthur BDC, Flixborough Industrial Estate, Bellwin Dr, Flixborough, Scunthorpe DN15 8SN, UK*
[4]*Inagro, Rumbeke-Beitem, Ieperseweg 87, 8800 Beitem, Belgium*
[5]*Flanders Research Institute for Agriculture, Fisheries and Food, Merelbeke-Melle, 9820 Merelbeke, Belgium*

Presenting author
Jos Houdijk. E-mail: jos.houdijk@sruc.ac.uk

Application
Toasting increases the soya bean meal replacement potential of faba beans for ruminants.

Introduction
The soya bean meal replacement potential of faba beans for ruminants is constrained by their lower levels of crude protein (CP; Premier Nutrition, 2025), and thus both rumen degradable protein (RDP) and digestible undegradable protein (DUP). Earlier studies demonstrated the potential of experimental pressure toasting to increase DUP in faba beans (Goelema et al., 1999). Heat treatments such as toasting are expected to reduce CP solubility. Here, the effects of two types of commercial scale toasting were assessed on CP solubility, rumen degradability and intestinal digestibility of faba beans.

Materials and methods
Faba beans (var Lynx, harvest 2023, Lincolnshire, UK) were either left untoasted (FB) or toasted in a commercial scale oil-fuelled or electric toaster. Nine temperature and duration combinations were chosen based on literature to create a wide matrix of toasting intensity; three designed temperature-duration combinations could not be achieved due to limitations of the electric toaster. All resulting samples were analysed for crude protein and protein solubility in potassium hydroxide. The latter informed selection of nine samples for RDP determination in ruminally fistulated cows (four nylon bags per sample per cow; 8×10 cm; 37 µm pore size; 3 g milled sample per bag) using an 8-h incubation protocol. Rumen undegradable CP (RUP) was estimated using linear regression from an unrelated data set of untoasted and toasted faba beans for which RUP was determined using a full degradation protocol (CBS, 2004). Intestinal CP digestibility was determined in duodenally fistulated cows, in which four bags with RUP material collected after 12-h rumen incubation were inserted every 20 minutes (3×7 cm; 37 µm pore size; 1 g milled sample per bag). Resulting residuals for both rumen and intestinal samples were pooled by sample pending CP analysis. These outcomes were used to calculate DUP levels by multiplying RUP with intestinal CP digestibility. Data were analysed through Pearson's correlations between protein solubility and the various CP degradability and digestibility parameters.

Results

Table 1 shows that toasting reduced protein solubility, by up to -25% and -31% for the oil-fuelled and electric toaster, respectively. The effect on protein solubility of the samples not selected to the animal study ranged from -6% to -19% for the oil-fuelled toaster, and from -8% to -21% for the electric toaster (data not shown). Table 1 also shows that toasting reduced RDP (up to -45%) and increased RUP (up to +94%), with effects generally increasing with increased toasting intensity (Table 1). Within each toasting type, the greatest toasting intensities (Temp H and Duration H) resulted in the greatest intestinal CP digestibility; the latter did not correlate with RUP (r=0.3006; P=0.47). Figure 1 shows strong negative correlations between protein solubility and DUP, which was stronger for the electric toaster (r=0.9965; P=0.004) compared to the oil-fuelled toaster (r=0.9091; P=0.012), though with a stronger slope for the oil toaster (-0.64) compared to the electric toaster (-0.51).

Table 1.

Crude protein (CP), CP solubility (Sol), ruminal degradable CP after 8 hours of incubation (RDP8), rumen undegradable CP (RUP) and intestinal CP digestibility of RUP (Dig) of untoasted faba beans (FB) or eight samples of FB toasted at different temperature and duration combinations in an oil-fuelled or electric toaster.

	Toasting conditions[1]			Protein parameters				
ID	Type	Temp	Duration	CP, %	Sol, % CP	RDP8, % CP	RUP, % CP	Dig, % CP
FB	-			24.1	95.2	73.1	21.8	92.4
TFB1	Oil	L	L	24.3	95.1	59.5	30.4	92.7
TFB2	Oil	L	H	24.9	79.7	49.4	36.7	90.4
TFB3	Oil	M	M	24.9	84.9	54.9	33.2	94.7
TFB4	Oil	H	L	25.4	78.1	52.5	34.7	94.0
TFB5	Oil	H	H	26.5	71.2	40.3	42.4	96.3
TFB6	Electric	L	L	24.8	84.8	68.1	24.9	94.0
TFB7	Electric	M	M	24.3	80.1	63.2	28.0	93.2
TFB8	Electric	H	H	25.2	65.9	49.6	36.6	95.2

[1] Toasting equipment names and processing parameters kept confidential due to commercial sensitivity

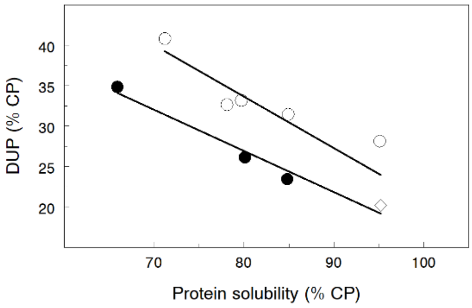

Figure 1. Relationship between protein solubility and digestible rumen undegradable protein (DUP) of faba beans var Lynx) toasted at different temperature and duration combinations in an oil-fuelled toaster (open circles) or electric toaster (closed circles), or untoasted (diamond).

Conclusion

This data supports the view that under the conditions used there was little evidence of post ruminal over-protection following toasting. Thus, toasting increases the level of DUP, which would as such increase its soya replacement potential in nutritionally balanced ruminant rations, However, the relationship between protein solubility and DUP seem to differ between the oil-fuelled and electric toasting equipment, which may have implications in terms of using protein solubility to predict final DUP levels, and the DUP yield per unit energy input.

Acknowledgements

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064

Effects of changing the human-edible fraction of dairy cow concentrates on milk production and feed efficiency

A. Cushnahan[1], A. Gordon[1], C. Ferris[1]
[1] *Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author

Aidan Cushnahan. E-mail: aidan.cushnahan@afbini.gov.uk

Application

Making increased use of low-human-edible feeds in dairy rations can contribute to the development of more sustainable milk production systems.

Introduction

The current study examined milk production and feed efficiency responses of dairy cows offered concentrates differing in human-edible fraction (HEF).

Materials and Methods

A three-period partial changeover design experiment using 32 early lactation dairy cows (mean of 50 days calved) examined the effects of offering four concentrates differing in HEF (proportionately 0.17, 0.28, 0.38 and 0.48 on a fresh basis; 0.17HE, 0.28HE, 0.38HE and 0.48HE respectively) with a grass silage based diet. The latter treatment most closely represented a "conventional dairy cow diet". Cows were offered 12 kg/day of their treatment concentrate via two out-of-parlour feeding stations. Two iso-energetic and iso-nitrogenous concentrate types were produced (0.17HE and 0.48 HE) and blended in different proportions through the stations to achieve the 0.28HE and 0.38HE treatments. The 0.48HE concentrate was based on cereals and soya-bean meal, while these ingredients were replaced with by-product feeds like sugar beet pulp and distillers dark grains in 0.17HE concentrate. The HEF of the diets were calculated as described by Wilkinson (2011). The forage component comprised grass silage and rotagrind straw (latter at 3.5% dry matter, (DM)), which was mixed in a diet-feeder before being offered to the cows. Cows also received 0.25 kg of a commercial concentrate at each milking. Feed intake, milk yield and milk composition was recorded during the fourth week of each period. Feed conversion rate (FCR) was calculated as energy corrected milk yield (ECM) per kg DM intake (DMI). Edible feed conversion rate (eFCR) was calculated as human-edible output divided by human-edible input per day. Net Food Production (NFP) was expressed as daily human-edible output minus daily human-edible input using HEF values described by Ertl et al. (2015) under current standard extraction allowances. Data was analysed using linear mixed model methodology (REML) with Cow and Period as a fixed effect. Linear and quadratic effects of treatment level were examined using polynomial contrasts.

Results

The silage had a DM, crude protein (CP) and lactic acid concentration and D-value of 283 g/kg, 157 g/kg DM, 121 g/kg DM and 711 g/kg DM respectively. Increasing the HEF of the concentrate tended to increase total DM intake (*P*=0.067, Lin) but reduced ECM yield (*P*<0.001, Lin.). Milk fat and protein concentration was unaffected by changes in HEF in the concentrate. However increasing the HEF reduced the concentration of total poly-unsaturated acids (PUFA) and conjugated linolenic acid (CLA) (*P*<0.001, Lin.) and reduced FCR, eFCR and NFP (P<0.001, Lin.).

Table 1. Performance of cows offered concentrates with differing levels of human-edible ingredients

	Treatment					P-value		
	0.17HE	0.28HE	0.38HE	0.48HE	SED	Treat.	Lin.	Quad.
Total DMI (kg/day)	23.2	23.6	23.8	23.8	0.28	0.205	0.067	0.297
ECM yield (kg/day)	39.0	37.7	37.0	36.1	0.73	0.002	<0.001	0.749
Milk fat (g/kg)	44.9	44.6	45.5	45.6	0.90	0.645	0.300	0.725
Milk fatty acid concentration (g/100 g total fatty acids)								
Total PUFA	2.4	2.4	2.3	2.2	0.04	0.003	<0.001	0.278
CLA	0.53	0.50	0.46	0.39	0.036	0.001	<0.001	0.407
Milk protein (g/kg)	32.4	32.5	32.6	32.9	0.35	0.822	0.957	0.394
FCR	1.68	1.60	1.54	1.52	0.033	<0.001	<0.001	0.314
eFCR (energy)	3.57	2.22	1.59	1.23	0.016	<0.001	<0.001	0.626
NFP(Protein)(g/day)	815	609	412	209	26.0	<0.001	<0.001	0.925

Conclusions

Increasing the HEF of the concentrate offered to dairy cows resulted in a reduction in milk yield and eFCR.

Acknowledgements

This project was funded by Department of Agriculture, Environment and Rural Affairs (DAERA).

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065

Milk production and feed efficiency of dairy cows offered concentrates differing in human-edible fractions and grass silage harvested at different stages of maturity

A. Cushnahan[1], A. Gordon[1], C. Ferris[1]
[1]*Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Aidan Cushnahan. E-mail: aidan.cushnahan@afbini.gov.uk

Application

The increased use of low human-edible feeds in concentrates can improve the sustainability of future milk production systems

Introduction

The study examined milk production responses of dairy cows offered concentrates differing in their human-edible fraction (HEF) and grass silages harvested at different stages of maturity.

Materials and Methods

Four treatments (2×2 factorial design) were examined in a three-period partial changeover design experiment using 32 mid lactation dairy cows (mean of 191 days calved). Factors comprised concentrates containing either a low (LE) or high level of human-edible ingredients (HE) and silages harvested at an early (EC) or later (LC) date (4 May and 17 May 2023). The HE concentrate contained significant quantities of cereals and soya-bean meal, while these feedstuffs were replaced with by-product feeds like sugar beet pulp and distillers dark grains in the LE concentrate treatments. The HEF of the LE and HE concentrates were calculated as described by Wilkinson (2011) and were 0.18 and 0.52 respectively. Rations were offered as a partial mixed ration (PMR) comprising 55% grass silage and 45% concentrate on a dry matter (DM) basis. Cows received 0.25 kg of a commercial pellet at each milking. Feed intake, milk yield and milk composition was recorded during the fourth week of each period. Feed conversion rate (FCR) was calculated as energy corrected milk yield (ECM) per kg DM intake (DMI). Edible feed conversion rate (eFCR) was calculated as human-edible output divided by human-edible input per day. Net Food Production (NFP) was expressed as daily human-edible output minus daily human-edible input using HEF values described by Ertl et al. (2015) under current standard extraction allowances. Data was analysed using linear mixed model methodology (REML) with animal and period fitted as random effects and tested for stage of maturity, concentrate type and interactions between these factors.

Results

The D-value of EC and LC silages were 709 and 690 g/kg DM respectively. Cows offered EC silage had a higher DM intake ($P<0.001$), ECM yields ($P=0.018$), milk fat content ($P=0.016$) and NFP ($P=0.015$) and a lower FCR ($P=0.005$) than cows offered LC silage. Concentrate type had no impact on DM intake, ECM yield, milk composition or FCR. Cows offered LE concentrates had a higher eFCR and NFP ($P<0.001$) than cows offered HE concentrate. There was an interaction between silage maturity and concentrate type for milk protein concentration, where cows offered EC silage and HE concentrate produced milk with a higher milk protein concentration than cows offered EC and LE concentrate (EC-LE, 36.7^{a}; EC-HE, 37.8^{b}; LC-LE, 37.2^{ab}; LC-HE, 36.7^{a}; SED, 0.43; $P=0.005$). In addition, cows offered EC silage and LE concentrate recorded a higher NFP value than cows offered the other treatments (EC-LE, 759^{c}; EC-HE, 24^{a}; LC-LE, 613^{b}; LC-HE, -1^{a}; SED, 42.8; $P=0.048$).

Table 1. Dairy cow response to concentrates differing in human-edible fraction and silage maturity

	Maturity	Conc.	P-value

	EC	LC	LE	HE	SED	Maturity	Conc.	Maturity × Conc.
Total DMI (kg/day)	22.9	20.2	21.4	21.8	0.60	<0.001	0.292	0.179
ECM yield (kg/day)	31.6	30.3	30.9	31.0	0.75	0.018	0.868	0.099
Milk fat (g/kg)	50.3	48.9	49.6	49.6	0.84	0.016	0.916	0.506
Milk protein (g/kg)	37.2	37.0	36.9	37.3	0.43	0.358	0.248	0.005
FCR	1.38	1.51	1.45	1.44	0.063	0.005	0.654	0.127
eFCR (Energy)	1.96	2.00	2.88	1.08	0.112	0.388	<0.001	0.948
NFP(protein) (g/day)	391	306	686	11	42.8	0.015	<0.001	0.048

Conclusions

Milk yields can be maintained and eFCR improved by offering concentrates based on low human-edible ingredients with grass silage harvested at differing stages of maturity.

Acknowledgements

This project was funded by Department of Agriculture, Environment and Rural Affairs (DAERA).

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Is there any potential for forensic determination of plant species ingested by cattle by proteomic investigation of faeces?

M. Matsuoka[1], M. McLaughlin[1], R. Burchmore[2], N. Jonsson[1]
[1]*School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube, Switchback Rd, Bearsden G61 1QJ, UK*
[2]*School of Infection & Immunity, College of Medical, Veterinary and Life Sciences, University of Glasgow, Garscube, Switchback Rd, Bearsden G61 1QJ, UK*

Presenting author
Miki Matsuoka. E-mail: 2265606m@student.gla.ac.uk

Application

Because of the public interest in the type and source of feedstuffs used in livestock production, we assessed the potential for faecal protein signatures to indicate whether specific plant sources were included in the diet of cattle. Many proteins derived from dietary plant materials were found in the faeces of cattle, but limitations on the depth of currently available plant specific information prevents a reliable identification of plant species.

Introduction

Our group has previously identified plant proteins such as serpin Z4 in bovine faeces (Huang et al., 2023). Concentrate feeds for cattle include cereals such as barley as a source of starch and energy, with supplementary protein, often as soyabean meal or as by-products of fermentation or oil extraction. However, there is particular public concern about the potential environmental effects of soyabean production (Wilkinson & Lee, 2018). We hypothesised that it might be possible to interrogate faecal proteomic signatures to forensically identify cattle that had been fed on specific plant species. The aim of the study was therefore to apply bioinformatic analysis to a pre-existing library of faecal proteins from barley-fed beef cattle to assess the reliability of assignment of plant species to proteins identified in faeces and to identify potential sources of bias.

Materials and Methods

The study was conducted using data generated previously by quantitative tandem mass tagged (TMT) mass spectroscopy of cattle faeces (Huang et al., 2023). In brief, 5 faecal samples were collected from 93 continental crossbred steers fed with an ammonia-treated barley-based diet (ATB) and 5 of 124 steers fed a propionate-treated similar diet (PTB). Both diets were barley cereal, soyabean meal, barley or wheat straw, and mineral premix. The presence of contaminating species was possible, but unlikely. The samples were analysed with TMT 10-plex by Huang et al. (2023). Here, MaxQuant (MQ) and Proteome Discoverer (PD) were both used to search against protein sequence databases including Swiss-Prot and TrEMBL downloaded from UniProt for each of 14 common feed plant species individually followed by quantification using Perseus after MQ. Searches were then conducted using a single, concatenated FASTA file with all 14 species. The abundance of each protein in faeces from cattle fed ATB and PTB was compared using Mann-Whitney U-test with Benjamini-Hochberg correction for FDR. Two potential sources of bias were assessed – the similarity of homologs and orthologs of proteins between species, and the richness of protein databases by species. The mean pairwise-distances of the sequences among 7 species of elongation factor Tu (EF-Tu, reported as present in all 7 species when searched individually), of ABC transporter domain-containing protein including ABC transporter G family member 31-like protein and pleiotropic drug resistance protein 1 and 2 (5 species), of germin-like protein (4 species), of hexosyltransferase (3 species), of pyruvate, phosphate dikinase (3 species), of JmjC domain-containing protein (1 species), and of trichome birefringence-like N-terminal domain-containing protein (1 species) was estimated with the Poisson model in MEGA 11. Spearman's rank correlation coefficient was determined for 1) the mean pairwise distances for each protein with the number of species in which each protein was found, 2) the total number of protein sequences in each species database with the number of identified proteins returned.

Results

More proteins were identified using PD than MQ. Individual database searches identified 8 and 35 barley proteins with MQ and PD respectively, compared to 1 and 11 in the single concatenated searches. No differences in protein abundance by barley treatment (ATB v PTB) were detected ($P > 0.05$). Each analysis identified serpin family proteins including serpin domain-containing protein, serpin Z4 and serpin Z7. The serpins were not included in the pairwise distance analysis because of the large number of potential orthologs and homologs. EF-Tu was identified from 5 and 7 multiple species in individual searches using both MQ and PD respectively. EF-Tu sequences were similar among 7 plant species, with an overall mean pairwise-distance of 0.17. In contrast, for trichome birefringence-like N-terminal domain-containing protein (found only in barley), the overall mean pairwise-distance was 0.6. The pairwise differences between barley and wheat for these two proteins were 0.004 and 0.09. The correlation between pairwise sequence distance and the number of species in which proteins were identified was -0.69 (not significant, $P = 0.09$). The number of sequences per species database correlated significantly with the number of identified proteins returned from individual species database searches and from the single concatenated database using PD ($r = 0.90$; $P < 0.001$, $r = 0.88$; $P < 0.001$ respectively).

Conclusions

Faecal proteomics identified highly abundant plant proteins that were likely to be in the diet, but it also identified proteins from species that were very unlikely to be ingested by cattle in this study. Each analysis found barley serpin Z4, which is the most abundant barley protein in faeces of cattle fed on the barley-based diet. Although the concatenated database search possibly improves peptide match while excluding highly conserved sequences, the number of sequences in the databases seems likely to bias identifications towards species with more sequences; proteins that are highly similar among species or orthologs are also likely to have incorrect species assignments. Current untargeted proteomic methods applied to faeces are unlikely to be useful for forensic assessment of plant species fed to cattle.

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Validation of Feed into Milk energy rationing models using data collated from production studies of Holstein dairy cows

X. Chen[1], A. Lavery[1], G. Scoley[1], C. Ferris[1], T. Yan[1]
[1]Sustainable Livestock Systems Branch, Agri-Food and Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author
Xianjiang Chen. E-mail: xianjiang.chen@afbini.gov.uk

Application

Feed into Milk (FiM, 2004) energy rationing models remain valid for rationing modern Holsten dairy cows.

Introduction

The FiM energy rationing models for dairy cows were developed over 20 years ago. Since then, dairy production in the UK has undergone considerable changes, with annual milk yields per cow having increased from approximately 6,000 litres in 2000 to 8,133 litres in 2023. The objective of the present study was to evaluate if energy rationing models within FiM remain valid for rationing modern Holsten dairy cows.

Materials and Methods

The present dataset was collated from 29 long-term indoor feeding studies (experiment periods ranged from >90 d to whole lactation) conducted between 2006 and 2023 at the Agri-Food and Bioscience Institute (AFBI) in Hillsborough, Northern Ireland. A total of 64 treatments were evaluated with more than 700 individual Holstein dairy cows. Feed intake, milk yield and liveweight of individual cows were recorded daily while milk composition was analysed weekly. Daily liveweight gain was estimated by linear regression of liveweight over time. All cows were offered grass silage-based diets, while maize silage and wheat straw were included in some rations. A total of 37,831 weekly mean data of individual cows were used in the present validation. Total daily ME intake (corrected for the effect of feeding level) was calculated from forage and concentrate DM intakes multiplied respectively by forage and concentrate ME concentrations. The ME concentrations of grass and maize silage were predicted using NIRS while ME concentrations of concentrates and straw were taken from the FiM database. Total ME requirement was calculated using FiM models as a sum of ME requirements for maintenance and milk production, liveweight gain, activity allowance and pregnancy. The validation was carried out by comparing total ME intake (actual intake) with total ME requirement (predicted intake).

Results

The data used in the present validation are presented in Table 1. The database represents a large range of dairy production conditions in terms of lactation No (1 to 10), days in gestation (0 to 236), weeks in milk (1 to 44), liveweight (370 to 853 kg), DM intake (10.0 to 40.0 kg/d) and milk yield (5.2 to 76.6 kg/d). Average ME requirement predicted using FiM energy rationing models is 256 MJ/d, which is only 1 MJ/d lower than the actual ME intake (corrected for the effect of level of feeding). The output shows a minor underprediction of 0.1% and a small mean prediction error (MPE) of 0.142. Most of the mean square prediction error (MSPE) was derived from the random factor (78.7%), with 21.3% from the line error, and 0% from the bias (difference between predicted and actual ME intake). A further evaluation using the linear regression of the prediction bias (difference between predicted and actual ME intake divided by actual ME intake, y) against liveweight (x) or milk yield (x) found that neither liveweight nor milk yield had a significant relationship with the prediction error.

Table 1. Data used in the present validation

	Mean	s.d.	Min	Max

Lactation No	2.5	1.42	1	10
Day in gestation	67	56.0	0	236
Week in milk	15	10.0	1	44
Liveweight (kg)	606	72.6	370	853
DM intake (kg/d)	21.7	4.19	10.0	40.0
Milk yield (kg/d)	34.2	8.78	5.2	76.6
Actual MEI (MJ/d)	257	49.3	101	458
Predicted MEI (MJ/d)	256	54.1	73	482

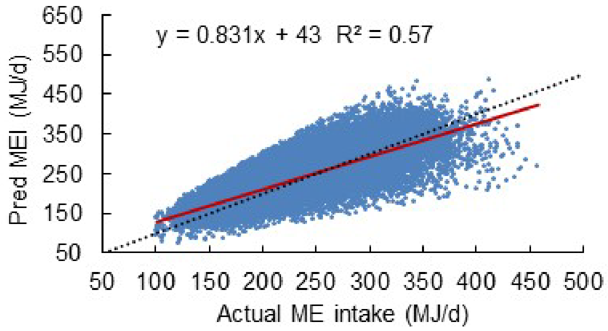

Figure 1. Actual vs. predicted ME intake (MJ/d)

Conclusions

The output of the present evaluation demonstrates that FiM energy rationing models developed over 20 years ago can accurately predict total ME requirements of the modern Holstein cow. The prediction accuracy remains similar across a large range of liveweights and milk yields.

Acknowledgments

This project was funded by the Department of Agriculture, Environment and Rural Affairs of Northern Ireland.

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Validation of Feed into Milk metabolisable protein rationing models using data collated from production studies of Holstein dairy cows

X. Chen[1], A. Lavery[1], G. Scoley[1], C. Ferris[1], T. Yan[1]
[1]Sustainable Livestock Systems Branch, Agri-Food and Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author
Xianjiang Chen.E-mail: xianjiang.chen@afbini.gov.uk

Application

Feed into Milk (FiM, 2004) metabolisable protein (MP) rationing models are still valid to ration modern Holstein dairy cows.

Introduction

There has been little attempt to validate the FiM MP rationing models using actual dairy production data since its publication over 20 years ago. This is especially relevant at present due to the intensification of dairy production within the UK and the associated need to reduce nitrogen excretion from dairy cows. Therefore, the objective of the present study was to evaluate if the MP rationing models within FiM were valid when tested on an experimental dataset collected from studies undertaken from 2006 to 2023.

Materials and Methods

Data used in the present study have been described by Chen et al. (2025). In summary, the dataset was collated from 29 long-term indoor feeding studies (64 experiment treatments), involving over 700 individual Holstein dairy cows. Studies were conducted between 2006 and 2023 at the Agri-Food and Bioscience Institute (AFBI) in Hillsborough, Northern Ireland. Feed intake, milk yield, milk composition and liveweight of individual cows were recorded. A total of 36,751 weekly mean data of individual cows were used in the present validation. The FiM MP rationing models were used to calculate total daily MP intake using measured daily forage and concentrate DM intakes, actual feed composition results, and DM and nitrogen rumen degradation variables taken from the FiM database. Total MP requirement was calculated using the FiM models as the sum of MP requirements for maintenance, milk production, liveweight change and pregnancy. Predicted milk crude protein (CP) yield was estimated as the difference between total MP intake and a sum of MP requirements for maintenance, liveweight change and pregnancy, divided by the efficiency of MP use for lactation. The validation was carried out by comparison of total MP intake (actual intake) with total MP requirement (predicted intake), and actual milk CP yield with predicted milk CP yield.

Results

Milk yield in the present dataset ranged from 6.6 to 76.6 kg/d (mean 34.4, s.d. 8.72 kg/d) and milk CP yield ranged from 0.22 to 2.66 kg/d (mean 1.18, s.d. 0.281 kg/d). Average MP requirement predicted using FiM models is 2.333 kg/d, which is 98 g/d less than actual MP intake. The output shows a underprediction of 4.0% and a small mean prediction error (MPE) of 0.138. Most of the mean square prediction error (MSPE) was derived from the random factor (90.5%), with 1.0% from the line error, and 8.6% from the bias (difference between predicted and actual MP intake). Similar prediction accuracy was also obtained for milk CP yield. Milk CP yield was overpredicted by 4.6% (54 g/d) with a MPE of 0.180. The MSPE was mainly derived from the random factor (67.9%) with 25.5% from the line error and 6.6% from the bias. A further evaluation using the linear regression of prediction bias for MP intake (difference between predicted and actual MP intake divided by actual MP intake, y) or for milk CP yield (difference between predicted and actual milk CP yield divided by actual milk CP yield, y) against live weight (x) or milk yield (x) found that neither liveweight nor milk yield had a significant relationship with the prediction error for MP intake or milk CP yield.

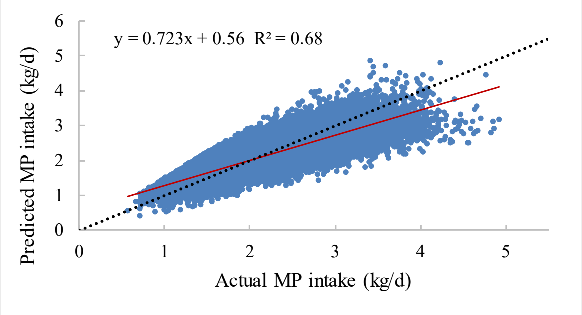

Fig 1. Actual vs. predicted MP intake

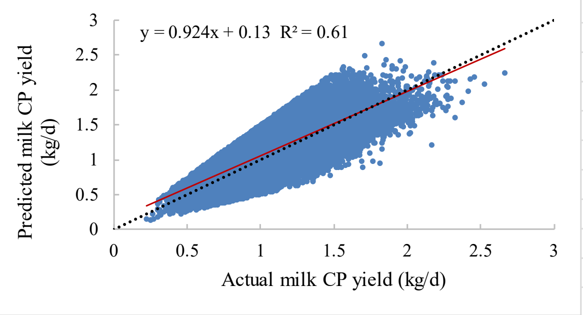

Fig 2. Actual vs. predicted milk CP yield

Conclusions

The FiM protein rationing models developed over 20 years ago were able to accurately predict total MP intake and milk CP yield within a varied historical dataset collected over the last decade. The prediction accuracy for MP intake or milk CP yield remains similar across a large range of live weights and milk yields.

Acknowledgments

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Genetic parameters for methane emissions in UK maternal sheep

K. Kaseja[1], E. Dods[2], J. Roden[2], N. Lambe[1]
[1]*Scotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3JG, UK*
[2]*Innovis, Peithyll Centre, Capel Dewi, Aberystwyth, Ceredigion SY23 3HU, UK*

Presenting author
Karolina Kaseja.E-mail: Karolina.Kaseja@sruc.ac.uk

Application

Reduction of the amount of greenhouse gas emissions from farm animals can be obtained by the appropriate breeding goals. Breeding for "low-emission" sheep can be the solution to mitigate the amount of methane produced on farms. Having a well phenotyped cohort of animals within a structured breeding program can allow for appropriate selection.

Introduction

Greenhouse gases (GHG) have become a major concern for society. Mitigation of GHG is the goal for many countries to combat climate change. The livestock sector contributes significantly to GHG emissions, particularly through methane production from enteric fermentation and nitrous oxide emissions from manure management. Mitigating these emissions is crucial for achieving climate change goals and ensuring the sustainability of livestock production systems. Thus, reducing methane production in farm animals (especially ruminants) has become one of the most important goals in animal production. If methane (CH_4) and carbon dioxide (CO_2) emissions are heritable, this could enable the development of breeding programs to reduce these emissions.

Materials and Methods

Portable Accumulation Chambers (PACs) provide a valuable tool for phenotyping individual animals (sheep) for methane and carbon dioxide emissions, as described by Jonker et al. (2018) and Jonker et al. (2020). A mobile trailer can travel from farm to farm, allowing for relatively easy measurement from grazing sheep. A dataset collected in PACs contained 3,150 records measured on 27 farms on 1,584 male and female lambs of maternal breeds, including Exlana, Lleyn, Dorset, Cheviot and composite lines, during two repeated rounds of measurements, approximately two weeks apart. All sheep were grazed on grass or forage with a minimum availability of 1500 kg DM for at least three weeks prior to measurement and for the two weeks between measurements. Lamb age ranged from 110 to 507 days (mean 2019) and live weight from 18 to 69.5 kg (mean 40.4 kg) at time of measurement. Age of dam (range 1 to 7), birth type (born in litter size of 1 to 4), rearing type (ranging from 1 to 4) and management group on the farm were also recorded. Animals were allocated the chambers randomly in a "lot" of 12 animals (one in each chamber). Sheep were weighed and kept off the pasture for at least one hour (maximum 4 hours) before the measurements were taken. Sheep stayed in the PAC for a period of 50 minutes, with the concentrations of methane, carbon dioxide and oxygen (O_2) taken at time 0, 25 and 50 minutes by the ENVCO Eagle 2 hand-held gas meter. All the data went through quality control, ensuring collected emissions of CH_4 and CO_2 increased with time, while the measure of O_2 decreased. Recorded (raw) CH_4 and CO_2 emissions were transformed to standardized units: grams per day (g/day). Transformation considered the pressure, temperature and average of gases produced by the "lot" of animals that were measured in PACs at the same time. Linear regression was used for repeatability estimation of the CH_4 phenotypes collected during round one and round two. Several models with different fixed effects were tested for significance in order to find the best model to estimate variance components for CH_4, CO_2 and gas ratio (molar proportion of methane to the sum of CH_4 and CO_2 emissions) – suggested proxy for methane emission relative to feed intake (Johanson et al., 2022). Genetic correlation between CH_4 and CO_2 emissions was also investigated.

Results

Quality control performed on transformed methane and carbon dioxide measurements indicated normality of data distribution (P <2.2e-16). Repeatability of CH_4 between measurement rounds was estimated at 0.41 (SE 0.02). A model incorporating fixed effects of animal sex, age of dam, time off feed, live weight, birth type, breed and management group was identified as optimal for both CH_4 and CO_2, with the majority effects being highly significant (P <0.001). Heritability estimates were 0.08 (0.02) for CH_4, 0.22 (0.04) for CO_2, and 0.23 (0.04) for the gas ratio. Fitting live weight was found to negatively impact (reduce) heritability of methane emission. No significant differences in heritability were observed between the two measurement rounds across all three traits. No significant difference from zero was found for the genetic correlation between CH_4 and CO_2 (0.01, SE 0.06), however a moderate phenotypic correlation of 0.27 (SE 0.01) was found.

Conclusions

This study conducts a comprehensive analysis into variance components of methane and carbon dioxide emissions for UK maternal sheep breeds. Moderate repeatability obtained between round one and round two measurements indicates that an altered phenotyping strategy, collecting one measurement only on more animals per sire, should provide sufficiently accurate data. Results obtained in this study were in line with the results from other research undertaken in New Zealand (Johnson et al., 2022) and Ireland (McHugh et al. 2022), proving that there is genetic variation for the examined traits and that levels of GHG produced could be successfully reduced in a breeding program with a goal to mitigate emissions from sheep. All the animals for which PAC phenotypes are collected are being genotyped and further research will focus on genomic selection for these traits. Furthermore, the next stage of this research will examine the correlations of PAC measurements with other important traits for which phenotypes are collected routinely (such as lamb and ewe production traits, health traits etc.).

Acknowledgements

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Ewe milk production and methane emissions: a simulation study

G. Valergakis[1], A. Kougioumtzis[1], D. Kalokairinou[1], A. Soufleri[1]
[1]*Department Of Animal Production, Faculty Of Veterinary Medicine, Aristotle University Of Thessaloniki, Thessaloniki 541 24, Greece*

Presenting author
Georgios Valergakis. E-mail: geval@vet.auth.gr

Application

Mitigation of methane emissions will improve the sustainability of the dairy sheep sector.

Introduction

Ewe milk is the leading dairy product of the Greek livestock sector. About 6 million dairy ewes are kept and their milk is used for the production of various types of cheeses, 21 of which retain a Protected Designation of Origin status. Among them, Feta is the most renowned, with 70% of its production being exported. Sheep milk production, while still retaining a rather natural profile, is not exempt from current public trends regarding greenhouse gas emissions and pressure is expected to increase in the future. Considering the significance of the dairy ewe sector, the aim of the present study was to identify factors and management practices that could potentially mitigate methane emissions.

Materials and Methods

A simulation study was designed to test the following hypotheses: i) Ewes with higher annual milk production emit lower amounts of methane per kg of milk produced. Recorded data regarding the dairy ewe breeds kept (Chios, Lacaune, Assaf-E) and their actual milk production (adjusted for fat and protein content) across Greece were used. ii) Rations with a restricted forage inclusion rate reduce methane emissions per kg of milk produced. iii) Use of forages with higher energy content result in rations with lower methane emissions per kg of milk produced. iv) Precision feeding resulting from feeding more homogenous groups results in lower methane emissions per kg of milk produced. Therefore, factors considered in this simulation study were: a) Milk yield type, five levels [based on bodyweight (kg) and annual milk production (kg), respectively]: 70/600, 65/500, 60/400, 55/300, 50/200. b) Forage inclusion in the ration: two levels, standard (mean 67%) and restricted (mean 44%). c) Forage energy concentration: three levels [low (alfalfa hay and wheat straw), intermediate (maize silage, alfalfa hay and wheat straw), high (maize silage and alfalfa hay). d) Grouping strategy: two levels (single group and high/low milk production groups). In total, a 5 x 2 x 3 x 2 factorial arrangement of effects was considered, yielding 60 different scenarios. For each scenario, 52 (weekly) rations were formulated, according to INRAe requirements (Hassoun et al. 2018) and the corresponding Feed Tables, for the lactating as well as the dry period, spanning a period of one year. Methane emissions were calculated using the following equations: (a) $CH_4/DOM = 45.42 - 6.6 \times FL + 0.75 \times FL^2 + 19.65 \times PCO - 35 \times PCO^2 - 2.69 \times FL \times PCO$ and (b) $CH_4 = DMI \times 0.001 \times DOM \times CH_4/DOM$ where CH_4/DOM is methane production per kg of digested organic matter, FL is the feeding level (dry matter intake as % of bodyweight), PCO is proportion of concentrate in the ration ($0 \leq PCO \leq 1$), CH_4 is methane production (g/day), DMI is dry matter intake (kg/day) and DOM is the digested organic matter (g/kg of dry matter) (Sauvant et al. 2018). From equation (b), yearly methane production was calculated; for those scenarios that included two groups of ewes, yearly emissions were weighted for the production of each group. Finally, emissions per kg of milk produced were calculated by diving total annual emissions by annual milk yield for each scenario. A general linear model was developed in order to study the effect of the above factors (included as fixed ones) on methane emissions (g/kg of ewe milk).

Results

All factors considered in the study had a statistically significant effect regarding methane emissions (Table 1). However, milk yield per ewe and amount of forage in the diet accounted for 82.1% and 15.3%

of the effect, respectively. Mean emissions from the five ewe types (50/200, 55/300, 60/400, 65/500 and 70/600) were 48.10, 36.46, 30.34, 26.71 and 23.52 g/kg of milk, respectively. Compared to 50/200 ewes, those of the 60/400 and 70/600 types emitted almost 37% and 51% less methane, respectively.

Table 1.
Factors affecting methane emitted per kg of ewe milk and corresponding adjusted Wald statistic (F). Adjusted $R^2 = 0.985$

Factor	F	P
Milk yield	911.801	<0.001
Amount of forage in the diet	170.379	<0.001
Type of forage in the diet	3.512	0.037
Grouping	24.737	<0.001

Conclusions

Increased milk production per ewe will result in reduced methane emissions per kg of milk, thus improving the sustainability of the sector. Genetic selection and management practices aiming to enhance ewe health, improve housing conditions and meet nutritional requirements while not overfeeding forages, should be the focus of sheep farmers and their consultants, alike.

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Genetic Gains, Greener Footprints: A novel life cycle assessment to demonstrate the effect of targeted genetic improvement on the environmental impact of beef x dairy production

E. Lai[1], A. Brown[2], M. Cleveland[1]
[1]*Genus, ABS Global, 1525 River Road, DeForest, Wisconsin, USA 53532*
[2]*Genus, ABS Global, 1525 River Road, DeForest, Wisconsin, USA 53532*

Presenting author

Ellen Lai. E-mail: ellen.lai@genusplc.com

Application

This is the first Life Cycle Assessment (LCA) that directly quantifies the environmental impact of beef-on-dairy (BxD) production in the UK and evaluates differences due to genetics. This novel method provides a framework for quantifying the environmental footprint resulting from genetic improvement, enabling the impact of a targeted breeding programme to be measured in terms of environmental outcomes.

Introduction

Amidst the rapid development of new sustainability interventions for livestock, genetic improvement remains the most impactful approach to permanently increase production efficiency. To fully realise efficiency gains, traits that impact animal performance from birth to slaughter must be considered for genetic selection, which is rarely accomplished in beef breeding programmes due to the segmented nature of the industry. BxD systems provide an opportunity to collect full lifecycle data, including traits related to growth, efficiency, and carcass characteristics, and comprise 35% of prime cattle slaughtered in Great Britain. Although genetic improvement is not a new technology, targeted index selection for BxD production is uncommon, and the effect of targeted breeding programmes on the environmental impact of livestock production remains largely unknown.

LCA is the gold standard for determining the environmental impact of livestock production. Several notable LCAs have assessed the environmental footprint of suckler beef (e.g., Putman et al., 2023), and one LCA has accounted for genetic merit in swine (Thoma et al., 2024); however, at present, no LCAs analysing BxD systems have been published.

The study objectives were to (1) provide a novel framework to include genetic merit in an LCA to quantify the environmental benefits achieved from genetic improvement, and (2) present the first environmental footprint for BxD production.

Materials and Methods

Attributional LCA models were developed for two UK BxD populations, each with distinct breeding goals: one targeted for commodity beef programmes emphasising age at slaughter, feed efficiency and carcass yield (HY), and the other for high-value Angus programmes emphasising growth and carcass quality (HQ). Within each population, two genetic levels were assessed, where levels were based on the programme-specific selection index of the sire: average and elite (^{3}1.8 SD above the mean) genetic merit. The system boundary included the dairy where the animal was conceived, the growing phases to reach slaughter weight – weaning, rearing, grazing (HQ only) and finishing – feed production and manure management from the growing phases, and the abattoir. LCA inputs comprised animal performance and feed data (N=10,214 HQ and N=3,132 HY). Data was aggregated from individual records in each growing phase for BxD animals in commercial settings. Traits included average daily gain, days in growing phase, and individual dry matter intake from feed intake trials (HY only). To account for genetic merit in the LCA, animal performance traits were averaged across genetic levels within each growing phase and population and used as inputs into the LCA. Impact assessment was performed according to the Product Environmental Footprint method 3.1 (European

Commission, 2021). Each environmental impact category was reported per one kg carcass weight (CW), the functional unit.

Results

The emission intensity for animals of average and elite genetic merit was 18.05 and 17.21 kg CO_2e/kg CW, respectively, for HQ (-4.66% reduction from average to elite) and 8.77 and 7.99 kg CO_2e/kg CW, respectively for HY (-8.82% reduction from average to elite). Manure management, feed production, and enteric fermentation were the primary contributors to emission intensity for both populations (Figure 1).

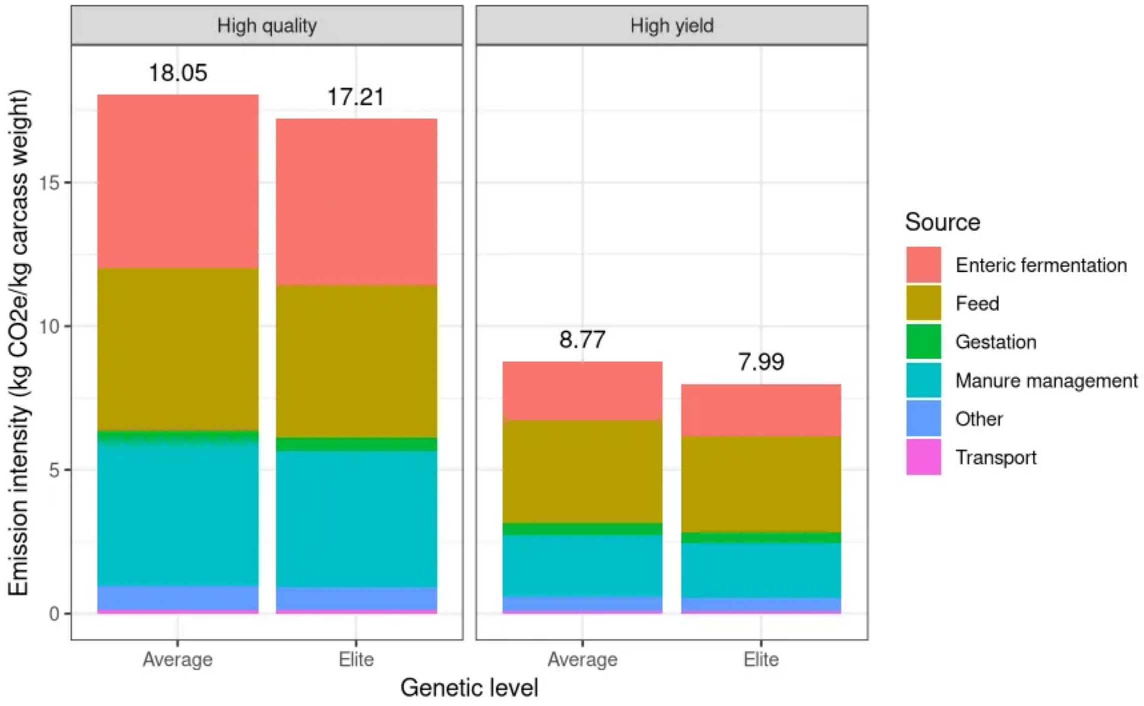

Figure 1 Emission intensity of each genetic level (average vs. elite) within each population (high quality vs. high yield), by emission source. Total emission intensity for each genetic level is above each bar.

Total emission intensity and enteric fermentation values from both average and elite genetic levels were lower than those previously reported for Northern European beef production (20.41 kg CO2e/kg CW from FAO, 2022), indicating an overall potential environmental advantage for BxD. Environmental impact of the elite genetic level was lower than that of the average genetic level in both populations. The larger impact in the HQ population compared to the HY population across all categories was driven by higher slaughter ages due to the required 180-day grazing period. Feed efficiency and days in the finishing phase were the primary drivers of impact.

Conclusions

The environmental impact of targeted breeding programmes can be assessed using LCAs that incorporate genetic improvement. Genetic selection aimed at the BxD value chain results in more efficient resource use and lower environmental impact of beef production.

LCAs developed for animal protein production should account for differences in genetic merit to capture the added environmental benefits that genetic improvement confers to the entire system. The environmental benefits achieved through genetic improvement, while incremental per generation, are permanent and cumulative over multiple generations. Consequently, continued genetic improvement will further reduce the environmental impact of BxD production relative to average BxD genetics, emphasising that designed genetic programmes are an integral part of developing protein production systems with lower environmental impact.

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Effect of tannins on reducing methane emissions in feedlot Nellore cattle

G. Pegoraro Rissi[1], J. S. Silva, A. A.G. Passerani, J. F. Bruno, I. C.S Bueno
[1]University of São Paulo, São Paulo - SP, 05508-220, Brazil

Presenting author
Guilherme Pegoraro Rissi. E-mail: guilherme.rissi@usp.br

Application

Tannins dose inclusion below 0.64% does not significantly reduce methane emissions in feedlot Nellore cattle.

Introduction

The increasing global population has heightened demand for animal protein production driving innovations in Mondial livestock production. Thus, the exploration of nutritional strategies, such as using natural additives, has gained prominence as it can increase the energy efficiency of animals and indirectly reduce methane emissions. These efforts are crucial to maintaining Brazil's position as the holder of one of the largest and most sustainable cattle herds globally, ensuring that the sector aligns with environmental goals while sustaining its economic importance. In this context, tannins, secondary plant compounds extracted from various parts, such as bark and leaves, have shown potential to improve animal performance by reducing the degradation of proteins and carbohydrates in the rumen, lowering enteric methane (CH_4) emissions, and enhancing the utilisation of nitrogen (Hassen et al., 2024). Therefore, this study aimed to evaluate the effect of tannins on methane emissions of feedlot Nellore cattle.

Materials and Methods

Sixteen non-castrated male Nellore cattle, approximately 18 ± 2 months old and with an average body weight of 387 ± 2 kg, were used. The animals were fed a basal diet with a roughage: concentrate ratio of 30:70, with the roughage portion consisting of corn silage (Table 1). The experimental design was completely randomized with four treatments: control (CTL), TAN16, TAN32 and TAN64, with additions of 0, 0.16, 0.32 and 0.64, per cent in dry matter, respectively. The animals were evaluated for 24 hours, five consecutive days, in the previously established periods for handling and methane collection: first period - day 4 to 8; second - day 43 to 47; third - day 77 to 81. Methane emissions (CH_4) were measured using the SF_6 tracer gas technique (Jonker et al., 2020), which uses a permeation tube emitting SF_6 gas in the rumen known constant flow. The eructated gases containing methane and SF6 were collected into evacuated canisters. The ratio of CH_4 to SF_6 was used to estimate methane emission CH_4, which were expressed as grams per day (g/day), kilograms of methane per kilogram of dry matter intake (kg/kg DMI), kilograms of methane per kilogram of average daily gain (kg/kg ADG), grams of methane per kilogram of body weight (g/kg BW), kilograms of methane per kilogram of carcass equivalent (kg/kg) and kilograms of methane per @ produced. The data obtained were analysed using the PROC MIXED procedure in SAS 9.4. The effects of tannins levels were evaluated through orthogonal polynomial contrasts.

Table 1.
Inclusion level of ingredients and chemical composition of diets

Ingredients (g/kg of DM)	**Treatments**			
	0.00	**0.16**	**0.32**	**0.64**
Corn silage	300.0	300.0	300.0	300.0
Corn meal	585.6	585.6	585.6	585.6
Soybean meal	68.1	68.1	68.1	68.1
Urea	11.9	11.9	11.9	11.9

Mineral[1]	28.0	28.0	28.0	28.0
Tannins	0.0	1.6	3.2	6.4
Kaolin	6.4	4.8	3.2	0.0
Chemical composition[2] (g/kg of DM)				
Dry matter (DM)	517.7	515.3	510.8	521.2
Crude protein (CP)	154.3	156.3	156.2	150.8
Ethereal extract (EE)	24.9	27.9	29.0	29.8
Neutral detergent fibre (NDF)	340.9	338.8	347.5	341.1
Acid detergent fibre (ADF)	131.3	119.6	123.9	130.9
Mineral matter (MM)	64.0	55.6	54.1	53.0
Crude energy (CE, MJ/kg)	69.2	68.5	69.2	69.6

[1] Mineral: Calcium: 170g; Phosphorus: 18g; Sodium: 50g; Magnesium: 14g; Sulfur: 20g; Potassium: 40g; Copper: 450mg; Manganese: 300mg; Zinc: 1300mg; Cobalt: 7.00mg; Fluorine: 180mg; Iodine: 25mg; Selenium: 12.60mg; 85,715UI of vitamin A; 14,000UI of vitamin D3; BHT: 100mg.

[2] Bromatological composition, determined at the Bromatology Laboratory of the Department of Animal Science of the FZEA of the University of São Paulo, Pirassununga.

Results

The addition of increasing levels of tannins did not significantly ($P > 0.05$) impact the values of CH$_4$ (g/kg DMI), DMI (kg), Ym (%), CH$_4$/ADG (g/kg) (Table 2). However, the CH$_4$ (g/day) altered quadratically as dietary tannins increased (P-value= 0.0368; $Y = 158.00 + 295.705 * dose - 393.0646 * dose^2$ with $R^2 = 0.1532$), as well as, CH$_4$ (g/kg BW, P-value= 0.0095; $Y = 0.3320 + 0.67766 * dose - 0.9092 * dose^2$ with $R^2 = 0,2223$) and CH$_4$ (g/kg BW$^{0.75}$, P-value= 0.0110; $Y = 0.3320 + 0.67766 * dose - 0.9092 * dose^2$ with $R^2 = 0,2223$). The results suggest that methane emissions tend to decrease at inclusion levels above 0.64%.

Table 2.

Methane production of Nellore cattle with increasing inclusion of tannins in the diet

Variable	Treatments (Tannins, %MS)				SEM	P-value		
	0.00	**0.16**	**0.32**	**0.64**		**L**	**Q**	**D**
CH$_4$ $_{g/day}$	160.70	188.07	217.78	185.36	13.88	0.10	0.04	0.30
CH$_4$ $_{g/kg\ DMI}$	16.50	15.32	20.39	16.30	1.53	0.52	0.35	0.03
DMI $_{kg}$	10.10	12.40	11.21	11.68	0.61	0.20	0.14	0.07
Ym $_{\%}$	3.03	3.09	3.69	2.95	0.27	0.76	0.15	0.13
CH$_4$ $_{g/kg\ BW}$	0.34	0.39	0.47	0.39	0.02	0.04	0.01	0.07
CH$_4$ $_{g/kg\ BW^{0.75}}$	1.59	1.83	2.20	1.82	0.12	0.04	0.01	0.09
CH$_4$ $_{g/kg\ ADG}$	127.78	122.10	199.02	155.24	26.43	0.17	0.47	0.09

DMI: Dry matter intake (kg/day); Ym: Conversion factor; BW: body weight; BW$^{0.75}$: metabolic weight; ADG: average daily gain; SEM: standard error of the mean.

Conclusions

Doses of tannins below 0.64% do not significantly impact methane emission reductions. Further research evaluating doses above 0.64% may help confirm the optimal inclusion rate to minimize emissions.

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The effect of a novel calcium peroxide-based dietary additive at various inclusion levels on the performance and methane emissions of growing beef cattle

O. Kalenikanse[1,2], V. O'Flaherty[2], S. Waters[2], D. Kenny[1]
[1]Teagasc Animal Bioscience and Grassland Research and Innovation Centre, *Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]University of Galway, *University Road, Galway H91 TK33, Ireland*

Presenting author

Omobolanle Kalenikanse. E-mail: ob.kalenikanse@teagasc.ie

Application

Inclusion of calcium peroxide (CaO_2) in concentrate feed can improve animal performance and reduce methane emissions of beef cattle.

Introduction

Irish agriculture accounts for over 38% of national greenhouse gas emissions with the greatest contribution resulting from enteric fermentation in ruminant livestock. Achieving a 25% reduction in Irish agricultural emissions by 2030 requires adopting feed management strategies that significantly reduce methane emissions from the national herd, ensuring sustainable livestock production in Ireland. Various feed additives such as 3-NOP (Stuart Kirwan et al., 2024), CaO_2 (Roskam et al., 2024), nitrates, lipids, and seaweeds (Beauchemin et al., 2022) have been found to reduce methane emissions in cattle by 15-30%. However, there is inconsistent evidence of the co-benefit of increased animal performance following the use of these feed additives. Moreover, these feed additives are mostly applicable within the context of total mixed ration which makes it unsuitable for lower intensity forage based production systems. This led to exploring a novel calcium peroxide-based dietary additive (CaO_2) which was developed by GlasPortBio, Ireland. A recent study conducted by Roskam et al., (2024) showed that CaO_2 reduces methane emissions by 16-29% and can be administered in pelleted format without affecting its anti-methanogenic potential. However, this study indicated that CaO_2 reduced nutrient digestibility, thus the need to optimise the inclusion level of the additive in cattle diet. As a result, the objective of this study is to evaluate the effect of dietary inclusion levels of a novel calcium peroxide-based feed additive on production performance and methane emissions of beef cattle.

Methods

Seventy-two spring-born Charolais cattle (n=36 steers and n=36 heifers) with an average body weight (±SD) of 455 ± 5.75 kg and age of 15 ± 0.4 months were enrolled in this study. The animals were blocked on body weight, baseline methane emissions and sex. Within each block, animals were randomly assigned to one of the four dietary inclusion levels of CaO_2, based on total dry matter intake: control with 0% CaO_2 inclusion (CON), a low inclusion diet with 0.4% CaO_2 (LID), a medium inclusion diet with 1.2% CaO_2 (MID), and a high inclusion diet with 2.0% CaO_2 (HID) (n=18). Animals received their respective treatments over a 12 week period, during which dry matter intake (DMI) (American Calan Inc., Northwood, NH), average body weight (ABW), average daily gain (ADG), feed efficiency (FCE) and gaseous emissions (C-Lock Inc., Rapid City, SD) were measured. All animals were fed a basal diet of grass silage and concentrate in a 60:40 forage to concentrate ratio. The average weekly individual DMI, growth and gaseous emissions data were analysed using REML. Fixed effects included treatment and weeks, while blocks was treated as random effects with animal as the experimental unit and week as the repeated measure. The statistical analysis was conducted using R software version 4.3.2.

Results

The daily DMI was affected by the dietary treatments with animals on MID having the lowest DMI when compared to other treatment groups ($P < 0.001$) (Table 1). Considering the ABW, ADG and FCE, animals on the HID treatment had the highest ABW and ADG when compared to other treatment groups but similar FCE with animals offered the MID dietary treatment ($P < 0.001$). Table 1 shows daily CH_4 values for the control group was 233 g/d, CaO_2 supplemented diets ranged from 209 to 228 g/d, resulting in daily CH_4 reductions of 2.2%, 9.4% and 10.3% for LID, MID and HID, respectively, compared to CON ($P < 0.001$). Animals offered HID has the lowest CH_4 yield with a reduction of 2.6, 2.3 and 0.6 g/kg DMI compared to the CON, LID, MID treatment group respectively ($P < 0.001$). Similarly, the animals on HID treatment group had lower CH_4 intensity up to 43, 52 and 13 g/kg of ADG when compared with CON, LID, MID treatment groups, respectively ($P < 0.001$).

Table 1. Effect of dietary calcium peroxide inclusion levels on production performance and gaseous emissions of growing beef cattle

Parameters	Dietary Treatments				SE	P-value	
	CON	LID	MID	HID		Treatment	Week
DMI (Kg)	9.10^{b}	9.01^{ab}	8.91^{a}	9.11^{b}	0.065	<0.001	<0.001
ABW (Kg)	493^{b}	490^{b}	484^{a}	507^{c}	5.770	<0.001	<0.001
ADG (Kg/d)	1.03^{a}	1.00^{a}	1.09^{b}	1.16^{C}	0.027	<0.001	>0.05
FCE (Kg ADG/Kg DMI)	0.11^{a}	0.11^{a}	0.12^{b}	0.13^{b}	0.003	<0.001	<0.001
CH_4 (g/d)	233^{b}	228^{b}	211^{a}	209^{a}	3.260	<0.001	<0.001
CO_2 (g/d)	7865^{b}	7654^{a}	7589^{a}	7687^{a}	81.20	<0.001	<0.001
H_2 (g/d)	0.308^{ab}	0.289^{a}	0.290^{a}	0.316^{b}	0.007	0.002	<0.001
CH_4 (g/kg DMI)	25.6^{c}	25.3^{c}	23.6^{b}	23.0^{a}	0.300	<0.001	<0.001
CH_4 (g/kg BW)	0.47^{c}	0.47^{c}	0.44^{b}	0.41^{a}	0.005	<0.001	<0.001
CH_4 (g/kg ADG)	231^{c}	240^{c}	201^{b}	188^{a}	7.770	<0.001	<0.001

$^{a\text{-}c}$ Means within a row with different superscripts differ ($P<0.05$). No significant treatment by week interactions

Conclusion

Supplementation of forage based diet with CaO_2 incrementally improved the growth rate and feed efficiency of growing beef cattle with a 2.0% inclusion level while reducing methane and methane intensity up to 10% and 19%, respectively when compared with unsupplemented animals.

Acknowledgments

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In vitro assessment of inert carriers to deliver antimethanogenic compounds in forage based diets

S. Payne[2], M. Callaghan[1], N. Tomkins[1], Z. Durmic[2]
[1]*Roam Agricultural Pty Ltd, NSW 2800, Australia*
[2]*University of Western Australia, 35 Stirling Hwy, Crawley WA 6009, Australia*

Presenting author
Nigel Tomkins. E-mail: nigel@roam-ag.com

Application

Ruminant livestock contribute significantly to agricultural greenhouse gas emissions. Direct abatement using antimethanogenic compounds could readily reduce emissions but must be stable and effective over time. Providing compounds in a stable form or carrier that can be routinely incorporated into diets provides a pathway to tangible mitigation outcomes.

Introduction

In Australia ruminant livestock contribute significantly to agricultural greenhouse gas emissions. Halogenated compounds such as bromoform ($CHBr_3$) have been shown to have potential as a feed additive for mitigating enteric methane but are inherently volatile due to their chemical characteristics. Nevertheless, if complexed into a stable carrier these compounds could be incorporated into various feed preparations and released in the rumen to achieve a direct abatement outcome and so contribute to reductions of agricultural emissions. The requirement for daily oral administration of compounds may limit utility in extensive production systems but readily aligns with intensive systems such as dairy and feedlot. The aim of this study was to identify a solid inert carrier used to stabilise the antimethanogenic compound bromoform that would deliver *in vitro* abatement and could progress to *in vivo* application.

Materials and Methods

Two solid inert carriers [activated carbon (AC), polymer resin (PR)] containing bromoform (15% w/w) were dosed daily *in vitro* at a low (L) or high (H) concentration, creating four treatments; ACL, ACH, PRL and PRH equivalent to 12.6 and 25.2 mg $CHBr_3$/kg DM for low and high doses, respectively. A substrate only treatment of 30 g ground oaten chaff was used as a control. Treatments were incubated in triplicate in 50 µm pore size nylon bags using an artificial rumen simulation technique (RUSITEC) for 14 days to assess methane mitigation. The RUSITEC system was initiated and maintained as described by Garcia *et al.,* (2019). A constant flow of buffer into each fermenter maintained pH of 6.0 – 7.0. Gas produced by each fermenter was measured daily, sub sampled for methane quantification, fermentation fluid collected for analysis of VFA at three time points over 14 days and *in vitro* NDF, ADF and DM degradability calculated. Data were analysed per timepoint using one-way ANOVA in JMP V15.20 (SAS Inst.) with treatments as fixed factors and methane concentration, production, total gas, and VFA profile as variables. A Tukey-Kramer HSD pairwise comparison was used when means differed ($P<0.05$).

Results

All carrier types supported significant reductions in methane concentration and yield ($P<0.05$). The AC carrier delivered the greatest and most persistent mitigation effect regardless of concentration with mean reductions of 89-91% in the first week and despite withdrawal of ACH during the second week, 97-99%, indicating a carry-over effect. Treatment PRH demonstrated a mean reduction in methane yield of 76%, during the first week, however, this diminished to 41%, in the second week. There was a delay of at least 72 h to achieve 16-18% methane reductions for PRL compared with other carrier combinations that demonstrated an *in vitro* effect within 48 h. Carrier type did not influence ($p>0.05$) NDF, ADF or DM degradation compared with the control across sampling timepoints. Total mean VFA concentrations were similar ($p>0.05$) between all treatments (104 mmol/L) and control (101 mmol/L) across sampling timepoints. An antimethanogenic activity of all treatments was apparent without a decrease in total gas production, total VFA concentration or substrate degradability *in vitro.* These preliminary results indicate that carrier type will influence the mitigation outcome. A 15% (w/w) $CHBr_3$ for the PR may have exceeded its actual carrying capacity thereby explain the diminishing effect over time. The polymer resin in this study was less effective and less consistent in terms of effects on methane production *in vitro*. Conversely the concentration of $CHBr_3$ on AC could be decreased given almost complete inhibition was achieved at the low concentration.

Conclusions

Bromoform stabilised onto an AC carrier demonstrated greater and more consistent effects on methane mitigation than PR, indicating it is a more promising candidate for progression to *in vivo* trials. The utility of AC to complex $CHBr_3$ in a stable form requires further validation beyond 14 days. Ultimately the commercial use of inert solid carriers such as AC is dependent on the demonstration of consistent and minimal level of methane mitigation over an extended time period for either a feedlot or dairy application.

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How UK sheep farming meets the sustainability agenda for the future – producing nutritious food, fantastic fibres and within a landscape to be enjoyed by future generations

N. Noble[1]
[1]*National Sheep Association, The Sheep Centre Malvern Worcestershire WR13 6PH*

Presenting author
Nicola Noble. E-mail: nicola@nationalsheep.org.uk

Sustainability is not just a buzz word but an imperative. Many are making sustainability claims without the independent evidence supporting them. NSA sees the need to accurately assess the current environmental, social, health and economic sustainability of UK sheep farming while also considering what changes the sector will need to adopt to improve further.
Its new report provides answers and direction, but also raises important questions and identifies areas where more can be done towards improving sustainability.
It summarises sustainability and the need for it being more than just a measure of carbon. Multifunctionality and holistic thinking are essential factors behind true sustainability, but these are concepts many people find difficult to understand, preferring to consider their specific area of interest in isolation from broader factors. Focusing on any individual sustainability metric in isolation is likely to lead to unintended consequences and does not take account of the trade-offs between the many outcomes arising from a surprisingly complex industry.

The report highlights all the positive attributes delivered by UK sheep farming above high-quality protein and a sustainable fibre production – such as a plethora of additional public goods (biodiversity conservation, improved soil fertility, carbon sequestration, air and water quality).
Current challenges with measuring sustainability and obstacles to overcome with carbon auditing tools are highlighted. The report justifies the need for more accurate measures of methane output and that a single metric will never do justice to the complexity and multifunctionality of UK sheep production systems.
Future challenges and solutions are discussed, demonstrating the need for policy and market support alongside adoption and attitude change to ensure food security and sustainability go hand in hand.
There is a focus on public goods provision from UK sheep farming with 21 case studies showcasing prime examples of sustainable UK sheep farming from across the breadth of the diverse stratified sheep system.
The report has over 150 references within the full evidence review which can be found at www.nationalsheep.org.uk/our-work/policy. Current sheep research projects the association is involved in will also be discussed.

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Prime lamb production from grazed grass - effect of ewe age and genotype on productivity and subsequent lamb performance

T. Keady[1], J. P. Hanrahan[2]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[2]*University College Dublin, Belfield, Dublin D04 V1W8, Ireland*

Presenting author
Tim Keady. E-mail: Tim.Keady@teagasc.ie

Application

Retaining ewes aged more than 7 years results in reduced lamb output due to increase barrenness and reduced litter size.

Introduction

Ewe genotype is a key determinant of ewe productivity (Hanrahan 2001, Keady and Hanrahan 2019). Keady (2014) reported that the mean cost of producing a replacement ewe, when joined for the first time at ~19 months, equated to approximately 25% of the value of her lifetime lamb-carcass output. Currently, 66% of lowland ewes in Ireland have been sired by one of the two main terminal sire breeds (Suffolk and Texel) (Keady et al 2019), both of which have inherently low productivity (Hanrahan 2001). The Belclare breed was developed from a range of genetic resources (Hanrahan 1989), has a litter size of approximately 2.2 under typical on-farm management conditions, and represents the sire of 9.5% of ewes in lowland flocks (Keady et al. 2019). The aim of the current study was to determine the effects of ewe age on productivity and on the performance of their progeny, using ewes from 3 genotypes which differ in prolificacy.

Material and methods

The study involved performance records between joining to lamb at 2 years of age until lambing at 8 years for a total of 424 ewes [157 Belclare (B), 114 B×Suffolk (B×S), 153 >75%S (S); 2 cohorts]. Ewes only left the flock when culled for physical reasons (e.g., udder and mouth issues, poor body condition) or they died. A random 50% of each genetic group had been joined at ~7 months and 86% of those lambed at 1 year. Oestrus was synchronized using progesterone-impregnated sponges. Ewes were joined with Charollais rams. All ewes were shorn at housing (early December) and offered grass silage *ad libitum* until lambing. A concentrate supplement was offered during the final 6 or 7 weeks of pregnancy; the level depended on forage feed value and expected litter size (ultrasonic scanning). Ewes rearing singles or twins received no concentrate supplementation post lambing while those rearing triplets received concentrate (0.5 kg/d) for 5 weeks, and their lambs had access to concentrate (up to 300 g/head daily) until weaning. Lambs were managed as a single flock post weaning and were all slaughtered prior to the end of the grazing season. Data were analysed using the MIXED and GLIMMIX procedures of SAS, as appropriate.

Results

For the Belclare, B×S and >75%S ewes, lambs reared/ewe-joined averaged 1.62, 1.70 and 1.34 (SE ≈ 0.045), respectively, with a significant heterosis effect; the effect of ewe age was quadratic ($P < 0.001$) (Figure 1). There were significant curvilinear age effects on ewe fertility (lambed or not; $P < 0.01$) and litter size ($P < 0.001$) (Figure 1), and these age effects exhibited interactions with the heterosis effect on these traits. There was a marked increase in the incidence of ewes that failed to lamb after 6 years of age (0.08, 0.10, 0.06, 0.08, 0.07, 0.12 and 0.24 for 2, 3, 4, 5, 6, 7 and 8 year old ewes, respectively; SE ≈ 0.016 up to age 6, 0.032 at age 7 and 0.061 at age 8), mainly due to an increase in incidence of barren ewes. Mean age at exit from the flock was 4.6, 4.9 and 4.8 years (SE = 0.17) for B, BxS and >75%S, respectively. There were significant ($P < 0.01$) curvilinear effects of ewe age for BCS. Body condition score post mating, at lambing, 5 weeks post lambing and at weaning increased until 4, 5, 3 and 3 years, respectively, (Figure 2) and then declined. Ewe BW at post mating, lambing and 5 weeks post lambing increased as ewes aged from 2 to 7 years, but decreased at 8 years.

There were significant ($P < 0.01$) curvilinear effects of ewe age on: lamb BW at birth and weaning, ADG from birth to weaning and birth to slaughter, and age at slaughter. Lamb growth rate from birth to weaning and birth to slaughter increased until ewe age 6 and 8, respectively; (for ages 2, 3, 4, 5, 6, 7 and 8 ADG from birth to weaning was 253, 294, 284, 282, 310, 306 and 291 (SE ≈ 3.4) g/d and from birth to slaughter was 221, 256, 269, 262, 280, 280 and 284 (SE ≈ 3.5) g/d, respectively) while age at slaughter averaged 207, 183, 175, 179, 172,171 and 167 (SE ≈ 2.6) d, respectively.

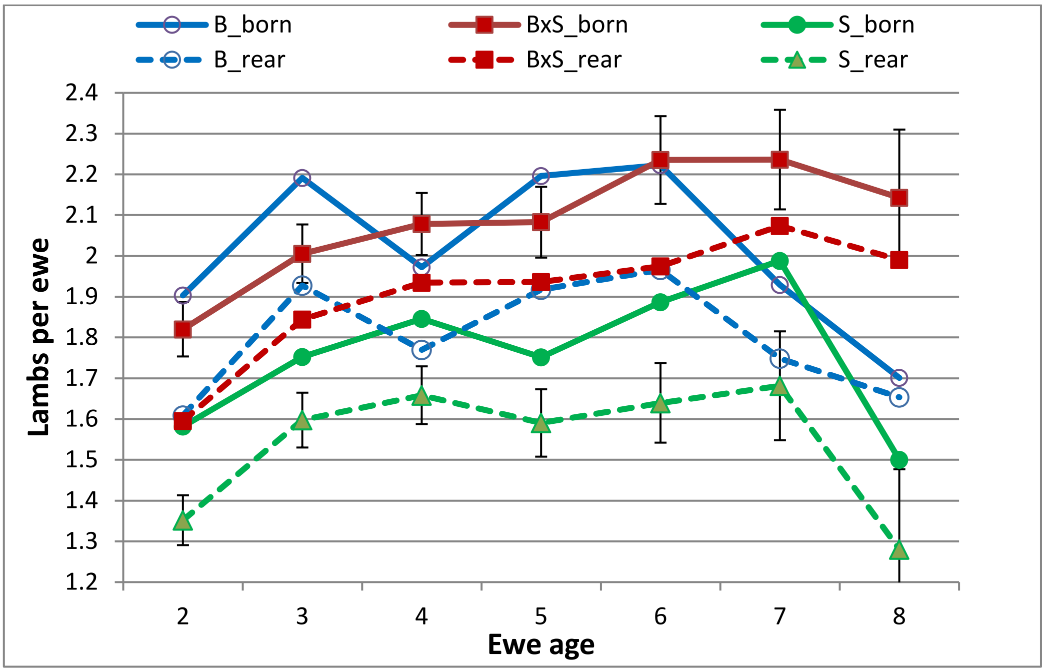

Figure 1. Effect of ewe age on litter size, number of lambs reared per ewe joined (B = Belclare, S = Suffolk) (vertical bars = SE)

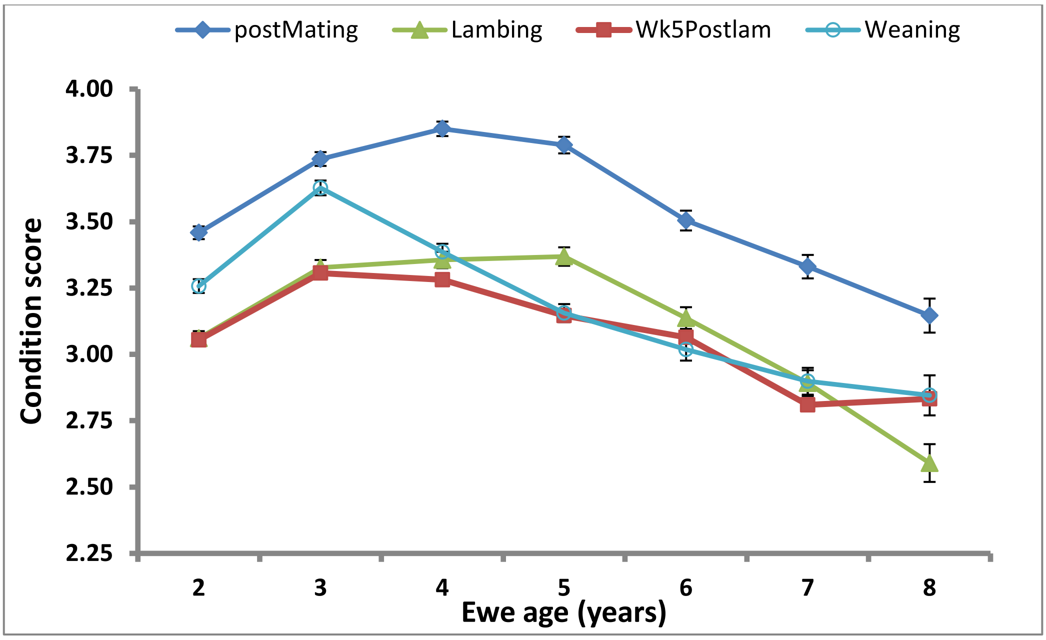

Figure 2. Effect of age on ewe body condition score at post mating, lambing, 5 weeks post lambing and weaning (vertical bars = SE)

Conclusions

Whilst ewe BW increased until 7 years, BCS declined from a younger age. The decline in the number of lambs reared per ewe joined at 7 and 8 years was due to increased barrenness and reduced litter size but the balance of causes varied among the genotypes.

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Updating economic values for the Irish sheep breeding index

E. O'Connor[1], J. Herron[2], L. Shalloo[2], F. McGovern[1], J. Anderson[3], N. McHugh[2]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[3]Sheep Ireland, Link Road, Ballincollig, Co. Cork P31 D452, Ireland

Presenting author
Edel O’Connor. E-mail: edel.oconnor@teagasc.ie

Application

The new economic values highlight the key traits driving profitability on Irish sheep farms and will allow for the national breeding index, the Euro-Star index, to be updated to reflect the importance of these key traits within the Terminal and Replacement indexes.

Introduction

An economic value is defined as the value of a unit change in an individual trait while all other traits are held constant (Hazel and Lush, 1942). Since its inception in 2019, the Teagasc Lamb Production Model (TLPM) has been used to calculate the economic values used in the current Irish sheep breeding objectives, called the Euro-Star index (Bohan et al., 2019). This stochastic whole-farm bio-economic model is used to calculate economic values across four broad animal characteristics, namely: maternal, lambing, production and health. Variable costs such as concentrates, fertiliser and veterinary costs that were used in the model have not been updated since 2017 (Bohan et al., 2019), however, across all Irish farms production costs have increased, an increase of 8% in 2023 (Teagasc NFS, 2024). Therefore, to ensure the economic values, and thus the national breeding objective, reflects current farm management practices both input and output costs used within the model required updating. The objective of this study, therefore, was to update all relevant costs and prices used within the TLPM and to recalculate the economic values for key traits within the Irish sheep breeding index. Of particular interest also was the impact of these changes on the national breeding objectives, particularly the relative emphasis placed on each trait.

Materials and Methods

The TLPM as described by Bohan et al. (2016) is a stochastic budgetary bio-economic model that describes an Irish lowland sheep production system. The model simulates the 12 month production cycle commencing at mating. The default scenario modelled, presenting a national average Irish lowland flock, contained 107 ewes, with a farm size of 13.27 ha, had a mean lambing date in early March, stocked at 7.5 ewes per hectare and weaning 1.5 lambs per ewe joined to the ram. All variable costs including fertiliser, concentrates, reseeding, silage making and veterinary costs were based on the average industry prices from 2021 to 2023. Monthly cull ewe price was based on historical data from 2021 to 2023, while monthly lamb price was based off future projections by the European Commission for 2025 to 2030 (EC, 2023). Economic values were simulated by modelling a one unit increase in each individual trait independently while holding all other traits constant. The difference in net profit between the changed (i.e., one unit increase per trait) and default scenario was used to calculate the economic values. An economic value was generated for a total of 14 traits, representing all the traits currently included in the national breeding objectives. Some of the key traits presented here include: maternal traits - number of lambs born and ewe mature weight, lambing - lamb survival, production –days to slaughter and pre-weaning weight, representing ewe milk yield, health - ewe and lamb lameness.

Results

All economic values increased from the previous versions reported by Bohan et al. (2019). The economic value for the number of lambs born was €56.72 per additional lamb born. Increasing number of lambs born increased lamb sales by €3,691 while also increasing variable costs such as concentrates (€385) and veterinary costs (€217). Ewe mature weight was calculated using three separate components including cull value (€0.16 per kg), maintenance (-€0.59 per kg) and replacement (-€0.09 per kg). Ewe cull weight was solely driven by the increase in cull sales (€170), while increasing ewe maintenance weight resulted in a €633 increase in variable costs. Increasing ewe replacement weight resulted in an increase in the cost of concentrate of €0.75 per ewe joined to the ram. The value of one additional lamb surviving past 24 hours was €81.90. This increased lamb sales by €239. Increasing days to slaughter by one additional day resulted in an economic value of -€0.39. Increasing days to slaughter resulted in an increase in concentrate costs, veterinary costs and the cost of depreciation of the shed used to finish the lambs by €8.20, €0.14 and €4.96 per lamb killed, respectively. Increasing lamb pre-weaning weight by one additional kilogram equated to an economic value of €2.99. This was achieved through a 4% increase in the milk yield of the ewes, resulting in a reduction of 14 day reduction in days to slaughter and an increased profit from surplus grass sold as baled silage while reducing veterinary and depreciation costs of the shed used to finish lambs. Lameness was calculated as a separate trait for both ewes and lambs, with economic values of -€0.31 and -€0.10 for ewes and lambs per one percent increase in lameness, respectively. Both traits resulted in increased labour and use of veterinary products.

Conclusions

Results from this study show that all of the economic values have increased from 2019 which can be explained by the increasing production costs and increased yearly lamb price over the last four years. These results will allow for the economic values within the current national breeding index to be updated while highlighting the new key traits driving profitability of Irish sheep farms.

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The role of a common sheep registration system in the efficiency of Icelandic sheep production

J. Sveinbjörnsson[1]
[1]*Agricultural University of Iceland, Hvanneyrabraut, 311 Hvanneyri, Iceland*

Presenting author
Jóhannes Sveinbjörnsson. E-mail: jois@lbhi.is

Application
The use of a common sheep recording system to facilitate common breeding work

Introduction
In Iceland there is only one breed of sheep, the Icelandic sheep. Of the North European short-tailed breeds, it has the greatest population size (Dýrmundsson and Niznikowski, 2010). A common sheep registration system has existed in Iceland since 1940. For the first decades only the most progressive breeders participated. The proportion of the sheep population registered slowly went up and was 46% by 2000. From year 2003, participation in sheep recording and other good farming practises became conditions for a part of the subsidies on lamb meat production in Iceland. By 2004, registration reached 81% of the sheep population, and since 2017 this number has been over 95%. The aim of this study was to investigate how these changes have affected the productivity and efficiency of Icelandic sheep production.

Materials and Methods
Data on the number of winterfed sheep and the total production of sheep meat in Iceland per year (1961-2023) were gathered from public records (statice.is). Data on production costs were obtained from yearly reports (1991-2019) of farm economics, i.e., the detailed economic data of a representative sample (5-10%) of Icelandic sheep farms through these years (Sveinbjörnsson & Kristófersson, 2021).

Results
In the years 1961-1980, the number of winterfed sheep in Iceland was between 736 and 896 thousand; the highest number occurred in 1978. During this period a considerable proportion of the meat was exported with subsidies. A quota system aiming on production only for the domestic market was introduced in 1983, and the sheep population decreased rapidly. In 1995, the quota system was abandoned, but export was not subsidised, so the number of winterfed sheep kept stable between 450 and 500 thousand in the years 1995-2002. The average yearly increase in sheep meat produced per winterfed sheep in Iceland was 0.06 kg in the period 1983-2002, but 0.24 kg in the period 2003-2023. The total sheep meat production was similar (8600 tons) from 385 thousand winterfed sheep in 2022 as from 474 thousand in 2002. The positive shift in productivity after 2003 is mainly related to the much greater participation in sheep registration and common breeding work, encouraged by the changes in the payment scheme. From 2003, a part of the subsidies was paid per kg of lamb meat produced only if farmers participated in sheep recording and other good farming practises. Analysis of yearly reports (1991-2019) of sheep farm economics show that the variable costs at farm level, adjusted for inflation, have remained stable throughout that period. The increase in productivity has counteracted new costs. Other benefits are also apparent from the major participation in sheep registration and common breeding work. In the late 20th century, too high a proportion of fat in lamb carcasses became a major drawback on lamb meat consumption in Iceland. The use of ultrasonic scanning for progeny testing since the 1990's, the adoption of EUROP classification system in 1997, use of inseminations and the overall great participation in the common breeding work since 2003 have resulted in great improvements. The average carcass is now 2 kg heavier, with a lower fat grade and considerably higher conformation grade than in the year 2000.

This has greatly helped maintain the domestic market for lamb meat. Recently, the common breeding work has become the basis for a national plan of eradication of scrapie by using genotypes protective against the disease. The ARR genotype was found for the first time in Iceland in January 2022, and now most Icelandic sheep farmers are active in this national plan.

Conclusions

By linking part of the subsidies on sheep production in Iceland to participation in a common sheep registration system, a very positive shift in productivity and carcass quality has been achieved.

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The effect of dam genetic merit on maternal traits including reproduction and ewe performance

A. Biggins[1,2], N. McHugh[1], T. Boland[2], H. Walsh[1], E. O' Connor[1], F. McGovern[1]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*

Presenting author
Anne Biggins. E-mail: anne.biggins@teagasc.ie

Application

Ewe prolificacy and other maternal traits of interest drive key determinants of farm productivity and profitability on sheep farms. Higher genetic merit sheep and their progeny outperform lower genetic merit ewes, therefore selecting high genetic merit animals will increase productivity.

Introduction

Genetics leads to improved animal performance across various species, including sheep. However, to sustain long-term genetic gain, selection indexes are required to aid producers in selecting animals to become the next generation's parents. The Irish €uro-star indexes are the national sheep breeding objectives that enable farmers to select animals based on their genetic potential. Similar breeding objectives exist for sheep in both Ireland and New Zealand, with both placing significant emphasis on maternal traits (Santos, et al., 2015); results from previous studies (Featherstone, et al., 2021) have shown that elite Irish or New Zealand maternal genetics could aid in improving Irish flock performance including ewe reproductive performance. This breeding study incorporates cross-breeding Irish and New Zealand genetics to obtain hybrid vigour. The objective of the present study was to compare the maternal performance of ewes and subsequent progeny performance based on their genetic merit and country of origin (Ireland or New Zealand).

Materials and Methods

This study took place from September 2021 until September 2024. Four genetic groups divergent in maternal genetics, including elite genetic merit Irish ewes (here on referred to as Elite Irish), low genetic merit Irish ewes (Low Irish), elite genetic merit New Zealand ewes (NZ) and a cross between elite genetic merit New Zealand and elite genetic merit Irish (NZxElite Irish) were used over the trial period. Animals were selected based on either the Sheep Ireland €uro-star Replacement index (Irish animals) or the New Zealand Maternal Worth (New Zealand animals). Twenty-five Suffolk and twenty-five Texel ewes were present in each group each year. Ewes were mated via laparoscopic artificial insemination (AI) using fresh semen in early October. Fourteen days post AI, rams were introduced for 42 days. Across all four years, the mean lambing date was 10th March and averaged 8 weeks in length. Ewe live weight and body condition score (BCS) were recorded at key time points, including pre-mating and 24 hr post lambing. Ewe's barren status and pregnancy scan size were recorded on each ewe. Lambs were weighed and tagged within 24 hours of birth. Lambing difficulty score and lamb vigour score were recorded on a four-point scale reflecting the scales currently used in the national genetic evaluations (www.sheep.ie). For lambing difficulty, one was unassisted, two had little assistance, three manual delivery okay, and four manual delivery difficult. For lamb's viability, one is unobserved, two the lamb sucked without assistance, three the lamb needed assistance, and four that the stomach tube was given. Data was analysed using a linear mixed model in PROC MIXED (SAS Inst. Inc., Cary, NC, USA) with breed (Texel or Suffolk), genetic group (NZ, NZx Elite Irish, Elite Irish or Low Irish), ewe parity, and year were included as fixed effects.

Results

Barren rate differed by genetic group ($P < 0.05$) and ranged from 2.6% (NZ) to 11.5% (Low Irish). Low Irish ewes had the lowest scan rate ($P<0.05$; Table 1), while all other genetic groups did not differ from each other. Ewe's live weight and BCS measured at both pre-mating and post-lambing differed by genetic group (Table 1). Pre-mating the Low Irish ewes had the lowest live weight ($P<0.05$). Lamb vigour score differed by genetic group where ($P<0.01$); lambs born to NZ ewes were the most vigorous, while lambs born to Low Irish ewes had the greatest lamb vigour scores, indicating they were the least vigorous ($P < 0.01$). Lambing difficulty differed by ewe genetic group ($P<0.001$; Table 1), with the Low Irish ewes having the highest lambing difficulty, which resulted in them having the highest percentage of ewes being assisted at lambing, 52% ($P < 0.001$; data not shown).

Table 1.
The effect of dam genetic merit on performance across the four genetic groups

	NZ[1]	NZ*Elite Irish[2]	Elite Irish[3]	Low Irish[4]	SEM	*P value*
Pregnancy scan rate	1.69^{a}	1.64^{a}	1.62^{a}	1.40^{b}	0.067	<0.05
Pre-mating live weight (kg)	83.14^{a}	83.97^{a}	82.41^{a}	79.60^{b}	0.711	<0.001
Pre-mating body condition score	3.57^{a}	3.54^{ab}	3.48^{b}	3.39^{c}	0.027	<0.001
Post- lambing live weight (kg)	82.17^{b}	85.28^{a}	82.49^{b}	81.26^{b}	0.899	<0.01
Post- lambing body condition score	3.04^{b}	3.17^{a}	2.94^{c}	2.90^{c}	0.049	<0.001
Lamb vigour score	2.31^{bc}	2.33^{c}	2.46^{ab}	2.53^{a}	0.095	<0.01
Lambing difficulty score	1.87^{c}	2.23^{b}	2.43^{a}	2.58^{a}	0.158	<0.001

[1] NZ = New Zealand, [2]NZ*Elite Irish = New Zealand X elite genetic merit Irish ewes, [3]Elite Irish= Elite genetic merit Irish ewes and [4]Low Irish= Low genetic merit Irish ewes

^{ab} Within rows mean with differing superscripts differ significantly

Conclusions

Results show that dam genetic merit influenced reproduction traits. New Zealand and Elite Irish ewes produced more lambs and achieved a greater pregnancy scan rate than ewes of low genetic merit. The crossbreeding of New Zealand and Elite Irish animals resulted in a lower proportion of ewes being assisted at lambing and greater lamb vigour at birth than the Irish groups.

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Ovine lameness - The ability of farmers to identify infectious lameness lesions and their subsequent treatment protocols - via survey

J. W. Delaney[1,2], E.. T. Kelly[2], J. W. Angell[3], F. Campion[1]
[1]*Teagasc Animal and Grassland, Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Veterinary Medicine, University College Dublin, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*
[3]*Wern Veterinary Surgeons, Station Road, Llanrwst Conwy Gwynedd LL26 0EH, UK*

Presenting author
Jake Delaney. E-mail: jake.delaney@teagasc.ie

Application

This study highlights the knowledge gap amongst farmers in identifying ovine infectious lameness conditions and utilizing appropriate treatment protocols at farm level. Future knowledge transfers activities should prioritize accurate lesion identification and appropriate lameness treatment protocols amongst farmers. This survey could also be refined and applied to other ovine infectious diseases or adapted to assess bovine lameness.

Introduction

Ovine infectious lameness represents a significant challenge within flocks due to its welfare and production limiting impacts. Infectious lameness conditions represent the majority of lameness cases within sheep flocks (Winter *et al.,* 2015) and comprise of three main disease types namely; Interdigital Dermatitis (ID), Footrot (FR) and Contagious Ovine Digital Dermatitis (CODD). All three conditions are linked, with Staton *et al.* (2021) reporting that 83% of ID/FR lesions can develop to CODD, where the causative bacteria is present and the lesion is allowed to progress. However, despite this each disease type requires varying treatment strategies for an effective resolution of the condition; while preserving antibiotic usage whenever possible in order to safeguard human and animal health against antimicrobial resistance (O'Neil., 2016). Foot bathing, for instance can be a useful method for the treatment of ID but offers limited efficacy for severe FR and CODD (Green *et al.,* 2018). There is a paucity of information on the ability of Irish farmers to identify infectious lameness lesions correctly in sheep and what subsequent treatment strategies are employed. The aim of this research was to assess the ability of Irish farmers to identify the types of infectious lameness lesions and to investigate the resulting treatment strategies that they employ on farm through a survey.

Materials and Methods

The survey was drafted in 2022 and was initially pilot tested by eleven Teagasc sheep specialists and technicians prior to its launch in January 2023. Once launched the survey remained live until May 2024 and was available in both online (via 'Survey Monkey') and paper based format. Online respondents were gathered through promotion on Teagasc social media. In-person respondents were collected at mart visits and attendance at Teagasc sheep events and responses were subsequently collated via 'Survey Monkey'. Following collation SAS Version 9.4 was used for all data screening and analysis.

Survey respondents were presented with images of ID, FR and CODD followed by a brief written description in random order and asked to identify the lesion. Questions were presented in multiple choice format and possible responses included: ID, FR, CODD, Toe Granuloma and Shelly Hoof.

Post lesion identification, respondents were asked to indicate if similar lesions had been observed within their flock and to detail the treatment methods they used on farm per lesion (use of multiple treatment methods permitted) and list any antibiotic treatment, if any, administered. Respondents

(14.5 %) who reported using lameness vaccinations were included in all analysis. Results are presented as the mean value with a -/+ 95% confidence interval.

Results

In total 364 Irish sheep farmers responded to the survey. Of which, 308 respondents answered the lesion identification questions. A total of 78.2% (95% CI: 73.6, 82.9), 77.6% (95% CI: 72.9, 82.3) and 61.7% (95% CI: 56.2, 67.1) of respondents identified ID, FR and CODD correctly, respectively. Out of 308 respondents, 148 (48.1%, SE= 0.04) identified all three lesions correctly. Of the respondents, 314 reported that they had identified at least one of the infectious lameness lesions on their farm. Wherein 90.8% (95% CI: 87.6, 94.0), 82.2% (95% CI: 77.9, 86.2) and 47.8% (95% CI: 42.1, 53.5) reported that they had seen ID, FR and CODD, respectively on their farm.

The most frequently utilised treatment method reported for the treatment of ID was the use of an antibiotic aerosol (71.2%; 95% CI: 65.1, 76.8). The second most frequently selected treatment method for ID was foot bathing (66.4%; 95% CI: 60.0, 72.3). In contrast, the administration of an antibiotic injection was the most frequent treatment method listed by respondents for FR (71.7%; 95% CI: 62.1, 80.0) and CODD (85.2%; 95% CI: 75.6, 92.1). Whilst, the use of an antibiotic aerosol was the second most frequently selected treatment method for both FR (61.3%; 95% CI: 52.1, 70.6) and CODD (49.4%; 95% CI: 38.1, 60.1). When asked to provide details of the antibiotic treatments administered the European Medicines Agency (EMA) Category 'D' class antibiotics (most frequently used – Tetracycline's) represented the majority of antibiotics administered for ID (82.2%), FR (76.5%) and CODD (64.7%). The remaining most frequently used antibiotics were from EMA category 'C' class antibiotics (most frequently used - Macrolides) and were administered as first line choices for ID (17.8%), FR (23.5%) and CODD (35.3%).

Conclusions

The majority of Irish flocks are faced with the challenge of infectious lameness annually. Contagious Ovine Digital Dermatitis, the least well-identified condition (61.7%), was reported to be present across 47.7% of flocks in this survey. The majority of respondents reported to use antibiotic aerosols most frequently to treat ID, in contrast to the use of antibiotic injections for the treatment of FR and CODD. The most common antibiotic class used for the control of ID, FR and CODD was EMA category 'D' antimicrobials, although there were a substantial amount of category 'C' class antibiotics used as first line treatments, contrary to veterinary treatment protocols for infectious lameness diseases.

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Adopting genomic technologies to provide insights on Microphthalmia in Texel sheep

E. Smith[1], J. Yates[1]
[1]British Texel Sheep Society, Stoneleigh Park, Unit 74, 4th Street, Kenilworth, Warwickshire CV8 2LG, UK

Presenting author
Ed Smith. E-mail: edsmith@texel.co.uk

Application

During 2023, a small number of cases of Microphthalmia were reported anecdotally by pedigree Texel sheep breeders in the UK. This disorder is a welfare issue because it can cause blindness in lambs who therefore do not survive to adulthood. Using an existing database of genomic data, animals that could produce affected lambs were identified. This information was then provided to pedigree breeders along with advice on breeding strategies to enable them to make more effective breeding decisions and reduce the chances of producing affected lambs.

Introduction

Microphthalmia in sheep is an autosomal recessive inherited condition within the Texel breed. It is characterised in affected animals by extremely small or no eyes and accordingly the animals are blind and do not survive to adulthood. The condition has been reported in Texel populations globally and is associated with a missense mutation (c.338G>C, p.R113P) in the *PITX3* gene that is involved in vertebrate lens formation (Becker et al. 2010; Goncalves et al. 2018). This single nucleotide polymorphism (SNP) is included in commercially available genotyping SNP arrays and can be reported on simply. Existing data can also be retrospectively analysed to provide information on previously genotyped animals. The aim of this work was to determine the level of carriage of the affected allele within the registered pedigree male population of British Texels (as only registered animals can be used for pedigree production), develop an effective mechanism of providing this information to pedigree breeders, and support the monitoring and removal of the carrier gene within the realms of Zootechnological legislation.

Materials and Methods

Low and medium density (15-50K) SNP genotypes (Ovine_15K, GGP_Ovine_15Kv2, GGP_Ovine_50K and GGP_Ovine_50Kv2) are stored commercially by Neogen Europe Ltd for all pedigree registered male Texel animals as part of Breed Society policy. The database includes 20,778 low and medium density individual animal genotypes and was retrospectively analysed to provide genotype information on the associated SNP in the *PITX3* gene in 2,746 animals. Data from animals born prior to 2018 were not included in subsequent analyses because information on fewer than 200 animals per year were available.These were predominantly male animals registered in the preceding three years and represented much of the *in-situ* pedigree male population. Carrier animals that could pass the condition on to their progeny were identified as heterozygous (C/G); whereas homozygous animals (G/G) were considered resistant. Homozygous (C/C) animals are clinically affected and do not survive. Additional genotypes were obtained for specific individuals that had been excluded from the initial analysis, to supplement the data. The change in breed registry policy to routine reporting of single-marker information from all commercially obtained genotypes, has provided further information on the prevalence of resistant and carrier animals in the pedigree population.

Results

To date 7,908 animals have a known *PITX3* genotype, including 6,914 males (5,795 registered and 1,119 unregistered) and 994 females (870 registered and 124 unregistered). In the registered pedigree male population, the overall proportion of carrier animals is 2.2%, however when split by year of birth, there is a noticeable increase in the rate of carrier animals born between 2020/2021 (Figure 1).

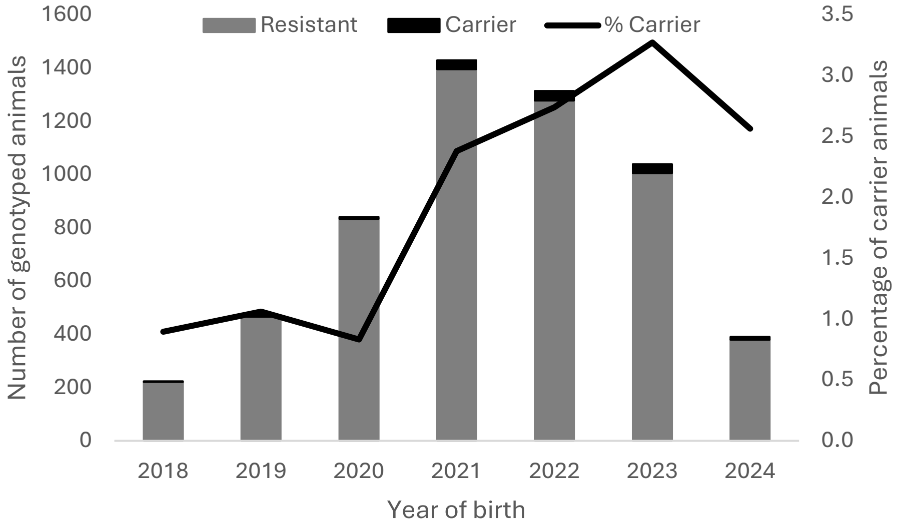

Figure 1. Numbers of pedigree registered male Texel animals genotyped and proportion of registered males that are Microphthalmia carriers, by year of birth.

Conclusions

The Breed Society has identified an increase in the proportion of Microphthalmia carrier animals in the pedigree registered male Texel population. This was precipitated by the widespread use of a small number of influential sires that were carriers. Routine genotyping of all registered sires (approx. 1400-1500 per year) has allowed the Breed Society to rapidly identify this trend. This has also led to the development of reporting systems that inform pedigree breeders of the status of their animals within the confines of Zootechnological legislation. The breed's online public database has been adapted to publish all genotype information available. This enables pedigree breeders and commercial producers to make informed breeding decisions on the use of registered and genotyped pedigree animals. Breeders are now more aware of the condition than previously and are more informed about their animals and those they may consider purchasing. This also supports a change of behaviour by breeders influencing the reduction of carrier animals in the male, and potentially the female, population. The identification of carrier animals for this deleterious gene and the facilitation of improved knowledge transfer by the Breed Society supports a responsible approach, increasing health and welfare in the pedigree population. Improving animal health is a prerequisite to sound animal husbandry, with positive knock-on effects on productivity and efficiency. It is now more likely that fewer lambs will be born blind than would have occurred without this intervention, resulting in lower levels of avoidable lamb losses.

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Heritability of wool shedding in mature ewes

S. Boon[1], T. White[3], M. Coffey[2], S. Id Lahoucine[2]
[1]*Agriculture and Horticulture Development Board, Middlemarch Business Park Siskin Parkway East Coventry CV3 4PE, UK*
[2]*EGENES SRUC, West Mains Road, Edinburgh, EH9 3JG, UK*
[3]*Sheep Improvement Group*

Presenting author
Sam Boon. E-mail: samuel.boon@ahdb.org.uk

Application

The ability to breed sheep that naturally shed their fleece is advantageous when the value of the fleece is less than the cost of shearing; wool shedding can enhance animal welfare and reduce labour costs.

Introduction

Breeders with wool shedding breeds are routinely scoring the ability of their sheep to shed their fleece. A 1-9 scale is used, with breeders asked to assess their flock when 50% of individuals are in the process of shedding their fleece; the point when the variation in shedding scores is greatest. Ideally breeding ewes are scored every year. Developing a breeding value for this trait will allow farmers to implement selection strategies to improve wool shedding attributes in their flock. The objective of this study was to estimate genetic parameters for this trait and thus develop a breeding programme for wool shedding.

Material and methods

The dataset included a pedigree of 10,608 individuals and 19,200 records collected from 7,512 sheep that had been scored between 2006 and 2022. Among them, 6,804 of the sheep measured (with 18,452 records) were Exlana; a wool shedding composite developed in the UK using Wiltshire Horn, Easycare, Kathadin and Dorper genetics. A further 699 of the sheep (with 727 records) were purebred Wiltshire Horns. The majority of shedding scores came from six flocks (7,243 measured sheep). The analysis used records for females over 300 days of age that had measurements taken between April and September. If multiple records were provided within the year, only the first record was used. This approach optimised the variation in the dataset as scores tended to become less variable over time. Scores had a non-normal distribution (see Table 1), which may have been expected as there had been phenotypic selection for the most desirable value (9=sheep that shed completely). However, it may also indicate the need for breeders to score their sheep earlier in the season to obtain greater variation in scores, with 46% of scores supplied being a 9.

Table 1. Breakdown of shedding scores

Shedding Score	Count of scores
1	1127
2	168
3	966
4	489
5	1643
6	1035
7	2357
8	2540
9	8875
Total	19200

For the analyses a repeatability model was implemented. This model includes 3 independent random effects (additive genetic, permanent environmental and residual) and the following fixed effects: litter size reared, age of the animal, flock-year-management contemporary groups. Heterosis and recombination were incorporated as covariates to take into account differences in breed composition. Note that litter size reared was included because this influences the onset of shedding, with those ewes rearing single lambs and in better body condition tending to shed earlier. Variance components of the model were determined using Restricted Maximum Likelihood (REML) estimation in ASReml (Gilmour et al., 2009).

Results

The variance component analysis indicated a moderate-to-high heritability for wool shedding with a genetic variance of 2.15 and a heritability of 0.44 (Table 2), supporting earlier findings by Pollott (2011) and the potential to use genetic selection to improve wool shedding in sheep.

Table 2. Variance components of wool shedding

Variance components	Variance	Heritability
Additive genetic effect	2.151	0.435
Permanent environmental effect	0.363	
Residual effect	2.434	

Breeding Values were generated for 10,609 animals that either had phenotypes or progeny with phenotypes. The average pedigree reliability was 0.58 (range 0.00 - 0.98). To assess the impact of wool shedding estimated breeding values (EBVs) at farm level the scores for ewes with a known sire (n=18,436) were split into those whose sire had an EBV in either the top or bottom 25% of the population. Within this sub-set of records, 46.2% achieved the top score of 9 (full wool shedding). For daughters of top 25% and bottom 25% sires this figure was 56.7% and 32.5% respectively.

Conclusions

The high heritability observed supports earlier studies by Pollot (2011) and shows that breeder-derived scores for wool shedding can be used to generate informative breeding values.

Acknowledgements

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CT scanning of live lambs is a useful tool for deriving carcase weight and composition in lamb growth rate studies

S. McGrath[1,2], T. Keogh[3], B. Holman[2,4], B. Allworth[1,2], H. Oddy[5]
[1]*Fred Morley Centre, Charles Sturt University, North Wagga, NSW, Australia*
[2]*Gulbali Institute, Charles Sturt University, North Wagga, NSW, Australia*
[3]*CSIRO Agriculture and Food, Clayton South Vic 3169*
[4]*NSW Department of Primary Industries and Regional Development, 105 Prince St, Orange NSW 2800, Australia*
[5]*NSW Department of Primary Industries and Regional Development, 105 Prince St, Orange NSW 2800, Australia*

Presenting author
Shawn McGrath. E-mail: shmcgrath@csu.edu.au

Application

Computerised tomography (CT) scanning of live animals can be used to compare the response of livestock to different nutritional treatments, and can be an alternative to comparative slaughter techniques for estimating carcase weight and tissue proportions in the carcase.

Introduction

Researchers often rely on growth rates to compare the response of livestock to different nutritional treatments, however differences in liveweight gain may not result in marked differences in carcase yield if the dietary effect is mainly on visceral mass (Keogh et al. 2023). CT scanning can be an important research tool for livestock production experiments, providing estimations of carcase fat, muscle, visceral fat, organ size, estimated maturity and allows repeat measurements on the same animal at different time-points, making CT scans a superior methodology to comparative slaughter (Keogh et al. 2024). The derived information can better inform predictive models for animal growth and improve models through more detailed energy transactions than measures of liveweight gain or comparative slaughter data (Oddy et al. 2019, Oddy et al. 2024). Some abattoirs in Australia now provide individual carcase feedback on sheep, including estimates of carcase muscle, fat and bone derived from DEXA, providing similar information to that derived from CT scanning. An advantage of post-slaughter DEXA scans compared with CT-scanning of live animals is that carcases are measured directly and therefore the mass of the animal's visceral tissues, skin, head and feet do not need to be estimated, resulting in lower risk of estimation errors compounding (Keogh et al. 2023). This paper reports a comparison between carcase weight and composition derived from CT scanning of live lambs and carcase feedback from a commercial abattoir.

Materials and Methods

Composite breed lambs (n=48) were drafted to one of three treatments for a 59 day feeding period: Treatment 1. Confined to individual pens and offered ad libitum feeding of either a barley grain-based pellet with a 30% inclusion of lucerne chaff; Treatment 2. Confined and offered a similar pellet without the inclusion of lucerne chaff, but with lucerne hay offered separately; Treatment 3. Grazing lucerne pasture as a group in a paddock. At the conclusion of the feeding period lambs were weighed and CT scanned after an overnight curfew. They were then put in a paddock as a single group with volunteer grasses and access to straw and water for 4 days before being trucked to a commercial abattoir and slaughtered after an overnight curfew. Empty body weight and hot carcase weights (HCW) were estimated using actual liveweight, stomach volume and viscera tissue weights derived from CT scans and estimated weight of fleece, head and feet (Keogh et al. 2023). Measurements at abattoir included the percentage bone, muscle and fat (via hot carcase DEXA scans) and HCW.

Data from the end of the feeding period and post-slaughter was analysed by generalised linear models using ASRemL. Lamb weights and empty body composition were modelled using a linear univariate model with fixed effect treatment.

Results

Lambs in Treatment 1 were heavier than lambs grazing lucerne at the conclusion of the feeding period and were fatter than other treatments; however treatments did not differ in non-visceral empty body mass (Table 1). Lambs grazing lucerne had greater visceral lean than lambs in Treatment 1 (Table 1). The estimation of HCW from CT scans showed general agreement with slaughter data ($R^2 = 0.80$). Fat and lean from CT scanning were well correlated with DEXA data, with estimates closest when fat content was higher or carcase lean was lower. (Fig. 1).

Table 1. Liveweight and estimated empty body (EB) component mass (kg) treatment means (± SE) of lambs at end of experimental period and hot carcase weights (HCW) derived from computerised tomography (estimated) of live lambs or weighed post-slaughter (actual). Different superscripts indicate treatment means differed significantly ($P<0.05$), (NV = non-viscera)

	Pelleted ration 1	Pelleted ration 2 + grain	Grazing Lucerne	p-value
Liveweight	51.9 ± 1.1^{a}	50.1 ± 1.1^{ab}	48.1 ± 1.1^{b}	0.041
EB weight	44.6 ± 1.0	43.0 ± 1.0	41.8 ± 1.0	0.145
Fat mass	14.7 ± 0.7^{a}	13.4 ± 0.7^{ab}	12.2 ± 0.7^{b}	0.039
Lean mass	25.5 ± 0.5	25.3 ± 0.5	25.0 ± 0.5	0.817
NVEB lean mass	20.2 ± 0.5	20.0 ± 0.5	19.3 ± 0.5	0.379
Viscera lean mass	5.3 ± 0.1^{a}	5.4 ± 0.1^{ab}	5.7 ± 0.1^{b}	0.042
HCW (estimated)	27.8 ± 0.7	26.7 ± 0.7	25.8 ± 0.7	0.108
HCW (actual)	27.1 ± 0.7^{a}	26.2 ± 0.7^{ab}	24.7 ± 0.7^{b}	0.041

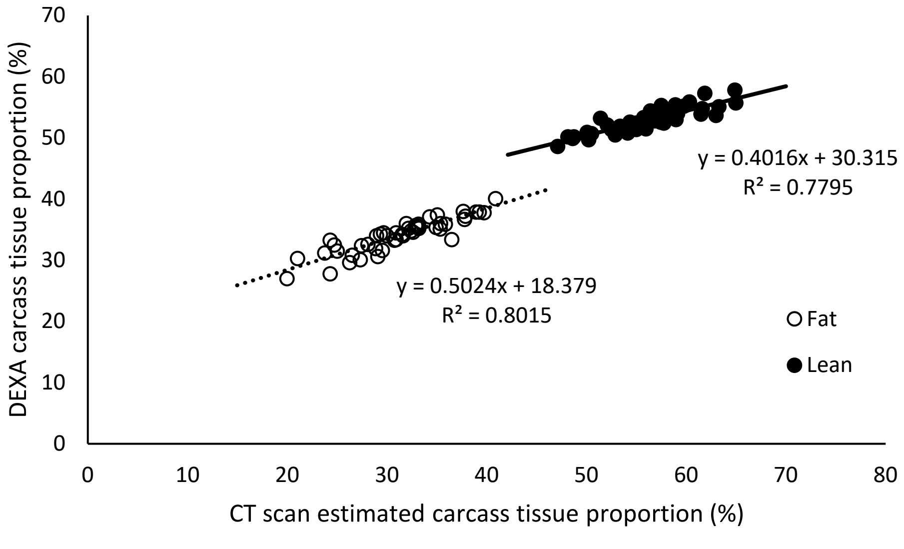

Figure 1. Comparison of the CT scan estimated proportion of fat and lean tissue in the lamb carcase and tissue proportions determined by DEXA scans of carcases after slaughter

Conclusions

Heavier liveweight at slaughter does not always result in greater non-visceral lean mass. Estimates of body composition on live lambs from CT scanning correlate well with DEXA measurements on carcasses post-slaughter. CT scanning may therefore be a useful method for comparing treatments in lamb production experiments as it enables the prediction of carcase weights at multiple timepoints whilst accounting for the effects of diverse nutritional treatments on gut fill and visceral organ mass.

Acknowledgements

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Effect of concentrate crude protein and amino acid supplementation on the milk production of late-lactation, grazing dairy cows of divergent genetic merit

R. van Emmenis[1,2], R. Fitzgerald[1], M. Chaize[1], M. Dineen[1]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]School of Biological Earth and Environmental Science, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland

Presenting author
Rochelle van Emmenis. E-mail: rochelle.r.vanemmenis@teagasc.ie

Application

Reducing the crude protein concentration of supplementary concentrate, offered to late-lactation grazing dairy cows, did not reduce milk production performance.

Introduction

Irish pastures typically provide sufficient CP, especially in autumn when pasture CP is naturally high. Consumption of high CP pasture, coupled with the cow's metabolisable protein requirements reducing during late-lactation (Bargo et al., 2003; Ipharraguerre & Clark, 2005), can lead to increased urinary nitrogen excretion. High urinary nitrogen excretion can increase the risk of nitrate leaching, especially under higher rainfall conditions (Selbie et al., 2015). To address this, low-CP concentrates offer a practical strategy to reduce nitrogen losses.

Previous research has examined the effects of lowering CP in concentrate feeds, though typically not at the reduced levels explored in this study. However, when dietary CP levels are low, specific amino acids may become limiting, potentially restricting milk production (Zhang et al., 2023). Furthermore, understanding how such reductions interact with the genetic merit of dairy cows is crucial for encouraging widespread adoption of low-CP strategies, as robustness to dietary changes may vary across different genetic lines (O'Sullivan et al., 2019). In this study, genetic merit was determined using the Economic Breeding Index (EBI), an Irish index that ranks dairy cows based on their genetic potential for profitability.

The objective of this experiment was to investigate the effect of concentrate crude protein and amino acid supplementation on the milk production of late-lactation, grazing dairy cows of divergent genetic merit.

Materials and Methods

An 8-wk experiment was conducted at the Dairygold Research Farm (Kilworth, Co. Cork, Ireland) from September to November, 2023. Eighty-eight Holstein Friesian cows were blocked based on pre-experimental milk production, parity and EBI and were then randomly assigned to 1 of 4 dietary treatments (n = 22). The dietary treatments consisted of supplemental concentrates with varying CP concentrations: 1) 170 g/kg of DM (H); 2) 130 g/kg of DM (M); 3) 95 g/kg of DM (L); and 4) 95 g/kg of DM with rumen-protected amino acids (LAA; 8.0 g/day absorbable Met and 7.2 g/day absorbable Lys). Cows received 1.79 kg of DM of concentrate daily in the milking parlour and grazed separately on swards consisting predominantly of perennial ryegrass. Milk yield was recorded daily (Dairymaster), with weekly analysis for constituents (MilkoScan 7). The two genetic groups (mean ± SD), high EBI (HEBI; €262.66 ± 32.92) and low EBI (LEBI; €185.02 ± 33.94), were balanced across dietary treatments. Data were analysed using the MIXED procedure in SAS (v9.4), with fixed effects of dietary treatment, week, dietary treatment by week interaction, genetic group, genetic group by dietary treatment interaction, and parity. An appropriate covariate adjustment was made per cow. Repeated measures were based on week with cow as a random effect. Significance was considered at $P \leq 0.05$.

Results

Reducing the concentrate CP concentration had no effect on milk production and milk composition (Table 1). Amino acid supplementation also had no effect, except for milk fat concentration, where cows fed LAA had greater milk fat concentrations when compared with H, but were similar to M and L (*P* = 0.02). Genetic group had an effect on all milk production outcomes except for lactose concentration (*P* = 0.08; Table 1). Cows of LEBI had greater milk yield when compared with cows of HEBI (*P* = 0.04). Cows of HEBI had greater milk fat and protein concentration, as well as, greater milk solids yield ($P < 0.01$). There was a genetic group by dietary treatment interaction for milk fat and protein concentrations (*P* = 0.01 and 0.04, respectively).

Table 1. Effect of concentrate crude protein on the milk production and composition of late-lactation, grazing dairy cows of divergent genetic merit

	Dietary treatment[1]					Genetic group[2]			*P*-values		
Item	H	M	L	LAA	SEM	HEBI	LEBI	SEM	DT	GG	DT*GG
Milk yield, kg/d	14.1	13.9	14.3	14.0	0.20	13.9	14.3	0.14	0.33	0.04	0.72
Fat concentration, g/kg	55.1^{b}	57.3^{ab}	55.5^{ab}	57.6^{a}	0.69	58.7	54.1	0.50	0.02	<0.01	0.01
Protein concentration, g/kg	44.5	44.2	44.6	45.2	0.28	45.9	43.4	0.20	0.09	<0.01	0.04
Lactose concentration, g/kg	45.0	44.7	44.9	44.6	0.15	44.6	44.9	0.11	0.15	0.08	0.06
Fat yield, kg/d	0.77	0.78	0.79	0.79	0.01	0.80	0.77	0.01	0.63	<0.01	0.48
Protein yield, kg/d	0.62	0.61	0.63	0.62	0.01	0.63	0.62	0.01	0.13	0.05	0.71
Lactose yield, kg/d	0.64	0.62	0.64	0.62	0.01	0.62	0.64	0.01	0.23	0.01	0.65
Milk solids yield, kg/d	1.40	1.39	1.42	1.42	0.02	1.44	1.38	0.01	0.60	<0.01	0.62

[1] Dietary treatments (DT) consisted of supplemental concentrates with decreasing crude protein concentrations: H) 170 g/kg of DM; M) 130 g/kg of DM; L) 95 g/kg of DM; or LAA) 95 g/kg of DM with rumen-protected amino acids.[2] Genetic groups (GG) were divergent in Economic Breeding Index (EBI) with high (HEBI) and low (LEBI) sub-groups

Conclusions

Reducing the CP concentration of supplementary concentrates below 170 g/kg of DM did not reduce milk production in late-lactation grazing dairy cows. Low CP concentrates can potentially reduce nitrogen intake, urinary nitrogen excretion and nitrate leaching during autumn without reducing animal performance. Supplementation with rumen-protected amino acids had no major effect on milk production, likely reflecting the reduced amino acid requirements of late-lactation cows. The overall outcomes of this experiment were consistent across cows of divergent genetic merit, with cows of HEBI outperforming cows of low EBI.

Acknowledgements

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Prediction of calcium and phosphorus availability in monogastric feed using marine sourced calcium and limestone

O. Demehin[1], S. O' Connell[2], V. Hala[3], A. Tischler[3]
[1]*Celtic Sea Minerals, Strand Farm, Currabinny, Carrigaline, Co. Cork, Ireland*
[2]*Munster Technological University, 46 Grand Parade, Cork City T12 VN56, Ireland*
[3]*Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, H-2100 Gödöllő, Hungary*

Presenting author
Olusoji Demehin. E-mail: o.demehin@marigot.ie

Application

The optimal utilisation of nutrients and the reduction of mineral interactions that inhibit nutrient digestibility in monogastric livestock can provide significant improvements in production efficiency.

Introduction

Phytate is the principal storage form of phosphorus (P) in feed material and has been shown to reduce mineral availability due to the chelating effect of its phosphate groups. The objective of the study was to determine how Marine mineral complex MMC, compared to limestone affects Ca-P interaction in monogastric nutrition leading to more available Ca and P. MMC is a marine derived Calcium-rich feed component sourced from *Lithothamnion glaciale* and delivers 74 additional minerals.

Methods

The study involved subjecting diet formulated with MMC or limestone and phytate as the only P source into an *in vitro* digestion simulation system to measure calcium and phosphorus released. The *in vitro* trial includes assessing the effect of different levels of calcium from MMC and limestone, different commercial phytases, and varying inclusion rate of phytase on Ca-P interaction. Also, an *in vivo* trial to evaluate digestible Ca and P in the ileum and faeces of pigs fed diet formulated with either MMC or limestone was performed. In both trial, calcium and phosphorus concentrations were analysed using an inductively coupled mass spectrometer. Statistical analysis of data was done on sigma plot version 12 software.

Results

The *In vitro* results showed a significant (P<0.05) increase in available phytate P for MMC (15%) compared to limestone (9%) and positive control (4%) diet formulated with phytate as the only P source in the presence of a technical grade phytase. The available calcium was not significantly affected. Reducing the level of calcium in the *in vitro* simulation caused a significant reduction in the available P in diet formulated with limestone compared to MMC at similar level. The *in vitro* study also showed that the type and inclusion rate of commercial phytases added to feed will affect Ca and P availability significantly. The *in vivo* trial involved feeding piglets a basic diet supplemented with MMC, limestone and Monocalcium phosphate (MCP) in four different proportion as follows: Diet A; 11.5% limestone, 0% MMC, and 1.7%MCP, Diet B; 6.9%Limestone, 0%MMC, and 1.7%MCP, Diet C; 4.63%Limestone, 2.5%MMC, and 1.8% MCP, Diet D; 5.39%Limestone, 2.5%MMC and 0%MCP. A total of thirty-two (32) piglets (initial body weight; 10kg) were divided into four groups and fed either of the four diets. The level of digestible phosphorus in the ileum of pigs fed diet B and D were comparable but significantly (*P*<0.05) higher than those fed diet A and C. While the total tract digestible phosphorus was significantly (*P*<0.05) higher in pigs fed diet B compared to those fed diet A, C and D. Those fed diet D was significantly higher compared to piglets fed diet A and B. Ilea digestible calcium was greater in pigs fed diet B compared to those fed diet A, C and D. However, there was no significant differences in the total tract digestible calcium in pigs fed diet B, C and D. Those fed diet A showed significantly lower total tract calcium compared to the other treatments. The *in vivo* data also suggested that most of the calcium from MMC are absorbed after the ileum as shown by increased total tract digestible calcium in pigs fed diet formulated with MMC.

Conclusion

In conclusion, our invitro result showed that MMC reduces Ca-P interaction allowing more phytate P to be available which could lead to safe removal of inorganic P source. This correlates with the in vivo findings when more P is made available in diet formulated with MMC without MCP.

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Effect of pH and feed substrate on in vitro protein digestibility of fish

C. H. S. S. Arachchilage[1], A. Alkith, A. Maxey, M. Scott
[1]*Nottingham Trent University, 50 Shakespeare St, Nottingham NG1 4FQ, UK*

Presenting author
Chamodi Harshani Senaweera Senaweera Arachchilage. E-mail: senaweeraharshani@gmail.com

Application

The significant interaction between feed and pH highlights the complexity of digestive processes and underscores the importance of considering multiple factors when evaluating feed quality.

Introduction

In vitro digestibility methods have become increasingly important in evaluating the nutritional quality of feed ingredients and diets for various species, including aquaculture (Moyano et al., 2015). These techniques aim to simulate the digestive processes that occur in the gastrointestinal tract, providing valuable insights into nutrient availability and digestibility without the need for extensive animal trials. However, the results obtained from the in vitro model maybe varies due to many factors including incubation pH. The goal of the study is to determine the effect of pH and feed substrate on nitrogen protein digestibility.

Material and methods

A 6 × 2 full factorial design was used in this study to evaluate the effects of incubation pH (1.36 vs. 3.13, representing the gut environments of salmonids and tilapia, respectively) and feed substrates (soybean meal, whey powder, black soldier fly larvae powder, waxworms, faba beans, and bioethanol yeast). Each substrate was tested in triplicate for each pH level.

The protein solubility of the feed was determined according to Araba and Dale (1990). In brief, 2 g of each dry feed substrate (in triplicate) and 0.08 g of pepsin were weighed into 15 mL tubes. Subsequently, 10 mL of HCl (0.0001 HCl for pH 3.13 samples and 0.1 HCl for pH 1.36 samples) was added to each tube. The tubes were vortexed then incubated in a water bath at 28°C for 18 hours and 24 minutes. After incubation, the samples were vortexed again, centrifuged at 4500 rpm for 5 minutes, and the supernatant was collected. Diluted samples were prepared by mixing 100 µL of supernatant with 900 µL of distilled water. From this, 25 µL of the diluted sample was pipetted into the wells of a microplate then, 200 µL of the working reagent (prepared using the Pierce™ BCA Protein Assay Kit) was added to each well. The microplate was incubated at 37°C for 30 minutes and subsequently analysed using a plate reader (Thermo Scientific Multiskan FC) at 620 nm against a standard curve. A two-way analysis of variance was performed, and means were compared using the Fisher's LSD test. Data of the study was analysed using R 4.4.2.

Table 1. Effect of feed and incubation pH on in vitro protein digestibility of fish.

	Protein solubility (µg soluble protein/g DM)	
Feed	pH1=1.36	pH 2=3.13
Bioethanol yeast	544c	420d
Black soldier fly larvae powder	602bc	540cd
Faba beans	699ab	557c
Soyabean meal	531c	472cd
Waxworms	691cabc	724b
Whey powder	743a	878a
SEM	45.2	
P		
Feed	<0.001	

pH	0.112
Feed*pH	0.04

pH: incubation pH. SEM: standard error mean. Means within a column with different superscripts are significantly different ($P \leq 0.05$).

Results

There were significant effects of feed ($P<0.001$), and the interaction between feed and incubation pH ($P= 0.04$) on protein solubility. At pH 1.36, faba beans had significantly higher protein solubility compared to soybean meal. However, these feeds did not significantly differ in protein solubility at pH 3.13.

Conclusion

The effect of protein source on in vitro protein solubility was influenced by incubation pH. Although significant differences in protein solubility were observed across feeds, it is important to note that these variations may not directly correspond to differences in fish performance.

Acknowledgments

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In vitro comparison of the binding capacity of three proprietary clay-based mycotoxin binders against standard 1m558 bentonite

H. Hall[1], E. Burroughs[1], A. Desbruslais[1], J. Apajalahti[2], G. Jurgens[2], K. Ülle[2], D. Wilde[3]
[1]Anpario plc, Manton Wood Enterprise Park, Worksop S80 2RS, UK
[2]Alimetrics Diagnostics Ltd, Koskelontie 19B, 02920 Espoo, Finland
[3]Massey Bros (Feeds) Ltd, Cranage Mill, Knutsford Road, Cranage, Crewe CW4 8EE, UK

Presenting author
Heidi Hall. E-mail: heidi.hall@anpario.com

Application
All monogastric species are affected by mycotoxins, produced by moulds found in feedstuffs and bedding. Clay based mycotoxin binders are commonly a low inclusion solution to mitigate the risk and effects on production and health.

Introduction
Mycotoxins are the secondary metabolites of fungi and with the effects of climate change (Hajnal *et al.,* 2023) are becoming increasingly prevalent in feed grains. In monogastrics, the effects of mycotoxin consumption include performance losses, damage to intestinal integrity, increased incidence of uterine prolapse and substantial increases in mortality, leading to reduced overall health and animal performance. With the impact of climate change it has been suggested that mycotoxin contamination is increasing (Hajnal *et al.,* 2023), so identifying and implementing effective solutions is essential for sustainable animal health and production. In-feed mycotoxin binders are a viable and cost-effective strategy for limiting animal exposure to mycotoxins. To assess the efficacy of different types of proprietary clay-based mycotoxin binders in comparison to a commercially available bentonite, an *in vitro* binding capacity study was performed.

Materials and Methods
Three proprietary clay-based mycotoxin binders (Anpro (AN), Anpro-UB (AUB), Anpro-NT (ANT) (Anpario plc)) and a commercially available bentonite (UK and EU approved 1m558) as a commercially relevant control (CTRL) were analysed in blind at an independent European laboratory as part of a larger study. Four replicates of one concentration (0.25 g/L), and one pH level (2.5) are reported here for brevity. The binders were tested against six common mycotoxins at the following concentrations (aflatoxin (AfB1), 10 µg/L, ochratoxin (OTA), 50 µg/L, zearalenone (ZEA), 100 µg/L, deoxynivalenol (DON), 100 µg/L, fumonisin B1 (FumB1), 100 µg/L, and T2, 100 µg/L). Each binder was mixed with the toxin solution or buffer to yield the required inclusion rate. The suspension of products was mixed then shaken for 90 min at 37 °C and 250 rpm. The suspension was transferred into Eppendorf tubes and centrifuged at 14,000 rpm for 20 min at 25 °C. The supernatants were filtered by regenerated-cellulose filters or PTFE filters (0.2 µm) and analysed for toxin content by high performance liquid chromatography (HPLC). The amount of mycotoxin bound to the materials, expressed as percent of adsorption, was calculated as the difference between the amount of mycotoxin in the supernatant of the blank tube (without a binder) and the amount found in the supernatant of the experimental tube (containing a binder), at the end of the test. Data was analysed using ANOVA in IBM SPSS.

Results
The proprietary blends (AN, AUB and ANT) bound significantly more AfB1 compared to the CTRL ($P<0.001$) (Table 1). There was no statistical difference in binding capacity between treatments and CTRL for DON, FumB1, and T2. AN and ANT bound significantly more OTA than AUB ($P<0.001$) and AN, AUB and ANT bound significantly more OTA than CTRL ($P<0.001$). All treatments (AN, AUB and ANT) bound significantly more ZEA than CTRL ($P<0.001$) with AN giving the highest binding capacity at 55.17%. AfB1 was the toxin bound most effectively by all treatments (over 90% of free toxin bound),

whereas none of the binders tested had a large binding capacity for DON (<5% of free toxin bound). All products were able to bind OTA and FumB1 between 50% and 81% effectively.

Table 1. Mycotoxin adsorption capacity at pH 2.5 (0.25 g/L) of product, displayed as a percentage

	Binder product (% of free toxin bound)					
Mycotoxin	CTRL[1]	AN	AUB	ANT	SEM[2]	P-value treatment
AfB1	81.8^{a}	90.44^{b}	90.08^{b}	90.20^{b}	0.695	**<0.001**
OTA	21.4^{a}	79.72^{c}	62.99^{b}	80.19^{c}	2.012	**<0.001**
ZEA	6.3^{a}	55.17^{c}	38.17^{b}	50.16^{bc}	3.248	**<0.001**
DON	2.9	1.58	2.05	3.42	1.907	0.915
FumB1	56.7	56.01	53.71	53.94	4.555	0.988
T2	35.1	56.57	34.50	43.87	10.219	0.633

[1] Control is a commercially available UK and EU approved 1m558 bentonite

[2] Standard error of the mean, a-c; differing letters denote significant differences

Conclusions

The products tested all show effective binding for the 6 toxins tested at pH 2.5 at commercially relevant doses. The three products tested (AN, AUB and ANT) all showed significantly improved efficacy for AfB1, OTA, and ZEA than the control (UK and EU authorised 1m558 bentonite). However binding efficacy is shown to be different for each of the products with some showing higher affinity for common toxins such as zearalenone, a common toxin linked with uterine prolapse in sows and gilts. Therefore, is it recommended that feed is analysed by HPLC to confirm the toxins present and a suitable toxin binder chosen based on these results.

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Benefits of essential oils in aquaculture

E. L. C. Ballester[1]
[1]Federal University of Parana, Rua XV de Novembro, 1299 - Centro, Curitiba - PR, 80060-000, Brazil

Presenting author
Eduardo Luis Cupertino Ballester. E-mail: elcballester@ufpr.br

In the last 15 years, Brazilian aquaculture has experienced significant growth. Currently, the production is estimated at more than one million tonnes, representing a gross revenue of around US$ 1,2 billion. Freshwater fish is predominantly produced, followed by marine shrimp, the main farmed species are Nile tilapia (*Oreochromis niloticus*), tambaqui (*Colossoma macropomum*) and the Pacific white shrimp (*Penaeus vannamei*). Together with this expansion, concerns about sustainability, the spread of diseases, overuse of antimicrobials, and animal health and welfare are raised. In aquaculture systems, routine husbandry practices such as handling, biometric measurements, and transport can cause stress to farmed animals, negatively affecting their immunological defenses, welfare and growth performance. Chronic stress impacts animals' state of physiological homeostasis and increases the production of reactive oxygen and nitrogen species (RONS). To mitigate these, torpor procedures (e.g., thermal shock) and chemical anaesthetic substances are used. Nevertheless, hypothermia may cause inappropriate analgesia and anaesthesia. Likewise, synthetic substances can cause adverse effects on both aquatic animals and farmers, as well as on the environment. Scientific advances, environmental protection, and consumers' preference shift towards food that is produced more sustainably and considers animal welfare are key elements that encourage the implementation of good practices in the sector. In this context, the use of natural substances is encouraged. Essential oils (EOs) are natural mixtures of compounds distilled from plant materials with broad biological activity, e.g., antibacterial, antifungal, antioxidant, and anti-inflammatory properties. Our research team have conducted several experiments with different EOs extracted from Brazilian plants, e.g., clove basil (*Ocimum gratissimum*) and lemon beebrush *(Aloysia triphylla*). Our results have shown the benefits of the EOs mainly in sedation, anaesthesia and the antioxidant systems of numerous farmed species of freshwater fishes and prawns. In this presentation, we are showing our main results.

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Evaluation of clove basil essential oil as a natural antioxidant in juveniles of Amazon river prawn

R. L. Ferreira[1], C. de Souza Valente[2], E. L. C. Ballester[1]
[1]Federal University of Parana, Rua XV de Novembro, 1299 - Centro, Curitiba - PR, 80060-000, Brazil
[2]University of Galway, University Road, Galway H91 TK33, Ireland

Presenting author
Cecilia de Souza Valente. E-mail: cecilia.souzavalente@universityofgalway.ie

Application

The present study validates the antioxidant potential of *Ocimum gratissimum* essential oil in aquatic organisms and it shows its potential use in the aquaculture sector. The *O. gratissimum* essential oil enhances prawns' innate immune system by stimulating the non-enzymatic antioxidant defence. Therefore, the *O. gratissimum* essential oil contributes to the health and welfare of farmed prawns.

Introduction

Freshwater prawn farming is among the main aquaculture activities. In South America, the *Macrobrachium amazonicum* is the major native species with aquaculture potential. It presents desirable performance and biological characteristics, including robustness, year-round breeding, and disease resistance. Although native species present lower environmental risks, farm confinement can cause animal stress and depress the immune system, leading to prawns being more susceptible to diseases. As a result, outbreaks may occur due to stress and, consequently, increasing the use of antibiotics. Antibiotics may lead to antimicrobial resistance and accumulation of toxic residues in the environment (Hassaan et al., 2021). Therefore, it is essential to search for sustainable alternatives to minimize the use of antibiotics. Among the promising phytotherapics as alternatives to synthetic drugs are essential oils. Current studies are focused on evaluating the analgesic properties of essential oils, and most of them are done with fish. Studies with essential oils and decapod crustaceans are still very scarce. Therefore, this research aimed to evaluate the antioxidant capacity of the clove basil (*Ocimum gratissimum)* essential oil (OG-EO) in the post-larvae (PL) of *M. amazonicum*. To date, there are no studies evaluating the potential antioxidant activity of OG-EO in *M. amazonicum*, which emphasizes the originality of the research.

Material and methods

To evaluate the antioxidant potential of EO-OG, a total of 360 PL of *M. amazonicum* (average weight 0.054 ± 0.010 g) was used (15 prawns per experimental unit). Prawns were obtained from the larviculture system of the Shrimp Culture Laboratory, Federal University of Paraná, Brazil. Different dietary inclusion levels of OG-EO were tested: 0%, 1%, 2%, and 3%. The experimental design consisted of 24 tanks (experimental units; *n* = 6) of 40 L (useful capacity), maintained with continuous aeration and constant water recirculation in a completely randomized design. Prawns were fed until apparent satiety four times a day (03:00; 09:00; 14:00; and 18:00). During the feeding trial, water temperature, dissolved oxygen and pH were analysed daily, while nitrite and total ammonia were measured weekly. For the analysis of prawn performance, the final weight, total length, biomass, biomass gain, survival, and feed conversion were evaluated. To evaluate the biochemical parameters, the hepatopancreas of 32 PLs (*n* = 8 per experimental group) were sampled, immediately stored in sterile 2 mL microtubes in liquid nitrogen, and sent to the Laboratory of Biochemistry and Genetics of the Federal University of Southern Border, Brazil. Tissues were then processed and used to evaluate the hepatopancreas' antioxidant capacity. All data obtained were evaluated for normality and homoscedasticity. Data when then subjected to one-way ANOVA followed by a mean comparison test (Tukey) at the 5% level of significance.

Results

All growth parameters analysed were similar among the experimental groups (Table 1). This result corroborated previous studies that showed that dietary Lippia alba essential oil (1% and 2%) has no impact on M. rosenbergii performance (De Souza et al. 2024).

Table 1. Growth performance of *Macrobrachium amazonicum* post-larvae fed diets supplemented with different levels of *Ocimum gratissimum* essential oil.

Growth parameters	**Experimental groups (g OG-EO kg diet $^{-1}$)**				
	0%	**1%**	**2%**	**3%**	***p*-value**
Weight (g)	0.32 ± 0.09	0.34 ± 0.08	0.31 ± 0.09	0.31 ± 0.07	*0.1734*
Total length (cm)	3.2 ± 0.38	3.3 ± 0.41	3.2 ± 0.44	3.1 ± 0.32	*0.0705*
Survival (%)	80 ± 12.15	80 ± 3.35	86 ± 5.40	80 ± 8.30	*0.4371*
Growth rate (g)	1.5 ± 0.15	1.5 ± 0.09	1.4 ± 0.07	1.2 ± 0.17	*0.2740*
Feed conversion	1.5 ± 0.30	1.4 ± 0.17	1.6 ± 0.16	2.1 ± 0.45	*0.2874*

Data presented as mean ± SD, *n* = 6. Absence of superscript letters on the same row indicates no significant differences between experimental groups (one-way ANOVA followed by Tukey test, *p*<0.05).

The non-enzymatic antioxidant molecule of reduced glutathione (GSH) was approx. 2.84-fold higher in prawns fed the diet supplemented with 3% OG-EO, with the enzymatic antioxidant defence and lipid peroxidation levels constant among all experimental groups (Table 1). Thus, OG-EO may present a non-enzymatic antioxidant bioactivity and contribute to the health and welfare of *M. amazonicum*.

Table 2. Antioxidant activity in the hepatopancreas of *Macrobrachium amazonicum* post-larvae fed diets supplemented with different levels of *Ocimum gratissimum* essential oil.

Antioxidant activity in prawn's hepatopancreas	**Experimental groups (g OG-EO kg diet $^{-1}$)**				
	0%	**1%**	**2%**	**3%**	***p*-value**
Lipid peroxidation	1.27 ± 0.45	1.47 ± 0.70	1.41 ± 0.64	1.39 ± 0.85	*0.9147*
Reduced glutathione	3.92 ± 2.33^{b}	8.75 ± 3.54^{ab}	9.75 ± 3.41^{ab}	11.13 ± 4.93^{a}	*0.0134***
Catalase	0.16 ± 0.04	0.17 ± 0.05	0.21 ± 0.13	0.20 ± 0.05	*0.5460*
Glutathione peroxidase	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	*0.1840*
Glutathione S-transferase	0.01 ± 0.00	0.02 ± 0.02	0.02 ± 0.01	0.017 ± 0.01	*0.3128*

OG-EG: *Ocimum gratissimum* essential oil. Data presented as mean ± SD, *n* = 8. Different superscript letters on the same row indicate significant differences between experimental groups (one-way ANOVA followed by Tukey test, *P*<0.05).

Conclusion

The inclusion of OG-EO in diets can increase the antioxidant status of *M. amazonicum*, particularly the non-enzymatic defence system. Thus, the dietary use of OG-EO as a natural antioxidant is beneficial and can improve the health and welfare of farmed prawns.

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Using cutting edge technology such as DNA sequencing etc to allow more insight into product mode of action

A. Palmonari[1]
[1]*University of Bologna, Via Zamboni, 33, 40126 Bologna BO, Italy*

Presenting author
Alberto Palmonari. E-mail: alberto.palmonari2@unibo.it

Ruminants play an important part in the food supply chain, and manipulating rumen microbiota is important to maximizing ruminants' production. Diet is a key factor that can manipulate rumen microbiota, and each variation of the physical and chemical composition creates a specific niche that selects specific microbes. Alteration in the chemical composition of forage, the addition of concentrates in the diet, or the inclusion of sugars or liquid feed, can induce a change in rumen microbiota. A balanced microbiota is crucial for ensuring proper fermentation of nutrients and milk production. These aspects become increasingly relevant in animal production, where milk and its derivatives are in high demand, with growing attention to high-quality and sustainable products. In this context, the current presentation aims to investigate the potential differences in the composition of the gastrointestinal microbiota in high and low-producing dairy cows fed and managed under the same conditions. A specific study was conducted at the Alma Mater University of Bologna dairy research farm involving 24 lactating cows, fed the same diet, but presenting different milk productions. Animals were then separated in two groups, high-producing cows (HY) and low-producing cows (LY). Rumen protozoa, ammonia (NH_3) concentration, and volatile fatty acids (VFA) were analyzed. DNA from rumen was extracted, purified, and sequenced with Illumina MiSeq. For VFAs, acetic and propionic acid were different between groups ($P \leq 0.05$), acetic being higher in LY, while propionic was higher in the HY group. No statistical difference was observed for the main protozoa genera and ammonia concentration. Ruminal microbiota composition revealed significant differences. The HY group showed a higher abundance of Lachnospiraceae ($P \leq 0.05$), and Veilonellaceae ($P \leq 0.05$), while Eubacteriaceae ($P \leq 0.05$), and RF16 spp. ($P \leq 0.05$) were higher in LY. A tendency ($P = 0.10$) was observed for the Prevotellaceae family, with higher values in LY compared to HY. Data obtained with the metataxonomic analysis are very interesting, since similar effects were observed in a previous in vitro study, in which we investigated the potential of molasses in modulating the rumen microbiota composition, fibre digestibility and in vitro volatile fatty acid (VFAs) production. Six different molasses-based liquid feed, equally representative of beet and cane molasses, were selected and incubated with rumen fluid for 24 hours in vitro. VFAs analysis was carried out by sampling incubation flasks at 1,2,3,4,6,8,24 h. For microbiota analysis, samples were collected after 24 h of incubation, and then subjected to DNA extraction. Obtained results showed how VFAs composition was affected by molasses inclusion: acetic acid levels were higher in the CTR group (73.5 mmol), while propionic acid being higher in beet and cane molasses treatments (19.6 mmol; 18.6 mmol, respectively). Molasses addition deeply influenced the in vitro composition of the rumen microbiota. Relative abundance of Veillonellaceae (6.48% and 8.67% + molasses compared to 4.54% in the CTR), Streptococcaceae (19.62% and 28.10% in molasses compared to 6.23% in CTR), and Fibrobacteraceae (0.90% and 0.88% in + molasses compared to 0.62% in the CTR) increased in beet and cane compared to the CTR group, while Prevotellaceae, the most predominant family in the rumen, decreased compared to CTR (37.13%, 28.88%, 49.6% respectively). The different composition of the microbial community also resulted in an improved fibre digestibility, with and increment of 17% and 20% compared to the control for beet and cane molasses, respectively. Results of both studies underline how the composition of the rumen microbiota could influence feed efficiency and production, and suggest that specific dietary addition would beneficiate microbial families positively associated with higher animal performances.

Designing and managing on farm trails as a costs effective way to support product and commercial development

S. Parker-Norman[1]
[1]*Big Sky, Upper Water Street, Newry BT34 1DJ, UK*

Presenting author
Sophie Parker-Norman. E-mail: Sophie.Parker-Norman@volac.com

From the perspective of the feed additive sector, commercial trials are a necessary tool for evaluating product performance within both the dairy and beef feed industries. Although these trials may not follow the rigid structure of controlled experimental methodology, they are routinely used alongside well-designed scientific studies to provide comprehensive evidence for customers. The practical, real-life nature of commercial trials allows assessment of product efficacy under real-world conditions, which is highly valued by end-users seeking relevant and applicable results.

To mitigate sources of error and improve reliability, steps such as robust herd selection, detailed data collection, and statistical adjustments for confounding factors are employed. In particular, robotic dairy herds have emerged as a valuable resource, offering precise and continuous data on feed intake, milk production, and animal behaviour. Similarly, the development of automated weighing systems and digital record-keeping in commercial beef herds has significantly improved the ability to conduct large-scale beef feeding trials. Regular weight measurements and performance tracking in intensive and semi-intensive beef systems allow for real-time assessment of feed efficiency, growth rates, and carcass yield, providing robust datasets to support product validation.

Nevertheless, these trials are not without challenges. Variability in herd management, environmental conditions, and genetics can complicate interpretation, while the need for large-scale studies can introduce logistical and financial constraints. Beef trials, in particular, require careful design and consideration of animal performance variability. Despite these obstacles, commercial trials remain a cornerstone of the feed industry, providing essential insights, often giving a 'feel' for a product, and ultimately aiding customers in their decision-making processes.

This presentation will elaborate on the challenges and best practices of commercial dairy and beef feeding trials, highlighting their role in advancing business and application development.

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The use in In vitro technology to provide quick and cost effective data to allow idea screening, support product development and product sales

J. Huntington[1]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*

Presenting author
James Huntington. E-mail: jhuntington@harper-adams.ac.uk

The use of in vitro techniques provides a rapid low cost means to assess the nutritive value of forages, individual feeds and mixed rations. However, rumen *in vitro* techniques cannot replicate rumen activity, merely model it. Tilley and Terry (1963) established the *in vitro* determination of diet digestibility. The end point model of Tilley and Terry (1963) has been adapted to measure rumen fermentation kinetics of feeds, feed combinations and the effect of dietary additives by using headspace gas pressure (Pell and Schofield 1993, Theodorou *et al*., 1994) and has been extensively reviewed by Rymer *et al.* (2005) and Getachew *et al.* (2005).

Absence of a fermentation*host interaction means that *in vitro* techniques are not confounded by host absorption facilitating a measure of VFA, NH_3, CO_2 and CH_4 production. However, the highly buffered nature of in *vitro* cultures makes detecting effects of treatment on rumen pH challenging. Some of these challenges may be overcome by weakening the buffer strength or increasing the readily fermentable fraction of the substrate to maximise opportunity of observing a treatment effect. Determination of protein degradability *in vitro* is however more complicated. The form of N fed to the culture in the diet will change to be partially (or totally) incorporated into microbial N such that, for a culture $N_{in}=N_{out}$. To be able to calculate diet protein degradability and microbial growth a determination of microbial N is therefore required. Methods exist for measuring *in vitro* microbial protein (Makkar *et al.,* 1982) but further validation is required.

Rumen *in vitro* techniques are ideal for testing feedstuffs, products and scenarios that would be difficult to test in any other way. Such as determining the fermentability/degradability of powders and liquids or implication of a range different silage fermentation stoichiometry on rumen function.

Other applications of rumen *in vitro* methods include:
- Bioavailability of dietary minerals and uptake by the rumen microbial population.
- Impact of including tannin in ruminant diets.
- Dietary factors effecting the rumen survival of *Treponema* spp. and the implications for animal health (interdigital dermatitis).
- Mode of action and rumen effect of essential oils (EO) and resilience of rumen protection methods for PUFA, EO and AA.

Yerby (in press) has taken a novel approach to *in vitro* techniques and employed them to investigate intestinal development in calves. Further, in other species intestinal fluid has been used as an inoculum source and applied to the investigation of caecal/hindgut fermentation in pigs (Anguita *et al.,* 2006) and horses (Bachmann *et al.,* 2020).

Testing novel rumen methane inhibitors that maintain diet degradability and a desirable rumen VFA profile is currently a major research objective. Using rumen *in vitro* techniques then a wide range of chemicals, compounds and natural products can be tested to determine their effect on rumen fermentation. Lower limits for effective response and upper limits to extremis can be determined, leading to the proposal of an effective dose levels for *in vivo* dietary inclusion without out compromising animal health. Inherent to these sorts of experiment is the collection of headspace gas. Total volume and composition can be measured. This is easier with the method of Pell and

Schofield (1993) rather than the automated equivalent of Theodorou *et al.* (1994). However, if measuring the pattern of methane evolution is the objective then manual gas production is the more reliable method.

Many *in vivo* studies are limited by animal numbers especially when using rumen fistulates. Whereas *in vitro* studies represent a boundless opportunity of experimental design. Rumen *in vitro* diet-type*treatment interactions and level treatment inclusion are possible even to extremis without compromising animal health. Increasing the number of interactions or factors increases statistical sensitivity but, the danger comes in designing studies with too many interactions/factors. Interpretation of two or three way interactions do not normally cause too much issue. However, because only your imagination, time to sample and physical capacity are your limitations it could be possible for example to increase the number of factors in a two way design (>4*4), or overlay a two or three way interaction experiment with a level of addition effect, or nest structures within a relatively simple two or three way design. This would not be wise and can be incredibly difficult to interpret in a meaningful way.

Some feed additives require a lead time of several days to exert their effects on the rumen microbiome. Therefore, adaptation of donor animal to the additive may be required or desired to maximise opportunity of observing an effect *in vitro*. This leads to the proposition of making the rumen fluid the test variable and substrate the standard. These experimental designs work best in conjunction with Latin square designed *in vivo* studies where the effect of different animals is removed. However, experience suggests that the effect being investigated needs to be quite large in order to be observed in a diluted and highly buffered situation of an *in vitro* analysis.

Treatment persistency in the rumen environment can be investigated using continuous culture techniques. These can be carried out in relatively simple reactors or in RUSITEC and permit many of the advantages of batch culture techniques whilst exploring the persistency of treatment effect. Continuous cultures also have the same advantage as batch cultures of being able to test dietary treatments to extremis without compromising animal health but, require more time to feed, deal with waste and prepare artificial saliva making them considerably more time consuming.

Standardised host diet, time of rumen fluid collection and test diet preparation facilitates an evaluation and comparison of TMR from different sources. Diet ingredient interactions within the rumen mean that the fermentation resulting from a TMR is not equal to the sum of its individual components. Using a standardised batch culture technique it is possible to determine how a diet will ferment, degrade, produce VFA (concentration and mix) and methane in the headspace. By extension this has enabled development of a method for evaluating diet compositions for reducing methane emissions.

In conclusion validation of treatment effects observed *in vitro* are ultimately necessary allowing *in vivo* studies to be designed with greater knowledge of treatment effects and limits.

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How Industry Uses Research Data to Drive Product Development and Sales

K. Farnan[1]
[1]*Adisseo, 180-182 High Street, Slough, SL1 1DN, UK*

Presenting author
Kirsty Farnan. kirsty.farnan@adisseo.com

Research and science are essential in developing and commercialising products, providing the foundation for innovation, improved efficiency, and enhanced functionality. Companies rely on scientific research to create new product ranges and improve existing formulations, addressing market demands and monetising viable solutions. Research data is fundamental in shaping product development, allowing for the examination and refining of multiple options. In vitro studies, for example, can be cost-effective in evaluating various formulations before advancing to field trials or commercial applications.

In ruminant nutrition, the selection of the base molecule is particularly significant, as it determines whether a product reaches the cow's system or is utilised by rumen microbes. Scientific data guides the formulation of products to ensure maximum bioavailability and efficacy. This approach is critical in various industries, where research-backed innovation leads to better-performing, sustainable, cost-effective solutions.

Beyond development, research data is integral to commercialisation, serving as the foundation for product positioning, marketing campaigns, and consumer engagement. Statistical insights derived from research findings are often used to highlight product benefits and differentiate them in competitive markets. However, the interpretation and presentation of research data must be scrutinised to prevent the overstatement of product efficacy. The use of sensationalised statistics can mislead consumers, making independent validation of scientific claims an essential component of ethical business practices.

Collaboration between academia and industry is a driving force behind innovation. Universities and research institutions work alongside commercial enterprises to develop, test, and refine new technologies and formulations. While these partnerships are crucial for scientific advancement, it is equally important that academic research remains objective and independent in evaluating commercial claims. Studies funded by statutory levy boards and similar organisations have identified cases where companies exaggerate the effectiveness of their products. Such findings highlight the necessity of rigorous, peer-reviewed research to hold companies accountable and ensure transparency in the industry.

Overall, research and science drive product development and safeguard consumer interests by ensuring that marketing claims are substantiated. As industries continue to evolve, the role of independent scientific validation will remain critical in maintaining trust, regulatory compliance, and the integrity of commercially available products. This abstract underscores the need for evidence-based innovation and ethical marketing practices to foster sustainable product development and commercialisation progress.

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An assessment of virtual fence technology to facilitate creep grazing in beef cow and calf systems

J. Brans[2], F. Lively[1], G. Arnott[2], G. O'Doherty[2], A. Gordon[1], C. Holohan[1]
[1]*Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*
[2]*The Queen's University of Belfast, University Road, Belfast BT7 1NN, UK*

Presenting author
Francis Lively. E-mail: Francis.Lively@afbini.gov.uk

Application

Virtual fence (VF) technology uses a combination of audio and electrical stimuli to contain grazing livestock within a boundary. The study demonstrated that VF can facilitate creep grazing and feeding of concentrates in beef cow calf systems.

Keywords: virtual fencing; cow; calf; creep grazing; management

Introduction

Virtual fencing (VF) is a novel technology which enables grazing livestock management without the need for physical fences. It offers flexibility and may deliver benefits for farm productivity and labour efficiency. Typically, VF systems use a GPS-enabled neck collar coupled with a mobile phone application through which the user sets the VF boundary. When the animal approaches the boundary, it receives an audio cue as a warning to turn around, and if it continues beyond the boundary it will receive an electric pulse. Through associative learning after a short training period, the animals learn the correct response to the audio cues (Lee et al., 2009). For suckler beef systems, this technology has the potential to enhance grazing management and improve animal performance. The aim of this study was to determine if VF could be used to adopt creep grazing in a beef cow and calf system.

Materials and Methods

Twenty-three beef cows and their calves were fitted with VF collars (Nofence, Norway). All 46 animals were initially grazed as one group for a 6 day training period with a single VF line. Following this, the animals were rotationally grazed with VF, with the calves having access to a larger pasture area than the cows; thereby restricting the cows and enabling the calves to creep graze. The VF boundaries were moved every 5 days to allow fresh grass allocation. After 14 days, an additional challenge was introduced, with calves being offered concentrates in a conventional feed trough in the 'creep area' for 19 days prior to weaning. The number of audio cues, duration of audio cues, number of pulses, and activity were obtained from the VF collars. The 'proportion of pulses' indicates the percentage of boundary interactions that resulted in a pulse. Data were analysed with R (version 4.2.2), using the non-parametric Dunn test from the "rstatix" package.

Results

Results outlined in Table 1 show that during the training period cows received more audio cues than calves ($P < 0.05$) which indicates that cows had more interactions with the VF. Despite this, the calves received more pulses ($P < 0.01$), which suggests calves were slower to learn the system than cows. During the subsequent periods, similar results were observed in the number of audio cues received and pulse proportion alongside an increase in the duration of the audio cues after the introduction of the concentrates. Increases in audio cue duration signify a less immediate response to the audio cue, and this coupled with the reduction in pulses in the period after training (decrease of 14 percentage points for cows and 17 percentage points for calves) suggests that the animals became more familiarized with the system.

Table 1. Variables collected by the collars, reported by period for cows and calves

Period	Training (day 1-6)			Creep grazing (day 7-21)			Creep grazing plus concentrates (day 22-39)			Whole experiment (day 1-39)		
	Cows	Calves	*P*	Cows	Calves	*P*	Cows	Calves	*P*	Cows	Calves	*P*
Audio cues per day	6.77	5.81	*	9.51	6.81	**	22.62	9.3	***	17.30	8.10	***
Audio cue duration (s)	61.17	67.25	NS	54.31	51.44	NS	156.2	71.98	**	113.92	61.71	***
Pulses per day	1.25	1.51	**	0.44	0.61	NS	0.51	0.44	*	0.47	0.52	NS
Pulse proportion (%)	18.42	26.06	NS	4.66	8.94	**	2.24	4.75	***	2.73	6.39	*

The data is presented as mean per day, P = probability, * indicates *P*<0.05, **indicates *P*<0.01, ***indicates *P*<0.0001 and NS indicates P>0.05.

Conclusions

This study demonstrated that VF can successfully facilitate creep grazing, and creep feeding of concentrates, in beef cow and calf systems. Animals adapted to the system with the number of pulses reducing after the initial training period. There were however differences observed between cows and calves, with calves having a higher pulse proportion. This might indicate a slower rate of learning among calves than their dams.

Acknowledgements

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Building the whole-herd machine vision monitoring platform at the John Oldacre Centre for Dairy Welfare & Sustainability Research

A. Dowsey[1], A. Montout[1], J. Gao[1], R. Bruce[1], M. R. Montes de Oca[1], A. Sharma[1,3], D. Baran[1], H. Liu[1], G.Richards[1], A. Bard[1], K. Reyher[1], M. Mendl[1], S. Mullan[1,2], D. E. Hidalgo[1], S. Held[1], T. Burghardt[1], N. Campbell[1]
[1]*University of Bristol, Beacon House, Queens Rd, Bristol BS8 1QU, UK*
[2]*University College Dublin, Belfield, Dublin 4 D04 C1P1, Ireland*
[3]*University of the West of England, Coldharbour Ln, Stoke Gifford, Bristol BS16 1QY, UK*

Presenting author
Andrew Dowsey. E-mail: andrew.dowsey@bristol.ac.uk

Application

Artificial intelligence with cameras and wearables has unlocked numerous applications in Precision Livestock Farming (PLF), facilitating farmers in timely management of production, health and welfare. Here we demonstrate its expansion to extensive longitudinal coverage of a whole dairy herd to underpin next-generation research in dairy welfare and sustainability.

Introduction

The John Oldacre Centre for Diary Welfare and Sustainability (JOC) at Bristol Veterinary School has been built on top of our established 200-cow commercial dairy farm where cows are milked in a herringbone parlour and housed all year round in a free stall system typical of UK dairy farms. Equally-sized groups (by age) are separated within the single barn, giving us the opportunity to achieve continuous video monitoring of all cows. Current PLF research and commercial systems focus on using one or a small number of cameras to directly detect the physical manifestations of later stage clinical disease, such as gait abnormalities characteristic of lameness. Conversely, spotting the early, subclinical stages of disease is essential for successful treatment, maintenance of high welfare standards and sustainable intensification (Held and Caplen, 2021). For research purposes, therefore, dynamic changes in activity, behaviour and social interactions are of fundamental importance, in terms of their links to subclinical disease and interplay with management practice, breeding, social hierarchy and emissions. Machine vision has the promise to characterise behaviour far more deeply than wearables or indoor positioning systems can, but requires several challenges to be overcome including: the reliable detection of each cow in crowded groups; the identification of each individual over long periods including the re-identification of the same cow between cameras; the accurate identification of behaviour patterns and the associated huge expert annotation burden needed to train fine-grained behavioural classifiers; and maintenance of reliable performance in diverse lighting and climatic conditions.

Material and methods

We have installed a total of 58 CCTV cameras with night vision and have optimised the dynamic range and shutter speed dependent on their locations.

- 6 wide-angle (2.4mm) 5MP ceiling-mounted cameras cover our transition pen, which provides a self-contained pilot study area with feeding, water and loafing.
- 6 further 2.4mm cameras cover cubicles in the main barn.
- 20 6MP fisheye cameras cover the remainder of the cubicles in the barn extension, needed as the ceiling is lower.
- 14 4MP cameras with 2.8mm lens cover the length of the feed face, angled to give a clear view of feeding behaviour.
- 5 ceiling mounted 2.4mm cameras monitor the cattle in the collecting yard before milking and a further 4 track them through the race after milking.

- 2 cameras are setup on the race optimised for manual body condition and mobility scoring.

A final camera uses Optical Character Recognition to read the output of the ear tag reader gate. This enables us to link the tracked movement data for each cow back to its veterinary and production records. The data collection platform architecture was designed to address the concerns of a privacy impact assessment as well as ensure data security. Each camera records motion-triggered video to its internal 256Gb encrypted SD card and is connected through a private network to a gateway server. Here, we have developed a load-balancing script to pull the encrypted video data from each camera through the server to a small cluster of workstations each containing 4 Nvidia RTX GPUs. The workstations run our cow tracking AI (Andrew *et al.,* 2021; Gao *et al.,* 2022; Sharma *et al.,* 2024), extracting only the motion of the cows themselves for archival and subsequent analytics.

Results

Our published cow tracking methodology relies on a continual reidentification approach, thus avoiding the classical tracking issues of objects losing their identifiers over long periods, particularly when moving between cameras. In addition, we spearheaded the deep metric learning paradigm for cattle re-identification that learns to differentiate between coat patterns generically rather than specifically differentiating between the cows in a training dataset. The system does not therefore need to be retrained when new cows transition into the herd (Andrew *et al.* 2021) and can also be trained on a new herd with little human involvement (Gao *et al.,* 2022). Single frame accuracy is now 99.91% (Sharma *et al.,* 2024), which leads to very few errors when results are integrated across video sequences, although more research is needed to determine the limits of the approach (e.g. when crowding occurs in the collection yard, infrared night-time tracking).

Conclusion

We have built a new research platform to link health, welfare and sustainability parameters to detailed longitudinally observation of a whole dairy cattle herd using an automated machine vision approach. Building on this, we are focussing on two aspects concurrently:

1. Implementation of a 20-week intensive data collection period across the camera network and including fortnightly mobility scoring, body condition scoring, and saliva and milk sampling (for salivary serum amyloid A and somatic cell counts as a markers for pre-clinical diseases and mastitis (Held and Caplen, 2021), as well as full midinfrared spectroscopy).
2. Development of a framework for fine-grained behavioural classification. Key aspects here are an AI-driven annotation tool for facilitating the generation of labels for supervised classifiers, and definition of a controlled vocabulary / ontology to describe the complex ethogram of behaviours that is shared between biological researchers and machine vision experts.

Acknowledgements

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Large scale use of Radio Frequency Identification (RFID) technology for a fully traceable bovine semen quality and field fertility management system at the National Cattle Breeding Centre in Ireland

G. Brychkova[2], M. O'Reilly[1], D. Corridan[1], S. Fair[3], C. Spillane[2], C. O Meara[1]
[1]*National Cattle Breeding Centre, M7 Business Park, Co. Kildare, W91 WF59, Ireland*
[2]*Plant and AgriBiosciences Research Centre, University Rd, Galway H91 REW4, Ireland*
[3]*Laboratory of Animal Reproduction, Department of Biological Sciences, University of Limerick, Limerick V94 T9PX, Ireland*

Presenting author
Ciara O Meara. E-mail: comeara@ncbc.ie

Application

The aims of the current study were to evaluate the effect of using LF-RFID chips for liquid semen traceability from the barn to the semen processing laboratory and to evaluate the effect of exposure of semen to HF-RFID chips within semen straws on sperm quality parameters post-thaw and field fertility (non-return rates) following Artificial Insemination (AI) for a fully integrated and complete RFID package from semen collection to AI.

Introduction

Low frequency (LF)-RFID chips are widely used in animal tracking for ear tags in bull studs, where their inability to identify multiple animals simultaneously reduces the risk of erroneous reads (Pinna et al., 2023). However, LF-RFID chips are less suited for high throughput environments such as tracking semen straws in goblets for efficient counting, storage, and distribution. High Frequency (HF)-RFID offers better performance due to their smaller size, making them more suitable for insertion into semen straws (Hoshino et al., 2011).

Materials and Methods

Semen was collected from Holstein Friesian (HO) bulls 1-3 days/week using an artificial vagina and partially diluted in aliquots of 10ml of egg-yolk based extender (Bullxcell, IMV technologies, Laigle, France) for transport to the laboratory and further dilution into semen straws (0.25ml) at 60 x 10^6 sperm/ml frozen. Inseminations were performed by AI technicians into HO (91%) or HO cross cows. Fresh semen assessment was performed on partially diluted semen. Sperm concentration assessment was performed on the raw semen sample using a coulter counter (Z Series Beckman Coulter, Beckman, Clare, Ireland). Total motility and progressive motility score were assessed routinely by a trained andrologist prior to freezing and immediately post-thaw using a phase contrast microscope at 20X (BX41, Olympus) and heated stage. Sperm kinematic parameters were measured after semen was diluted in easy buffer B (IMV Technologies®, France) and placed on a pre-warmed glass chamber (4-well, 20-mm depth; Leja, IMV) maintained at 37°C and 1000 sperm were analysed using a computer assisted sperm analyser (CASA system, v.14; IVOSII, IMV Technologies).

In Experiment 1, Semen ejaculates (n=2/bull) from 5 bulls travelled to the laboratory either in the presence (Treatment A) or absence (controls; Treatment B) of a large LF-RFID chip. Following semen assessments Treatment A was also subjected to the presence of a HF-RFID chip (Treatment A1) or no HF-RFID chip (Treatment A2) while Treatment B was subjected to presence of a HF-RFID chip in the straw (Treatment B1) or no chip (Control; Treatment B2). All treatments were assessed *in vitro*. In Experiment 2, semen ejaculates from bulls (n=6) were subjected to presence of HF-RFID chips in the straws or controls with no chips. Straws were cryopreserved and used for AI in cows (n=625 and n=631 for control and HF treatment groups, respectively).

Statistical significance was analysed by linear mixed-effect model followed by PostHoc Tukey Games-Howell test or ANOVA with PostHoc Dunnet's test. Data were considered to differ significantly, if $P<0.05$.

Results and Discussion

No adverse effects of LF-RFID or HF-RFID were observed on semen quality parameters *in vitro* or on fertility *in vivo*, as measured by the 56-day non-return rate in conventional programs ($p > 0.05$). These findings are in contrast with Pacchierotti *et al*. (2021), where they suggested that exposure of semen to LF waves could cause decreases in sperm motility and cell viability. The biggest limitations were the capacity for consistent multiple readings with HF-RFID chip straw groups following cryopreservation (and immersion in liquid nitrogen) and Bluetooth connectivity with AI technician handheld devices in field situations.

Conclusion and Implications

RFID technology in bovine semen is a promising technology for more accurate sample labelling as it can improve on errors encountered with barcode traceability systems. New advances in reading RFID under liquid nitrogen could change the landscape in this field of work.

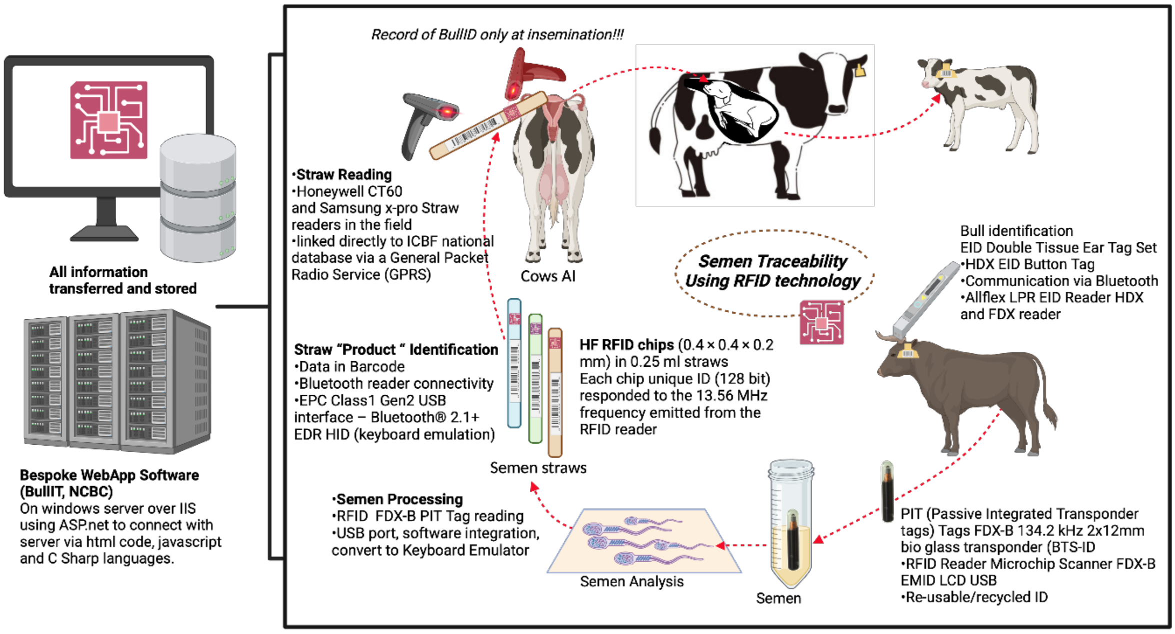

Figure 1. Technological hardware and software considerations for using Low Frequency (LF)-RFID chips to label the liquid semen and for using High Frequency (HF)-RFID chips for labelling straws for freezing and dispatch for field fertility

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Universal Bovine Identification via Depth Data and Deep Metric Learning

A. Sharma[1,3,4], L. Randewich[4], W. Andrew[1,4], S. Hannuna[4], N. Campbell[4], S. Mullan[1], A. Dowsey[1,2], L. G. Smith[3], M. Hansen[3], T. Burghardt[4]
[1]Bristol Veterinary School, University of Bristol Langford House, BS40 5DU, UK
[2]Department of Population Health Sciences, University of Bristol, Oakfield House, Oakfield Grove, BS8 2BN, UK
[3]Centre for Machine Vision, University of the West of England, Bristol, Coldharbour Lane, BS16 1QY, UK
[4]School of Computer Science, University of Bristol, Merchant Venturers Building, Woodland Road, BS8 1UB, UK

Presenting author
Asheesh Sharma. E-mail: as16542@bristol.ac.uk

Application

This study introduces a depth-only deep learning system for bovine identification, enabling breed-agnostic monitoring, critical for precision livestock farming. We show that depth, as a biometric, can potentially broaden the real-world applicability of our coat-pattern based method to the majority of UK cattle breeds that lack distinctive coat patterns.

Introduction

In the dairy industry, monitoring individual cows is essential for improving farm productivity and managing disease outbreaks through contact tracing and social interactions. Individual identification allows tailored care and boosts production by enabling body condition scoring and yield monitoring. However, as herd sizes increase, the skewed cow-to-human ratio complicates manual monitoring tasks, therefore automated systems are in demand.

Traditional approaches to cattle identification, such as RFID tags, face financial and welfare challenges (Awad, 2016). Additionally, reliance on coat patterns limits the application our previous approach to certain cattle breeds, such as Holstein-Friesians (Andrew et al., 2023). This study introduces a depth-only biometric identification system that uses commercial 3D depth cameras to address these limitations. This method uses body morphology as a biometric measure, overcoming the constraints of coat-pattern biometric. Deep learning architectures, including ResNet and PointNet, are employed to differentiate individuals using depth data, offering a scalable solution for real-world farm environments.

Materials and Methods

The CowDepth2023 dataset consists of 21,490 synchronised dorsal view colour-depth image pairs from 99 Holstein-Friesian cows monitored over 14 days (Sharma et al., 2024). We used a Kinect V2 depth camera placed 4 meters above the ground. The camera recorded depth and RGB streams at 30 Hz. Depth maps were pre-processed to isolate individual cows through thresholding (2–3.4 meters range) and background subtraction. Images were manually annotated with identification labels through visual comparison with coat patterns. Depth maps were converted to 3D point clouds using camera, ensuring consistent mapping from pixel coordinates (u, v, z) to physical units (x, y, z). Point clouds were uniformly resampled to 2048 points using the farthest point sampling algorithm (Eldar et al., 1994). The ResNet-50 architecture was employed with a Spatial Context Module (SCM) to prioritise regions in depth maps. The model generated 128-dimensional embeddings representing individual identity. PointNet was used to process point clouds directly. The architecture employed Multi-Layer Perceptrons to create embeddings invariant to the order of input points. Metric learning was employed to train the models and differentiate between depth maps/point clouds of the different animals. The metric learning framework ensures that embedding formed clusters of examples belonging to the same individual while maintaining separation between other individuals. The CowDepth2023 dataset was split into training (70%), and testing (30%) sets for evaluation. Open-set validation removed temporal neighbours to simulate real-world variability. The k-Nearest Neighbours (kNN) algorithm was used to classify the embeddings to cow labels.

Results

Model performance was quantified with classification accuracy and evaluated across five randomised splits. According to Table 1, the baseline ResNet model achieves a kNN mean accuracy of 99.88% with colour images of coat patterns. In comparison, our ResNet model with depth map as input performs similarly well on the test data, with only 0.05% less accuracy. Furthermore, including the SCM layer does not yield any significant improvement; in the case of colour images, the accuracy slightly increases by 0.03%, and decreases by 0.01% in the case of depth maps. The performance of PointNet model is sensitive to the number of input 3D points. Overall, both ResNet and PointNet models can generalise well on depth maps and point clouds, respectively, and exhibit performance that is comparable to models that use colour images of coat patterns.

Table 1. kNN test accuracies for various models. For all the models arranged in rows, the third column reports the difference in accuracy from the best-performing baseline model – ResNet with SCM (colour).

Model	**Mean and range**	**Diff. from baseline**
ResNet-50 (Colour)	99.88\%, (-0.13, +0.08)	-0.04
ResNet-50-SCM (Colour)	**99.91\%, (-0.13, +0.06)**	0.00
ResNet-50 (Depth)	**99.83\%, (-0.17, +0.07)**	-0.08
ResNet-50-SCM (Depth)	99.82\%, (-0.19, +0.09)	-0.09
PointNet (2048 points)	**99.09\%, (-0.70, +0.19)**	-0.82
PointNet (64 points)	87.58\%, (-1.64, +0.62)	-12.33

Conclusions

Utilizing depth data captured by 3D cameras, ResNet and PointNet architectures demonstrated high accuracy for individual identification. The proposed methodology leverages dorsal-view body morphology as a biometric measure, enabling identification for breeds lacking distinct coat patterns, which constitute a significant portion of cattle populations in the UK. This depth-only approach demonstrates practical potential for enhancing precision livestock farming and personalised animal welfare. Future work will explore longitudinal studies to test the system's reliability over time, paving the way for more comprehensive applications in welfare monitoring.

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Validation of IceQube step-counts on dairy cows grazing ryegrass/plantain swards

R. Mott[1], L. Chesney[1], D. Patterson[1]
[1]*Agri-food And Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Richard Mott. E-mail: richard.mott@afbini.gov.uk

Application

The IceQube motion detection system provides an extremely good correlation between actual and recorded steps of dairy cows at pasture, but consistently underestimates the number of steps taken. A simple correction algorithm can be applied to improve the agreement between actual and recorded steps.

Introduction

The IceQube motion detection system is used extensively both on-farm and in research settings to make inferences about dairy cow behaviour and, by extension, their welfare. The original IceTag system has been validated in housed dairy systems (Nielsen et al., 2018; Shepley et al., 2017) and the more recent IceQube system has been validated in a pasture setting (Charlton et al., 2022). All previous studies have shown a good correlation with observed data but with the devices consistently underestimating the number of steps. The consistency of this underestimation presents the opportunity for a correction algorithm to be designed to provide greater agreement between actual and reported step-counts. In the context of a wider project investigating behavioral differences between two different sward types, ryegrass and ryegrass/plantain, the objective of this study was to validate the number of steps recorded by the IceQube against the observed number of steps for the same period and to design an algorithm to improve the agreement between the two data sets.

Materials and Methods

Dairy cows grazing on swards of ryegrass (n=22) and ryegrass/plantain mix (n=22) were each individually observed once for 15 min, and a step count recorded. Steps were recorded only from the leg that had the sensor attached (rear right) and a step was defined as the hoof fully leaving contact with the ground and being replaced in a different position (forward, backward or sideways). Manually recorded data were than modelled against the IceQube output for the same time-points. An Interclass Correlation (ICC2) and a Bland Altman test of agreement were performed on these data. A linear model was used to examine the relationship between the two data sets, and the intercept and slope from this model used to derive a correction algorithm (corrected steps = intercept + (slope x IceQube steps)). The ICC2 and Bland Altman test were run again, this time comparing the observed data with the corrected IceQube data. Acceptable agreement was specified *a priori* as a bias of ±5% and the 95% CI encompassing zero (Lenoir et al., 2017).

Results

The correlation (ICC2) between the manual observation and the IceQube step count was excellent (0.97), however, the IceQube significantly under-estimated (34 ± 6.5 SE vs 44 ± 4.6 SE, $P<0.001$) the actual number of steps for each 15 min period (Figure 1) and gave poor agreement (bias = 10.02, 95% CI 7.35 to 12.69). Applying a correction algorithm based on the intercept (5.21) and slope (1.13) from the linear model generated corrected IceQube data that had the same ICC2 and an acceptable agreement with number of steps visually observed (bias = 0.02, 95% CI -2.43 to 2.47) (Figure 2). There was no effect of age/parity of cow, ground conditions or time of day on the accuracy of the IceQube.

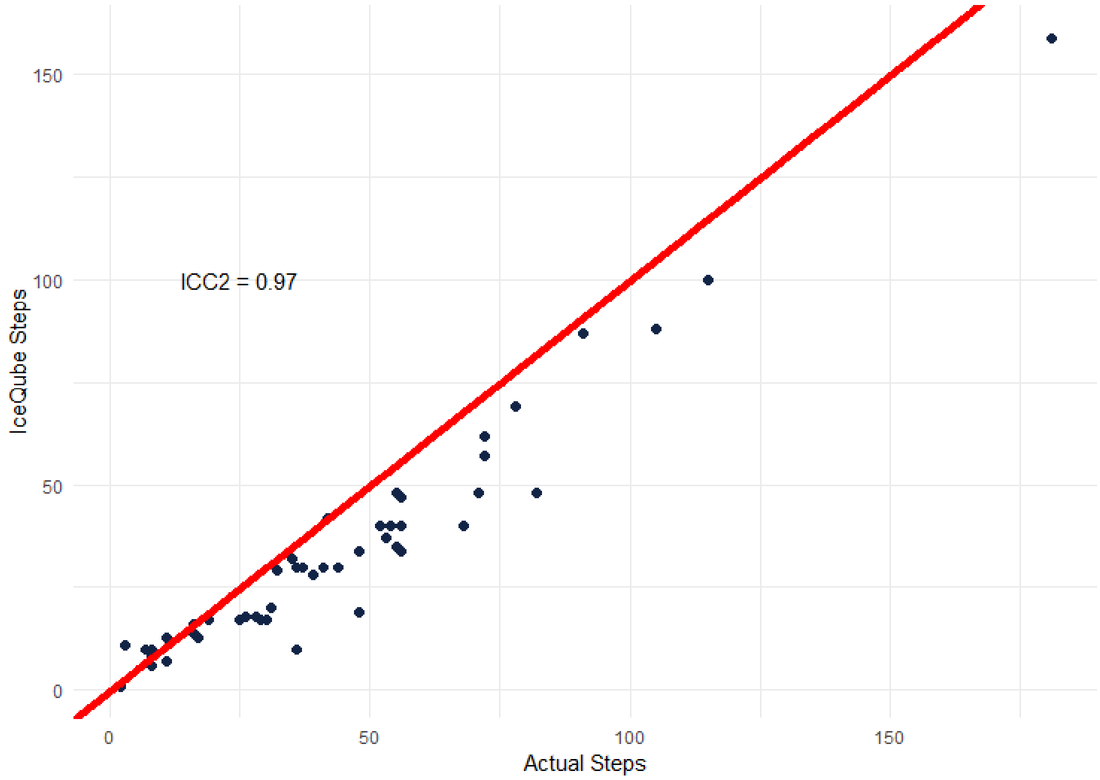

Figure 1. Actual (observed) step-count verses IceQube reported step-count showing a correlation of 0.97 but a consistent and significant (p=0.12) underestimation shown by the deviation from perfect agreement (red line – intercept = 0, slope = 1)

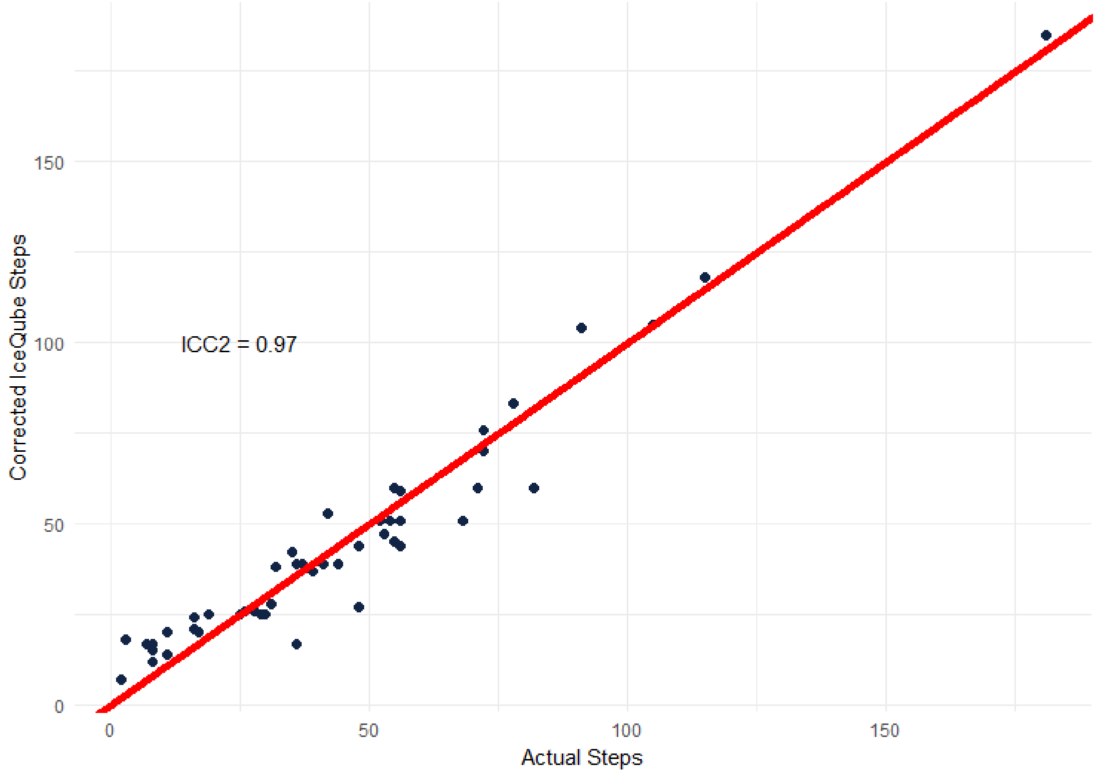

Figure 2. Actual (observed) step-count verses corrected IceQube reported step-count showing a correlation of 0.97 and now an acceptable agreement between observed and reported data (red line – intercept = 0, slope = 1)

Conclusions

In pasture grazing conditions, the IceQube provides an excellent correlation with the actual step count, allowing the magnitude of any differences between treatment groups to be reliably reported. Using the correction algorithm presented here, the number of steps reported by the IceQube for a 15 min period can be corrected to give an acceptable agreement with the actual number of steps taken.

Acknowledgements
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Research and Development at HCC to underpin marketing of red meat from Wales as a sustainable and essential food source for the human diet

H. McCalman[1]

[1]Hybu Cig Cymru (Meat Promotion Wales), Ty Rheidol, Parc Merlin, Glanyrafon Industrial Estate, Aberystwyth SY23 3FF, UK

Presenting author

Heather McCalman. E-mail: hmccalman@hybucig.cymru

HCC is funded through a levy to promote red meat from Wales. Research and Development is a fundamental part of underpinning marketing strategies by providing evidence of sustainability and meat quality credentials. It is important to support innovation and work with the industry to develop solutions to the many challenges and opportunities that are faced. This presentation will review HCCS recent and current initiatives in this field working with many collaborators.

Impact of prolific breed type on ewe and lamb performance, production efficiency and carcass output in a pasture-based sheep meat production system

T. Boland[1], F. McGovern[2], S. Lott[1], M. McEvoy[3], B. Lynch[4], J. Higgins[1]
[1]University College Dublin, Belfield, Dublin D04 V1W8, Ireland
[2]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[3]Germinal Ltd, Camp Road Witham St. Hughs Lincoln LN6 9QJ, UK
[4]Teagasc, Oak Park, Carlow R93 XE12, Ireland

Presenting author
Tommy Boland. E-mail: tommy.boland@ucd.ie

Application

Increasing ewe prolificacy is a key strategy to increase flock output and profitability. Across the three breed types examined (Lleyn, Belclare and Mule) no differences were recorded in ewe efficiency metrics, but the progeny of mule ewes reached target slaughter weight 10 days sooner than the progeny of Belclare ewes. This reduced age at slaughter could contribute to the environmental sustainability of lowland sheep production.

Introduction

Globally, the demand for sheep meat is predicted to rise by 15.7% by 2030 in line with a growing global population. Increases in production to match this demand must be achieved in the context of economic and environmental sustainability. Farmers are exploring various ways to improve the efficiency of their farming enterprises including through: increasing stocking rate (Earle et al., 2017b), replacing monocultures with multispecies swards (Grace et al., 2019) or replacing concentrate feeding with brassica crops (Dolan et al., 2022). Increasing output per ewe increases profitability per ewe and per ha (Bohan et al., 2018) and reduces the carbon footprint per kg of meat produced (Jones et al., 2014). Highly prolific ewe breed types currently present in the Irish sheep flock include Belclare, Lleyn, Blue-faced Leicester, Galway ewes (Bohan et al., 2017) and Mule ewes (F1 cross between Blue-faced Leicester ram and Black-faced mountain ewe). Combined, these breed types represent the primary maternal genetics of only 16% of ewes within Ireland (Bohan et al., 2017). The objective of this study was to compare the three most common prolific ewe breed types in Ireland in terms of ewe and lamb performance and subsequent ewe efficiency parameters in a single management system over four production years.

Materials and Methods

The study commenced in October 2017 and concluded in January 2022 incorporating four full production years (from mating until subsequent lamb slaughter). Three hundred and fifty-four ewes, divided across three breed types: Belclare X (n=119), Lleyn X (n=120), and Mule (Blue-faced Leicester × Black-faced Mountain; n=115), were sourced from numerous commercial sheep flocks nationally in August 2017. These ewes consisted of breeding ewe lambs (born spring 2017; 1-year old at parturition n = 103), breeding hoggets (born spring 2016; 2-year old at parturition; n = 92), and mature ewes (born Spring 2015 or earlier; n = 170). Additional breeding ewes were purchased in August each year (to replace dead and culled ewes) and joined the ram at the point of mating (October, each year). In total n = 208 Belclare X, n = 199 Lleyn X, and n = 201 Mule ewes were included over the four-year period. Ewes were mated to Charolais rams following heat synchronisation and all ewes were housed at day 105 post ram introduction. All ewes lambed indoors, were turned out to pasture at approximately four days post partum and were weaned at 14 weeks post partum. Lambs were slaughtered at a target live weight of 46.5kg.
Animal performance data were analysed using the MIXED procedure of SAS (SAS, version 9.4, Inst. Inc., Cary, NC, USA), with the individual ewe as the experimental unit for all parameters. The model

contained the fixed effects of ewe breed type, ewe age, time of first lamb crop, lambed to first service and the 2-way interactions of ewe breed type × ewe age and their interactions included in the statistical model. Combined litter weight was included as a covariate in the model and experimental year (production cycle) was included as a random term. Mean values were considered to be different when $P < 0.05$ and considered a tendency when $P \geq 0.05$ and < 0.10.

Results

Ewe type had no impact of efficiency parameters ((kg lamb weight/kg ewe live weight at mating) *100) at weaning or slaughter. Per ewe mated Mule ewes had higher ($P<0.05$) numbers of lambs scanned, birthed and weaned than Lleyn ewes while Belclare ewes were intermediate and not different from Mule or Lleyn ewes ($P>0.10$). Progeny of mule ewes had a higher ($P<0.05$) growth rate from birth to weaning and birth to slaughter than the progeny of Belclare ewes, resulting in a reduction of 10 days (195 vs 205 days: $P<0.05$) in the number of days to reach target slaughter weight.

Conclusions

Mule ewes as a prolific dam type results in lambs reaching slaughter weight 10 days earlier than Belclare ewes though there were no differences over all in ewe efficiency parameters of the three breed types investigated.

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Effects of grazing contrasting grass swards on lambs' performance and an approach to calculate their grass intake

O. Cristobal Carballo[1], X. Chen[1], D. Patterson[1], S. Huws[2], K. Theodoridou[2], A. Aubry[1], T. Yan[1]
[1]*Agri-food And Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*
[2]*Queen's University of Belfast, University Rd, Belfast BT7 1NN, UK*

Presenting author
Omar Cristobal Carballo. E-mail: omarcristobal.carballo@afbini.gov.uk

Application

Grazing multispecies swards increases the rate of weight gain and final body weight of finishing lambs. Estimations of total ME requirement and forage ME content is a useful tool to estimate grazed grass intake of lambs.

Introduction

In the UK, grass-based systems are fundamental to the sustainability and production of the ruminant livestock sector. The simplification of grass seed mixtures used has resulted in monoculture grazing systems. The productivity and reliability of these systems are heavily dependent on high inputs of nitrogen fertilisers, which increases the risk of air and water pollution, greenhouse gas emissions and biodiversity loss. In grazing swards, the inclusion of legume and/or herbs has been shown to reduce the dependency on nitrogen input whilst increasing animal production efficiency. However, there is only limited evidence of the effects of grazing multispecies swards on the performance of finishing lambs and no evidence of predicting grazed grass intake whilst grazing these contrasting sward types. This study aimed to assess the effect of three sward compositions on animal performance and herbage intake of finishing lambs during the mid to late growing season.

Materials and Methods

Sixty crossbred ewe lambs were randomly allocated to one of the three grazing groups (n= 20 lambs), which were balanced in terms of initial body weight and date of birth. The grazing swards consisted of: perennial ryegrass (RG, control); perennial ryegrass/white clover (R/WC); and multispecies mixed sward (MSS; perennial ryegrass, white clover, red clover, timothy, plantain, chicory and trefoil). Lambs were grazed for nine weeks under a rotational grazing management system. The herbage biomass of each sward was estimated using the rising platemeter (RPM) and 0.25m^2 quadrat clips, and then used to calculate the grazing area allocation for each lamb grazing group as part of the rotational grazing system. Representative sub-samples of herbage from each sward were collected, dried and milled periodically for NIRS and wet chemistry analyses. Measured grazed herbage intake (kg DM/d), in each group, was estimated using the difference of sward height before and after grazing (using the RPM) and the biomass density of the sward. From each group, individual lamb herbage intake (kg DM/d) was then calculated using the group measured grazed herbage intake (kg DM/d) value multiplied by the proportion of individual sheep ME requirement over the sum of group total ME requirements. The individual sheep ME requirement was predicted as a sum of ME requirements for maintenance (Yang et al., 2020), live weight gain and grazing allowance (AFRC, 1993). Live weight was recorded weekly in the mornings. Response variables were analysed using a linear mixed model via REML, with grazing sward as the fixed effect and lamb ID as a random effect. Pairwise differences between treatments were examined using the Tukey's test ($P \leq 0.050$).

Results

Performance results are summarized in Table 1. At the start of the study, the average body weight of lambs was 34.1 kg, with no differences ($P = 0.940$) between lambs allocated to each of the sward types. After 9 weeks of grazing, lambs in the MSS showed a ~30% higher ($P < 0.001$) rate of average daily gain (ADG) when compared to the RG and R/WC. Pairwise comparisons showed 4.4-4.7%

heavier final BW (*P*≤0.0495) in MSS lambs than their counterparts in the RG or R/WC. Despite the greater ADG for lambs on MSS, their DM intake (DMI) was similar to lambs on RG, whilst DMI of lambs grazing R/WC was 17% and 20% lower (P < 0.001) that the RG and MSS, respectively.

Table 1. Effects of grazing perennial ryegrass (RG), ryegrass/white clover (R/WC) and multispecies (MSS) swards on body weight (BW), average daily gain (ADG) and grazed herbage DM intake (DMI; measured grazed herbage) measured by finishing ewe lambs

	RG	**R/WC**	**MSS**	**SED**	**P-value**
Initial BW (kg)	34.19	34.04	34.05	0.446	0.933
Mean BW (kg)	36.97	36. 74	37.55	0.611	0.386
Final BW (kg)	40.08a	40.18a	41.95b	0.895	0.071
ADG (kg/d)	0.126a	0.119a	0.165b	0.0120	<0.001
DMI (kg/d)	1.277a	1.096b	1.319a	0.0599	<0.001

Figure 1 shows the estimated grazed herbage DM intake calculated from herbage ME content and ME requirements for maintenance, body weight gain and activity allowance, against measured grazed herbage DM intake as described previously. The estimated DMI was 11% lower (*P*<0.050) than measured DMI for RG, whilst no significant differences (*P*>0.050) were observed for R/WC (-1.0%) and MSS (3.0%).

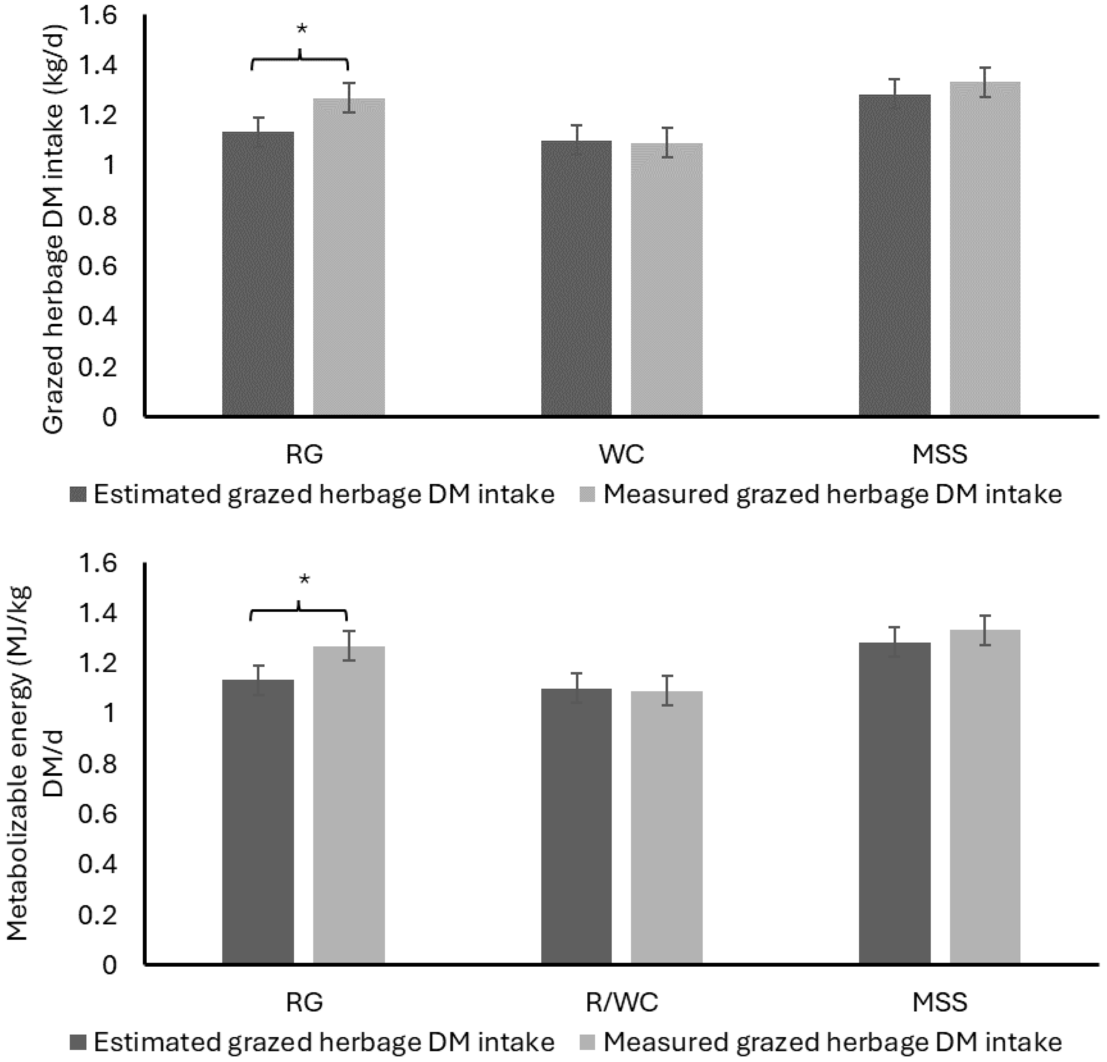

Figure 1. Estimated grazed herbage DM intake (dark grey bars: calculated from herbage ME content and ME requirements for maintenance, body weight gain and activity allowance) against measured grazed herbage DM intake (clear grey bars: calculated from group intake measured by rising platemeter and quadrats, multiplied by the proportion of individual sheep ME requirement over the sum of group total ME requirements).

Conclusions

Grazing multispecies swards in finishing ewe lambs increase the ADG resulting in heavier lambs at slaughter when compared to ryegrass and ryegrass/white clover swards. The grazed herbage DM intake calculated from the ME requirement of lambs and forage ME content and that estimated from rising platemeter and quadrats are comparable and the former method can be used to predict grazed herbage DM intake for grazing lambs.

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Does the inclusion of white clover or plantain to a perennial ryegrass sward enhance lamb performance during the post-weaning period?

R. Rooney[1,2], S. Woodmartin[1], A. Monaghan[1], T. Boland[2], P. Creighton[1]
[1]*Teagasc Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*

Presenting author

Rebecca Rooney. E-mail: rebecca.rooney@teagasc.ie

Application

Enhancing growth rates in perennial ryegrass lamb finishing systems through the addition of herbs and legumes to the sward.

Introduction

Pasture based systems are fundamental to ruminant production in temperate regions. Pasture based systems are dominated by perennial ryegrass which offers a favourable early summer growth pattern and high dry matter (DM) yields. Pre weaning it can support the nutritional needs of lactating ewes and their lambs (Grace et al., 2019).

In many pasture based sheep systems, post-weaning lamb performance is sub optimal as grass quality reduces as the grazing season progresses, requiring costly concentrate supplementation to achieve optimum finishing weights. The addition of summer active legumes and herbs to monocultures of perennial ryegrass can boost sward quality in the post weaning period (Golding et al., 2011), while also supplementing the seasonal growth pattern of perennial ryegrass swards.

Method

Three sward types were investigated using a randomized block design. Sward types investigated included perennial ryegrass (*Lolium perenne L.*; PRG), PRG plus white clover (*Trifolium repens* L; PRG + WC) and PRG, WC plus plantain (Plantago *lanceolate* L.; PRG + WC + Plan). Companion forage content was calculated as a percentage of the total sward on a dry matter basis. Three farmlets were established to investigate each sward treatment. Each flock consisted of 100 ewes with an average litter size of 1.8 lambs/ewe, with a mean lambing date of the 8th of March. Lambs were turned out to their respective treatment groups 24 - 36 hours post lambing and remained in these groups until drafted for slaughter. Each flock was managed as a rotational grazing system pre-weaning and a leader follower grazing system was implemented post-weaning, where lambs were grazed ahead of the ewes and were removed from the paddock when a target post grazing sward height of 6 cm was reached. Ewes were then introduced to graze down to a residual of 4.5 cm. Lambs were weaned at an average of 14 weeks of age. Lambs were weighed fortnightly from the point of weaning and drafted accordingly to achieve a target carcass weight of 20 kg.

Data were analysed using a linear mixed model, PROC MIXED in SAS. Fixed effects of treatment, litter size and sex were included in the model for all lamb performance traits.

Results

Results are presented in Table 1. Lamb weaning weight was similar across sward treatments ($P > 0.05$) with an average weight of 28.7 kg. Lambs grazing PRG + WC and PRG + WC + Plan had a higher post weaning average daily gain (ADG) ($P < 0.01$) of +17.5 g/day, on average. Lambs grazing PRG + WC + Plan had a higher lifetime ADG ($P < 0.05$) than those grazing PRG. Slaughter weight was unaffected by sward treatments averaging 46.5kg. Pre-grazing yields for the PRG, PRG + WC and the PRG + WC + Plan swards were similar across treatments averaging 1311kg DM/ha . The average WC content of the PRG + WC sward post weaning was 9 %, while The PRG + WC + Plan sward had a WC content post weaning of 10 % and a Plan content of 14 %, giving a total companion forage content of 24 % in the PRG + WC + Plan sward.

Table 1 The effect of sward type on lamb growth performance in the post-weaning period.

	Sward Type[1]				
	PRG	PRG + WC	PRG + WC + Plan	SEM	P-value
Lamb performance					
Weaning weight	29.5	28.2	28.5	0.53	NS
Post-weaning ADG[2]	162^{a}	179^{b}	180^{b}	0.50	<0.01
Lifetime ADG	207^{a}	213^{ab}	216^{b}	0.30	<0.05
Slaughter weight	46.2	46.6	46.9	0.40	NS
Sward parameters					
Pre-grazing yield, kg DM/ha	1332	1327	1275	87.57	NS
Post grazing sward height (cm)	6.4	6.6	6.1	29.31	NS

$^{a\text{-}b}$ Values within rows with different superscripts differ at $P < 0.05$; [1]PRG = perennial ryegrass, PRG + WC = perennial ryegrass and white clover, PRG + WC + Plan = perennial ryegrass, white clover plus plantain; [2]ADG = average daily gain.

Conclusion

The results from this study support the use of more diverse swards compared to PRG monocultures in the post-weaning period to support enhanced animal performance. However, incorporating Plan into the PRG + WC sward did not result in significant additional benefits for animal performance compared to the PRG + WC sward alone. The plantain content in the sward may have been insufficient to promote further lamb growth.

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Comparison of nonlinear functions to model the growth profiles of female lowland sheep

N.Sharif[1,2], F. McGovern[1], N. McHugh[3], T. Pabiou[4], D. Berry[3]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]University College Dublin, Belfield, Dublin D04 V1W8, Ireland
[3]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[4]Sheep Ireland, Link Road, Ballincollig, Co. Cork P31 D452, Ireland

Presenting author
Numan Sharif. E-mail: numan.sharif@teagasc.ie

Application
Growth curve parameters are important indicators of animal performance and can be used to compare the growth rate of different animals. They can be helpful in developing the best management, breeding and feeding plans.

Introduction
Growth functions can condense longitudinal body weight data into parameters with biological significance (Lupi et al., 2015), which are important for comparing the growth rate of different animals. Individual animal model parameter values can be useful for farmers to help make informed decisions regarding the management and nutrition of animals, thereby optimising production efficiency. The selection of an appropriate function to model the growth profiles of animals mainly depends on the structure of the data (Sharif et al., 2021) but also on how well the function fits the data as well as the potential application of the function. Several different growth functions exist each with their only merits and shortcomings. This study aimed to evaluate different growth functions with two, three or four parameters to model the growth of female lowland sheep.

Materials and Methods
A total of 990,306 body weight records from 228,390 female lowland sheep born during the years 2010 to 2020 were available from the Sheep Ireland national database. Records were discarded if the number of animals per flock per year was <20. Outliers per day of age were identified as weight records heavier than the third or lighter than the first quartile by more than two times the interquartile range. Subsequent to this, any weight exceeding the mean weight per day by three standard deviations was discarded. After these edits, only sheep with a minimum 1 weight measurement in the first week of life, 2 measurements during 8 to 180 days, 2 measurements between 181 and 999 days of age and 1 measurement after 999 days of age were retained. The number of animals for the final analysis was 13,090 with 158,463 body weight records.
Six nonlinear functions (Brody, Gompertz, Logistic, Negative exponential, Richards and von Bertalanffy) were fitted to the longitudinal body weight data of individual animals using the NLIN procedure of SAS 9.4 (SAS Institute Inc.) and the parameter values for the parameters A (asymptotic weight), B (integrated constant) and K (maturity rate) for each animal estimated. Outliers for each parameter of each function were identified based on the interquartile range method and were not included in calculating the summary statistics of each parameter. The fit of each function was evaluated based on the root mean square error (RMSE) and coefficient of determination (R^2) per animal as well as the ease of convergence and the estimation of biologically plausible parameter values.

Results
The Logistic and Negative exponential functions achieved convergence for all animals, while only 82.39% of animals converged for the Richards functions. Hence, the Richards function was not considered useful. All the other studied functions achieved convergence for ≥99.91% of animals. Individual animal parameter values were most sensible for both the Gompertz and von Bertalanffy

functions. The Brody function yielded the greatest mean R^2 (0.96) and the smallest mean RMSE (3.98 kg), while the Logistic function provided the smallest mean R^2 (0.94) and the greatest mean RMSE (5.25 kg) across the population. The mean R^2 value for all the remaining functions was 0.95. The modelled body weights by the studied functions are shown in Fig. 1.

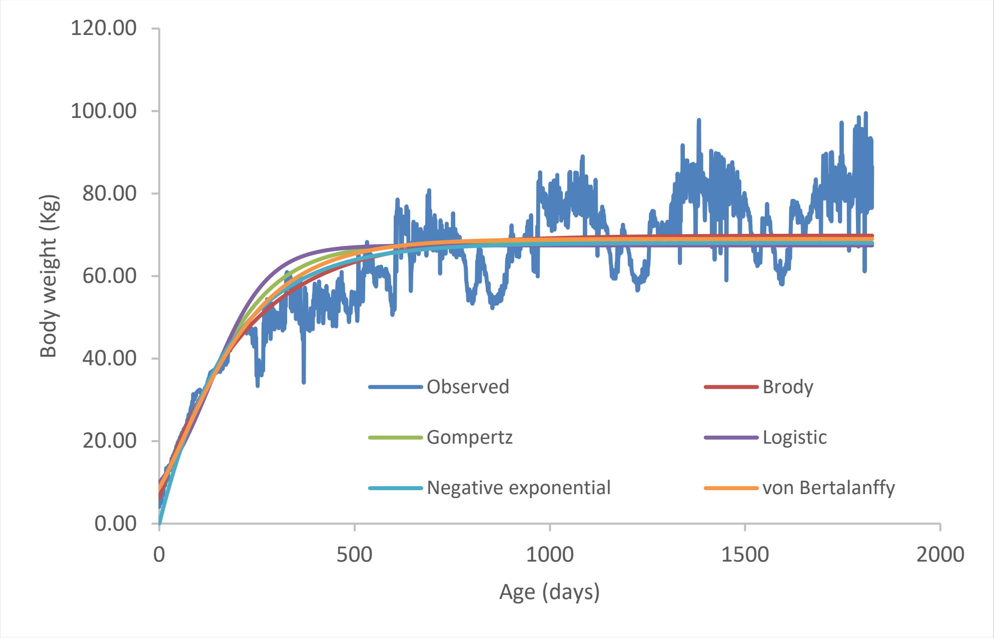

Figure 1. Observed and modelled longitudinal body weights of female lowland sheep across five growth functions.

The mean value for the A parameter estimated by the different functions ranged from 67.42 kg (Logistic) to 69.80 kg (Brody). The mean value for the B parameter also varied between the functions with the Logistic function being the highest (5.76) and the von Bertalanffy function the lowest (0.50). The range of the K parameter was between 0.0046 kg/day per kg mature weight (Brody) and 0.0135 kg/day per kg mature weight (Logistic). The A parameter within all the functions was negatively correlated with both the B (r = -0.23 to -0.13) and K parameters (r = -0.55 to -0.41), while the B parameter within all the functions was strongly positively correlated (r = 0.56 to 0.79) to the K parameter.

Conclusions

The results suggested that the Richards function cannot be used to model growth curves of female lowland sheep due to its difficulty in achieving convergence for a large proportion of the animals. All the other studied functions were capable of describing the growth profiles of animals. The Gompertz and von Bertalanffy were identified as being the best functions based on their ease in convergence and yielding the greatest number of animals with biologically sensible parameter estimates.

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Effects of supplementing Moringa oleifera leaf meal in liquid solution on the performance, rumen metabolites and methane emission in growing Yankasa rams

F. A. Adejoro[2], T. A. Ibrahim[1], M. Lawal[3], S. B. Abdu[1], H. M. Yusuf[1], S. F. Dodo[1]
[1]Department of Animal Science, Ahmadu Bello University, Sokoto Road, Samaru, Zaria, Kaduna State, Nigeria
[2]School of Biosciences, University of Nottingham, Nottingham NG7 2RD, UK
[3]Agricultural Education Department, Federal College of Education Katsina, Dutsin-ma Road, Katsina State, Nigeria

Presenting author
Festus Adeyemi Adejoro. E-mail: festus.adejoro@nottingham.ac.uk

Application
The utilization of Moringa leaf meal offer potential for improving sustainable livestock production. Adding powdered Moringa leaf to drinking water is a novel strategy that is adoptable among smallholder farmers within the tropics and sub-tropics.

Introduction
The use of natural feed additives in animal nutrition has gained significant interest due to their potential benefits in enhancing animal health and performance. The leaves of the *Moringa oleifera* plant are particularly valuable due to their high protein, vitamin, mineral, and bioactive compounds (Gopalakrishnan et al., 2017), which make it a good dietary supplement for livestock (Nehorai and Shahar, 2018). *M. oleifera* has been extensively studied for its rich nutritional profile, medicinal, rumen modulating and methane reducing properties (Ibrahim *et al.,* 2022).

The impacts of *M. oleifera* supplementation on rumen metabolites and methanogenesis represent a work in progress (Makkar and Becker, 2016). Methane (CH_4) emissions from ruminants are a significant contributor to greenhouse gas, accounting for approximately 14% of global emissions from agriculture. In a recent research by Ibrahim *et al*. (2022), extracts have been obtained from *M. oleifera* leaves using aqueous organic solvents, leaving behind the residues unutilized, despite some nutritional value the residues possess. The use of organic solvents such as methanol, ethanol and acetone to get liquid extracts from leaves may not be economically feasible for some farmers who may be interested in exploring the potential of *M. oleifera* leaves at improving animal performance and mitigating greenhouse gases such as methane. Strategies to reduce methane emissions without comprising the animal performance are critical. However, there is a paucity of research examining the impact of different inclusion levels of liquid moringa leaf meal on performance, rumen metabolites and enteric methane emissions of growing Yankasa rams. Understanding these relationships is essential for developing effective feeding strategies that enhance productivity and reduce the environmental footprint of livestock farming.

Materials and Methods
Sixteen (16) growing Yankasa rams with average weight of 16.6 kg were used for the study. Following prophylactic treatment, the rams were randomly allotted into four treatment groups with four replicates per treatment. The animals were housed in individual pens and provided with individual drinker and feeding trough. After two weeks adaptation, the rams on three treatment groups received Moringa leaf meal solubilised in water. Moringa leaf meal was milled into powder and stored and each day, 250 g was solublised in 1000 mL water and each animal was drenched via oral gavage at 4, 8 and 12 g/kg dry matter intake per day based on previous weeks' total feed intake. The animals in control group received only water drenching and all animals received these treatments for a period of 85 days.

The rams were fed a formulated diet containing soybean shell, maize offal, groundnut cake, parboiled rice offal, limestone, bone meal and common salt in a total mixed ration. Each ram was fed twice daily a total of 5% of its live body weight with a fourth nightly adjustment according to the new body weight taken. At the 9th week of the animal trial, rumen gases were collected and analysed for methane using a portable IRCD4 Biogas Analyser. Data collected were subjected to statistical analysis using a General Linear Model (GLM) procedure of (SAS, 2002) and treatment means were compared using the Tukey Test.

Results

The *M. oleifera* leaf has 284 g/kg crude protein, 211 g/kg crude fibre, and 452 g/kg soluble carbohydrate. The supplementation of growing Yankasa rams with LMLM increased weight gain and efficiency of feed utilization compared to the rams on the control group but not in a dose-dependent pattern. The nitrogen intakes were higher ($P<0.05$) in rams supplemented with LMLM compared to the rams on the control group in a non-dose dependent pattern.

Although, there was no difference ($P>0.05$) in the rumen enteric methane production for the rams that received LMLM supplementation compared to the rams on the control group, the ratios of the methane to feed intake, was lowered ($P<0.05$) in the rams supplemented with LMLM compared to the rams on the control group. Supplementation of LMLM at 12 g kg^{-1} DM intake of growing Yankasa rams reduced CH_4/feed intake by 17.4%.

Table 1. Effect of different dosage of Moringa leaf solution on intake, growth and rumen methane of growing Yankasa rams

Parameters	**0**	**4**	**8**	**12**	**SEM**	**P value**
Initial weight (kg/head)	16.6	16.8	16.6	16.4	0.87	0.782
Final weight (kg/head)	22.5^{b}	25.3^{a}	24.^{5a}	24.4^{a}	1.16	0.030
Average daily gain (g/head)	69.1^{b}	100.0^{a}	92.7^{a}	94.1^{a}	8.29	0.012
Feed conversion ratio	14.3^{a}	10.8^{b}	11.3^{b}	11.5^{b}	1.29	0.019
Methane (g/100 g)	18.5	19.3	20.8	18.3	2.13	0.301
Methane/feed intake (g/kg DM)	23.0^{a}	20.8^{b}	20.9^{b}	19.0^{b}	0.71	0.041

Means with different superscripts across a row differ significantly ($P < 0.05$); SEM = Standard error of mean.

Conclusions

It could be concluded that liquid *M. oleifera* leaf meal supplementation up to 12 g kg^{-1} DM intake holds potential for drenching growing Yankasa rams as a dietary supplement, particularly improving feed intake, nutrient digestibility, nitrogen utilisation and weight gain with no additional increase in rumen enteric methane emission. This may be particularly useful in heat-stressed rearing conditions commonly experienced in the tropics.

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The effect of sward type on growth performance, enteric methane output and the rumen microbiome in lambs during the post-weaning period

S Woodmartin[1], P. Creighton[1], T. Boland[2], A. Monaghan[1], P. Smith[3], E. Dunne[1], F. McGovern[1]
[1]Teagasc Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland
[3]Teagasc Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author
Sarah Woodmartin. E-mail: Sarah.Woodmartin@teagasc.ie

Application

This study suggests that diversifying the grazed sward, through legume or herb inclusion, can enhance the growth performance of lambs while directly or indirectly reducing methane output. These results could inform sustainable farming practices, guiding pasture management strategies that improve animal productivity and environmental sustainability. Furthermore, the study provides a basis for developing recommendations at farm level to optimize pasture-based lamb-finishing systems.

Introduction

Increasing lamb growth rates directly from pasture and reducing enteric methane (CH_4) emissions are key drivers to enhancing production efficiency and achieving more sustainable lamb-finishing systems. Pasture-based production systems in temperate climates are predominated by perennial ryegrass (*Lolium perenne L.*), a productive pasture species characterized by its high forage yields and nutritive value. Grazed grass incurs lower production costs, offering a unique economic advantage over alternative feed sources. However, while perennial ryegrass monocultures can maintain productivity year-round, their chemical composition, digestibility and growth exhibit seasonal variations, often becoming notably suboptimal during summer and autumn, hindering intake and performance of grazing animals. Botanically diverse pastures containing legumes and herbs can boost sward quality in the latter half of the grazing season and have demonstrated significant potential for enhancing animal performance (Cranston et al., 2015). Furthermore, manipulation of the diet to reduce CH_4 emissions is an important strategy available to livestock farming, however strategies that require daily supplementation of the basal diet are not currently feasible in most pasture-based systems. Implementing strategies to increase the daily live-weight (LW) gain of growing animals at pasture, such as improved grassland management and sward diversification can potentially reduce age at slaughter, thereby decreasing the animal's lifetime emissions. Hence, the objective of this study was to assess the influence of binary sward mixtures of perennial ryegrass plus a companion forage, on growth performance, enteric CH_4 output and the rumen microbiome in lambs post-weaning.

Materials and Methods

A randomized block design was employed to investigate five sward treatments, namely, perennial ryegrass (PRG), PRG plus white clover (*Trifolium repens L.;* PRG+WC), PRG plus red clover (*Trifolium pratense L.;* PRG+RC), PRG plus chicory (*Chicorium intybus L.;* PRG+Chic) and PRG plus plantain (*Plantago lanceolate L.;* PRG+Plan). A rotational leader follower grazing system was implemented post-weaning. A subset of 120 lambs, *circa* 15 weeks of age (n=24 per treatment) were selected at weaning in 2021 and 2022 for CH_4 measurements using portable accumulation chambers. Groups were balanced for weaning LW, sex and reared litter size (LS). Six CH_4 measurements were obtained from each lamb between weaning and slaughter. Rumen fluid was harvested using a transoesophageal sampling device. Lambs were drafted for slaughter once they reached a minimum LW of 45kg. Average sward companion forage content was 28% on a dry matter basis.

Data were analysed using a linear mixed model, PROC MIXED in SAS. Fixed effects of treatment, birth LS, reared LS, sex and dam parity were included, while dam was included as a random effect for all lamb performance traits. Fixed effects of treatment, dam parity, lamb LW, time off feed, temperature, pressure, humidity and chamber were included for CH_4 g/day, CH_4 g/kg LW and CH_4 g/kg average daily gain (ADG). Contemporary group accounted for date and lot number and was included as a random effect for CH_4 traits. Fixed effects of treatment, lamb age and LW were included for microbiome analysis.

Results

Lambs grazing PRG+WC, PRG+RC or PRG+Chic had a higher post-weaning ADG ($P<0.001$) of 29-60 g/day and an increased lifetime ADG ($P<0.001$) of 24-46 g/day, compared to lambs grazing PRG. The addition of any companion forage reduced age at slaughter, with reductions of 39, 50, 30 and 16 days achieved for lambs grazing PRG+WC, PRG+RC, PRG+Chic and PRG+Plan respectively ($P<0.001$). Lambs grazing PRG+WC, PRG+RC or PRG+Plan ranked lower for CH_4 g/day, CH_4 g/kg LW and CH_4 g/kg ADG compared to lambs grazing PRG ($P<0.001$). There was a lower relative abundance of *Methanobrevibacter* ($P<0.001$) in the rumen of lambs grazing PRG+RC than those grazing PRG, PRG+Chic and PRG+Plan.

Table 1. Effect of sward type on performance, CH_4 output and the relative abundance of *Methanobrevibacter* in the rumen of lambs post-weaning.

	Sward Type^{1}						
	PRG	PRG+ WC	PRG+ RC	PRG+ Chic	PRG+ Plan	SEM	P-value
Lamb performance							
Post-weaning ADG^{2}	120^{d}	163^{ab}	180^{a}	149^{bc}	131^{cd}	0.7	<0.001
Lifetime ADG	162^{c}	195^{ab}	208^{a}	186^{b}	173^{c}	0.4	<0.001
Age at slaughter	252^{d}	213^{ab}	202^{a}	222^{b}	236^{c}	4.0	<0.001
CH_4^{3} output							
CH_4, g/day	14.3^{a}	13.4^{bc}	12.4^{d}	13.9^{ab}	13.2^{c}	0.30	<0.001
CH_4, g/kg LW^{4}	0.40^{a}	0.37^{bc}	0.34^{d}	0.38^{ab}	0.36^{c}	4.1	<0.001
CH_4, g/kg ADG	109.6^{a}	88.8^{b}	77.6^{c}	92.3^{a}	98.9^{b}	0.01	<0.001
Rumen archaea							
Methanobrevibacter	89.0^{b}	84.4^{ab}	81.4^{a}	87.1^{b}	86.6^{b}	1.24	<0.001

$^{a\text{-}d}$Values within rows with different superscripts differ at $P<0.05$;^{1}PRG=perennial ryegrass, PRG+WC=perennial ryegrass and white clover, PRG+RC=perennial ryegrass and red clover, PRG+Chic=perennial ryegrass and chicory, PRG+Plan=perennial ryegrass and plantain.^{2}ADG=average daily gain.^{3}CH_4=methane.^{4}LW=live weight.

Conclusions

Enhancing production efficiency in tandem with a reduction in enteric CH_4 emissions will enhance the profitability and environmental sustainability of pasture-based lamb production. This study demonstrates that incorporating herbs and legumes into perennial ryegrass swards significantly enhances lamb performance post-weaning, reducing age at slaughter compared to those finished on perennial ryegrass monocultures. Additionally, these findings indicate that more diverse swards offer the potential to achieve direct and indirect reductions in CH_4 output while manipulating the population of methanogens within the rumen, potentially offering a more favourable microbial signature to reduce CH_4 output. This research supports the use of binary sward mixtures in intensive pasture-based sheep production systems as a practical and effective strategy to improve production efficiency and mitigate CH_4 emissions in lamb-finishing systems.

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Performance characteristics of Scottish Blackface and Texel x Scottish Blackface lambs offered forage brassica, perennial ryegrass or cereal based concentrate diets

F. Campion[1], T. Boland[2], N. Claffey[1], M. Dolan[1,2]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]University College Dublin, Belfield, Dublin D04 V1W8, Ireland

Presenting author
Frank Campion. E-mail: francis.campion@teagasc.ie

Application

This study demonstrates that using alternative forages such as forage brassicas has a role to play in lamb finishing systems. The results demonstrate the type of performance achievable when grazing during the winter months and how this compares to other systems.

Introduction

Scottish Blackface (SBF) lambs can grow up to 240 g/day and attain a carcass weight of 118-23 kg when offered ad-libitum concentrates while using terminal sires crossed with SBF ewes has also been shown to increase lamb growth rates and lamb carcass output in comparison to purebred SBF lambs (Claffey et al., 2018). However, ad-libitum concentrate systems are not always practical for some producers and not all hill ewes can be crossbred. Finishing these type of lambs using forage based diets has the potential to increase the options for lamb finishers but there is a paucity of information on these finishing systems. The objectives of this experiment was to compare the performance of SBF lambs and Texel X SBF (TXSB) lambs when grazed on either perennial ryegrass based pastures, forage brassica crops or offered ad-libitum concentrates indoors.

Materials and Methods

This study was performed over three production cycles (October to February each year) and was a 3x6 factorial design. Factors were lamb type and diet type. Lambs were blocked by live weight (within lamb type category) and balanced for source farm with 25 TXSB entire male, 25 SBF entire male and 25 SBF castrate male lambs randomly allocated to one of six diet types; ad-libitum concentrates, forage rape (FR), hybrid brassica (HB), kale (K), permanent pasture (PP) and perennial ryegrass re-seed (RS). Lamb live weight was recorded every 14 days until lambs were drafted for slaughter. Lambs were drafted for slaughter at ≥40 kg live weight with a target carcass weight >18kg. Carcass conformation was scored using the EUROP grid system (E= excellent and P= poor) and carcass fat cover was scored using a one to five scoring system (1=low fat cover; 5 = high fat cover; Hopkins, 2021) with a target fat score of three. Data residuals were examined for normality using the UNIVARIATE procedure of SAS (Version 9.4) and data was analysed using the MIXED procedure of SAS (Version 9.4). Individual lamb was the experimental unit for all animal parameters analysed and was included as a random effect. Lambs offered PP and RS are excluded from the post slaughter lamb performance analysis, as 6% and 14 % respectively were drafted for slaughter over the three years creating an un-representable sample for the analysis.

Results

As presented in Table 1. TXSB, SBF entire male and SBF castrate male lambs all had higher average daily gains (ADG) when offered ALC in comparison to all other diet types ($P < 0.01$). There was a lamb type by diet type interaction. Texel cross Scottish Blackface lambs achieved a higher ADG compared to either SBF entire male or SBF castrate male lambs offered ALC, FR, K, HB and RS ($P < 0.05$). Scottish Blackface entire male lambs achieved a higher ADG compared to SBF castrate male lambs offered ALC, FR and PP ($P < 0.05$). There was no difference in ADG between SBF entire male and SBF castrate male lambs offered HB, K and RS (*P* > 0.05). Lambs offered ALC had higher slaughter weights and carcass weights compared to lambs offered FR, HB and K (*P* < 0.01). Kill out percentages were similar among diet types, with no difference observed between lambs offered ALC, FR, HB and K (*P* > 0.05). There was also no difference in carcass fat or conformation scores across all diet types (*P* > 0.05).

Table 1. Effect of treatment diet on average daily gain (g/day) of TXSB, SBF entire male and SBF castrate male lambs offered ALC, FR, HB, K, PP and RS

	ALC	FR	HB	K	PP	RS	SEM	P-value
All lambs	243.7^{a}	129.5^{b}	132.6^{b}	126.2^{b}	36.8^{d}	73.7^{c}	4.25	< 0.01
SBF Entire	241.7^{a}	130.8^{b}	127.8^{b}	122.5^{b}	37.2^{d}	67.0^{c}	6.57	< 0.01
SBF Castrate	195.7^{a}	103.2^{b}	109.4^{b}	114.0^{b}	16.8^{d}	54.8^{c}	7.17	< 0.01
TXSB	292.4^{a}	152.4^{b}	158.2^{b}	144.5^{b}	53.3^{d}	95.0^{c}	7.12	< 0.01

a–d Means within a row with common superscripts do not differ (P > 0.05)

TXSB = Texel x Scottish Blackface entire males, SBF E = Scottish Blackface entire males and SBF C = Scottish Blackface castrate males

ALC = ad-libitum concentrate, FR = forage rape, HB = hybrid brassica, K = kale, PP = permanent pasture, RS = reseed

Conclusions

The current study demonstrates that SBF and TXSB lambs perform best when offered ALC but forage brassica crops are a viable alternative. In contrast, perennial ryegrass swards alone were not suitable for finishing lambs in the autumn/winter period due to the decline in forage quality and subsequent effects on lamb performance. Lambs grazing forage brassica crops performed to a satisfactory level throughout the winter period which conveys the potential in using these crops in lamb finishing systems

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Welfare pens for dairy cows in automatic milking systems – a descriptive study

F. Johansen[1,3], C. Barry[2], G. Arnott[1], S. A. F. Buijs[3], C. Kielland[2]
[1]School of Biological Sciences, Queen's University Belfast, University Rd, Belfast BT7 1NN, UK
[2]Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Elizabeth Stephansens v. 15, 1433 Ås, Norway
[3]Animal Welfare Unit, Agri-Food and Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author
Francesca Johansen. E-mail: francesca.johansen@teagasc.ie

Application
Welfare pens are used within Norwegian automatic milking system dairies. These pens are designed to provide prioritised access to resources for certain 'high-risk' cow groups (e.g., lame), and may positively influence animal welfare. This study describes welfare pens, their typical usage, benefits, challenges, and costs, and our results can be applied to automatic milking systems worldwide.

Introduction
Automation of the milking process by automatic milking systems increases the autonomy of the cow, with potential welfare and productivity benefits. However, these benefits are diminished if the cows fail to visit the milking robot frequently and regularly enough to maintain production (Jacobs and Siegford, 2012). Certain 'high-risk' cow groups (e.g., lame, low-ranking, primiparous), have a reduced ability to compete for access to resources, and typically have lower voluntary visit frequencies and prolonged milking intervals. Thus, they may produce less milk, have poorer udder health, and may experience compromised welfare. Systems which improve access to resources may help mitigate these issues. In Norway, a system called a welfare pen is commonly used within automatic milking system herds. These systems create two pens, one for the 'main' group and a smaller pen for 'high-risk' cows. Theoretically, the smaller pen houses fewer cows, resulting in less competition for resources. Despite their popularity in Norway, little empirical evidence exists regarding the structure, usage, benefits, challenges, and economic implications of welfare pens. The aim of this descriptive study was to 1) describe welfare pens and their integration into Norwegian automatic milking system barns, 2) outline how welfare pens are used by Norwegian dairy farmers, 3) report the perceived benefits and challenges of welfare pens, and 4) outline the economic implications of including a welfare pen in an automatic milking system barn.

Materials and methods
The study consisted of three components: an online questionnaire, on-farm data collection, and semi-structured interviews. The questionnaire was distributed to Norwegian farmers with automatic milking systems and gathered information pertaining to farm and welfare pen management, benefits and challenges of welfare pens, and opinions on welfare pens. The questionnaire was available online for approximately seven weeks during October-November 2023 and 132 responses were collected. The on-farm portion of the study took place on five farms with welfare pens in southeast Norway. The farms were issued a logbook where they recorded events of cows being moved into the welfare pen, when and why they were moved, and when and why they were removed from the welfare pen. A total of 137 logbook events were registered over a three-month period from October 2023 to January2024. Semi-structured interviews were conducted with three Norwegian agricultural building advisors in August 2024. The interviews explored the advisors' perception of, and experience with, welfare pens, particularly relating to economic considerations and practical implementation. Descriptive data analysis was conducted, and open-ended question responses were thematically analysed.

Results

Figure 1A illustrates the typical structure of a welfare pen and its integration into a Norwegian automatic milking system barn. The welfare pen was reported by a majority of questionnaire respondents to accommodate 10-15% of their productive herd. Pregnant cows and heifers approaching calving and freshly calved cows were reported both in the questionnaire and in the logbook data to be the most common reasons for moving cows to the welfare pen, with management of sick and lame cows ranking second. The decision to remove cows from the welfare pen depended on the reason for being placed into the welfare pen, with a combination of predetermined criteria and direct observation being used to decide when to remove cows from the pen.

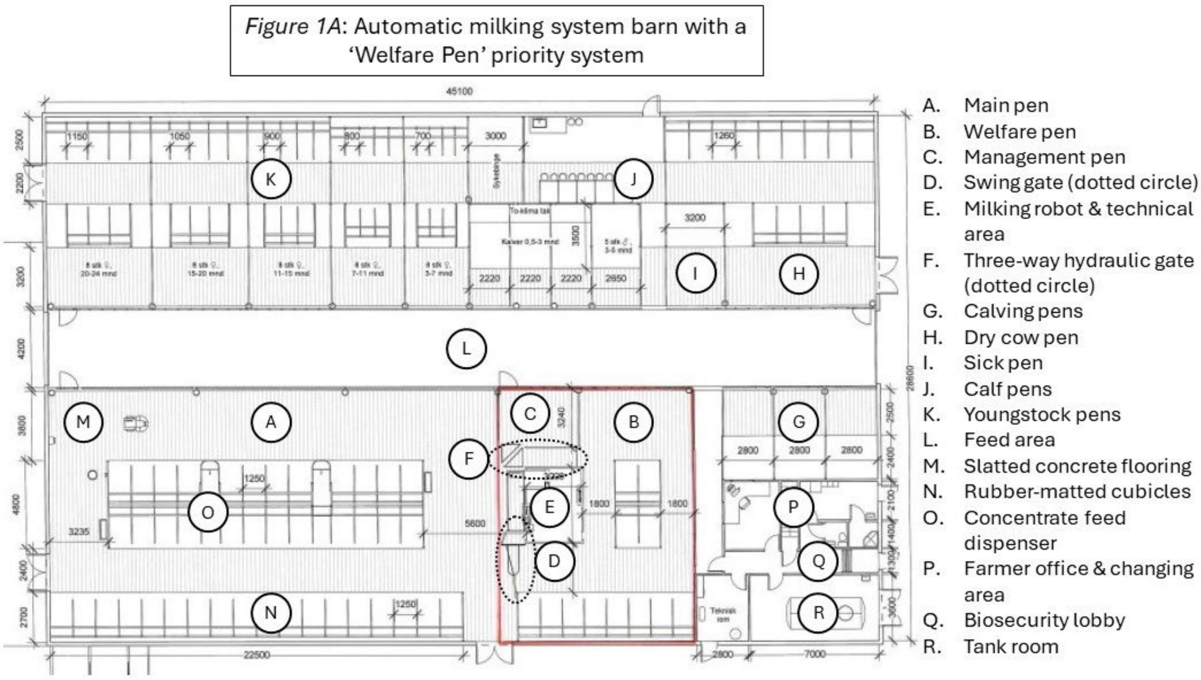

Figure 1. Automatic milking system barn with a welfare pen priority system

Most survey respondents (71%) felt that the welfare pen had reduced their labour requirements, and overall, respondents felt that animal welfare on the farm was improved. Of respondents without a welfare pen, 65% reported that they would consider installing one. Concerns regarding welfare pens were linked to practical design and management issues, and cost. The building advisors were largely positive towards welfare pens and regularly recommended them. However, they highlighted challenges regarding costliness, particularly when retrofitting existing buildings to accommodate welfare pens. Furthermore, they emphasised that the benefits of installing a welfare pen can be challenging to quantify, as there is no counterfactual for comparison.

Conclusion

Welfare pens are a potentially beneficial addition to automatic milking systems and are generally perceived by users to reduce labour and increase welfare. They are a flexible solution which can be tailored to an individual management system. Though most often used for managing cows in the periods before and after calving, they can be used for several other cow groups and practical applications. The system is particularly applicable when building a new barn for a single robot in systems with limited access to pasture. Further research is necessary to fully understand the benefits, or lack thereof, of welfare pens.

Acknowledgements

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Cow time budget: a novel method of data collation to support analysis of individual cow performance under pasture-based robotic milking systems

F. Carnovale[1], N. Rutherford[1], T. Takahashi[1]
[1]Agri-Food Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author
Francesca Carnovale. E-mail: francesca.carnovale@afbini.gov.uk

Application

A high degree of inter-animal variability in cow performance while grazing often encourages adopters of robotic milking systems to confine their animals indoors throughout the year. This study proposes a new data collation method to identify the drivers of such variability, with a higher-level aim of facilitating the industry transformation to more pasture-based systems in the future.

Introduction

Automatic milking robots (AMR) are increasingly becoming integrated into pasture-based dairy systems in recent years to improve labour productivity. However, animal performance under a pasture-based AMR system is often lower than a housed AMR system, for which the technology was originally developed (Lyons et al., 2014). In Northern Ireland, where ~92% of farmed land area is grassland, recent research has also shown that grazed cows, on average, experience lower milk yields, milking frequency and number of non-milking visits to AMR ('refusals') than their housed counterparts. Nonetheless, some cows adapted better to a pasture-based AMR system and indeed outperformed most housed cows, thereby displaying a strong potential to effectively combine the AMR technology with rich grassland resources locally available (Rutherford et al, 2024). To facilitate this transformation requires a better understanding of the drivers behind the inter-animal variability in cow performance under grazing and, ultimately, identification of optimal intervention strategies to minimise such variability. As a first step towards this goal, the present study developed a novel method of data collation to elucidate the high-resolution (minute by minute) time budget allocation between activities by individual cows milked by AMR.

Materials and methods

The experiment was conducted at Agri-Food & Biosciences Institute, Hillsborough, over a 83-day period in the summer of 2020. Twenty-four (24) Holstein-Friesian cows were given daytime access to pasture and 24-hour access to AMR. The animals were subject to two-way grazing on a 21-day rotation, with access to block A from 04:00 to 12:00 and access to block B from 12:00 to 20:00. To mirror the typical land constraint faced by a local dairy farm (insufficient pasture for the ongoing stocking rate), all animals were kept indoors overnight. Cows had 24-hour access to the housing (i.e. an option *not* to be grazing during daytime) and total mixed ration (TMR) therein. Cow activities were continuously monitored through three data sources: (i) milking behaviour using AMR (Lely Astronaut, Lely, The Netherlands); (ii) grazing time and duration using gate sensors (Lely Grazeway, Lely, The Netherlands); and (iii) TMR intake using feedboxes (Feed Intake Controlling & Recording System, Bio-Control, Norway). Following the experiment, the three datasets were programmatically combined using R version 4.3.0 to create a complete time budget (24-hour behaviour diary) for each cow. These integrated data were further linked to the farm management database so that cows' grazing patterns could be compared against animal-level profitability.

Results

The final dataset thus compiled consisted of ~60,000 activity records (across 24 cows and 83 days), including milking, refusals, grazing, TMR visits, transition periods from one activity to the next, and time spent indoors but outside AMR/TMR (e.g. resting and ruminating). While the full data analysis is beyond the scope of the present study, a case study using the cow which recorded the lowest margin over feed costs (MOFC) is provided here to illustrate the potential value the final dataset. The cow (#352) had a mean daily milk yield of 22.3L and MOFC of £4.60. Two random dates within the same lactation stage were chosen for detailed examination: 15th and 20th August 2020. Milk yields on these dates were 26.7L and 22.5L, respectively. Figure 1 depicts the total daily time this cow spent on various activities on the two dates. A notable difference in grazing time was observed, particularly in the afternoon. In contrast, the percentages of time spent milking and consuming TMR were similar on both days. Further insights into these differences are provided in Figure 2, which summarises the chronological patterns in cow behaviour. On both days, the cow exhibited a consistent pattern of successful milking sessions. However, on the low-yield day (20th August), there was a notable reduction in afternoon grazing time and fewer TMR visits, accompanied by an increase in multiple refusals. These non-milking visits occurred during periods when the cow might otherwise have been grazing. By contrast, the cow spent more time outdoors on the high-yield day (15th August).

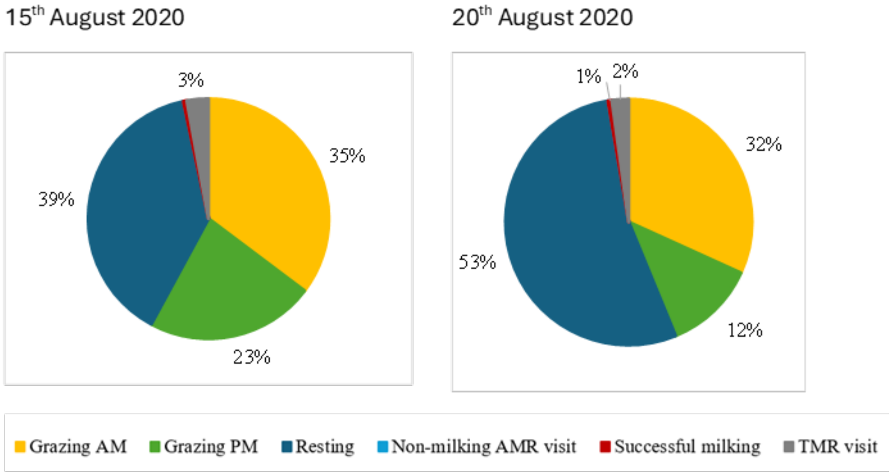

Figure 1. Examples of daily time budget (cow #352, 15th/20th August 2020).

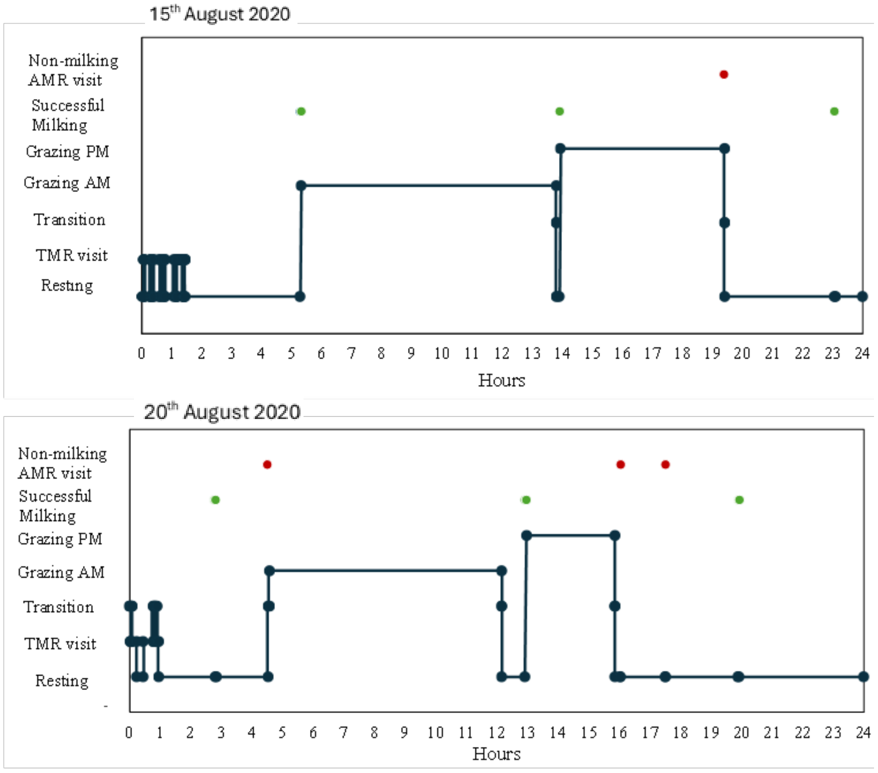

Figure 2. Examples of daily timeline (cow #352, 15th/20th August 2020).

Conclusions

Maximising pasture utilisation is essential to for grassland-dominated regions to capitalise on their comparative advantage. It is hoped that the methodology developed herein will help identify key efficiency barriers in pasture-based AMR systems and provide opportunities for dual optimisation of feed and labour inputs. The initial case study seems to indicate the importance of time spent grazing, both in the morning and in the afternoon.

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Associations between daily lying times, transition cow management and early lactation health outcomes in multiparous dairy cows

J. Cook[1,2], P. Pepler[2], L. Viora[2], D. Hill[2]
[1]*World Wide Sires, Beachin Stud Lea Lane, Aldford, Chester, Cheshire CH3 6JQ, UK*
[2]*University of Glasgow, Switchback Rd, Bearsden G61 1QJ, UK*

Presenting author
John Cook. E-mail: john.cook14@btinternet.com

Application

Immediate improvements in cow health can be made by encouraging farmers to fully facilitate lying duration. Optimising lying duration should also be prioritised during the planning and construction of new facilities.

Introduction

Restricted lying times in dairy cows can alter feeding behaviour, as cows deprived of lying time will compensate with substantial reductions in feeding time, which in turn can result in health disorders. Lying times can be influenced by management factors as well as animal specific factors including illness and injury, age and parity, reproductive status and lactation stage of the cow (Tucker et al., 2021). Length of stay in a close-up pen prepartum (days in close up, DCU), prepartum stocking density, calving density and time from the last pen filling event to calving are management factors considered important in ensuring transition cow health (Cook and Nordlund, 2004). Failure to optimize these factors may also cause forced reductions in lying duration with negative consequences for animal health. This study therefore explores the associations between management factors considered important for transition cow health and daily lying times and evaluates their association with disease outcomes.

Materials and Methods

Daily lying duration for 21-d prior to calving until 21-d post calving was recorded for 499 cows located in a single UK herd calving over a 12-month period. For each cow, data were recorded on the length of stay in the prepartum pen, prepartum stocking density, calving density and number of days from the last pen filling event to calving. The number of cows in the prepartum pen on each day was counted and used to calculate the average daily stocking density experienced by each cow from 8 d to 2 d prior to calving. Calving density was calculated as the number of cows calving each week during the study period divided by the overall average number of cows calving per week for the same period. The number of days from the last pen filling event to calving was calculated for each cow by subtracting each cow's calving date from the date of the last additions to the pen. Health events and treatments were recorded by farm staff in accordance with written protocols. Linear regression was used to assess the association between the selected management factors and daily lying times. Logistic regression was then used to model the odds of disease (any single case or combination of ketosis, mastitis, metritis, milk fever, and displaced abomasum) during the first 30 days in milk. We considered 3 ways to include daily lying duration in our models: daily lying duration, 3-d averages, and 7-d averages. Weekly averages, for the 3-wk before calving, were chosen because these smoothed out the variability of daily lying duration and avoided overfitting. Stepwise variable selection based on Akaike's information criterion was used to select the 7-d average lying duration to include in the final model. The disease model was based on mean pre-calving daily lying durations over the period of 8 d to 2 d prior to calving, days spent in close-up and gestation length (GL). To avoid multicollinearity issues, we used the scores for the first principal component (PC1) of the covariance matrix of DCU and GL as a proxy variable to simultaneously adjust for DCU and GL.

Results

Daily lying duration was 745 ± 115 min\d (mean ± standard deviation). No association was found between prepartum stocking density, PC1, calving density and time from the last pen filling event to calving and daily lying duration in prepartum cows. Both lactation group (*P* < 0.05) and season of calving (*P* < 0.05) were associated with daily lying duration, with cows in the 3rd lactation and above lying for 23 min\d longer than those in the 2nd lactation, and those calving in autumn/winter lying for 22 min\d longer than those calving in spring/summer.

Figure 1 shows the daily lying duration prepartum for diseased and non-diseased cows. Daily lying duration was negatively associated with the odds of disease (*P* = 0.03); however, the positive coefficient for its quadratic term (*P* = 0.04) indicates that the rate of reduction decreased as lying time increased, meaning that odds of disease had a minimum value at a daily lying time of 860 min.

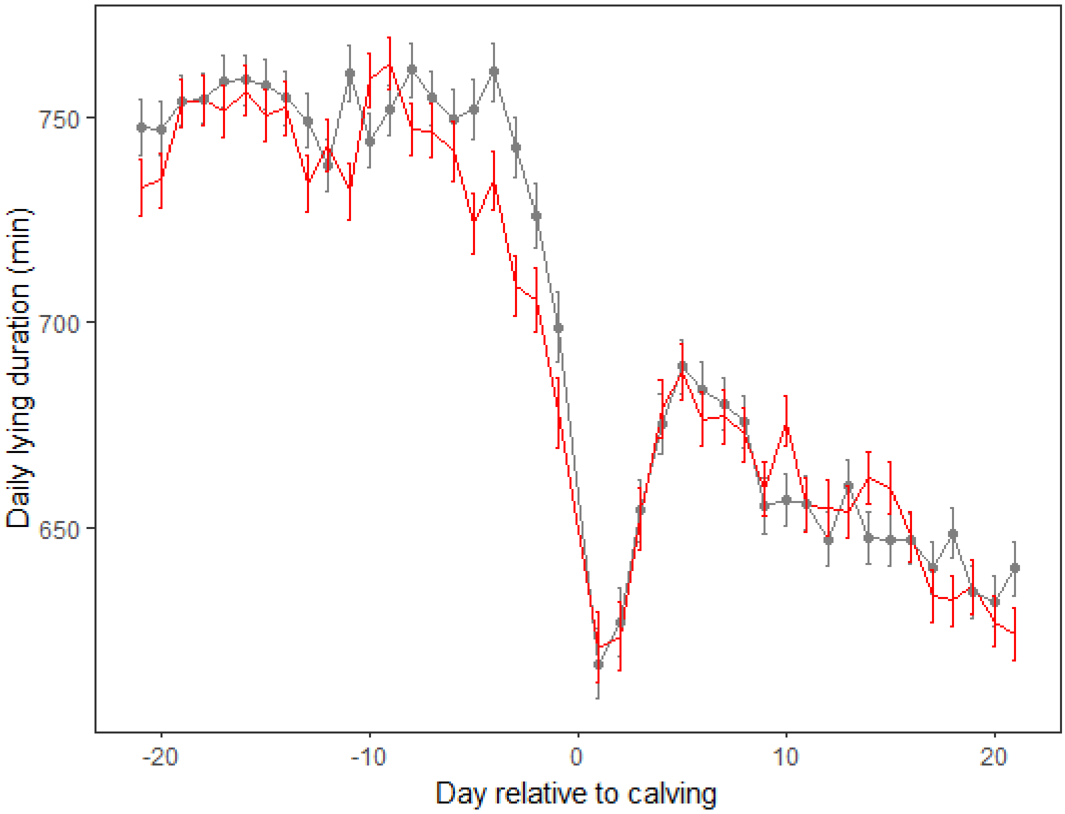

Figure 1. Daily lying duration around calving for cows with no disease (grey line; error bars show standard error) versus cows with at least one case of disease (red line; any single case or combination of ketosis, mastitis, metritis, milk fever, and displaced abomasum) during the first 30 days in milk.

Conclusions

Lying duration was not associated with the management factors considered important for transition cow health but was associated with parity group, with cows in parity 3 and above lying down for longer than cows in parity 2. Cows lay down for longer in the cooler seasons of autumn/winter than spring/summer. Daily lying duration during the transition period are however associated with health outcomes in multiparous cows. This information can be used to inform farmers during the construction of new facilities, as well as in the daily management of current buildings. For example, in deciding the dimensions and size of cubicles and bedded areas, or the frequency of addition of bedding materials and increased bedding depth.

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Social behaviour changes as a result of disease in dairy cows

D. Baran[1], A. Dowsey[1], S. Held[1], D. E. Hidalgo[1], S. Mullan[1]
[1]Bristol Veterinary School, University of Bristol, Langford House, Bristol BS40 5DU, UK

Presenting author
Daria Baran. E-mail: dariab97@hotmail.com

Application

The growing dairy herd sizes as well as concerns over antimicrobial use on farms calls for early disease detection to prevent negative welfare and economic impacts. This systematic review aims to summarise what is already known about changes in social behaviour of dairy cows with disease and its potential as an early indicator of disease using automated methods in the emerging field of precision livestock farming (PLF) in agriculture.

Introduction

Early detection of disease in dairy farming is of major interest in animal welfare research to aid in early intervention from farmers to prevent the negative welfare and economic impacts (af Sanderberg et al., 2023). There is a growing interest in utilising social behaviour changes of dairy cows for early disease detection, using automated methods, on the basis of sickness behaviour. Sickness behaviour is an adaptive response by the individual caused by the interaction between the central nervous system and the immune system mediated by cytokines which are released upon infection/inflammation or injury (Dittrich, Gertz and Krieter, 2019). This communication between the brain and the immune system is essential to maintain survival of the individual (Dantzer and Kelley, 2007). Previous studies have shown animals are motivated to perform these sickness behaviours, for example inactivity in rats, which aids in conserving energy (Sepulveda-Varas et al., 2013). Those behaviours not critical to the immediate survival of the animal, such as social interactions (e.g displacements) or grooming are expected to change before changes in essential behaviours, as those are predicted to have a higher sensitivity to a disease challenge(Caplen and Held 2021). Not all aspects of sickness behaviour have yet been investigated completely or explained, however, some of these behaviours provide an opportunity for the early detection of sick individuals (Dittrich, Gertz and Krieter, 2019) before clinical signs of disease and the detection of subclinical conditions.

Materials and Methods

The objective of this systematic review was to summarise the changes in social behaviour with disease occurrence in dairy cows. The search for relevant literature was conducted following the PRISMA guidelines using two electronic databases, CAB abstracts and Web of Science, accessed between January and April 2024. The initial search yielded 1934 papers, through initial abstract and title screening, papers were included if the abstract reported social behaviour changes with disease in adult dairy cows. Review papers, those that did not compare healthy and sick individuals and papers that included an additional intervention, for example, effect of group size on behaviour without disease comparison were excluded. This resulted in 13 papers being included in this review

Results

All of the included studies have focused on naturally occurring cases of disease, eight studies reported a decrease in actor displacements for sick individuals and three papers reported a decrease in reactor displacements. On the other hand, four papers reported an increase in reactor displacements for sick individuals. Seven papers have utilised cameras to record social interactions while five studies used electronic feed-bins. Majority of the studies have also focused on changes in behaviour during the transition period, comparing matched healthy and sick individuals.

Conclusions

The reported changes show conflicting results and may be attributed to confounding factors such as regrouping, social rank, or competition for resources, although the intra-individual differences need to be considered for future research on behavioural changes with disease as well as inter-individual differences (Thomas et al., 2024). Where possible future research should consider analysing multiparous and primiparous cows separately, analyse individuals in a stable social environment, and include a variety of social interactions rather than just focusing on displacements at the feed-face. The reviewed papers raise a few important and yet unanswered questions such as, to what extent is the social behaviour of individuals impacted by the disease versus the normal daily disturbances and to what extent is the behaviour change associated with pain or the systemic response to disease. An automatic detection system that tracks individuals and identifies their behavioral events is needed and this systematic review will be used to aid in the development of automatic detection algorithms on farm using cameras for early and subclinical disease detection.

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Analysing the impact of the temperature-humidity index on Beef cattle welfare across three different systems

A. A. Khan[1], C. Morten, D Enriquez-Hidalgo, J. Rivero
[1]*Rothamsted Research, West Common, Harpenden AL5 2JQ, UK*

Presenting author
Afsal Ayoob Khan. E-mail: afsal.ayoob-khan@rothamsted.ac.uk

Application

The present study is relevant for improving animal welfare protocols and providing better animal management practices in the livestock sector.

Introduction

Various climatic variables influence animal welfare aspects (Gauly et al., 2013). Understanding the behavioural dynamics of cattle in response to environmental changes is pivotal for optimizing animal welfare and farm management practices. The Temperature-Humidity Index (THI) is a relevant indicator commonly used to assess the environmental stress in livestock, particularly under extreme environmental conditions (Dunn et al., 2014). Qualitative Behavioural Assessment variables (QBA), first introduced by Wemelsfelder et al. (2000) to assess the welfare of farm animals, incorporate data on animals' body language. The objective of the present study was to analyse the relationship between THI and the QBA descriptors.

Materials and Methods

We conducted QBA on beef cattle across three farmlets such as two pasture-based (based on permanent pasture (PP), and based on reseeded grass-clover swards (GWC); cattle housed at weaning and turned out to pasture in spring) and one intensive finishing system (IFS; 40% concentrate, 60% silage; cattle housed at weaning and kept indoor until finishing) from January to December 2023 at the North Wyke Farm Platform (NWFP), Rothamsted Research, UK. We assessed animal welfare through QBA as adapted by Cooke et al. (2023), recorded between weekly and monthly (varying between months) and evaluate the influence of THI on the QBA descriptors, such as activity levels and social behaviours in beef cattle over the three systems. The THI was calculated from the air temperature (Ta) and relative humidity (RH) data recorded automatically every 15 min at the NWFP met station. Pearson correlation coefficients were computed to measure the strength and direction of the relationships between THI [$THI= (0.8*Ta + (RH/100) * Ta – 14.4)- 46.4$] (Habeeb et al., 2018), and QBA descriptors. Principal Component Analysis (PCA) was employed to reduce the dimensionality of the QBA descriptors and to identify patterns of variation among the behavioural responses. Further, PCA biplots were created to visualize the clustering of farmlets and the contributions of QBA variables to the principal components. In addition, an ANOVA was performed to examine the effects of farmlets, THI, and their interaction on the QBA variables.

Results

The mean THI was 62 in the summer and 48 in the winter (Figure 1). The analysis revealed several negative ($p<0.05$) correlations between THI and QBA variables in the three systems. Notably, ‘Calm’ (-0.52, -0.17, -0.25), ‘Active’ (-0.46, -0.54, -0.44), ‘Lively’ (-0.36, -0.44, -0.30), and ‘Playful’ (-0.37, -0.41, -0.40). Negative correlation between THI and ‘Agitated’ (-0.38, -0.25, -0.26), ‘Apathetic’ (-0.38, -0.45, -0.35) and ‘Distressed’ (-0.38, -0.38, -0.42) (correlation values were indicative to IFS, PP and GWC, respectively) were also found, indicating decreased behavioural expression in animals with higher THI levels. However, ‘Indifferent’ showed a positive correlation with THI in farmlets IFS, PP and GWC (0.13, 0.41, 0.38). Moreover, the absence of a considerable negative correlation of THI with the remaining QBA variables does not indicate that these variables are irrelevant but rather that they may indicate adaptive behavioural responses to THI during the study period. Farmlets-specific effects were not significant, suggesting that treatments across the three farmlets did not influence the QBA variables (p = 0.340). The PCA biplot (Fig 2) provides a comprehensive visual representation of the relationships between QBA descriptors and the grouping of farmlets (IFS, PP and GWC). Dim1 (27.5% of variance explained) and Dim2 (16.4% of variance explained) collectively account for 43.9% of the variability. The overlap of the farmlets within the PCA biplot ellipses indicates that behavioural patterns among the three groups are not distinctly separated. This finding suggests that farmlets conditions may not influence the overall QBA of animals, which is further supported by the results of the ANOVA analysis (p = 0.557). Hence, the study highlights the impact of THI on various aspects of beef cattle behaviour.

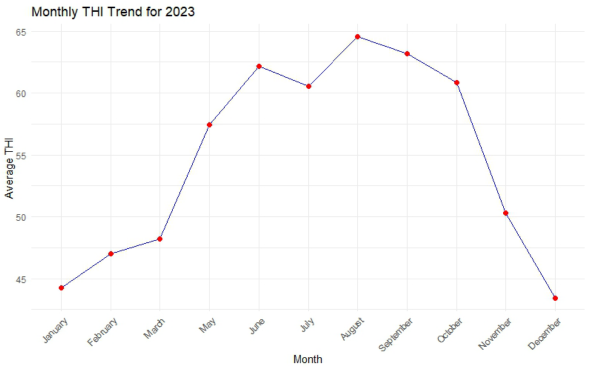

Figure 1. Temperature and Humidity Index (THI) during the assessment period

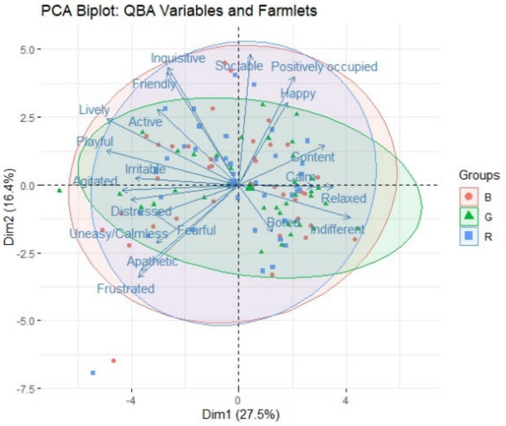

Figure 2. Principal Component Analysis Biplot of QBA Variables across three Different Farmlets Systems [IFS (R), PP (G), GWC (B)]. Dim1 (27.5%) and Dim2 (16.4%) Variability Explained.

Conclusion

It is evident that THI had a relationship with the QBA descriptors reflected in the behaviours across all three farm systems that were investigated. Given that all the farmlets demonstrated a negative correlation across QBA variables, were influenced by changes in THI, and no PCA difference among farming systems, the findings underscore the importance of incorporating environmental considerations into animal welfare strategies in all farming systems.

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A high-resolution analysis of feeding behaviours: do seconds matter in understanding feed intakes of beef heifers?

G. A. Wager-Jones[2,3], M. Butler[2], E. Harris[2], E. Bleach[1], M. Rutter[1], K. Behrendt[1]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*
[2]*Agriculture Data Science Research Centre, Edgmond, Newport TF10 8NB, UK*
[3]*Peacock Technology Ltd, Alpha Centre, Stirling University Innovation Park, Stirling FK9 4NF, UK*

Presenting author
Georgina Anna Wager-Jones. E-Mail: georgina_anna@outlook.com

Application

Using vision technologies to measure feeding behaviours and predict feed intakes in beef heifers can provide high resolution feeding behaviour and real time insights that can be used to support sustainable livestock management practices.

Introduction

Precision livestock technologies are essential for advancing animal health and welfare, these technologies enable continuous monitoring to identify issues such as lameness, oestrus and diseases. However, many existing Precision Livestock Farming (PLF) technologies focus on a limited set of variables. In feeding behaviour studies, systems often capture basic metrics like presence of an animal at the feeder and visit frequency at an hourly or daily rate, but do not track head movements whilst the animal is at the feeder. This is of importance because feeding behaviour has been linked to feed intake (Schwartzkopf-Genswein *et al.*, 1999). While traditional observation methods can provide insights, they are labour-intensive and prone to errors (Sowell *et al.*, 1998). Camera systems offer a less intensive solution, capable of monitoring behaviours of a group of animals at high resolutions, in real time, without physically attaching devices to animals (Porto *et al.*, 2015). The integration of computer vision with high-resolution behavioural monitoring offers an opportunity to capture more nuanced behavioural patterns efficiently. The objective of this study was to utilise vision technologies to analyse feeding behaviour at a focused timescale and evaluate the predictive power for estimating feed intake in beef heifers. This work aims to contribute to the development of precision livestock farming techniques that can improve resource efficiency and sustainability in the beef industry.

Materials and Methods

An automatic recording system was developed to monitor the actions of five British Blue heifers, weighing 368.6 ± 27.1 kg, fed a total mixed ration (TMR). Images captured by the recording system were processed using computer vision techniques as outlined in Wager-Jones *et al.* (2024). The processed data from the vision system were linked with feed bin weight measurements, resulting in each second of observation having a corresponding image frame and feed bin weight. The frames were grouped over time to calculate durations of two variables: down events and down duration. For example, 10 consecutive frames showing the heifers with its head down were grouped as a 10-second duration, with the corresponding feed bin weight calculated as the mean of smoothed bin weights to reduce noise inherent in such systems. Observations of the heifers at the feed bin were further refined: if a "no_animal" event occurred but lasted 5 seconds or less and was flanked by the same heifers' "head down" events on either side, the "no_animal" event was integrated into the same observation rather than treated separately. In total, 108 unique observations were obtained, with each observation representing a single feed bunk visit. For each observation, variables were calculated. The down_events variable represented the total number of head-down events during the visit, while down_duration captured the cumulative duration of all head-down events. The estimated_intake was derived as the difference in feed bin weight (grams) before and after each visit. The relationship between estimated intake and the predictors, down_events and down_duration, was analysed to assess their significance. F-statistics were used to evaluate the overall significance of the model, and ANOVA was performed to determine the individual contributions of each predictor to the model's fit.

Results

The linear mixed-effects model evaluated the relationship between estimated feed intake and the predictors, down_events and down_duration, while accounting for random variability across individual heifers (heifers_id). The model explained a substantial proportion of variance, with a marginal R^2 of 0.686 and a conditional R^2 of 0.689. Among the fixed effects, down_events showed a significant positive association with estimated intake (Estimate = 53.43, $P<0.001$), indicating that an increase in the number of downward events is strongly correlated with higher feed intake. Conversely, down_duration was not a significant predictor (Estimate = 0.51, P=0. 470). The random effect for heifers_id had a standard deviation of 24.06, reflecting moderate variability in intake across individual heifers. ANOVA results confirmed that down_events was a significant contributor to the model (F= 37.33, $P < 0.001$), while down_duration didn’t contribute significantly.

Conclusions

This analysis underscores the significant role the number of down events during a feed bunk visit have in predicting feed intake. The lack of a significant relationship between down duration and feed intake implies that the total time spent with the head down is less predictive of intake than the occurrence of specific down events. These findings highlight the value of vision-based technologies in capturing detailed behavioural metrics that can be used to obtain predictions of feed intake from feeding behaviours and contribute to calculations of feed efficiency. Further research should investigate the specific characteristics of down duration and consider the potential influence of other behaviours that may occur during a down event, which could explain why down duration is not a reliable predictor of feed intake.

Acknowledgements

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Feeding behaviour in Holstein cattle fed different milk allowances as calves

S. O'Callaghan[1,2], G. Arnott[1], O. C. Carballo[2], L. Leal[3], G. Scoley[2], S. Stergiadis[4], D. Seymour[3,5]
[1]Queen's University Belfast, University Rd, Belfast BT7 1NN, UK
[2]Agri-Food and Bioscience Institute, 18a Newforge Ln, Belfast BT9 5PX, UK
[3]Trouw Nutrition R&D, Blenheim House, Blenheim Road, Ashbourne, Derbyshire DE6 1HA, UK
[4]School of Agriculture, Policy and Development, University of Reading, Whiteknights House, Reading RG6 6UR, UK
[5]Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, 50 Stone Rd E, Guelph, ON N1G 2W1, Canada

Presenting author
Sinéad O'Callaghan. E-mail: socallaghan08@qub.ac.uk

Application

Increasing preweaning nutritional levels can shape feed behaviours of Holstein cattle, particularly in their first lactation.

Introduction

Early life is a critical time in a calf's life in terms of health and long-term performance. Adequate nutrition in early life is vital to ensure a calf is primed to reach its growth potential and appropriate milestones at the most beneficial time for the animal and the farmer. Currently, the industry standard is to feed calves 10% of their birth body weight (~4 L milk replacer/day). However, increasing evidence indicates that increasing this to 20% of the calf's birth body weight (~8 L milk replacer/day) can increase the animal's performance (Leal et al., 2021). There is a lack of longitudinal studies investigating the behavioural impact of early-life nutritional quantity during the animal's later life. This study aims to investigate if the preweaning nutritional level affects the feed behaviour of Holstein cattle during their first and second lactation. The hypothesis is that the level of nutrition in early life will influence later life feed behaviour.

Materials and Methods

At birth, 86 Holstein heifer calves were individually housed and blocked into pairs; each pair received identical colostrum. Within a block, each calf was assigned one of two treatments: the conventional level of milk replacer (4 L/day) or an elevated level of milk replacer (8 L/day). All calves were weaned at week 8 after one week of being stepped down. Calves were housed in pens of 4-6 animals from week 10 onwards and managed similarly regardless of treatment. The animals were followed through their first (n = 77) and second lactations (n = 61). During both lactations, cows were housed in a free-stall barn and fed ad libitum partial mixed ration (PMR) via electronic feed bunks (RIC; Hokofarm Group B.V. Emmeloord, the Netherlands). They also received two types of lactation concentrate from out-of-parlour feeders (Fullwood Packo). A high-protein concentrate was limited to 2.3 kg/day for all cows, and a high-energy concentrate was adjusted depending on the individual's milk output. Feed intake data was collected using the automatic feeders. Every visit to an automatic feeder recorded information including the animal's ID, feed type, date, start and end time of visit, the cow's parity, the days in milk (DIM), and the amount of feed consumed during the visit for both first and second lactation. Response variables, including feed intake, number of visits to the feeders and the time (secs) spent at the feeders, were analysed using the GLIMMIX procedure of SAS v9.4 (SAS Institute., Cary, NC) with fixed effects of week, treatment and the interaction of week and treatment, and the random effects of block and cow within block. Repeated measures were modelled using a first-order autoregressive covariance structure. The SLICE option of the LSMEANS statement was used to test for treatment differences at specific weeks of lactation. These models were run for concentrate and PMR in both first and second lactation, with each lactation being limited to 44 weeks.

Results

The week of lactation was significant for the average concentrate intake in both lactation 1 (F = 45.74, *P* <0.001) and lactation 2 (F = 29.77, *P* < 0.001). The average PMR intake revealed that the week of lactation was significant in the first lactation (F = 1.51, *P* = 0.016). Cattle fed the elevated treatment in preweaning, had a higher number of concentrate eating bouts in the first lactation compared to cattle fed the conventional treatment in early life (F = 8.11, *P* = 0.005). Within the first lactation, the average number of concentrate and PMR eating bouts revealed that the week of lactation was significant (concentrate: F = 5.31, p <0.001; PMR: F = 1.82, *P* = 0.001). Week of lactation was also significant for the average number of concentrate and PMR eating bouts in the second lactation (concentrate F = 1.80, *P* = 0.001; PMR F = 1.57, *P*= 0.009). In first lactation, cattle fed the conventional treatment spent significantly more time (seconds) at the feeder than cattle fed the elevated treatment (Elevated = 175.90 secs vs Conventional = 208.62 secs, *P* = 0.020). Examination of the post hoc least square means tests showed there were significant treatment effects from weeks 24 – 43, as seen in Figure 1, with the conventional treatment spending longer time eating than the elevated treatment. This pattern did not cross into the second lactation; only the week of lactation was significant for the average time spent eating concentrate in the second lactation (F = 4.18, *P* < 0.001). The week of lactation for average time spent eating PMR was significant in both the first lactation (F = 3.00, *P* <0.001) and the second lactation (F = 2.72, *P* < 0.001).

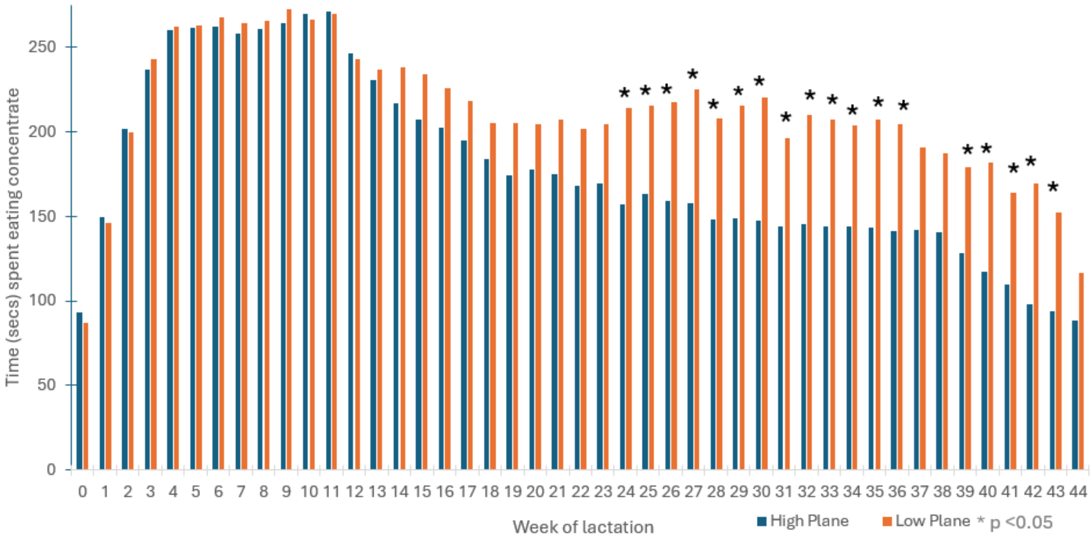

Figure 1. Average time (seconds) spent eating concentrate in the first concentrate

Conclusions

As expected, the cows' feeding patterns were significantly influenced by the week of lactation, for both concentrate and PMR across both lactations. The treatment differences were primarily seen in the first lactation, affecting the number of concentrate eating bouts and the time spent eating concentrate, especially between weeks 24 and 43 (Fig. 1). The time spent eating during the second lactation was only influenced by the week of lactation, showing that both treatment groups had a more consistent intake pattern at this stage. This study's results indicate that preweaning nutritional level can shape feed intake behaviour, particularly in the first lactation.

Acknowledgements

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Feeding on the Moove: Assessing the effect of on-board feeding during long-distance road-ferry-road transport on the blood physiology of unweaned calves

L. van Dijk[1,2], S. Siegmann[1,3], P. Bennett[1], K. Sugrue[1], K. van Reenen[3], E. A.M. Bokkers[3], G. Sayers[2], M. Conneely[1], N. L. Field[1]
[1]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[2]*Munster Technological University Kerry, Tralee, Co Kerry V92 HD4V, Ireland*
[3]*Wageningen University and Research, Droevendaalsesteeg 4, 6708 PB Wageningen, Netherlands*

Presenting author
Luca van Dijk. E-mail: Luca.vanDijk@Teagasc.ie

Application

Using an on-board milk replacer feeding system on a ferry measurably improved the blood physiology of unweaned calves the next day, but improvements were not maintained until later timepoints.

Introduction

Europe and other parts of the world are currently seeing increased societal concern regarding the welfare of calves and other animals during transport. One of the largest welfare risks in transported calves is caused by prolonged fasting durations. This study aimed to assess changes in the blood physiology of unweaned calves as a result of using an on-board milk replacer feeding system during a road/ferry/road journey from Ireland to the Netherlands.

Materials and Methods

Calves were transported from an assembly centre in Ireland, via a lairage in France, to a veal farm in The Netherlands. A total of 100 Holstein Friesian (HF) or HF cross calves (average age 27.6d, range 14 – 42d) were enrolled in the trial; 40 calves were fed on board the truck during the ferry section of the transport between Ireland and France using an on-board feeding system (FEED), and the remaining 60 calves were included as controls (CONT). Calves were blood sampled prior to departure at the assembly centre in Ireland (Day 0), upon arrival at the lairage in France (Day 1), upon arrival at the veal farm in The Netherlands (Day 2), and one week post arrival at the veal farm (Day 9). The transit from Ireland to France lasted approximately 22h, they remained at the lairage in France for 13h, after which the transit to The Netherlands lasted approximately 12.5h. Both FEED and CONT calves were fed milk replacer prior to departure at the assembly centre in Ireland, after sampling at the lairage in France, and following the sampling upon arrival at the veal farm in The Netherlands. At the veal farm, calves were fed milk replacer twice daily. CONT calves were fasted for approximately 28h between Ireland and France and for approximately 27h between France and The Netherlands. In contrast, FEED calves were fasted for approximately 15h between Ireland and feeding on the ferry, approximately 13h between feeding on the ferry and France, and for approximately 27h between France and The Netherlands. Blood samples were analysed for glucose, beta-hydroxy-butyrate (BHB), non-esterified-fatty-acids (NEFA), total protein, urea, lactate, and creatine kinase. Generalised linear mixed models were used in SAS on Demand to analyse the effect of feeding or not feeding on the ferry on changes in the blood physiology of calves. Statistical models accounted for differences in weight, age, and breed. An interaction between treatment (FEED, CONT) and time (Day 0 – Day 9) was included to determine differences between treatment groups at different time points.

Results

At the lairage (Day 1), glucose was higher (4.8 vs. 4.0 mmol/L; $P < 0.01$) and BHB (0.20 vs. 0.34 mmol/L), NEFA (0.52 vs. 0.71 mmol/L), urea (3.06 vs. 3.49 mmol/L), and lactate (0.85 vs. 1.25 mmol/L) were lower for FEED calves compared to CONT calves (all $P < 0.01$; Figure 1). No blood variables differed between treatment groups on arrival at the veal farm. Total protein was within the reference range for both groups at all times and did not differ between treatment groups on any Day (all *P* > 0.1). Creatine kinase was higher than the reference range on all days, possibly affected by muscle damage as a result of transport. Creatine kinase did not show interactions between treatment and Day (*P* = 0.09), regardless of Day, creatine kinase did not differ between treatments (*P* = 0.99). No variables differed between treatment groups at Day 9 (all *P* > 0.1).

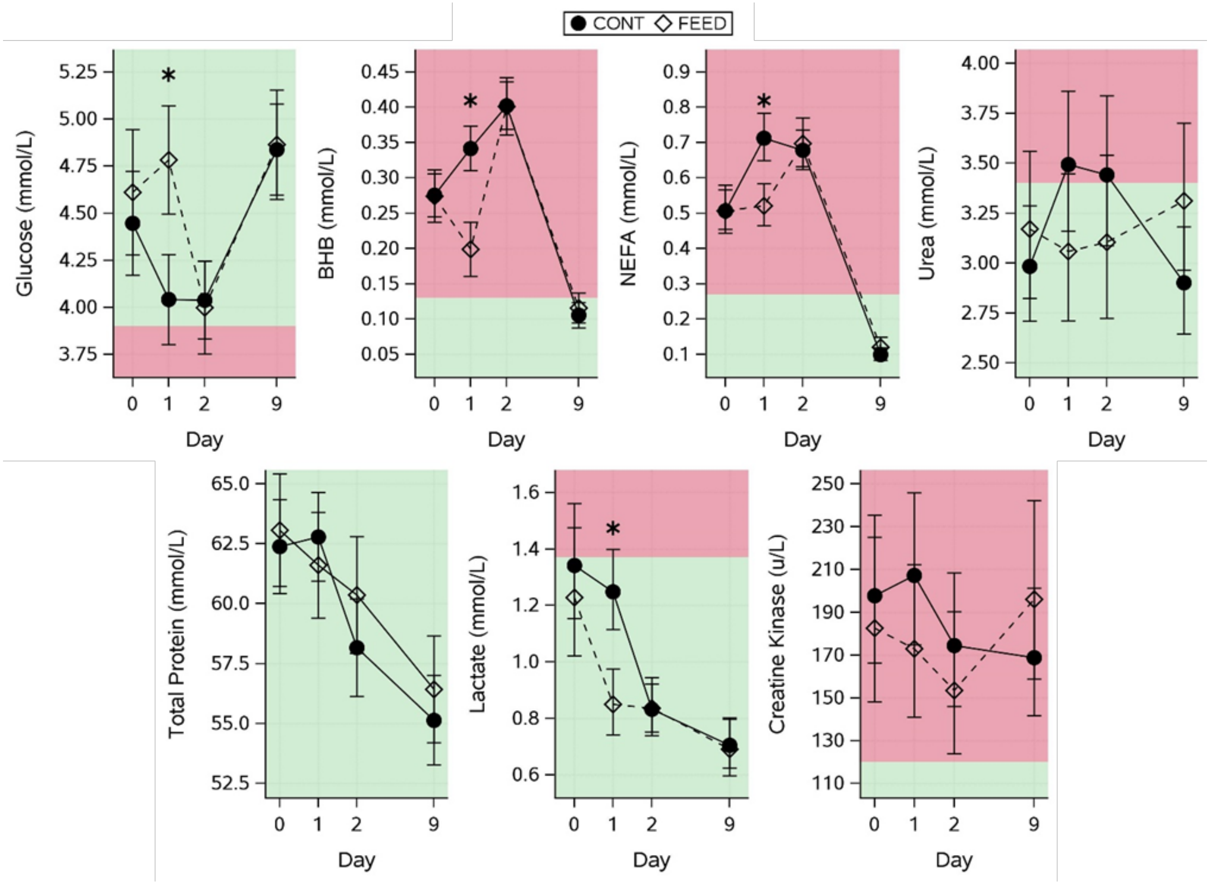

Figure 1. Effect of treatment (CONT, FEED) on blood variables (glucose, BHB, NEFA, total protein, urea, lactate, and creatine kinase) on days relative to departure (Day 0: assembly centre in Ireland, Day 1 = lairage in France, Day 2 = arrival at veal farm in The Netherlands, Day 9 = 7 days post arrival on veal farm). All variables are presented as means ± confidence interval. Red (dark) shaded areas represent areas outside of normal reference limits. * Significant difference (p < 0.05) between treatment groups within a day.

Conclusions

In general, most blood variables were better for FEED calves than for CONT calves at the first sampling after feeding on the ferry, but no differences between treatment groups were apparent on arrival at the veal farm. On-board feeding of calves has the potential to improve the blood physiology of calves but more work is needed to avoid blood physiology indicators falling out of reference ranges.

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Animal welfare incidents that occur during and after transport to Australian export abattoirs: an evaluation of government reports

F. Carnovale[1], D. R. Arney[2], D. Lowe[3], C. J. C. Phillips[4]
[1]*Agri-Food Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*
[2]*Estonian University of Life Sciences, F. R. Kreutzwaldi tn 1a, 51006 Tartu, Estonia*
[3]*Animals' Angels, Westminster, MD 21158 USA*
[4]*Curtin University Sustainability Policy, Perth WA 6845, Australia*

Presenting author
Francesca Carnovale. E-mail: francesca.carnovale@afbini.gov.uk

Application

This research investigated the information available from Animal Welfare Incident Reports to determine the prevalence of conditions reported at export abattoirs, the timing of reporting and the actions taken, to reduce the suffering of animals who are unfit to transport and sell.

Introduction

Maintaining high welfare standards for farmed animals is essential at all life stages, including pre-transport, during transport, and post-transport periods. During these stages, animals face multiple stressors such as hunger, thirst, exhaustion, and inadequate space and rest during transit (EPRS, 2021). In Australia, the transportation of farmed animals faces considerable challenges stemming from long distances, the remoteness of many farming operations, and their distance from markets and abattoirs. Thus, animal welfare issues are increasingly apparent within the food chain, highlighting the need for effective regulation and operational guidance. According to the Australian Standard (AS) 4696:2023, farmed animals suspected of disease, illness, or injury affecting their slaughter suitability must be assessed by the on-plant veterinarian (OPV) to ascertain their progression through the slaughter process (Australian Standards, 2023). The determination of an animal's entry into the food chain depends on its ability to ensure the integrity of meat and meat products. Consequently, if the animal condition fails to meet the AS for a welfare concern, an Animal Welfare Incident Report (AWIR) is initiated by staff at the export abattoir or by the OPV. It is a tool for recording incidents involving animals sent to meat export abattoirs and markets and includes measures to reduce suffering and evaluate if State or Territory agencies were informed of the incident. This research examined data from AWIRs, evaluating the prevalence of conditions, timing, and actions undertaken. The study also explored the relationships among these aspects. Consequently, the findings of this research are expected to offer insights into the welfare of farmed animals and their transportation not only in Australia but also in a broader context.

Materials and Methods

A synthesis was performed on 571 of 631 incident reports from 2020/2021, obtained from the Australian government following a Parliamentary Inquiry prompted by media allegations of non-compliance with animal welfare standards in the Australian meat processing sector. Additionally, National Vendor Declarations (NVD) linked to the AWIRs were analysed, providing insights into the food safety status and traceability of animals within the food chain. The following information was extracted from the AWIR and the accompanying NVDs, where available: total and affected number of animals in incidents in the current transport, number of incidents to date for that abattoir that year (termed number of previous incidents), species (cattle, pigs, sheep, and horses), gender (female, entire male, and castrated male), age (the average age in months for each gender), departure and arrival locations, body condition score (BCS; poor, acceptable or good), behaviour (normal/calm, mildly stressed or severely stressed/distressed), timestamps for transport and action-related events, Corrective Action decisions (emergency killing, EK, Priority slaughter PS, no corrective action NCA, or dead on detection/arrival, DoD/A), and identification of the responsible party for declaration.

Spearman's correlation coefficient was utilised to evaluate the relationships between these variables based on the raw and untransformed data. A Principal Component Analysis (PCA) was performed to assess relationships among variables, identify redundant descriptors, and facilitate interpretation.

Results

Among the 571 AWIRs examined, 367 pertained to cattle, 162 to sheep, 35 to pigs, and seven to horses. In terms of transported animals in affected consignments, sheep were the most numerous at 63,883, followed by cattle at 25,486. Sheep were the most impacted animals, followed by cattle and pigs. Most incidents related to pigs involved handling issues that did not require EK. The primary reported issue for cattle was lameness and injuries sustained during transport. The BCS in cattle was reported as poor relative to sheep, which had an acceptable BCS, while pigs and horses exhibited good BCS. Cattle exhibited mild distress, whereas sheep remained predominantly calm. Pigs and horses exhibited the highest levels of distress. The predominant outcome after detection was EK, especially following extended journeys. The detection time upon arrival at the abattoir was consistent across all species, at 12 hours, indicating a notable effect on welfare. Action was promptly taken following detection. A notable percentage of individuals identified with welfare conditions were referred for PS in the kill queue. Animals DoD/A were predominant among cattle.

The correlation analysis revealed a higher number of affected animals in larger consignments. A positive correlation exists between extended transportation time and the number of affected animals with an EK decision. Older animals and females exhibited a higher number of EKs. Animals subjected to handling issues were more prone to being designated as NCA. Extended detection-action intervals were associated with heightened PS and reduced EK. Older males exhibited a higher prevalence of genitourinary issues. Older females exhibited a higher likelihood of having cancer and being EK.

The PCA analysis revealed the relationship between voyage characteristics and corrective actions. The first principal component explained 23.83% of the data variation and also demonstrated a significant correlation with the total number of affected animals and the assignment of an animal to an EK. Extended action times exhibited an inverse correlation with the execution of PS. Finally, voyages involving substantial animal populations correlated with extended delivery-detection times and a reduced incidence of animals exhibiting normal or calm behaviour.

Conclusions

The recorded incidence of animal welfare conditions of concern was highest for pigs, which were also most likely to be stressed and suffer from handling-problems. Cattle had the second highest incidence of welfare conditions of concern and the most incident reports. Sheep were most likely to be lame. The most common outcome following detection was EK, particularly in long journey. The time to detection following arrival at the abattoir was 8-12 h, which represents a significant impact on welfare. The findings indicate the necessity of implementing measures to enhance the enforcement of standards concerning the suitability of animals for loading and sale, thereby preventing the transport of unfit animals.

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Exploring the role of miR-127 in dairy cattle: associations with ageing, milk production, and stem cell function

M. Macleay[1], G. Banos[1,2], F. X. Donadeu[1]
[1]Roslin Institute, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, UK
[2]Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK

Presenting author
Madison Macleay. E-mail: s1204766@ed.ac.uk

Application

A circulating microRNA, miR-127, was associated with dairy cow performance and affected bovine stem cell function in vitro. miR-127 may be a potential biomarker for performance, providing information to support selection, thus improve herd health, fertility, and profitability.

Introduction

Dairy cattle performance mainly comprises early growth, fertility, disease resistance, milk production, and lifespan. These traits are intercorrelated and cow performance could be improved by utilising biomarkers for these traits (Guinan et al., 2023). Circulating microRNAs are ideal biomarkers because they affect many cellular processes, where they repress mRNA translation (Friedman et al., 2009), are relatively stable, and easily measured in biofluid samples (Ioannidis et al., 2018). The present work investigated associations between plasma microRNAs and cow age and performance traits, using longitudinal sampling, and potential microRNA function. The potential roles of one microRNA identified, miR-127, in bovine tissue development was investigated by determining the effects of manipulating miR-127 levels in bovine mesenchymal stem cells (MSCs) on proliferation, differentiation, and putative gene targets.

Materials and Methods

Levels of 378 miRNAs were quantified by PCR array in plasma from 12 calves that were previously identified as having extremely different early performance. Four calves had infections and died, four had poor growth and fertility, and four had good growth/fertility with no health issues. Growth and fertility in the latter two groups differed in mean average daily gain before weaning (0.5 vs 0.9), one year bodyweight (332kg vs 385kg), age at first service (505d vs 429d), and number of services to conception (2.25 vs 1.25). Eighty-five microRNAs were differentially expressed between groups or were associated with a performance trait. Nine of these were validated by RT-qPCR using longitudinal samples at calf (<1 month old), heifer (14-23 months), and cow (29-35 months) stages from 91 animals. Spearman pairwise correlations were applied to microRNA levels. General(ised) linear mixed models (GLMM) tested associations between individual microRNA levels or microRNA ratios and age or performance, including growth, health, survival, fertility, and milk production in the first two lactations. Fixed factors included miRNA expression and factors that influence the trait, as chosen by backwards selection, including breed, year of birth or calving, month of birth or calving, and length of lactation as applicable. The random term was animal ID with associated pedigree. microRNA levels were also quantified in 19 body tissues from three 5-month-old male calves. Then, microRNA level was analysed by GLMM in R (fixed term: tissue type; random: ID). miRPath, TargetScan, and miRTarBase were used to identify putative genes and pathways targeted by miR-127.

Bovine MSCs were extracted from subcutaneous adipose tissue or skeletal muscle from four 5-month-old male Holstein-Friesian calves. Cells were transfected with miR-127 mimic, miR-127 inhibitor, or scramble nucleotide sequence control using HiPerFect transfection reagent (Qiagen), using the manufacturer's fast-forward protocol. To quantify cell proliferation, MTT assay was performed and absorbance read at 550nm. For adipogenesis, induction media was applied for 14 days containing DMEM, 10% foetal bovine serum (FBS), 100 IU/ml penicillin, 100μg/ml streptomycin, 1μM dexamethasone, 10μg/ml insulin, 0.5mM 3-isobutyl-1-methylxanthine, and 100μM indomethacin. Lipids were stained using oil red O and quantified as the proportion of the image stained. For myogenesis, induction media containing DMEM, 2% FBS, 100 IU/ml penicillin, and 100µg/ml streptomycin was applied for four days. Cells were stained for nuclei (DAPI) and myosin heavy chain. Fusion index was calculated as the proportion of nuclei located within myotubes. Levels of miR-127, its predicted targets, and lineage-specific markers were quantified by RT-qPCR. MTT absorbance, immunostaining, and qPCR data were analysed using GLMM (fixed terms: treatment and time; random term: ID).

Results

Figure 1 shows a graphical representation of results for miR-127. Additionally, among nine studied miRNAs, eight were associated with age, longitudinal miR-126-3p levels were associated with first lactation somatic cell count (mastitis indicator) and second lactation milk yield, and cow miR-142-5p levels were associated with calving interval. Seven ratios in total were associated with a performance trait. miR-127 was widely expressed across calf tissues, with no significant associations between tissue type and miR-127 expression. In addition to the microRNAs shown in Figure 1, miR-127 was significantly ($P<0.05$) negatively correlated with miR-126-3p and miR-34a. In MSCs, adipogenesis and MAPK4 expression were not affected by miR-127 mimic or inhibitor.

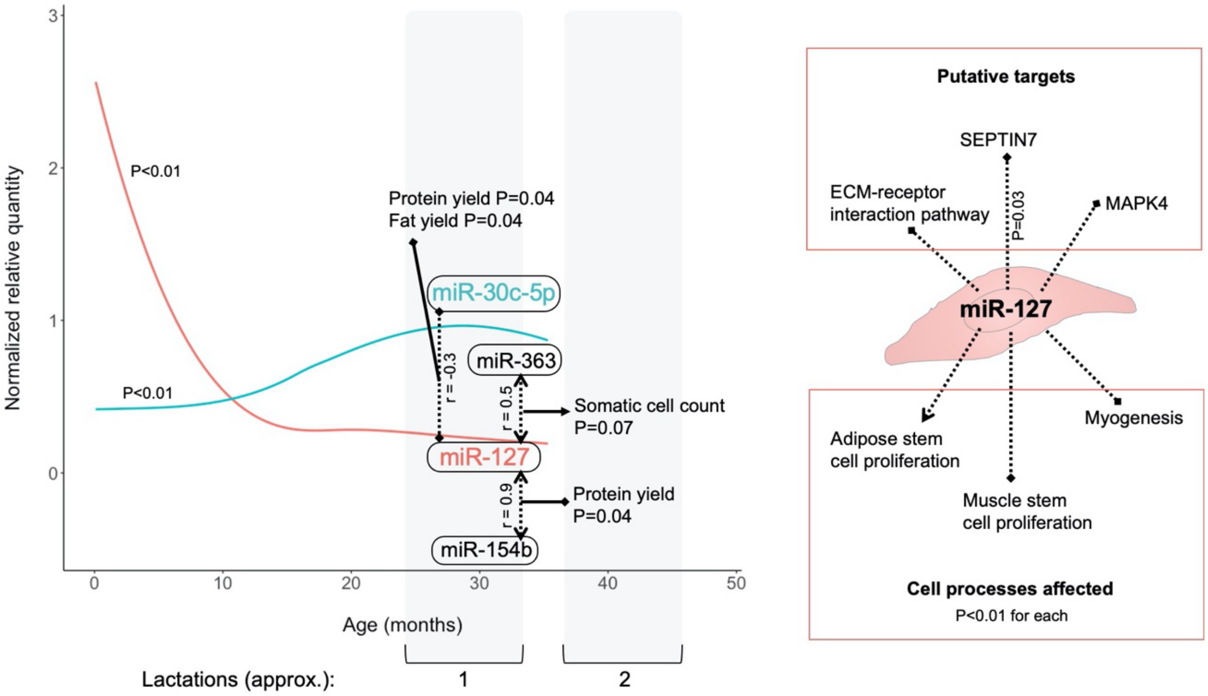

Figure 1. Graphical summary of miR-127 results. Lines with arrows indicate positive associations and lines with clubs indicate negative associations. Left: plasma miRNA quantity plotted against age. Loess curves show that on average, miR-127 decreased with age (red) and miR-30c-5p increased (blue). Correlations with some miRNAs are denoted by dotted lines with Spearman's rho. Loess curves of miR-154b and miR-363 closely resemble miR-127 (not shown). Solid lines indicate associations between the ratio of those two miRNAs, quantified in first lactation, with a trait. Right: potential targets and functions of miR-127 in bovine MSCs. Altering miR-127 levels during myogenesis significantly reduced fusion index and SEPTIN7 expression.

Conclusions

miR-127 was expressed widely in calves, decreased with age, and ratios involving miR-127 were associated with milk production or somatic cell count, suggesting potential as a biomarker. Together with results from bovine MSCs, this supports a role for miR-127 in stem cell function and warrants further investigation.

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Exploring relationships between milk urea nitrogen, blood urea nitrogen and nitrogen-use-efficiency using a multi-experiment dataset

A. Lavery[1], A. Gordon[2], C. Ferris[1]
[1]*Agri-food And Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*
[2]*Agri-Food and Biosciences Institute ,18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Anna Lavery. E-mail: anna.lavery@afbini.gov.uk

Application

Relationships between MUN and NUE within the current dataset were poor, suggesting that MUN could not be used as an 'on-farm' proximate measure of 'herd' NUE. The addition of data on dietary and cow factors might improve the prediction.

Introduction

Improving nitrogen-use-efficiency (NUE: defined as milk N output/N intake) is key to reducing the environmental impact of dairy systems. However, while NUE can be easily measured on research farms where information on N intake is available, on commercial farms NUE must be predicted. Both blood urea N (BUN) and milk urea N (MUN) are known to be indicators of protein nutrition status in dairy cows and as such have been used as predictors of NUE. While the use of BUN requires invasive blood sampling, MUN is normally determined within routine test-day milk sampling. While previous predictions have been developed using short-term relatively homogenous datasets, this study examined relationships between MUN, BUN and NUE using a multi-experiment dataset developed over almost three decades, and which involved large variability in both diet type (all grass silage-based) and cow genotype. Relationships were examined at both an individual 'cow' level and 'treatment' level.

Materials and Methods

This study involved a statistical re-assessment of aggregated individual cow data obtained from 39 studies conducted between 1996 and 2023 at the Agri-Food and Bioscience Institute (AFBI) in Hillsborough, Northern Ireland. Individual cow MUN data were obtained from monthly test-day milk sampling undertaken within studies (from 2008 onwards), while individual cow BUN data were obtained from occasional blood sampling undertaken within studies (according to protocols within individual experiments). Individual cow NUE was determined using daily data (N intake and milk N output) averaged for the 7-day period (day of sampling ± 3 days) around the date of blood/milk sampling. Treatment datasets were determined by averaging BUN, MUN and NUE data for all individual cows on each treatment for each week. The BUN dataset represented 1396 individual cows (704 primiparous, 983 multiparous) cows, while the MUN dataset represented 905 individual cows (408 primiparous and 662 multiparous cows). The BUN and MUN treatment mean dataset represented 116 and 56 treatments, respectively. Relationships between MUN, BUN and NUE were examined using individual cow data and treatment mean data (mean for each week) using regression analysis in Genstat v21 (VSN International Ltd).

Results

As expected, there were positive relationships between BUN and MUN levels with both the individual cow and treatment datasets, with a 1 mg/dl increase in BUN associated with a 1.75 (individual) and 1.65 (treatment) mg/dl increase in MUN content (Table 1). With the individual cow data there were weak negative relationships for both BUN and MUN, and NUE, with adjusted R^2 of 4.4 and 6.4, respectively. Similar relationships existed when treatment mean data was examined, with a 1 mg/dl increase in BUN associated with a proportional decrease of 0.009 in NUE. Similarly, a 1 mg/dl increase in MUN was associated with a proportional decrease of 0.004 in NUE. While assessing the data on a treatment mean basis did improve the adjusted R^2 of all regression equations, the R^2 of all relationships are low (R^2 <0.5) suggesting that they could not be used to provide an accurate prediction of NUE at farm level. Given that the current dataset encompasses 39 studies conducted over almost three decades, variations in diet and cow factors over that period may have contributed to the weak relationships observed. Previous studies suggest that MUN as a biomarker of NUE is most accurate when used under similar nutritional circumstances as those under which the models were developed (Godden et al., 2001). Ongoing work is examining if the inclusion of dietary variables and cow factors in the model can improve the relationships observed.

Table 1. Linear relationships between milk urea N (MUN, mg/dl), blood urea N (BUN, mg/dl) and nitrogen-use-efficiency (NUE, milk N/N intake) for individual cow and treatment mean datasets

Dataset	Equation†	*P*-value	Adjusted R^2‡
Individual cow			
	MUN = 3.756(0.245) +1.747(0.066) BUN	<0.001	34.6
	NUE = 0.344(0.001) +-0.006(0.002) BUN	<0.001	4.4
	NUE = 0.346(0.002) +-0.003(0.002) MUN	<0.001	6.4
Treatment means			
	MUN = 4.085(0.323) +1.646(0.088) BUN	<0.001	40.5
	NUE = 0.358(0.002) +-0.09(0.0005) BUN	<0.001	15.8
	NUE = 0.361(0.003) +-0.004(0.0003) MUN	<0.001	15.7

†Standard errors shown in parentheses

‡Adjusted R^2 determined from the percentage variance

Conclusions

When relationships were examined using the current long-term and diverse dataset, both BUN and MUN appeared to have limited potential to predict NUE, either at an individual cow or treatment level.

Acknowledgements

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Residual Metabolizable Protein Intake as a Potential Indicator of Variation in Dietary N Use Efficiency Between Lactating Dairy Cows

C. Reynolds[1], L. Crompton[1]
[1]*University Of Reading, Reading, Berkshire RG6 6AH, UK*

Presenting author
Chris Reynolds. E-mail: c.k.reynolds@reading.ac.uk

Application

Estimates of residual metabolizable protein (MP) intake (MPI) were highly correlated with dietary N use efficiency (NUE) and feed efficiency (FE), and largely determined by N intake (NI) and milk protein yield, suggesting genetic selection for feed efficiency and milk protein yield will have positive effects on NUE in dairy cattle.

Introduction

Dietary NUE (milk N/NI) in lactating dairy cattle is relatively low, partly because of protein degradation in the rumen, with typically 75% or more of NI excreted in faeces and urine of lactating dairy cows. These manure N losses contribute to inventories of nitrate, nitrous oxide, and ammonia associated with milk production. As NUE is inversely related to NI, decreases in diet crude protein (CP) concentration are invariably associated with increased NUE. In previous studies (Reynolds et al., 2016) we observed considerable variation in NUE between individual animals, and the range in NUE between animals was similar for diets differing in CP concentration. This variation in NUE between animals suggests there is a genetic component to NUE as has been demonstrated for feed efficiency (milk energy yield/kg feed dry matter intake [DMI]) (Liu and VandeHaar, 2020). Residual feed intake (DMI minus predicted DMI based on estimated metabolizable energy [ME] or net energy requirement) is used to compare FE between individual animals. We hypothesized that calculation of residual MPI might provide a more appropriate estimate of animal variation in protein use efficiency than NUE, as animals do not have a CP requirement per se. Therefore, our objective was to compare estimates of residual MPI with NUE and FE and use multivariate regression to evaluate their primary determinants.

Materials and Methods

A total of 42 Holstein cows (mean parity and days in milk 3.6 and 131, respectively) were individually fed using Calan gates for 12 weeks to provide measurements of daily DMI and milk yield and weekly milk composition and body weight. Cows were offered a 16% CP diet during a 3-wk covariate period and then changed to one of 2 diets (n=21 each) formulated to provide 90% (14% CP) or 105% (18% CP) of MP requirements (Reynolds et al., 2016) for a further 9 weeks. Residual MPI was calculated as measured MPI minus MP requirements (milk protein production, maintenance, body weight change, and gestation) using weekly averages and Feed into Milk (FiM; Thomas, 2004). Residual ME intake (MEI) as a measure of FE was also calculated using MEI and FiM estimates of ME requirement. Pearson correlations of efficiency estimates and production parameters were determined using SAS. Weekly averages were analysed using Mixed Models procedures testing fixed effects of diet CP, week, and their interaction and random effects of cow, with week as a repeated effect. The mixed model also included average protein intake, milk yield, milk fat, protein, lactose, urea, and somatic cell concentrations, body weight (BWT), and parity as covariates.

Results

Within treatment diets, relative variation for residual MPI between cows was similar to variation for NUE and residual MEI. Pearson correlation analysis showed significant correlations between residual MPI and NUE (-0.710; $P < 0.001$; Figure 1) and residual MEI (0.836; $P < 0.001$). Residual MPI was greater ($P < 0.012$) for the 18% CP diet than the 14% CP diet (266 vs 35 g/d, respectively; SEM = 48), reflecting greater MPI relative to requirement for the higher CP diet, with covariate effects ($P < 0.007$) of protein intake, milk yield, milk protein, fat, and urea concentration, and BWT. Residual MEI was lower ($P < 0.001$) for the 18% CP treatment (-29.9 and 33.5 MJ/d for 18 and 14% CP diets, respectively), reflecting the increase in milk solids yield with higher diet CP. Using means for the last 3 weeks of measurements, the overall relationship between residual MPI and residual MEI had a relatively low R^2 (0.367), but within diet CP treatments there were positive relationships with higher R^2 values (0.874 and 0.691 for 14 and 18% CP, respectively). This is similar to the findings of Lui and VandeHaar (2020), who also observed a higher correlation between residual DMI and NUE within diet CP treatment groups.

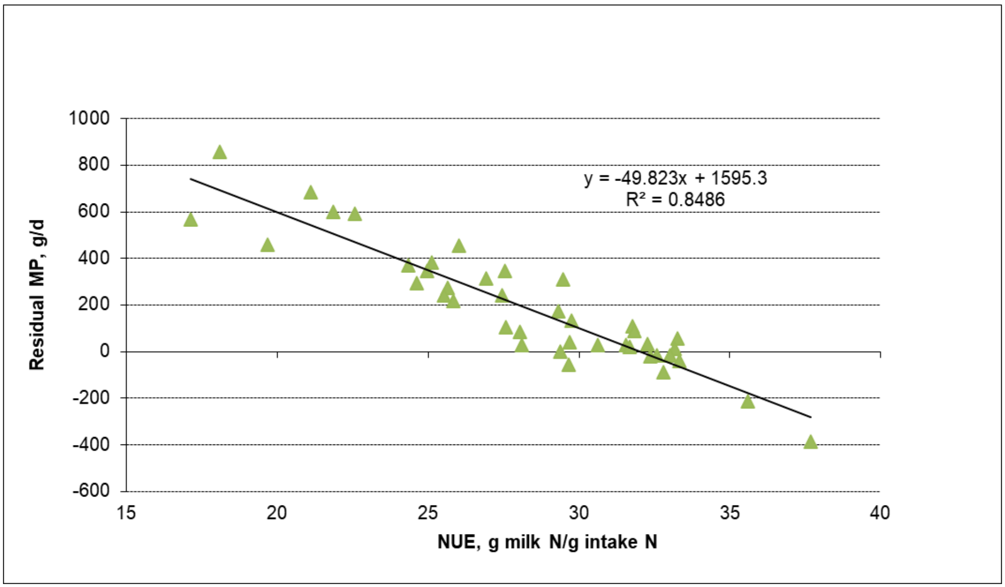

Figure 1. Relationship between residual metabolizable protein (MP) intake and N use efficiency (NUE) in lactating Holstein cows. Data are averages for the last 3 weeks of treatments.

Conclusion

The correlation between residual MPI, residual MEI, and NUE efficiency indicates that milk yield and protein concentration relative to DM and protein intake are shared primary determinants. Although residual MPI is a better reflection of the biological basis of differences in NUE between cows, these results suggest that it can be reliably estimated using measurable parameters (N intake and milk N yield). The correlation between NUE and milk yield, milk protein concentration, and feed efficiency suggests that continued genetic selection for these traits will contribute to improved breeding value for NUE.

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Effect of Injectable Trace Mineral Supplementation on Transition Health, Antioxidant and Trace Mineral Status in Dairy Cows

M. J. Ashar[1], P. Gonzalez-Rivas[2,3], F. Dunshea[3,4], C. Marth[5], S. Chauhan[1]
[1]School of Agriculture, Food and Ecosystem Science, University of Melbourne, Parkville VIC 3010, Australia
[2]Virbac Australia Pty Ltd, 361 Horsley Road, Milperra 2214 NSW, Australia
[3]School of Agriculture, Food and Ecosystem Science, University of Melbourne, Parkville VIC 3010, Australia
[4]Faculty of Biological Sciences, University of Leeds, Woodhouse, Leeds LS2 9JT
[5]Veterinary Biosciences, University of Melbourne, Parkville VIC 3010, Australia

Presenting author
Mohammad Javed Ashar. E-mail: jashar@student.unimelb.edu.au

Application

Injectable trace mineral supplementation pre-calving enhances antioxidant levels in dairy cows, potentially improving animal health, fertility and farm sustainability.

Introduction

The transition period, which extends from 3 weeks before to 3 weeks after parturition, is marked by a decrease in dry matter intake, high incidences of metabolic and infectious diseases, inflammation, immune dysregulation, and oxidative stress (Abuelo et al., 2019). Trace minerals are essential in dairy cows' metabolism, supporting immune and reproductive function, hormone synthesis, and energy and protein metabolism. Copper (Cu), zinc (Zn), manganese (Mn), and selenium (Se) are crucial components of metalloenzymes that assist in free radical detoxification and chromium (Cr) plays role in insulin function and stress mitigation. Injectable trace mineral supplementation (ITMS) is used as nutritional tool to improve mineral status of cattle prior to challenging events such as calving (Arthington and Ranches, 2021). The study hypothesized that pre-calving ITMS would enhance antioxidant status and improve the status of biomarkers of negative energy balance (NEB) and plasma trace minerals in multiparous dairy cows and heifers. The objective was to assess ITMS effects on plasma non-esterified fatty acid (NEFA), plasma beta-hydroxybutyric acid (BHBA), serum haptoglobin, erythrocyte glutathione peroxidase (GPx), and plasma trace minerals.

Materials and Methods

Fifty-five Holstein Friesian cows (41 multiparous and 14 heifers) were enrolled in a study at the University of Melbourne dairy farm, Dookie Campus, Southeastern Australia. The cows were stratified by body condition score and parity into two groups. During the dry-off period, they were fed cereal hay and pelleted feed, and after calving, they received a pelleted concentrate feed during milking alongside pasture grazing, without additional mineral supplements. Sixty days before expected calving, cows in the treatment group (ITMS) received a subcutaneous injection of trace mineral solution (Multimin Chrome, Virbac Australia; 1 mL/75 kg body weight for heifers under 2 years and 1 mL/100 kg for cows), while the control group (CNTRL) received an equal volume of isotonic saline. Blood samples were collected 60- and 21-days pre-calving and 7- and 14-days post-calving. Data were analysed using R v.4.3.2 with linear mixed-effects models. Baseline values from the first sampling were used as covariates, with group, sampling day, and parity as fixed effects, and animal ID as a random effect. Estimated marginal means (EMMs) were calculated for all factors and interactions, with pairwise comparisons conducted without multiple comparison adjustments. Trace mineral status was compared between 60 days pre-calving and 14 days post-calving.

Results

Plasma NEFA concentration was lower in the ITMS compared to the CNTRL group (*P* < 0.01). No significant differences were observed between treatment groups across different sampling days in multiparous cows. However, in heifers, NEFA concentration was lower in the ITMS compared to the CNTRL group with a tendency of lower NEFA 7 days after calving (*P* = 0.083) and significantly lower values at 14 days after calving (*P* < 0.05). Plasma BHBA concentration was unaffected by treatment overall, but the multiparous cows of the ITMS group exhibited a marked decline in BHBA on day 7 after calving (*P* = 0.018) with a trend towards a reduction on day 14 after calving (*P* = 0.063). Plasma BHBA was unaffected for heifers between the treatment groups across different sampling days. Erythrocyte GPx activity was significantly increased by ITMS (*P* < 0.0001). In multiparous cows, GPx was significantly higher in the ITMS than the CNTRL group at 14 days after calving (*P* < 0.001). In heifers, GPx was higher in the ITMS group on 21 days before calving, and 7 and 14 days after calving. Serum haptoglobin concentration was not affected by treatment overall, although it was higher in multiparous cows in the ITMS group 7 days after calving. No appreciable variation in haptoglobin was observed in heifers between the ITMS and the CNTRL group across different days of sampling. Overall, plasma Se concentration was higher in the ITMS compared to the CNTRL group (*P* < 0.05). Injectable trace mineral supplementation was found to significantly elevate Se 14 days after calving in both multiparous cows and heifers (*P* < 0.05 for both). Concentration of Cu, Zn and Mn in plasma was not affected by treatment.

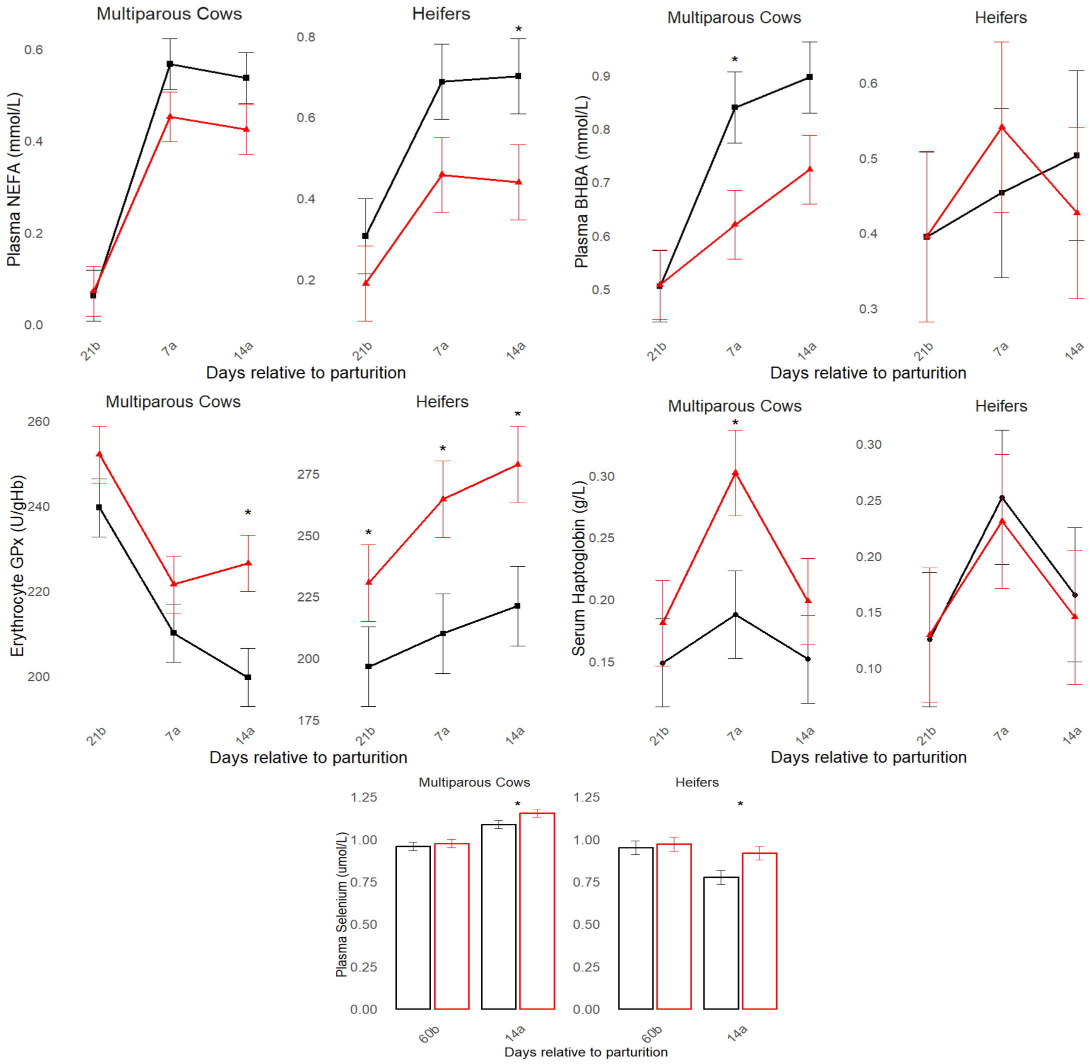

Figure 1. Effect of treatment on plasma NEFA, plasma BHBA, erythrocyte GPx, serum haptoglobin and plasma Se concentrations; red lines/bars show estimated marginal means (±SE) for cows treated with injectable trace minerals (ITMS) at dry-off, while black lines/bars represent control group (CNTRL); an asterisk (*) indicates p < 0.05. X- axis scale labels: 60b – 60 days pre-partum, 21b – 21 days pre-partum, 7a – 7 days post-partum and 14a – 14 days post-partum.

Conclusions

Pre-calving ITMS improved the post-partum metabolic and antioxidant profile of transition cows. Treatment reduced overall NEFA concentrations with a significant post-partum reduction in heifers, decreased post-partum BHBA concentrations in multiparous cows, increased Se concentrations and enhanced erythrocyte GPx activity thereby improving the antioxidant status. This reduction in NEFA and BHBA and improvement in the antioxidant status may lead to improved health, production and fertility outcomes.

Acknowledgements

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The effect of a water-based delivery of an Ascophyllum nodosum extract on milk production and nitrogen balance in mid-late lactation Holstein Friesian dairy cows

A. Casey[1], S. Vigors[1], T. Boland[1], F. Godwin[1], M. Markiewicz-Keszycka[1], M. Garcia-Vaquero[1], P. Hennessy[2], J.ohn O'Sullivan[3], Z. McKay[1]
[1]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*
[2]*Terra NutriTECH, Moone, Athy, Co. Kildare R14 YV25, Ireland*
[3]*BioAtlantis, Clash Industrial Estate, Clash East, Tralee, Co. Kerry V92 RWV5, Ireland*

Presenting author
Aaron Casey. E-mail: aaron.casey@ucdconnect.ie

Application

Understanding how supplementing dairy cows with an *Ascophyllum nodosum* extract via a water delivery system affects intake, milk production and nitrogen excretion can help optimize nutrient utilization and reduce environmental nitrogen losses in dairy systems.

Introduction

Nitrogen (N) losses from dairy production contribute to environmental pollution through nitrates in groundwater and emissions of ammonia and nitrous oxide into the atmosphere (Lahart et al., 2021). Ascophyllum nodosum (ASC) can reduce N degradation and ammonia-N accumulation *in vitro* by limiting ruminal proteolysis and N degradability (Wang et al., 2008, Belanche et al., 2016). However, limited research exists on the *in vivo* effects of ASC on N excretion and milk production (Antaya et al., 2019), while no studies have explored the use of extracted bioactive compounds from ASC administered to dairy cows. Furthermore, delivering an extract via a water-based delivery system offers a novel and more consistent and targeted approach, useful for pasture-based systems such as those common in Ireland. This study aimed to evaluate the effect of administering an ASC extract through a water-based delivery system on feed intake, milk production and total N excretion.

Materials and Methods

Nine multiparous Holstein Friesian dairy cows (*Bos taurus*) from the Spring calving dairy herd at UCD Lyons Farm were used in a replicated 3 × 3 Latin Square ($n = 9$). Cows were balanced for parity (3 ± 0.87), days in milk (179 ± 27), milk solids yield (2.03 ± 0.41 kg/d), and body condition score (3.03 ± 0.20). Cows were then randomly assigned to one of three dietary treatments: partial mixed ration diet (PMR) with no ASC extract (**Control**), or PMR with ASC extract at either 61.5 g DM/d (**Low**) or 125.3 g DM/d (**High**). The PMR comprised of grass silage and soybean meal, with 3.54 kg/d of concentrate supplemented during milking. Each 21-d period included 13 days of dietary acclimatization in a free-stall barn, followed by 8 days in metabolism stalls for environmental acclimatization and sampling. Seaweed extracts were administered via individual water troughs using a controlled water system. The PMR was offered twice daily using a Keenan diet feeder. Cows were milked twice daily, with milk samples collected for milk composition analysis. To facilitate the separate collection of urine and faeces, cows were fitted with harnesses, and daily samples of urine and faeces were collected for chemical analysis. Data were analysed using the MIXED procedure of SAS® studio. The model included the fixed effects of treatment, period and their interaction while pre-experimental body weight was included as a covariate.

Results

There was no effect of treatment on water or dry matter intake ($P > 0.05$). Cows offered the High treatment had greater yields of milk, fat, protein, milk solids and energy corrected milk relative to both the Control and Low treatment cows ($P < 0.05$). There was no effect on milk composition parameters ($P > 0.05$). There was an increase in milk N excretion, partitioning and nitrogen utilization efficiency

(NUE) for cows offered the High treatment relative to both the Control and Low treatment cows ($P < 0.05$). Urinary N excretion and partitioning was greater for cows offered the Low treatment in comparison to both the Control and High treatment cows ($P < 0.05$). Treatment did not impact faecal N excreted or partitioned ($P > 0.05$).

Table 1. The effect of *Ascophyllum nodosum* extract on water and feed intake, milk production, and milk composition parameters.

	Treatments[1]				*P*-value		
	Control	Low	High	SEM	Treatment	Period	Interaction[2]
Drinking water intake (L/d)	87.8	86.4	86.9	3.15	0.862	0.011	0.186
Total DMI[3] (kg/d)	20.7	20.5	20.9	0.37	0.376	0.001	NS
Milk production (kg/d)							
Milk yield	19.94^{a}	19.91^{a}	20.83^{b}	0.734	0.047	<0.001	NS
Fat	1.20^{a}	1.16^{a}	1.25^{b}	0.059	0.001	0.002	NS
Protein	0.82^{a}	0.83^{a}	0.87^{b}	0.033	0.003	0.017	0.174
Lactose	0.90	0.90	0.94	0.030	0.076	<0.001	0.215
Milk solids	2.04^{a}	2.00^{a}	2.13^{b}	0.093	0.005	0.007	NS
Energy corrected milk	27.5^{a}	27.5^{a}	29.3^{b}	1.28	0.002	0.002	NS
Milk composition (%)							
Fat	5.99	5.86	5.79	0.155	0.430	0.012	NS
Protein	4.13	4.15	4.17	0.037	0.306	<0.001	NS
Lactose	4.50	4.51	4.50	0.040	0.644	NS	NS

$^{a-b}$ Within a row, means with different superscripts differ ($P < 0.05$).

[1] Control = 0 g/kg DM of ASC extract; Low = 2.93 g/kg DM of ASC extract; High = 5.88 g/kg DM of ASC extract.

[2] Treatment x Period; [3] Dry matter intake.

Table 2. The effect of *Ascophyllum nodosum* extract on nitrogen excretion.

	Treatments[1]				*P*-value		
	Control	Low	High	SEM	Treatment	Period	Interaction[2]
Intake (kg/d)							
Feed N	0.598	0.590	0.597	0.0110	0.524	<0.001	NS
N output (kg/d)							
Milk	0.129^{a}	0.129^{a}	0.136^{b}	0.0052	0.005	0.019	0.139
Faeces	0.162	0.158	0.168	0.0046	0.093	NS	NS
Urine	0.199^{a}	0.229^{b}	0.198^{a}	0.0059	0.005	NS	0.078
N partitioning							
Milk	0.219^{a}	0.219^{a}	0.228^{b}	0.0076	0.044	NS	NS
Faeces	0.272	0.268	0.281	0.0066	0.165	0.027	NS
Urine	0.345^{a}	0.381^{b}	0.334^{a}	0.0100	0.013	0.034	0.100
NUE[3] (%)	21.72^{a}	21.81^{a}	22.66^{b}	0.752	0.035	0.161	NS

$^{a-b}$ Within a row, means with different superscripts differ ($P < 0.05$).

[1] Control = 0 g/kg DM of ASC extract; Low = 2.93 g/kg DM of ASC extract; High = 5.88 g/kg DM of ASC extract.

[2] Treatment x Period; [3] Nitrogen utilization efficiency

Conclusions

Including an *Ascophyllum nodosum* extract through a water system to mid-late lactation dairy cows did not impact water or dry matter intake. Milk production parameters and NUE were improved for cows offered the High treatment while urinary N excretion was increased for cows offered the Low treatment.

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The effect of concentrate crude protein concentration and supplementation level on the milk production of autumn-calving, late-lactation, grazing dairy cows

N. Maher[1,2], A. Lawless[3], K...M Pierce[2], M. Dineen[1]
[1]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin D04 V1W8, Ireland*
[3]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*

Presenting authors
Neil Maher. E-mail: neil.maher@teagasc.ie

Application

When autumn-calving, late-lactation, grazing dairy cows were offered low concentrate supplementation levels, concentrate crude protein concentration had no effect on milk production. However, when cows were offered higher supplementation levels, reducing the concentrate crude protein concentration reduced milk protein concentration, milk protein yield and milk solids yield.

Introduction

Reducing the crude protein concentration of the diet of dairy cows has been shown to decrease nitrogen excretion, lowering the potential for negative environmental impact (Hynes et al., 2016). However, there is a concern that reducing dietary crude protein may lead to a limitation in the supply of specific amino acids and therefore affect milk production. Previous experiments have demonstrated no effect of reducing the concentrate crude protein concentration on milk production when fed to grazing spring-calving cows during early-mid lactation (Mulligan et al., 2004; Hynes et al., 2016), during late-lactation (Doran et al., 2022) or autumn-calving cows during mid-lactation (Burke et al., 2008). These experiments reported a range in pasture crude protein concentration typically higher than the cows' requirement (180-240 g/kg DM); however, during the summer period, pasture crude protein concentration can be quite lower. When evaluating the effect of low crude protein concentrates, it is important to consider the supplementation level as greater supplementation levels will have a greater effect on overall dietary crude protein concentration. It is also critical to quantify the milk response and economic viability of concentrate supplementation to late-lactation cows within pasture-based systems. Therefore, the objective of this experiment was to investigate the effect of concentrate crude protein concentration and supplementation level on the milk production of autumn-calving, late-lactation, grazing dairy cows.

Materials and Methods

This experiment was conducted at the Teagasc Johnstown Castle Farm (Co. Wexford, Ireland) from May to July, over 2 grazing seasons (2023 and 2024). Sixty-four multiparous and 20 primiparous autumn-calving, late-lactation (211 ± 23 days in milk) Holstein-Friesian dairy cows were enrolled in a 2-wk covariate period. At the end of the covariate period, cows were blocked based on pre-experimental milk production and parity and were randomly assigned to 1 of 4 dietary treatments (n=21). The data collection periods lasted 8 and 7 weeks during 2023 and 2024, respectively. The dietary treatments were pasture supplemented with; 1) 0.89 kg of dry matter (DM)/cow per day of concentrate with a crude protein (CP) of 95 g/kg DM (**LL**); 2) 0.89 kg DM/cow per day of concentrate with a CP of 170 g/kg DM (**LH**); 3) 2.67 kg DM/cow per day of concentrate with a CP of 95 g/kg DM (**HL**); and 4) 2.67 kg DM/cow per day of concentrate with a CP of 170 g/kg DM (**HH**). Individual daily milk yields were recorded. Weekly milk samples were analysed for composition using a MilkoScan 7 (Foss Electric). All data were analysed in a repeated measures model using the MIXED procedure of SAS (version 9.4). The model included the fixed effects of diet, week, their interaction and parity, with an appropriate covariate adjustment applied for each cow. Experimental week was included as a repeated effect and cow was included as a random effect. Multiple comparisons between treatment means were made using the Tukey–Kramer method. Significance was considered if $P \leq 0.05$.

Results

Cows fed HH had greater milk yield when compared with cows fed LL and LH ($P < 0.01$) but were similar to cows fed HL. There was no effect of diet on milk fat concentration; however, milk protein concentration was greater for cows fed HH when compared with cows fed HL ($P = 0.045$). Cows fed HH had greater milk fat yield when compared with cows fed LL but were similar to cows fed LH and HL. Cows fed HH had greater milk protein yield and milk solids yield when compared with cows fed LL, LH and HL ($P < 0.01$). Cows fed LL, LH and HL had similar milk production and composition (Table 1).

Table 1. Effect of concentrate crude protein concentration and supplementation level on milk production and milk composition of autumn-calving, late-lactation, grazing dairy cows

	Diet[1]					
Item	LL	LH	HL	HH	SEM	*P*-value
Milk yield, kg/d	17.3^{b}	17.8^{b}	18.0^{ab}	18.8^{a}	0.30	<0.01
Fat, g/kg	45.5	45.9	45.0	44.6	0.69	0.23
Protein, g/kg	39.0^{ab}	38.6^{ab}	38.4^{b}	39.1^{a}	0.29	0.05
Fat yield, kg/d	0.78^{b}	0.81^{ab}	0.80^{ab}	0.83^{a}	0.02	0.02
Protein yield, kg/d	0.67^{b}	0.68^{b}	0.69^{b}	0.73^{a}	0.01	<0.01
Milk solids yield, kg/d	1.45^{b}	1.49^{b}	1.49^{b}	1.56^{a}	0.02	<0.01

[1] LL = pasture + 0.89 kg of DM/day of a 95 g/kg of DM CP concentrate; LH = pasture + 0.89 kg DM/day of a 170 g/kg of DM CP concentrate; HL = pasture + 2.67 kg DM/day of a 95 g/kg of DM CP concentrate; HH = pasture + 2.67 kg DM/day of a 170 g/kg of DM CP concentrate

[a-b] Means within row with different superscripts are significantly different ($P < 0.05$)

Conclusions

During the summer period, when autumn-calving cows were in late-lactation, lowering the crude protein concentration of the concentrate reduced milk production performance when cows were offered 2.67 kg DM/day of concentrate. There was no effect when cows were offered 0.89 kg DM/day of concentrate. Further analysis is required to determine the nutritive value of the pasture offered, as well as, the metabolic nitrogen status of the cows.

Acknowledgements

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The relationship between milk fat globule size, structure of its membrane and the dairy cow diet

K. Kliem[1], S. Bellamy[1]
[1]*University Of Reading, Reading, Berkshire RG6 6AH, UK*

Presenting author
Kirsty Kliem. E-mail: k.e.kliem@reading.ac.uk

Application

There is current interest in the human health benefits of the milk fat globule (MFG) and its membrane. It has been suggested that MFG size and composition could be influenced by dairy cow diet. This pilot study reported a relationship between globule size and certain membrane fatty acids (FA), the availability of which could be changed by altering the cow diet.

Introduction

Fat in milk is present in the form of a MFG, which comprises a triglyceride core surrounded by a phospholipid-rich membrane (MFGM). Some of these phospholipids have been reported to have a wide range of beneficial effects on human health (Anto et al., 2020). Milk is a good dietary source of these phospholipids, and certain cow diets have been found to increase milk phospholipid content (Argov-Argaman et al., 2014), possibly by decreasing MFG size. In addition, recent research has reported that smaller MFG from unhomogenised milk can have a direct beneficial effect on the gut microbiome, through provision of metabolic support for bacterial growth via metabolites in the MFGM (Raz et al., 2025). It has been hypothesised that the presence of certain MFGM FA, such as *cis*-9 18:1, may drive the mechanism that increases the size of the globule (Cohen et al., 2015), but this has not been fully elucidated. This pilot study aimed to determine whether there was a relationship between MFG size and FA profile of the MFGM phospholipids (in particular *cis*-9 18:1), in milk from cows fed two different diets.

Materials and Methods

Milk was sampled during afternoon milking from 20 cows at the Centre for Dairy Research, University of Reading. The cows were consuming one of two diets; fresh grass only (n=10; mean ± s.e.m. 309 ± 22.2 days in milk), or a total mixed ration (TMR, 50:50 forage:concentrate) diet (n=10; 82 ± 10.4 days in milk). Within 24 h of collection milk was analysed for MFG size using laser diffraction. The remaining milk was analysed for phospholipid FA profile, with initial extraction using diethyl ether/hexane (Kliem et al., 2013), solid phase extraction to isolate the phospholipids and then methylation of extracted FA (Burdge et al., 2000). Methylated phospholipid FA were separated and identified using gas chromatography (Kliem et al., 2013). The effect of diet on MFG size and phospholipid FA profile was assessed using a one-way ANOVA , and the relationship between MFG size and phospholipid FA profile across all samples was determined using Pearson's correlation coefficient. Results were reported as significant when $P < 0.05$.

Results

There was no effect ($P > 0.05$) of diet on MFG size or total milk fat (Table 1). Diet did however affect phospholipid FA profile, with milk from grass-fed cows containing a greater ($P < 0.05$) proportion of 18:3 n-3 and n-3 polyunsaturated FA, and TMR-derived milk containing a greater ($P < 0.05$) proportion of 20:3 n-6 and 20:4 n-6 (Table 1). There was also a tendency ($P = 0.088$) for TMR milk to have a higher proportion of 18:2 n-6 in the phospholipids (probably due to presence of maize silage and fat supplement in the TMR), and for grass milk to have a higher ($P = 0.087$) proportion of conjugated linoleic acids. There were correlations between some of the FA proportions and MFG size. Higher proportions of 16:0 in milk phospholipids were associated with larger MFG sizes (Pearson r =

0.601; Figure 1a) whereas higher proportions of *cis*-9 18:1 were associated with smaller MFG sizes (Pearson r = -0.649; Figure 1b).

Table 1. Effect of dairy cow diet on milk fat globule size, milk fat content and milk fat phospholipid fatty acid (FA) profile.

	Diet[1]			
	Grass	TMR	SEM	*P*[2] Treatment
Milk fat globule size (uM)	4.4	4.4	0.25	0.984
Milk fat (mg/g milk)	46.4	43.9	2.18	0.507
Phospholipid FA profile (g/100 g FA):				
12:0	2.1	2.2	0.18	0.893
14:0	10.6	10.9	0.30	0.680
16:0	37	39	0.7	0.194
18:0	8.8	8.4	0.26	0.494
18:1 *cis*-9	21	19	0.8	0.258
18:2 n-6	2.7	3.0	0.09	0.088
18:3 n-3	0.56	0.45	0.023	0.021
20:3 n-6	0.13	0.17	0.006	0.011
20:4 n-6	0.19	0.21	0.008	0.026
20:5 n-3	0.11	0.08	0.013	0.229
22:5 n-3	0.10	0.11	0.010	0.537
Σ CLA[3]	0.39	0.34	0.015	0.087
Σ n-3 PUFA[3]	0.88	0.71	0.032	0.015
Σ n-6 PUFA	3.4	3.7	0.09	0.113

[1] Cows consumed either fresh grass only, or TMR – total mixed ration.

[2] Significance of the effect of diet treatment for n=10 cows.

[3] CLA – conjugated linoleic acids; PUFA – polyunsaturated fatty acids.

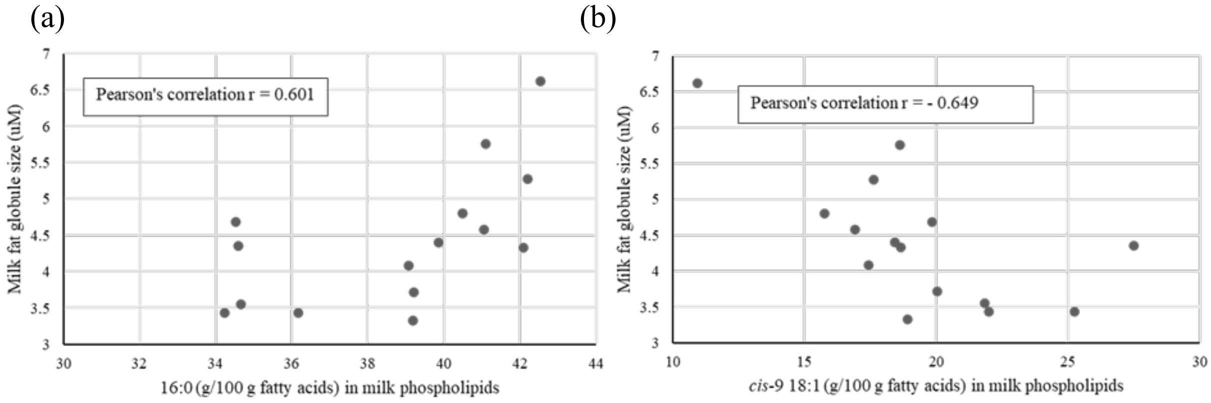

Figure 1. Correlation between milk fat globule size and milk phospholipid proportion (g/100 fatty acids) of (a) 16:0, and (b) *cis*-9 18:1, in milk from 20 dairy cows.

Conclusions

In this pilot study, there was no effect of the two diet types on MFG size. However there were correlations between FA profile and MFG size which were not consistent with hypotheses from previous studies; this could be due to the presence of other FA in mammary epithelial cells which contribute to the synthesis of MFGM. Future research into optimising MFG size and phospholipids needs to include an assessment of the phospholipid families present in MFG, particularly in milk from cows fed diets high in different unsaturated fatty acids.

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Enhancing solid-state fermentation of sunflower meal: optimizing duration of fermentation with synergistic probiotic strains

A. Abdulrahman[1,2], K. Kliem[1], C. Rymer[1]
[1]*University Of Reading, Reading, Berkshire RG6 6AH, UK*
[2]*University of Duhok, Zakho Rd, Duhok, Duhok Governerate 1006 AJ, IQ*

Presenting author
Ahmed Abdulrahman. Email: a.m.abdulrahman@pgr.reading.ac.uk

Application

Solid-state fermentation with Bacillus subtilis enhances the nutritional value of sunflower meal for use in poultry feeds by lowering fibre content, providing a cost-effective protein alternative to soyabean meal. This approach reduces production costs and supports environmental sustainability.

Introduction

Feed expenses represent about 60-70% of the total costs in poultry farming (Adesehinwa, 2007), with soybean meal (SBM) traditionally used as the main protein source. However, reliance on SBM has become problematic due to environmental issues, including deforestation and substantial water usage (Ferreira et al., 2016), making it essential to explore other options. Sunflower meal (SFM) is emerging as a more affordable alternative (Ciurescu et al., 2019), but its high fiber content poses challenges in poultry diets by reducing nutrient absorption and potentially affecting bird growth and health (Villamide & San Juan, 1998). A promising solution to improve the nutritional profile of SFM involves fermenting it with microorganisms such as bacteria and yeast. A study we carried out in our lab found that fermenting SFM with a *Bacillus subtilis* preparation reduced the total fibre content and increased levels of certain essential amino acids, compared to samples without added microorganisms. This experiment aimed to optimize the duration of SSF for sunflower meal using various probiotic strains both individually and in combination. Neutral detergent fiber (NDF) used as an indicator, and amino acid content was also measured as part of the evaluation.

Materials and methods

This experiment aimed to compare two probiotic strains, *Bacillus subtilis* (treatment A) and *Saccharomyces cerevisiae* strain K5-5A (B); both obtained from the National Centre for Biotechnology Education, University of Reading, with two commercial strains, 11CFT (C) and 11GFT (D); purchased from Corteva Agriscience). Individual cultures (A and B) were inoculated at 5% (v/w) per 5 g sample, whereas commercial probiotics (C and D) were inoculated as per manufacturers' recommendations (2.4% v/w). For treatment combinations the equivalent doses were used (e.g., for ABCD 5+5+2.4+2.4%). Sunflower meal was adjusted to 80% moisture and autoclaved. Treatments and combinations (including controls of just SFM) were incubated at 30°C in duplicate, with sampling every two days, over a 14-day period. Samples were then freeze-dried and stored at -20°C until analysis. NDF was determined following a protocol using Ankom 200 Fiber Analyzer. Amino acids were analysed after hydrolysis in 6M HCl in an atmosphere of nitrogen, with separation based on mass-to-charge ratio (m/z) using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer with Nexera UPLC, without derivatization. Data were analyzed using one-way ANOVA within the GLM procedure in Minitab version 22.1, with Tukey's test for post hoc comparisons. The independent variable was treated as a fixed factor. Differences were considered significant if $p < .05$.

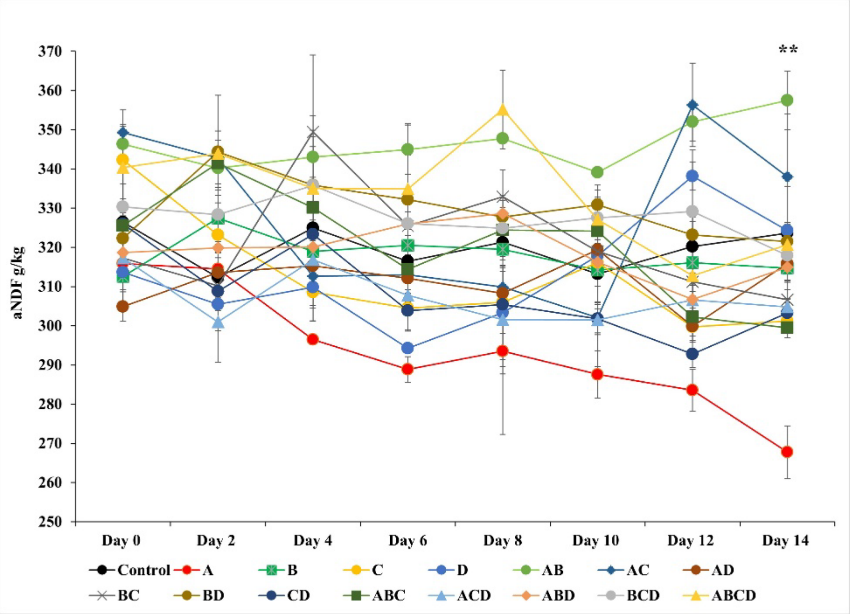

Figure 1. Impact of 14-day fermentation and microbial inoculum on the aNDF content in Sunflower Meal. **A:** *Bacillus subtillis*, **B:** *Saccharomyces cerevisiae* Strain K5-5A, **C:** 11CFT (*Lactobacillus buchneri* ATCC PTA-6138, and *Lactobacillus plantarum* ATCC 55944), **D:** 11GFT (*Lactobacillus buchneri* ATCC PTA-6138, *Lactobacillus plantarum* ATCC PTA-6139, and *Lactobacillus plantarum* ATCC 55944). **aNDF:** neutral detergent fibre corrected for ash. ** significant differences at p < .001.

Table 1. Effect of microbial inoculum (alone and in combination) on the amino acid and aNDF contents (g/kg DM) of sunflower meal following a two-week period of solid-state fermentation.

Treatments	Control	A	B	C	D	AB	AC	AD	BC	BD	CD	ABC	ACD	ABD	BCD	ABCD	SEM	*p-values*
aNDF	324^{abc}	268^{d}	315^{bc}	301^{bcd}	324^{abc}	358^{a}	338^{ab}	316^{bc}	307^{bc}	322^{abc}	303^{bcd}	300^{cd}	305^{bcd}	315^{bc}	318^{bc}	321^{abc}	6.64	<0.001
Lysine	14.9^{bcd}	13.3^{e}	13.90^{de}	13.5^{e}	11.3^{f}	13.1^{e}	12.9^{e}	15.2^{bc}	15.9^{ab}	16.7^{a}	15.6^{ab}	14.2^{cde}	15.2^{bc}	15.5^{abc}	15.0^{bcd}	13.7^{de}	0.26	<0.001
Arginine	35.9^{a}	16.3^{g}	31.8^{bc}	30.1^{cd}	24.3^{ef}	31.4^{bc}	23.0^{f}	31.2^{bc}	33.6^{ab}	33.4^{ab}	32.2^{bc}	32.9^{abc}	29.9^{cd}	31.6^{bc}	32.1^{bc}	27.2^{de}	0.58	<0.001
Threonine	15.7^{abc}	11.6^{g}	15.6^{abc}	14.2^{cdef}	15.3^{bcd}	15.1^{bcde}	11.8^{g}	13.2^{efg}	16.7^{ab}	17.4^{a}	16.0^{abc}	14.6^{cdef}	15.9^{abc}	15.8^{abc}	13.0^{fg}	13.5^{defg}	0.38	<0.001
Valine	18.2^{efg}	16.2^{h}	18.5^{def}	18.1^{efg}	16.3^{h}	18.8^{bcde}	16.5^{h}	18.7^{cde}	19.2^{bcd}	19.6^{b}	19.4^{bc}	17.5^{g}	18.2^{efg}	19.7^{b}	21.3^{a}	17.8^{fg}	0.17	<0.001
Leucine	18.2^{d}	15.0^{g}	18.4^{d}	18.5^{cd}	16.6^{ef}	17.1^{e}	14.4^{g}	16.0^{f}	20.5^{b}	20.5^{b}	20.6^{b}	17.9^{d}	19.1^{c}	20.6^{b}	24.0^{a}	20.7^{b}	0.13	<0.001
Isoleucine	18.0^{de}	14.9^{i}	18.2^{de}	18.5^{cd}	16.6^{gh}	17.0^{fg}	14.6^{i}	16.0^{h}	20.4^{b}	20.6^{b}	20.4^{b}	17.6^{ef}	19.2^{c}	20.7^{b}	24.0^{a}	20.6^{b}	0.13	<0.001
Histidine	9.7^{ab}	5.4^{f}	8.7^{cd}	9.0^{cd}	8.4^{d}	9.3^{bc}	6.6^{e}	8.8^{cd}	10.0^{ab}	10.3^{a}	10.3^{a}	8.9^{cd}	9.8^{ab}	10.2^{a}	9.7^{ab}	8.4^{d}	0.13	<0.001
Phenylalanine	16.4^{d}	13.4^{f}	16.9^{c}	17.2^{bc}	15.2^{e}	13.7^{f}	12.0^{g}	13.3^{f}	17.3^{bc}	17.4^{b}	17.4^{b}	16.1^{d}	16.1^{d}	17.3^{bc}	20.6^{a}	17.6^{b}	0.08	<0.001

Data were analysed using one-way ANOVA within the GLM procedure in Minitab version 22.1. Different letters within the same row indicate significant differences at p<.05. Tukey's test was used for post hoc comparisons.

Results

During the first 12 days of fermentation, there was no difference between treatments for NDF content (Fig. 1). However, by day 14, *Bacillus subtilis* fermentation resulted in lower ($P<0.05$) NDF content compared with the control (Table 1). After 14 days fermentation lower ($P<0.05$) lysine concentrations were observed for treatments A, B, C, D, AB, AC, ABC, and ABCD, relative to the control. Arginine concentrations were also lower ($p < .05$) across most treatments, particularly in groups A, D, AC, and ABCD. Similarly, SFM threonine and histidine content was lower (P<0.05) with treatments A and AC, compared with control. Conversely, the combination fermentation of *S. cerevisiae* and the two commercial strains (treatment BCD) improved (P<0.05) the concentration of valine, leucine, isoleucine, and phenylalanine.

Conclusion

After 14 days of fermentation, *B. subtilis* (A) decreased the NDF content of sunflower meal, and there was no effect of other treatments either alone or in combination. However, essential amino acids were also lower in several treatment groups (including *B. subtilis* fermentation). Although *B. subtilis* shows promise as an effective microbial candidate to improve the nutritional quality of SFM for poultry diets, changes in essential amino acid content must be considered.

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In vitro fermentation of high-fibre sunflower meal: a potential protein source for poultry feed

A. Abdulrahman[1,2], K. Kliem[1], C. Rymer[1]
[1]*University Of Reading, Reading, Berkshire RG6 6AH, UK*
[2]*University of Duhok, Zakho Rd, Duhok, Duhok Governerate 1006 AJ, IQ*

Presenting author
Ahmed Abdulrahman. E-mail: a.m.abdulrahman@pgr.reading.ac.uk

Application

Fermenting high-fibre sunflower meal with *Bacillus subtilis* offers a sustainable, cost-effective protein alternative to soybean meal in poultry feed.

Introduction

Feed costs represent 60–70% of poultry production expenses (Adesehinwa, 2007), with soybean meal (SBM) being a key protein source. However, SBM is environmentally unsustainable due to issues like deforestation and high water use (Ferreira et al., 2016), necessitating viable alternatives. Sunflower meal (SFM) offers a cost-effective option (Ciurescu et al., 2019), but its high fibre content limits digestibility and may impair poultry performance (Villamide & San Juan, 1998). Fermentation using microorganisms such as bacteria and yeast has emerged as a promising method to enhance SFM's nutritional profile by breaking down fibre and improving digestibility. This study explored the in vitro fermentation of high-fibre SFM with microorganisms, aiming to develop it as a sustainable alternative protein source for poultry.

Materials and methods

This experiment followed a 4x2x2 factorial design to investigate the effects of three microorganisms (*Bacillus subtilis*, *Cellulomonas sp.*, and *Saccharomyces cerevisiae* strain K5-5A) compared with a negative control. Two moisture levels (80% and 100%) were applied during *in vitro* fermentation of SFM, with fermentation durations of 4 and 8 days. Microbial slope cultures (obtained from the National Centre for Biotechnology Education, University of Reading). Samples in triplicate were inoculated with 10% (v/w) active yeast/bacteria; controls were inoculated with sterilised water. Flasks were incubated at 30ºC. Samples were collected on days 4 and 8, freeze dried for 65 h, and stored at -20°C for further analysis. Crude protein (CP) content was determined using reference method AOAC 2001.11 (AOAC, 2005), while phytic acid and total dietary fiber (TDF) were determined using a commercially available kit (K-PHYT and K-RINTDF, Megazyme, Ireland). Neutral detergent fiber (NDF) was measured using an Ankom 200 Fiber Analyzer. Amino acid content was analysed after hydrolysis with 6M HCl in an atmosphere of nitrogen, followed by separation using Shimadzu LCMS-8050 triple quadrupole mass spectrometer with Nexera UPLC based on their mass-to-charge ratio (m/z) without derivatisation. Data were analysed using a three-way ANOVA within the GLM procedure in Minitab version 22.1 with fixed effects of microorganisms, moisture levels, and fermentation durations, with Tukey's test for post hoc comparisons. The independent variables were treated as fixed factors. Differences were considered significant if *p* < 0.05.

Table 1. Effect of fermentation conditions (microorganism, moisture level and period) on chemical composition of *in vitro*-fermented high fibre sunflower meal (g/kg DM).

	Microorganisms						Moisture level				Fermentation period			
Composition	Control	*B. subtilis*[1]	*S. cerevisiae*[2]	*C. sp.*[3]	SEM	*P-values*	80%	100%	SEM	*P-values*	4 days	8 days	SEM	*P-values*
CP	237^{b}	243^{a}	239^{ab}	238^{b}	1.19	0.014	240	239	0.84	0.298	250	229	0.84	<0.001
TDF	659^{a}	645^{b}	649^{ab}	650^{ab}	2.63	0.004	652	650	1.86	0.468	628	674	1.86	<0.001

aNDF	490	483	484	487	3.52	0.441	482	490	2.49	0.020	470	502	2.49	<0.001
PA	30.7^{a}	30.4^{ab}	27.1^{c}	29.5^{b}	0.27	<0.001	29.6	29.2	0.19	0.164	28.7	30.2	0.19	<0.001

Data were analyzed using a three-way ANOVA within the GLM procedure in Minitab version 22.1. Different letters within the within each of micro-organisms, moisture level, fermentation period significant differences at p < .05. [1] *Bacillus subtilis*. [2] *Saccharomyces cerevisiae* Strain K5-5A. [3] *Cellulomonas sp*. CP: crude protein, TDF: total dietary fibre, aNDF: neutral detergent fibre corrected for ash, PA: phytic acid.

Table 2. Effect of interactions between fermentation conditions (microorganism and period) on selected amino acid contents of *in vitro*-fermented high-fibre sunflower meal (g/kg DM).

Interaction: Microorganisms × Fermentation period

	Control		*B. subtilis*[1]		*S. cerevisiae*[2]		*C. sp*[3]		SEM	*P-values*
	4 d	8 d	4 d	8 d	4 d	8 d	4 d	8 d		
Lysine	7.2^{a}	5.9^{bc}	7.8^{a}	5.8^{bc}	7.4^{a}	6.2^{b}	6.1^{b}	5.4^{c}	0.12	0.002
Arginine	7.9^{a}	4.8^{de}	7.6^{a}	5.7^{c}	7.9^{a}	5.1^{d}	6.7^{b}	4.4^{e}	0.10	<0.001
Valine	10.1^{a}	6.2^{ef}	9.2^{bc}	7.2^{d}	9.7^{ab}	6.9^{de}	8.8^{c}	5.9^{f}	0.15	<0.001
Histidine	4.3^{a}	2.9^{d}	4.2^{a}	3.3^{c}	4.4^{a}	3.3^{c}	3.8^{b}	2.8^{d}	0.06	0.004

Data were analyzed using a three-way ANOVA within the GLM procedure in Minitab version 22.1. Different letters within the same row indicate significant differences at p < .05. Tukey's test was used for post hoc comparisons. **Con.** (control: no probiotics added)**, A:** *Bacillus subtilis.* **B**: *Saccharomyces cerevisiae* Strain K5-5A. **C:** *Cellulomonas sp.*

Results

Fermenting sunflower meal (SFM) with *Bacillus subtilis* significantly increased crude protein and reduced total dietary fibre compared to unfermented SFM, while fermentation with *Saccharomyces cerevisiae* significantly reduced phytic acid content (P<0.05, Table 1). Total dietary fibre (TDF), neutral detergent fibre (aNDF), and phytic acid (PA) increased with 8 days of fermentation. No significant interactions were observed between microorganism, moisture level, and fermentation period for chemical composition. However, interactions between treatments influenced specific amino acids (Table 2). Four-day fermentation with *B. subtilis* or *S. cerevisiae* significantly enhanced lysine, arginine, and histidine content compared to fermentation with *Cellulomonas sp.* (*P*<0.05). Essential amino acid levels decreased after 8 days of fermentation compared to 4 days (*P*<0.05). Fermentation with *B. subtilis* at both moisture levels yielded significantly higher leucine, isoleucine, and phenylalanine compared to other microorganisms or unfermented SFM (*P*<0.05).

Conclusion

Fermenting high-fiber SFM with *Bacillus subtilis* enhances crude protein and reduces dietary fiber, while *Saccharomyces cerevisiae* decreases phytic acid levels. Fermentation for 8 days significantly increased TDF, aNDF, and PA. This might be attributed to reduced crude protein after 8 days, altering nutrient concentrations, as TDF was adjusted for ash and protein, while aNDF was corrected for ash. Four-day fermentation boosts essential amino acids like lysine and arginine, particularly with *B. subtilis*, making it a promising approach to improve SFM's nutritional value as a poultry protein source.

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The association of intestinal dysbiosis, gut permeability and systemic bacterial infection in pedigree broiler chickens

M. Vasilogianni[1], D. Stuart[3], R. Hawken[4], J. Williams[1], D. Xia[1], A. Psifidi[2], D. Blake[1]
[1]Royal Veterinary College, Department of Pathobiology and Population Sciences, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK
[2]Royal Veterinary College, Department of Clinical Science and Services, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA, UK
[3]Cobb Europe, Oyster House, Severalls Lane, Colchester, Essex CO4 9PD, UK
[4]Cobb-Vantress, Siloam Springs, AR 72761, US

Presenting author
Marili Vasilogianni. E-mail: mvasilogianni21@rvc.ac.uk

Application
This work attempts to understand the nature of a poor gut health phenotype present in a pedigree broiler chicken line and test the association of intestinal dysbiosis with gut permeability and systemic bacterial infection.

Introduction
Poor gut health poses a serious welfare consideration for broiler chickens. Intestinal dysbiosis (or dysbacteriosis) (Teirlynck et al., 2011) describes an ill-defined syndrome that is generally characterized by disruption of the gut microbiome, and typically occurs around the first 20-30 days of life. Intestinal dysbiosis has been associated with gut leakage and increased bacterial colonization of the liver (Tellez et al., 2014; Di Vincenzo et al., 2023). This project aims to study naturally occurring intestinal dysbiosis in broiler chickens reared under farm conditions, using the natural phenotypical variation observed within a pedigree chicken line.

Materials and Methods
A group of 210 one-day-old male pedigree broiler chickens were housed in a single pen under controlled commercial conditions. They were allowed to grow with ad-libitum access to a standard broiler diet for a period of five weeks, including starter, grower and finisher phases. Blood samples were collected from every chicken on days 15 and 28 via the brachial vein, and on days 35 or 36 from the heart immediately after euthanasia. The occurrence of dysbiosis was evaluated at the time of post-mortem with the use of a macroscopic evaluation scoring system (Teirlynck et al., 2011). Liver tissue was collected immediately post-mortem for assessment of bacterial translocation. Whole blood and homogenised liver tissue sample dilutions were cultured under aerobic conditions using MacConkey and 7% sheep blood agar for 20h at 37°C. Blood and liver bacterial colony forming units (CFU) were counted and compared between chickens with high and low dysbiosis scores using the non-parametric Mann-Whitney test. Selected colonies from liver and blood samples were identified with the use of MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry)(Tsuchida et al., 2020). A subgroup of 20 randomly selected chickens were also subjected to an in-vivo gut permeability assessment using fluorescein isothiocyanate - dextran (FITC-d) on days 15, 22, 28 and 36 of the study. FITC-d blood concentration levels were compared between single score dysbiosis groups (scores 1-9) using two-way ANOVA paired with Fischer's LSD multiple comparisons test.

Results
The results showed the presence of bacteria in the blood and liver of chickens with high and low dysbiosis scores (Figure 1A). The total number of culturable bacteria in the blood, cultured with the use of blood agar, differed between the chickens with low and high dysbiosis on days 22 and 35-36, but not on day 28. However, there was no significant difference in the number of coliforms, which was assessed with the use of MacConkey agar. No significant difference was detected in bacterial

numbers identified in the liver. The colonies isolated for identification were chosen as representatives of commonly encountered morphotypes. In total, 17 species of bacteria were identified, of which five were found in both biological samples, five were only found in blood and seven only in liver samples (Figure 1B). FITC-d gut permeability assessment showed variable outcomes between low and high dysbiosis chickens (Figure 2).

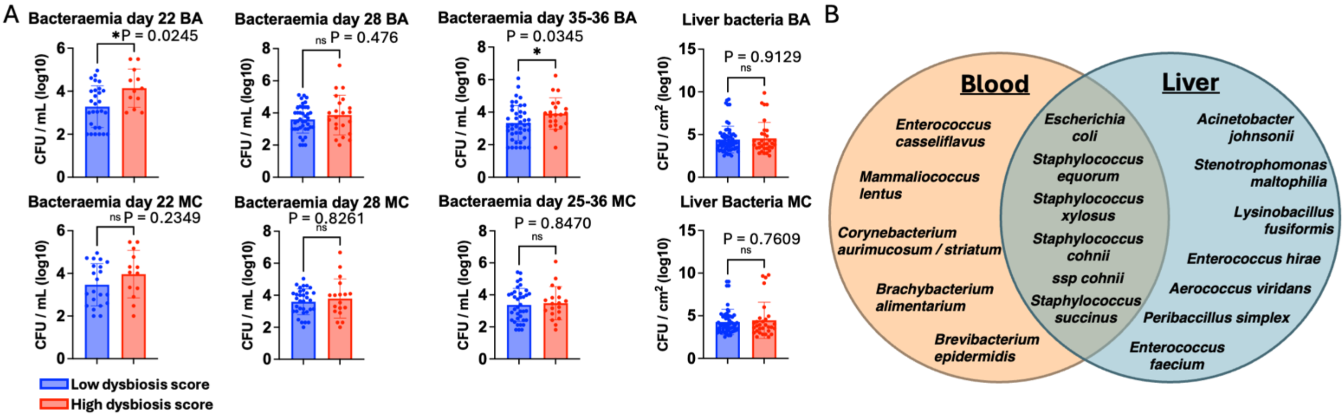

Figure 1. A: the number of CFUs in the blood and liver of chickens with low and high dysbiosis scores at different timepoints cultured at 37 °C using blood agar (BA) and MacConkey agar (MC) under aerobic conditions. B: Bacterial species identified in the blood and liver cultures.

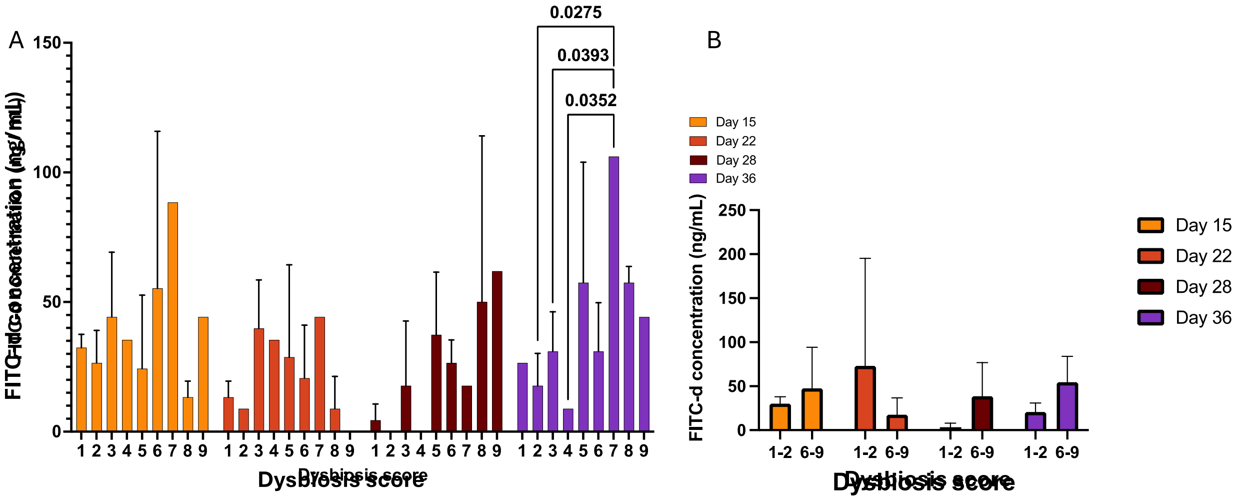

Figure 2. FITC-d concentration in the serum of chickens grouped by score (A), and by low and high dysbiosis score groups (B).

Conclusions

Macroscopic dysbiosis scores assigned during post-mortem were not directly reflected by total bacterial or specific coliform counts in the blood and liver, or FITC-d concentration in the blood, suggesting that intestinal dysbiosis is not always directly associated with gut leakage. The detection of non-gut-associated bacteria, such as *Staphylococcus xylosus*, in the blood and liver of these chickens suggests multiple sites of infection within this pedigree broiler line. This study underscores the complexity and multifactorial nature of the intestinal dysbiosis and provides a foundation for investigating the mechanisms underlying this syndrome, including the roles of microbial diversity, host genetics and extraintestinal infections.

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Lycopene and African walnut extract improved seminal antioxidative enzymes and serum biochemical Indices of heat-stressed cockerels

O. A. Ojo[1], K. Okukpe[2], O. A. Jimoh[3], A. O. Akintunde[4], U. U. Carols[5]
[1]Department of Animal Production, Kwara State University Malete Nigeria, Kwara State University Rd, Malete 241103, Kwara, Nigeria
[2]Department of Animal Production University of Ilorin, 1515, P.M.B, Ilorin, Nigeria
[3]Department of Animal Production Technology, Federal Polytechnic, Unwana, Afikpo North Local Government Area, Ebonyi State, Nigeria
[4]Department of Agriculture and Industrial Technology Babcocke University, Ogun, 121003 Nigeria
[5]University of Agriculture and Environmental Sciences, Umuagwo, Nigeria

Presenting author
Olayinka Abosede Ojo. E-mail: ykmelodya1@yahoo.com

Keywords: Lycopene, African walnut, Cockerels, seminal plasma, heat stress

Application
Herbal supplementation during climatic stress could alleviate neuronal cell damage, prevent tissue retention of synthetic pharmaceuticals and enhance better health status of animal product consumers.

Background
Incidence of global warming has adversely influenced animal health and reproductive performance more prominently under tropical climate. According to Hassen et al (2022), the recent ban of synthetic pharmaceuticals has necessitated the use of herbal supplements, which are cheap and naturally available. Shafe et al. (2024). reported that lycopene and African walnut leaf extract (AWLE) have been proven to contain bioactive components with antioxidant capability needed to improve seminal plasma biochemical indices of cockerels under tropical environment.

Objective
This study was conducted to determine the effect of lycopene and AWLE on seminal plasma biochemistry indices heat-stressed cockerels.

Materials and methods
This research was approved by ethical approval committee of the Kwara State University, Nigeria and carried out at the Poultry Unit of the Teaching and Research Farm, Kwara State University, Malete. Lycopene extraction was carried out according to Roldan-Gutierrez *et al.* (2007) procedure.
30-weeks old cockerels (n=54) were used for this experiment (10 weeks) with treatments stated as follows; **Group 1** - 250ml of water, no inclusion (control), **Group 2** - 7.5 ml of lycopene / 250 ml of water, **Group 3** - 15 ml of lycopene / 250ml of water, **Group 4** - 7.5 ml of AWLE / 250 ml of water, **Group 5 -** 15 ml of AWLE / 250 ml of water, **Group 6 -** 7.5 ml of lycopene + 7.5 ml of African walnut leaf extract / 250 ml of water, **Group 7 -** 15ml of lycopene + 15ml of leaf extract / 250 ml of water, **Group 8 -** Vitamin C 0.1g / 250ml of water, **Group 9 -** Cold temperature and 250 mls water (20°C).

Semen was collected using Bakst and Long (2010) methodology. All data collected were subjected to Analysis of Variance using Statistical Analysis System software. Means were separated using Duncan Multiple Range Test.

Results

The table of result is as shown in Table 1. Observation showed that cockerels under group 5 experienced a significantly depressed Malondialdehyde (0.61 ± 0.09 U/mg), while showing significant (*P*<0.05) elevation in seminal catalase value (741.27±21.01 U/mg). It was also noted total protein value (9.75 ± 0.23 mg/dl) was significantly (*P*<0.05) increased under group 9, which is statistically similar with values recorded for groups 4 and 8. According to Asfandiyar et al. (2024), malondialdehyde is a biomarker of oxidative stress (OS), which leads to molecular damage and lipid peroxidation. Catalase protects cells from oxidative damage caused by reactive oxygen species (ROS). A reduction in MDA value and an increase in catalase under group 5 could be due to the influence of bioactive components present in AWLE. Seminal plasma proteins (SPP) enhance sperm protection, transport and fertility via improved capacitation and acrosome reaction.

In conclusion, oral administration of 15 ml of AWLE / 250 ml of water (Group 5) while maintaining a controlled thermoneutral zone temperature for cockerels (18-22^{0}C) might improve reproductive performance under heat-stress condition.

Table 1. Effect of lycopene and AWLE on seminal plasma biochemistry of heat stressed cockerels

TREATMENT	T - CHOL (mg/dl)	TOTAL P (mg/dl)	SOD U/mg	CAT U/mg	ARGIN ng/nl	GPx U/mg	MDA U/mg	TAC (mg/dl)
1	208.67±92.78	8.67±0.06^{d}	203.53±44.07	624.98±393.98^{ab}	83.39±4.02	478.67±329.58	0.98±0.13^{ab}	152.68±48.51
2	182.66±41.38	8.78±0.41^{cd}	195.93±2.92	357.64±43.89^{ab}	91.44±36.89	768.54±254.71	1.05±0.09^{a}	195.16±60.97
3	101.16±10.36	9.51±0.08^{abc}	228.10±124.89	276.62±118.42^{ab}	82.52±20.44	800.36±214.55	0.69±0.37^{ab}	110.55±21.25
4	137.58±114.23	9.68±0.62^{ab}	181.10±30.79	334.58±140.60^{ab}	102.20±19.26	699.34±97.63	0.70±0.21^{ab}	136.48±23.43
5	143.35±97.08	8.85±1.09^{cd}	187.05±34.22	741.27±450.70^{a}	113.61±28.65	794.16±303.95	0.61±0.21^{b}	194.47±54.88
6	224.56±210.92	8.88±0.26^{cd}	171.54±23.78	149.11±11.91^{b}	108.72±80.95	842.67±800.88	1.05±0.17^{a}	88.17±29.32
7	194.22±42.33	8.98±0.35^{bcd}	190.69±41.57	673.17±12.46^{ab}	97.30±9.33	696.38±87.57	0.78±0.00^{ab}	134.54±0.12
8	186.99±74.46	9.26±0.32^{abcd}	172.62±19.26	442.65±235.84^{ab}	135.35±11.14	474.34±220.71	0.91±0.10^{ab}	142.12±135.61
9	43.93±4.68	9.75±3.23^{a}	150.27±116.32	170.94±12.88^{b}	131.65±10.34	85.47±15.67	0.75±0.00^{ab}	188.73±0.00

T-CHOl= total cholesterol, Total P = total protein, SOD = Superoxide dismutase, CAT = Catalase, ARGIN = arginase, GPx = Glutathione peroxidase, MDA = Lipid peroxidation, TAC = total antioxidant capacity.; T1=0ml of extract / 250ml of water, T2= 7.5ml ml of lycopene / 250ml of water, T3=15ml of lycopene / 250ml of water, T4=7.5mlin full then of AWLE 250ml of water, T5=15ml of AWLE / 250ml of water, T6 =7.5ml of lycopene + 7.5ml of AWLE / 250ml of water, T7=15ml of lycopene + 15ml of AWLE / 250ml of water, T8=Vitamin C 0.1g per 250ml of water, T9= Cold temperature + Cold water (20^{o}C); Means within the column with different superscript ^{abcd} are significantly different (p < 0.05).

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Statistical sexual discrimination in Japanese quail using body morphometric and meristic measures

K. Kareem-Ibrahim[1], K. Kareem-Ibrahim[1], B. Adegbenro[1], F. Olukoya[2]
[1]Lagos State University, Lasu Main Rd, Ojo, Lagos 102101, Lagos, Nigeria
[2]Federal University of Agriculture, Abeokuta, Ogun State, 110111, Nigeria

Presenting author
Dr Khadeejah Kareem-Ibrahim. E-mail: kf_abdkareem@yahoo.com

Application

Statistical sexual discrimination using bird morphometrics and meristic measures without expending huge resources associated with conventional sexing of quail birds.

Introduction

Quail birds are mostly kept for their eggs, which is dependent on the flock size and proportion of female birds within the flock. Many bird species are morphologically monomorphic and are only sexually dimorphic in adult stages, which makes it difficult to determine their sexes accurately at younger ages. Discrimination of sex in poultry is done using different methods such as examining of feet, neck, feather colour and length as well as cloaca control, radiographic examination, footprint and hormonal or operational method. Morphometrics is a concept that encompasses both the size and shape of an organism and phenotypic characterization precedes all other forms of characterization, upon which genetic and molecular characterization is built (Ali et al., 2021). Important body morphometrics in quails are body weight (BW), body length (BL), shank length (SL), body girth (BG), and wing length (WL) (Botwe et al., 2023). Vent sexing or venting is the most used traditional method of determining sex in day-old birds, along with sexing from the primary and secondary wing feathers, whereby qualified and trained professionals can distinguish differences in males and females, by visual examination of the cloaca of the animal. However, this method is both complex and requires huge investment in time, training, and economic resources. Thus, it becomes imperative that some other cost efficient and reliable methods of sex identification and discrimination be developed to achieve that purpose. This study aims to develop a novel approach to sexual discrimination by evaluating differences in morphometric measure and using statistics to build a discriminant function along the sexual divide.

Materials and Methods

This study was conducted at the Poultry Unit of the Livestock Farm, Lagos State University, Epe Campus, Lagos Nigeria at latitude 6.586892N and longitude 3.997527E.

A total of 150 quail birds were reared intensively in 15 cages comprising eight female and two male birds, from hatch to sexual maturity, and eggs were collected at 15-18 weeks old, which were set and hatched in the incubator. The birds were assigned unique ID from hatch, and the morphometrics recorded against their IDs. These measurements were used as training data to build a discriminant function along sex lines. Morphometric measures and meristic of the new hatchlings numbering 106 were collected as test data and the data was subjected to linear and quadratic discriminant functions established with the training data. A digital weighing scale sensitive to 0.00g was used to weigh the Body Weight (BWT), while Body Length (BLT), Wing Length (WLT), Shank Length (SLT), Chest Length (CLT) and Leg Length (LLT) were measured with a flexible measuring tape (mm), while Number of Secondary Feathers (NSF) and Number of Scales on a leg (NSL) were counts. All measurements and counts were used as predictors for the discrimination. Recorded morphometric and meristic measures were entered on a Microsoft Excel Spreadsheet and analysed using the Minitab® Statistical Software. Statistical analyses included descriptive statistics, t-test for independent samples, linear and quadratic discriminant functions and reclassification based on sex.

Results

Only two [Body weight (BWT) and Leg length (LLT)] of the eight variables investigated were statistically different (P<0.05) due to sex, while sex was not significant (P>0.05) on the other six variables studied. The largest difference due to sex was observed in the mean values for body weight where the female birds were 14.7% heavier than male birds (Figure 1). The female birds consistently had higher values than male birds in all variables studied.

Out of the 106 birds tested on the linear discriminant function, the overall accuracy was 68.9% with 73 birds accurately classified to their true groups. This comprised of 34 out of 51female accounting for 66.7% accuracy and 39 out of 55 male birds representing 70.9% accurately classified.

The quadratic discriminant function had a better accuracy with an overall accuracy of 72.6%, while there was improved accuracy for both female (70.6%) and male (74.5%) birds classification based on the morphometric and meristic measures.

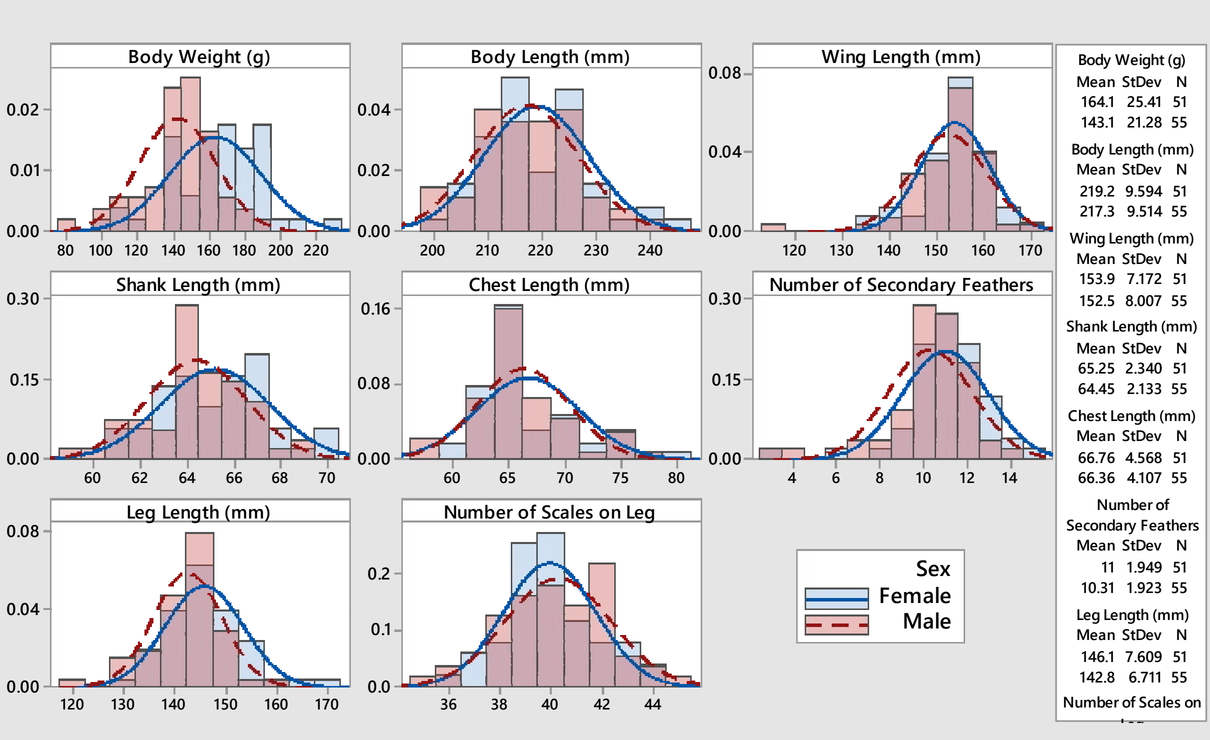

Figure 1: Histogram of Morphometric and Meristic Measures with Normal fit

Conclusions

Morphometric and meristic measures are veritable discriminatory variables that can be used for sex-based discrimination of quail birds without incurring additional expenses for experts and specialists that engage in traditional vent inspection for sexing.

Male birds had lower misclassification values compared to female birds and the quadratic discriminant function provide a more accurate classification when compared to the linear discriminant function.

Shank length (SLT) had the greatest impact on the discriminant function for groups, while body weight and body length had the least in classifying sexes. The discriminant function provides a reliable platform for classification of quail chicks at earlier stages, thereby providing a cost-effective method compared to conventional methods of sexing.

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Relationship between village chicken availability and dietary diversity along a rural-urban gradient

C. Mseleku[1], M. Chimonyo[2], R. Slotow[3], M. Ngidi[4]
[1]*University Of KwaZulu-Natal, Durban 4000, South Africa*
[2]*University of Venda, University Rd, Thohoyandou, 0950, South Africa*
[3]*University of KwaZulu-Natal, Durban 4000, South Africa*
[4]*University of KwaZulu-Natal, Durban 4000, South Africa*

Presenting author

Cresswell Mseleku. E-mail: 212504154@stu.ukzn.ac.za

Application

An increase in village chicken flock sizes is closely related to dietary diversity and, hence, food and nutrition security.

Introduction

Dietary diversity refers to different food items a household eats over a particular reference period (Verger *et al.,* 2021). It is one of the fundamental factors of nutritional security, and a proxy used to measure diet quality (Jones, 2017; Ali *et al.,* 2019). The dietary diversity scoring method, which is a count of food items consumed by a household over the preceding 24 hours, is the preferred measure of estimating dietary diversity (Verger *et al.,* 2021). The objective of the study was to investigate the relationship between village chicken flock sizes and dietary diversity of households in urban, peri-urban and rural environments. These different environments are usually reflected as the distance from the city centre, forming a rural-urban gradient (Chagomoka *et al*., 2015).

Materials and Methods

Ethical approval for the study was provided by the University of KwaZulu-Natal (Reference number: HSSREC/00005927/2023). Twelve sites were selected in urban, peri-urban and rural environments to create a rural–urban gradient on dietary diversity scores. The sites representing the urban settlements lay within 10 km from the city centre, while the peri-urban residential areas were located between 10 and 40 km from Pietermaritzburg city. The rural settlements were between 40 and 70 km from the city centre. Face-to-face interviews were conducted using a structured questionnaire on rural (n = 100), peri-urban (n = 100), and urban (n = 100) areas of Pietermaritzburg uMgungundlovu District, KwaZulu-Natal in South Africa. The questionnaire captured household demography, chicken flock sizes and composition, reasons for keeping village chickens, and the household's daily diet composition and diversity. Dietary diversity was assessed using a 24-h dietary recall and a dietary diversity scoring method.

Results

There was a linear increase in the flock size of village chickens with the distance from the city centre. An increase of 1 km from the city centre increased village chicken flock size by 30.4 (P<0.01). Village chickens contributed positively to household income from chicken sales and manure. A negative quadratic relationship was observed between distance from the city centre and the number of livestock-derived foods (LDFs) that were consumed ($y = -1.06x^2 + 4.31x - 1.38$; P<0.05). There was a linear increase in distance from city centre and consumed vegetables. The number of consumed vegetables increased with an increase in distance from city centre by 1.33 (P<0.01). A negative quadratic relationship was observed between distance from the city centre and consumed pulses ($y = -1.02x^2 + 4.27x - 2.13$; P<0.001). The negative elations suggest that the benefits of large flocks in rural areas are compromised by reduced levels of income. The number of consumed LDFs increased by 0.45 (P<0.05) as the flock size increased. The number of consumed vegetables increased with an increase in village chicken flock sizes by 0.68 (*P*<0.01). There was a linear increase in the dietary diversity score with distance from city centre. Dietary diversity score increased by 0.24 (*P*<0.05). There was a positive linear relationship between village chicken flock sizes and dietary diversity. Dietary diversity score increased with an increase in village chicken flock sizes by 1.50 (*P*<0.05).

Conclusions

Availability of village chickens improves household's dietary diversity and alleviate poverty in both urban and rural areas. Expanding village flock sizes could, therefore, enhance food and nutrition security at the household level.

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Insights on Animal Production in Brazil: Trends, Challenges, and Opportunities

L. F. Araújo[1]
[1]*University of São Paulo, 374 - Butantã, São Paulo - SP, 05508-220, Brazil*

Presenting author
Lúcio Francelino Araújo. E-mail: lfaraujo@usp.br

Brazil's agribusiness plays a crucial role in both the domestic market and the global economy, particularly in the crop and livestock sectors. The country is one of the world's largest producers and exporters of a wide range of agricultural products, including poultry, pork, beef, dairy and sheep products. Poultry is an important part agro-industry, and the country is the world's largest exporter and second largest exporter of chicken meat. In addition, it is also a world leader in pork production. It is one of the world's largest pork producers and exporters, particularly to China, which is one of the main buyers. Brazil is the world's largest exporter of beef, and the cattle industry is the backbone of its agribusiness. With vast pastures and a favorable climate, it can produce beef efficiently and on a large scale. Major export markets include China, the US and the European Union. Concerning dairy products, Brazil is a major producer particularly milk and cheese. The country has a large domestic market for dairy products and is increasing its presence in world markets. Although not as large as the poultry, pork and beef industries, sheep production is still an important part of the agro-industry particularly in the southern states where the climate is more suitable for sheep farming. Brazil's agribusiness is vital not only to the Brazilian economy, but also to global food security. The country's ability to meet the demands of rapidly growing populations, particularly in emerging markets, has made it a critical player in the global food supply chain. As global demand for protein continues to rise the country has a profound economic impact both domestically and globally. It creates jobs, boosts exports and strengthens the country's geopolitical position in global trade, particularly in food and agriculture.

Brazilian Beef: Challenges and Opportunities in Meeting Global Demand for High-Quality Meat

S. da Luz e Silva[1]

[1]*University of São Paulo, 374 - Butantã, São Paulo - SP, 05508-220, Brazil*

Presenting author

Saulo da Luz e Silva. E-mail: sauloluz@usp.br

Brazil plays a pivotal role in the global beef industry, supplying diverse markets with high-quality meat. A key factor behind this success is the widespread use of Bos indicus cattle, particularly Nelore, which are well adapted to tropical climates. Their resilience to heat and parasites, combined with efficient forage utilization, enhances sustainability while maintaining competitive production costs. These factors directly influence meat characteristics, such as leaner carcasses and distinct marbling patterns, which cater to specific consumer preferences worldwide. Advances in genetics, nutrition, and pasture management have further improved meat quality and productivity. Despite its strengths, the Brazilian beef industry faces significant challenges, including environmental concerns, land use regulations, and the need to enhance production efficiency while reducing carbon emissions. Addressing these issues through science-based strategies—such as integrated crop-livestock forest systems and precision livestock farming—is essential for ensuring sustainable growth At the same time, Brazil's progress toward becoming officially recognized as free of foot-and-mouth disease presents a major opportunity for expanding market access. This achievement has the potential to open new trade routes, strengthen consumer confidence, and enhance the country's competitiveness in premium beef markets. This presentation will explore the unique characteristics of Brazilian beef production, highlighting both the challenges and opportunities in meeting the growing global demand. By leveraging its tropical advantages, embracing innovation, and improving animal health status, Brazil continues to solidify its position as a leading supplier of high-quality beef worldwide.

Sustainable Sheep Production in the Americas: Challenges and Innovations

S. B. Gallo[1]
[1]University of São Paulo, 374 - Butantã, São Paulo - SP, 05508-220, Brazil

Presenting author
Sarita Bonagurio Gallo. E-mail: saritabgallo@usp.br

The main product of sheep production in Brazil is the young lamb, followed by the sale of wool and, more recently, an increase in milk production. The Brazilian flock is estimated at 21,792,139 head. However, the distribution of animals is not homogeneous, with a greater number of animals in the Northeast and South regions of the country. Sheep meat production in Brazil began to grow in 2010 and continues to do so. The main breeds raised are the Santa Ines (a native breed), the Dorper, the Suffolk, the Texel, the Ile de France and the Corriedale. The number of breeders of wool producing breeds has decreased. Although the herd is small, dairy breeds such as the Lacaune have been imported. Brazil has few breeding projects, which are more often carried out by a few producers. The most common production system is for the animals to be grazed only, with supplementary feeding during times of low food availability. However, in some regions, lambs are finished in confinement. The most commonly used forage crops are tropical. The most commonly used grains are corn, soybean, cotton and citrus co-products. Sheep production in Brazil still faces many problems, such as the disorganization of the production chain, low reproduction and production rates and the lack of breeding programs. In addition, there is a lack of information about the production system in general. Breeders need better access to technical training. Despite the difficulties, sheep production is growing, mainly due to the short production cycle, which is suitable for small farms and family work. In addition, consumers are interested in sheep meat and milk, which makes it easier to sell at high prices. In conclusion, the Brazilian sheep industry faces challenges, but also has a promising future.

Brazilian Livestock and Global Warming: Impacts and Mitigation Strategies

I. C. da Silva Bueno[1]

[1]*University of São Paulo, 374 - Butantã, São Paulo - SP, 05508-220, Brazil*

Presenting author
Ives Cláudio da Silva Bueno. E-mail: ivesbueno@usp.br

Brazilian livestock farming plays a vital role both in the country's economy and in global food supply. However, it also faces significant challenges concerning global warming. Livestock production contributes to greenhouse gas (GHG) emissions, primarily methane, which is released during the digestive process of ruminants.

Nevertheless, it is important to highlight that Brazil has adopted several mitigation strategies to address these environmental impacts. One notable initiative is the use of integrated crop-livestock-forestry (ICLF) systems, which promote more sustainable and efficient production by integrating agricultural, livestock, and forestry activities within the same area. This system helps reduce GHG emissions and increases carbon sequestration in the soil.

Another strategy is the genetic improvement of herds, aiming to produce more productive animals with lower methane emissions. Additionally, soil and pasture management practices, such as pasture rotation and the use of green manure, also contribute to reducing emissions and improving soil quality. The adoption of technologies such as precision livestock farming, which uses sensors and digital tools to monitor and optimise production, has also shown positive results in reducing environmental impact.

In summary, despite the challenges, Brazilian livestock farming has demonstrated a growing commitment to sustainability and mitigating global warming. By adopting innovative and integrated practices, the sector can continue to significantly contribute to the economy and food security while reducing its environmental impact.

Code EFABAR: Supporting Responsible and Sustainable Livestock Breeding in Society

G. Cartick[1]
[1]*EFFAB-FABRE TP, Rue de Trèves 61, 1040 Bruxelles*

Presenting author
Geena Cartick. E-mail: geena.cartick@effab.info

Application

The Code EFABAR sets a European standard for responsible farm animal breeding practices, guiding breeders in balancing productivity with ethical and sustainable objectives. Through structured principles, Code EFABAR supports breeders in addressing modern challenges in livestock production, ensuring practices meet societal demands for sustainable, welfare-conscious livestock systems.

Introduction

Code EFABAR has evolved over two decades in response to advancements in animal genetics and shifting societal expectations, moving beyond a narrow focus on productivity to embrace a balanced approach that addresses animal welfare, environmental impact, and public health (Smith et al., 2018; European Forum of Farm Animal Breeders, 2020). Initially established to foster transparency and ethical breeding, Code EFABAR's timeline reflects key milestones: in 2001, the code set foundational guidelines; by 2010, it expanded to include environmental and resource efficiency goals (Jones & Taylor, 2012); in 2015, it integrated genetic diversity and public safety considerations (European Commission, 2016). Today, Code EFABAR is an internationally recognized framework, setting standards for breeders to support livestock's role in a sustainable global society.

Materials and Methods

Code EFABAR is structured around six foundational pillars: animal welfare and health, environmental impact mitigation and adaptation, efficient resource use, genetic diversity, product quality and quantity, and public and food safety (European Forum of Farm Animal Breeders, 2020). These principles are applied through a detailed assessment process, where breeders adhere to standards that guide responsible breeding, ensuring animal well-being and sustainability (Thompson et al., 2021). The code emphasizes using genomics and phenoty ping tools to mitigate negative genetic correlations between functional and productive traits (Johnson et al., 2019). Additionally, breeders adopt management practices, including animal welfare during transport, that support the well-being of livestock across various production systems.

Results

The adoption of Code EFABAR across breeding organizations has led to measurable improvements in breeding outcomes, including enhanced animal welfare and efficiency in resource utilization (European Livestock Breeding Association, 2022). By prioritizing genetic diversity and environmental sustainability, breeders have achieved a more balanced approach, aligning livestock production with the needs of society and the environment (Martinez & Lee, 2023). Code EFABAR has established itself as a benchmark for transparency, with breeders engaging with stakeholders to communicate their commitment to ethical practices.

Conclusions

Code EFABAR stands as a critical framework for responsible breeding, supporting livestock's role in a global society that values sustainability and ethical practices. By promoting transparency and setting high standards for animal welfare and environmental responsibility, Code EFABAR ensures that breeding practices are aligned with the long-term interests of animals, people, and the planet. The code not only guides breeders but also fosters public trust and supports a sustainable future for livestock production (European Forum of Farm Animal Breeders, 2020; Smith et al., 2018).

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Exploring the genetic landscape of indigenous sheep breeds: unveiling population structure and ancestral connections

M. Kyritsi[1], E. Pavlou[1], A. Tsoureki[1], A. Argiriou[1,2], S. Michailidou[1]
[1]*Centre For Research And Technology Hellas - Institute Of Applied Biosciences, 57001 Thermi, Thessaloniki, Greece*
[2]*University of the Aegean - Department of Food Science and Nutrition, Metropolite Ioakeim 2, 81400, Myrina, Lemnos, Greece*

Presenting author
Maria Kyritsi. E-Mail: kyritsimaria@certh.gr

Application

By studying the genetics of indigenous populations, we offer valuable data to guide breeding strategies that ensure sustainable, profitable sheep farming.

Introduction

Autochthonous sheep breeds are well-adapted to local environments and agro-ecological conditions compared to cosmopolitan breeds, making them preferred in Mediterranean countries like Greece. Preserving rare alleles of indigenous breeds is essential for their resilience and adaptability in harsh environments, poor-quality pastures, and extreme temperatures. In this context, we analysed the most numerous Greek sheep breeds to unveil their genetic variation patterns, through population structure, ancestry, evolutionary history, and inbreeding levels. Three cosmopolitan breeds frequently reared in Greece in large flocks were included, to identify possible admixture patterns and ancestral connections with Greece's national flock. We also examined whether geographical barriers, transhumance or human management have shaped the existing genetic material of sheep flocks in Greece over the years.

Materials and Methods

A total of 384 sheep samples originating from nine Greek sheep breeds (Boutsko, Chios, Kalarritiko, Karagkouniko, Katsika, Lesvou, Pelagonias, Serres, Thrakis) and three cosmopolitan breeds (Assaf, Lacaune, Charollais) were analysed using different versions of Illumina's OvineSNP50K bead arrays (v1, v2, and v3). We retained only the common SNPs among versions (N=53,137 SNPs). Quality filtering was performed in PLINK v1.90 (Chang et al., 2015), setting the thresholds as follows: SNP call rate at 98%, minor allele frequency at 1%, and deviations from Hardy-Weinberg equilibrium at a p-value of 1x10-6. Additionally, samples with SNP missingness >10% were removed. The genetic background of sheep populations was tested both within and between breeds. Within each breed we analysed the i) observed (Ho) and expected (He) heterozygosities to calculate genetic diversity within each breed using the ARLEQUIN software v3.5.2.2 (Excoffier and Lischer, 2010), ii) Wright's inbreeding coefficient FIS (Individual within Subpopulation) to measure excess of homozygotes or heterozygotes using PLINK v1.90, iii) effective population size (Ne) over 1,000 generations to assess relevant diversity histories using SNeP (Barbado et al., 2015) and iv) Runs of Homozygosity (ROHs) to identify inbreeding levels using PLINK v1.90, for a total length of 1Mb ROH, 15 variants in the scanning window, allowing 1 heterozygous SNP and 1 missing call to estimate homozygosity. Population structure was assessed by principal component analysis (PCA), while admixture analysis was performed to test for the genetic structure of the populations using the ADMIXTURE v1.3 software (Alexander and Lange, 2011) and the cross-validation (CV) error method, assuming a number of subpopulations (K) ranging from 2 to 12. The genetic differentiation of breeds was assessed using Wright's pairwise FST values. Historical geneflow and ancestral connections were investigated between breeds using the Treemix software v1.12 (Pickrell and Pritchard, 2012) for different migration events, starting from 0 to 20 migration events, by grouping SNPs in windows of 500.

Results

Overall, the national flock of Greece's sheep is characterized by moderate levels of genetic heterozygosity with mean values for the nine populations being Ho=0.356 and He=0.362. Chios breed, the only sheep breed that has been subjected to genetic improvement breeding schemes over the last decades in Greece, presented the lowest number of Ho (0.326) and He (0.344) values. On the contrary, the highest values were acquired for Karagkouniko breed (Ho=0.378, He=0.372). Within breed heterozygosity results were also verified by mean FIS values with the highest value obtained for Chios breed (FIS=0.155) and the lowest for Karagkouniko breed (FIS=0.022). Patterns of homozygosity and inbreeding levels revealed that Thrakis breed had on average the greatest number of ROH length (8.25Mb), followed by Lesvou (7.49Mb) and Serres (7.32Mb) breeds, suggesting more recent inbreeding events. Population structure analysis revealed the genetic proximity of Boutsko and Kalarritiko breeds, as anticipated based on historical data and the region of origin of these breeds (mountainous areas in Western Greece). Admixture analysis revealed a partitioning of the Serres, Kalarritiko, and Pelagonias breeds, corresponding to the farms from which the individual animals originated. Ancestral Ne displayed a gradual decreasing trend over the last 1,000 generations for all the studied populations. In the distant past, for Kalarritiko breed Ne was estimated to be 3,411, gradually reaching Ne=153 13 generations ago. All Greek breeds presented a wide genetic pool of considerable sizes ranging from 66 (Boutsko and Katsika) to 153 (Kalarritiko), assuring for long-term population viability. Genetic differentiation of breeds and geneflow analysis revealed three distinct clusters of sheep breeds, confirming also population structure results. In particular, sheep that originate from the mountainous areas of Western Greece, were found closely related (Kalarritiko, Boutsko, Katsika, Pelagonias), whereas, breeds from Eastern Greece and the Aegean Sea were found in close proximity. The greatest genetic distance was acquired in all pairwise comparisons of Greek breeds with the Charollais breed. Among Greek breeds, the greatest genetic differentiation was observed between Chios and Boutsko breeds, which is expected based on their phenotypic, historical, and pedigree data. Geneflow analysis indicated that Chios and Lesvou partially shaped the Karagkouniko population through distant gene flow events.

Conclusions

To address the genetic landscape of Greek sheep breeds we analysed ~50K SNPs to unveil their population structure and ancestral connections. To date, our work comprises the most comprehensive genetic characterization of sheep diversity in Greece. Our results expand our knowledge on the molecular variability of sheep breeds and present data on geographical partitioning and gene flow events. Although Greece's national flock is characterized by large introgression from cosmopolitan breeds, we conclude that long-term preservation of sheep diversity is feasible by designing the appropriate breeding schemes.

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A genetic analysis of farmer assigned causes of death in lambs

K. Hay[1], N. Lambe, J. Roden, S. Jarvis, C. Dwyer
[1]*Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK*

Presenting author
Kim Hay. E-mail: kimhay@innovis.org.uk

Application

Genetic selection on maternal breeding values could be used to reduce stillborn lambs and death from dystocia in maternal breeds.

Introduction

Poor lamb survival is a major cause of productive inefficiency in the sheep sector. Survival rates vary dramatically between flock and system; however, it is estimated between 10-15% of all lambs born die every year before weaning in the UK and around the world (Dwyer et al, 2016). Investigating why these lambs die could help inform future breeding decisions.

Materials and Methods

Lamb records (n= 58,851) for survival to 48 hours and 8 weeks, were collected over 7 years, across 22 farms and 7 different breed lines that make up the Innovis breeding program. Farmers recorded the cause of death. The most common identified causes of death; dystocia, starvation/mismothering/exposure or stillbirth, were analysed. Significance of fixed effects were assessed using general linear regression in GenStat (VSN International, 2022). Significant fixed effects were flock, lamb sex, lamb breed, lamb birth type and birthweight was a significant covariate. All significant 2-way interactions were fitted. Predicted means for each level of fixed effect were calculated and pair-wise t-tests assessed if differences were statistically significant. Heritability and maternal heritability were estimated by fitting a univariate animal model, with the above fixed effects and covariates, in ASReml, using 160,482 pedigree records over 17 generations.

Results

Nine hundred and twenty lambs were assigned as being stillborn (1.5% of all lambs in the dataset), 568 lambs were assigned a cause of death as dystocia (0.9%) and 575 lambs were assigned starvation/mismothering or exposure as a cause of death (1.0%). There were significant differences between males and females for all causes of death with males more likely to die than females from all causes. For birth type there were significant differences between single born lambs, twin born lambs and lambs born in litters of 3 or more. There was a significant difference between all birth type categories for starvation/mismothering or exposure, with triplets most likely to die. There was a significant difference between single lambs and higher litter sizes for lambs dying from dystocia, with singles more likely to die. Triplets were significantly more likely to be stillborn than singles or twins. Cause of death heritabilities were low and not significantly different from zero (Table 1), but stillborn and dystocia had a significant maternal heritability.

Table 1. Variance components (σ^2_a = additive variance, σ^2_m = maternal variance, σ^2_p = phenotypic variance) and heritability estimates (h^2 = heritability, h^2_m = maternal heritability) for farmer assigned causes of death with standard errors.

Trait	σ^2_a	σ^2_m	σ^2_p	h^2	h_m^2
Stillborn	0.14±0.13	0.33±0.16	5.04±0.15	0.03±0.03	0.06±0.03
Dystocia	0.17±0.18	0.45±0.26	4.47±0.33	0.04±0.04	0.10±0.06
Starvation/Mismothering or exposure	0.15±0.19	0.09±0.16	4.65±0.20	0.03±0.04	0.02±0.03

Conclusions

To reduce deaths from dystocia and reduce stillborn lambs, maternal selection is required. Direct and maternal heritability for starvation/mismothering or exposure were not significant, so selective breeding is not likely to reduce these deaths; management decisions are likely to be more effective. These causes of death could be used in the future to increase accuracy of genetic evaluation of lamb survival traits.

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Characterisation of Copy Number Variants in a multi breed sheep population using high-density genotypes

A. Dunne[1,2], N. McHugh[1], T. Pabiou[3], D. Purfield[2]
[1]*Teagasc Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*Munster Technological University, 46 Grand Parade, Cork City T12 VN56, Ireland*
[3]*Irish Cattle Breeding Federation, Link Road, Ballincollig, Cork P31 D452, Ireland*

Presenting author
Adam Dunne. E-mail: adam.dunne@teagasc.ie

Application

Characterisation of copy number variants has heretofore not been available on the Irish sheep population, this coupled with knowledge of their impact on animal performance may lead to greater genetic improvement for the Irish sheep industry.

Introduction

The advent of low cost DNA genotyping for ruminants with the primary objective of improving genetic evaluations and providing accurate parentage has resulted in the genotyping of large numbers of animals (including sheep). However, a multitude of additional uses for high density genotypes exist; one such application is copy number variant (CNV) detection. A CNV is a structural change in DNA, normally defined to be > 1kb in length, where a region of DNA is duplicated or deleted. Copy number variants have been extensively studied in cattle with associations observed across a multitude of production traits. In comparison there is a paucity of such studies in sheep; with a small number of studies showing an association between CNVs and milk and growth traits (Di Gerlando et al., 2019; Ladeira et al., 2022). However, these studies focused on a single breed and used a relatively low density single-nucleotide polymorphism (SNP) panel, with a limited number of studies on sheep using a higher density SNP panel for CNV detection. Due to cost restraints, the lower density panels tend to be more widely used for sheep and no previous study has compared the role of panel density on CNV detection. The objective of the current study was to characterise and investigate the functional impact of CNVs in a multi-breed sheep population comprising of eight breeds. A secondary objective was to validate the impact of panel density on CNV identification.

Materials and Methods

Genotype data, including genotyping intensity values, was available for 174 Irish sheep from 8 breeds genotyped on the Illumina OvineSNP600K BeadChip platform. Only genotypes from animals with a SNP call rate of ≥0.9, and SNPs with a call rate ≥0.9 were retained for analysis. Only autosomal SNPs with a known chromosome and position were retained. After edits, 561,891 SNPs on 174 animals were available for analysis. To investigate the capacity of low density SNP panels to identify CNVs, a SNP panel resembling the Ovine SNP50 panel with 41,458 autosomal SNPs common to both the OvineSNP600K panel and the OvineSNP50K was generated for each animal, this panel will be hereafter referred to as the SNP50 panel. For both the SNP50K and SNP600K panels, CNVs were called using an integrated hidden Markov model within the PennCNV software (Wang et al., 2007). Only CNVs with a length of 3 or more SNPs were retained across both panels. To remove potential false positive CNV calls, animals with a log R ratio (LRR) standard deviation > 0.3 , B allele frequency (BAF) drift > 0.01, or absolute waviness factor > 0.05 were removed from further analysis (37 animals were removed). A CNV was determined to be common to an animal across both genotyping panels if the CNV was detected on both the SNP50K and SNP600K panel. Overlapping CNVs that overlapped by at least one base pair were grouped into regions known as CNVRs using the software HandyCNV and were classified into three categories: deletions, duplications or mixed (containing at least one deletion and one duplication). CNVRs were generated both across all breeds and within breed. Sheep QTLdb was then used to identify CNVRs that are associated with economically important traits.

Results

A total 5,201 CNVs were called from the SNP600K panel across 137 animals, consisting of 3,447 deletions and 1,754 duplications. Across animals, there was a median of 13 deletions and 9 duplications detected per animal. Deletions had a mean length of 66.15 kb and duplications had a mean length of 55.11 kb. In comparison only 244 CNVs were called from the SNP50 panel comprising 146 deletions and 98 duplications. The distribution of CNVs was found to differ across breeds, which may be due to their variations in origin and breed management. The mean length of CNVs called from the SNP50 panel was greater than those called by the SNP600K panel for both deletions and duplications. There was a median of 2 deletions and 1 duplication per animal using the SNP50 panel. Only 25% of CNVs called by the SNP50 panel overlapped with a CNV called by the SNP600K panel and had a mean length of 267.31 kb whereas CNVs with no overlap had a mean length of 140.17kb. Copy number variants called from the SNP600K panel were grouped into 2041 CNVR. The most common CNVR, found in 66 animals, was located on chromosome 10, between 71,051,498 and 72,205,564 bp and has been shown to overlap with quantitative trait loci (QTL) associated with carcass traits. In total there were 131 CNVR found in more than 5% of animals.

Conclusions

Copy number variants are a common feature of the sheep genome that are not accounted for in breeding programmes. The distribution of CNVs varies among different breeds, and these CNV regions have been found to overlap with areas containing genes associated with various functional traits. Low density panels, such as the SNP50K, have limited capacity to accurately identify CNVs; they typically identify longer CNVs and tend to have a high false discovery rate.

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Alternative Definitions of Methane Emissions in Sheep Production Systems.

D. Kelly[1], N. McHugh[2], P. McCarron[1], E. O' Connor[1], D. Purfield[3], F. McGovern[1]
[1]*Teagasc Mellows Campus, Athenry, Co. Galway H65 R718, Ireland*
[2]*Teagasc Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[3]*Munster Technological University, 46 Grand Parade, Cork City T12 VN56, Ireland*

Presenting author
Dermot Kelly. E-mail: dermot.kelly@teagasc.ie

Application

By exploring the utility of various methane metrics, this research will identify the most pertinent definitions of methane emissions in sheep. These insights can then inform breeding programs focused on selecting low emission animals.

Introduction

Methane is a potent greenhouse gas with a warming potential eighty times greater than carbon dioxide over a shorter timeframe, with ruminant livestock accounting for approximately 30% of global emissions 73% of these emissions due to enteric fermentation (Black et al., 2021) (EPA, 2013), thereby making methane a critical target for climate change mitigation. Previous studies have shown that genetic, dietary and management factors influence the efficiency of methane production, allowing some animals to produce less methane without compromising productivity (Islam & Lee, 2019; Johnson et al., 2022; Mahala et al., 2022). Advances in methane quantification techniques for small ruminants, such as Portable Accumulation Chambers (PAC), have made it feasible to measure methane emissions in ruminants on a large scale and offer scalable and practical alternatives to labour intensive, limited throughput respiration chambers (O'Connor et al., 2021). Defining methane in a meaningful way is a challenge, as no consensus exists in the literature. Various methane definitions exist with some focusing on absolute emissions and others adjusting for efficiency. This is one of the first studies to comprehensively examine a range of methane emissions in sheep.

Materials and Methods

A total 13,695 records of methane emissions data was available from 7,204 animals including on growing animals (4 to 19 months) and on ewes (lactating and dry); collected from 2019 – 2024. Further phenotypic data included live weight (recorded on the day of PAC measurement), body condition score, and dry matter intake (DMI) (both recorded within 30 days of PAC measurement). Dry matter intake was measured using the n-alkane technique when animals were grazing outdoors and via the feed and weigh method when housed indoors. For growing animals information was available on carcass weight, carcass fat %, carcass muscle %, and average daily live weight gain. Various methane emission metrics were explored, including ratio based traits such as methane per kg of bodyweight (reflects methane emissions relative to the animal’s total body weight), methane per kg of metabolic bodyweight (MBW) (adjusts emissions for metabolic activity, scaling by bodyweight raised to the power of 0.75), and methane per unit dry matter intake (DMI) (Relates emissions to the animals feed consumption efficiency). Pearson correlations were used to examine the relationships between these various traits/metrics.

Results and Discussion

For ewes, the mean daily methane emissions were 21.0 (SD = 9.4) g/day. Their mean DMI was 1.8 (SD = 0.7) kg/day. For growing animals, the mean daily methane emissions were lower, at 13.7 (SD = 6.6) g/day, while their mean DMI was 1.2 (SD = 0.5) kg/d.

For growing animals daily methane emissions showed a moderate positive correlation with body weight and DMI, r = 0.37, CI: 0.26-0.48 ($P < 0.001$) and r = 0.43, CI: 0.32-0.53 ($P < 0.001$), respectively. Methane per kg of body weight was strongly correlated with daily methane emissions (r = 0.81, CI:

0.79-0.82, $P < 0.001$). Similarly, methane per kg MBW also showed a strong correlation with daily methane emissions (r = 0.89, CI: 0.88-0.90, $P < 0.001$). Methane per unit DMI showed a moderate positive correlation with daily methane emissions (r = 0.58, CI: 0.49-0.66, $P < 0.001$)

In ewes body weight and DMI showed a weak to moderate positive correlation with daily methane emissions, r = 0.16, CI: 0.14-0.19 ($P < 0.001$) and r = 0.13, CI: 0.02-0.22 ($P < 0.001$) respectively. Methane per kg of body weight showed a strong positive correlation with daily methane emissions (r = 0.95, CI: 0.95-0.96, $P < 0.001$), as did methane per kg MBW (r=0.97, CI: 0.97-0.98, $P < 0.001$). Methane per unit DMI had a moderate positive correlation with daily methane emissions (r = 0.40, CI: 0.31-0.49, $P < 0.001$). These results indicate that body composition metrics such as body weight and metabolic bodyweight are key predictors of methane emissions across life stages, with metrics of DMI showing a strong but lesser association.

Conclusion and Implications

This study represents the first to comprehensively explore a range of alternative methane definitions for sheep, revealing that each metric captures distinct dimensions of methane efficiency. This suggests that no single metric fully encompasses the complexity of methane emissions. By identifying this, this study supports a more targeted approach, tailoring methane metrics to the unique aspects of an animal’s growth or production phase.

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Gene expression changes in nucleotide metabolism and cell adhesion pathways contribute to altered methane emissions in sheep

S. R. C. Kaitholil[1,2], M. Mooney[1], A. Aubry[2], V. Razban[2], F. Rezwan[3], M. Shirali[1,2]
[1]Queen's University, University Rd, Belfast BT7 1NN, UK
[2]Agri-food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK
[3]Aberystwyth University, Penglais, Aberystwyth SY23 3FL, UK

Presenting author
Steffimol Rose Chacko Kaitholil. E-mail: schackokaitholil01@qub.ac.uk

Application

Methane emissions from ruminants pose significant challenges to environmental sustainability and energy efficiency in sheep farming. This study investigates the blood transcriptome of lambs with varying methane emissions to identify genes linked to methane production, paving the way for the genetic selection of low methane-emitting animals as a cost-effective and sustainable strategy.

Introduction

The growing demand for meat drives increased sheep farming, contributing to higher methane emissions and other negative environmental impacts. While promising strategies to reduce methane emissions are being explored, including feed adjustments and additives (Hristov, 2024), genetic selection for low methane-producing sheep offers the potential of sustained long-term benefits (Worku, 2024). However, understanding of the genetic and biological mechanisms of methane production remains limited, with most research focused on rumen ecosystems (Asselstine et al., 2021) rather than live animals. This study aims to identify differentially expressed genes by utilizing blood transcriptome, exploring how methane emissions are related to physiological and metabolic pathways.

Materials and Methods

Twenty-four male weaned lambs (Texel x Scottish Blackface, average weight 30 kg) were randomly assigned to four dietary groups, each receiving a total mixed ratio diet of 50:50 grass silage and concentrate, with varying amounts of microalgae oil (*Schizochytrium* sp.) supplementation levels: 0g, 5.4g, 10.8g, and 16.2g per kg dry matter. Dry matter intake (DMI) and body weight (BW) were recorded daily and weekly for nine weeks. Methane emissions were measured for 48 hours at the end of week 9 using individual respiration chambers. Blood samples collected at the end of experiment were analyzed via RNA sequencing, using an Illumina NovaSeq platform. Bioinformatic data analyses included sequence data quality checks, read alignment, normalization, differential expression analysis, weighted gene co-expression network analysis (WGCNA) and functional enrichment analysis. WGCNA module genes were further analyzed through protein-protein interaction network (PPI) construction to identify highly interconnected gene network associated with methane production.

Results

Microalgae oil supplementation had no significant effect on body weight (P= 0.25) or dry matter intake (DMI) (P= 0.07*)*, indicating no impact of microalgae oil on growth or feed consumption. Methane emissions at the end of the study (week 9) varied between 19.50 and 47.86 g/d, negatively correlating with microalgae oil levels (R = -0.52, P = 0.01), suggesting microalgae oil effect in reducing methane emissions (Cristobal-Carballo et al., 2021). Differential expression analysis revealed seven significant genes linked to methane production, including upregulated *NME4, LOC121819234, MARCHF3*, and *PLXNB3*, and downregulated *LOC105603087, LOC132657460*, and *LOC101116551*. *NME4* and *PLXNB3* are involved in nucleotide metabolism and axonal guidance pathways. *NME4* upregulation, related to coenzyme-A (CoA) metabolism and lipid accumulation, was likely driven by microalgae oil supplementation, resulting in altered methane emissions. *PLXNB3* upregulation activates *PAK5 (P21 Activated Kinase 5),* a regulator of barrier function, and epithelial cell migration, also impacting the signalling pathways associated with gut inflammation. WGCNA revealed significant correlations between four gene modules (total of 3,671 genes) and methane emissions, with enriched pathways such as focal adhesion, efferocytosis and gap junctions. These pathways are crucial for cellular communication, inflammation resolution, and epithelial homeostasis, and have been reported to be influenced by diet by impacting nutrient absorption rates. Ten highly connected hub genes (*Histone H4(LOC101108868), Talin 1(TLN1), Histone H3.1 (LOC101118484), Cofilin 1(CFL1), Filamin A(FLNA), Calponin 2(CNN2), Actin gamma 1(ACTG1), Histone deacetylase 5(HDAC5), Histone H2A**X** (LOC101106791),* and *Actin beta (ACTB))* identified through PPI network analysis, were linked to histone and actin network families, which play roles in gene expression regulation, cell adhesion, and immune responses, and all positively correlated with methane production.

Conclusion

This study has identified key genes associated with methane production in lambs, and involved in immune regulation, cellular differentiation, and cell adhesion. These findings offer insights into the molecular mechanisms of methane emissions and highlight candidate genes for marker-assisted selection of low methane-emitting sheep. Further research is needed to explore how these genes interact with microbiota-driven changes in the gut and to assess the long-term effects of microalgae oil supplementation on gene expression and methane emissions.

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The significance of Anaplasma phagocytophilum in Irish sheep

J. Gilmore[1], K. O Keefe[2], C. Hurley[2], M. Hennessy[2], A. Naranjo-Lucena[3,4], M. Casey[4], C. Mc Dermott[4], K. Beirne[4], S. Fagan[5], J. F. Mee[6], T. Keady[7], S. Mc Gettrick[8], A. M. Flaherty[8], R. Froehlich-Kelly[8], M. Sheehan[9], A. Zintl[4]

[1]*Farmlab Diagnostics, Lodge Veterinary Centre, Emlagh, Co. Roscommon, F45 VW89, Ireland*
[2]*Department of Agriculture, Food and the Marine, Blood testing Laboratory, Agriculture House, Kildare Street, Dublin, Ireland*
[3]*Department of Agriculture Food and the Marine, National Reference Laboratory for Antimicrobial Resistance, Backweston Laboratory Campus, Celbridge W23 VW2C, Ireland*
[4]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland*
[5]*Department of Agriculture, Food and the Marine, Regional Veterinary Laboratory, Hebron Road, Kilkenny, Ireland*
[6]*Teagasc, Mellows Campus, Athenry, Co. Galway H65 R718, Ireland*
[7]*Teagasc, Moorepark, Fermoy, Co. Cork P61 C996, Ireland*
[8]*Department of Agriculture Food and the Marine, Regional Veterinary Laboratory, Backweston Campus, Celbridge, Co. Kildare W23 X3PH, Ireland*
[9]*Department of Agriculture Food and the Marine, Regional Veterinary Laboratory, Backweston Campus, Celbridge, Co. Kildare W23 X3PH, Ireland*

Presenting author
John Gilmore. E-mail: john.gilmore@farmlab.ie

Application
Anaplasma phagocytophilum is present in the Irish sheep flock and should be considered as a contributor to morbidity and mortality in tick infested areas.

Introduction
Anaplasma phagocytophilum causes the disease known as Tick Borne Fever in ruminants. It is associated with general malaise, lack of production, fever, respiratory signs, and immunosuppression leading to co-infection with other infectious diseases such as Louping Ill and Tick Pyaemia in sheep. It is also a cause of infectious abortion in sheep. Little is known about the prevalence and significance of *A. phagocytophilum* in the Irish national sheep flock. The aims of this study were two-fold. Firstly, to assess the prevalence of *A. phagocytophilum* in lamb carcases submitted to 3 Irish Regional Veterinary Laboratories in 2021 and 2022 using real-time qPCR and to determine if there was an association between PCR positivity and co-morbidities. Secondly, to estimate the flock-level prevalence of *A. phagocytophilum* in Irish sheep flocks using a commercial inhibition elisa.

Materials and methods
A sample from the spleen of every lamb submitted to three Irish Regional Veterinary Laboratories (Sligo, Athlone and Kilkenny) in the calendar years of 2021 and 2022 was analysed by real-time qPCR. Postmortem data, detailing age and diagnosis were recorded for each animal. Chi-square and Multiple Correspondence Analysis (MCA) was carried out to identify relationships between *A. phagocytophilum* PCR status and other concomitant findings.
To determine the flock seroprevalence of *A. phagocytophilum*, pools of 5 sera from 376 flocks collected in 2019 were analysed using the Anaplasma antibody competitive elisa Test Kit, (VMRD, Pullman, WA 99163 USA). 36 positive and 36 negative flocks were subsequently tested individually to determine within-flock prevalence. ROC and AUC characteristics were then employed to determine the optimal cut-off for use on pooled samples.

Results

PCR analysis of lambs submitted for PM analysis showed that pneumonia accounted for 22.8% of the primary cause of death in TBF-positive lambs and 13.9% in TBF-negative lambs. Pneumonia causing bacteria from the family Pasteurellaceae were identified in 33.3% of TBF positive samples and 17.7% of TBF negative samples.

ROC and AUC analysis indicated that using a 2% inhibition cut-off was optimal when using the commercial ELISA to test pooled serum samples compared with a 30% cut-off for use on individual samples recommended by the manufacturers. When a 2% pooled cut-off was employed the national flock level seroprevalence was 28.2% with positive flocks tending to be located in areas where ticks are known to be present.

Conclusions

A. phagocytophilum is a significant disease in Irish sheep flocks located in areas suitable for ticks. This study suggests that infection with the organism may lead to an increased susceptibility to particular infectious diseases such as pneumonia caused by *Mannheimia haemolytica*.

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Exploring the faecal microbiome of weaned lambs with high and low gastrointestinal nematode burden, grazing perrenial rye grass and mixed swards

C. Reigate[1], J. Jefferson[2], T. U. Webster[2], K. Wells[2], J. Rivero[1]
[1]*Rothamsted Research, West Common Harpenden AL5 2JQ, UK*
[2]*Swansea University, Singleton Park, Sketty, Swansea SA2 8PP, UK*

Presenting author
Claire Reigate. E-mail: claire.reigate@rothamsted.ac.uk

Application

This project was funded by the BSAS Steve Bishop Net Zero Award. This pilot study investigates the parasitic gastrointestinal nematode burden and the faecal microbiota of weaned lambs grazing two sward types. Exploring the interaction between parasite load, diet and the gut microbiome may help in our understanding of host-susceptibility to infection.

Introduction

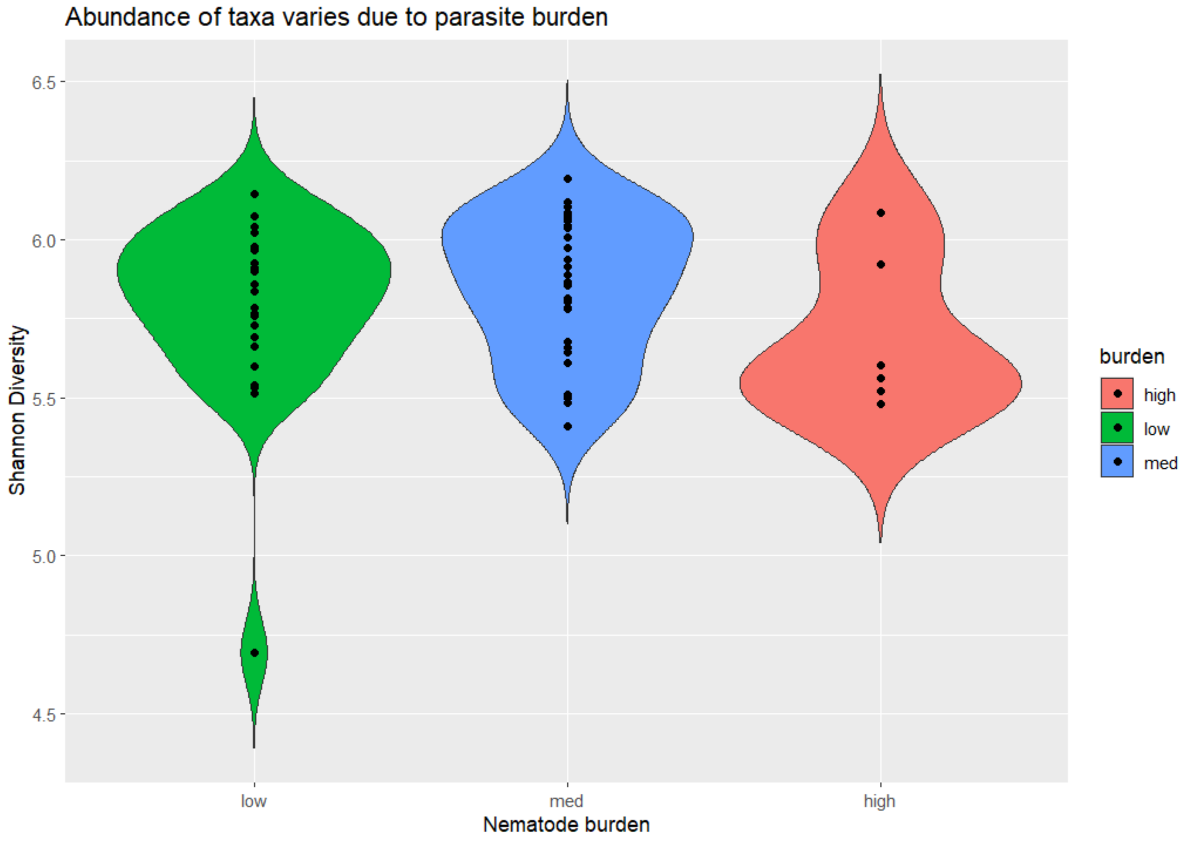
Parasitic gastrointestinal nematodes (GIN) can negatively impact animal health and production and can be an important constraint to efficient sheep production. GINs share their environment with the gastrointestinal microbiome. GINs induce pathological changes to the gut mucosa and their presence is likely to impact upon the microbial community. Diet is also a distinct driver of diversity and abundance of intestinal microbiome. Previously, sheep experimentally infected with *Haemonchus contortus* have been shown to exhibit a variation in gut bacterial composition associated with high or low levels of infection (Mamun et al., 2020). However, this association has not been explored in naturally infected animals who will inevitably harbour mixed GIN infection. Therefore, this preliminary study was designed to investigate the gut microbiome of weaned lambs naturally infected with gastro-intestinal parasites grazing two different sward types.

Materials and Methods

Naturally voided faecal samples were collected from five Suffolk mule X Charollais cross lambs grazing four plots, established with two pasture types (perennial rye grass, and a mixed sward of 2 grasses, 2 legumes and 3 forbs), at three post-weaning time points between July and September (d0, and d35 and d57). Sixty faecal samples were collected from the ground into sterilised labelled plastic pots and transferred back to the lab for analysis. From each faecal pat, three sub samples (0.5g) were transferred into sterilised 3ml Eppendorf tubes containing 2ml RNAlater. These were left at room temperature for 24h then stored at -20°C until further analysis. The remaining sample was used for faecal egg counts (FEC). Faecal egg counts were determined using the FECPAK S5 method (Techion Ltd, UK), the presence of coccidia, tapeworm and strongyloides were recorded. DNA was extracted using the prepGEM Bacteria kit (MicroGEM, Charlottesville, VA, USA). Two-step PCR was performed targeting the V4 region of 16S rRNA gene. During the second PCR, sample-indexing was performed using the Nextera ® XT Index Kit (Illumina, Inc., San Diego, CA, USA). Final libraries were normalised to 4 nM and sequenced on an Illumina MiSeq platform (paired 300 bp reads). Sequencing reads were processed using QIIME2 2024.5.0 {Bolyen, 2019 #65008} to obtain a list of amplicon sequencing variants (ASVs) for each sample. The ASV taxonomy was classified by training a naïve-Bayes classifier on the SILVA reference database in release version v.138 {Quast, 2012 #65016} for 16S rRNA gene sequences, and ASVs assigned to chloroplasts or mitochondria were removed. Chao1 and Shannon Diversity indices from rarified samples were used to estimate microbial per-capita species richness and diversity. For the egg count data, generalised linear mixed effect models were fitted using the 'glmmTMB' function for R (R core team 2015). The number of nematode eggs detected was the response variable, and explanatory variables were month of sampling and sward type with individual lamb as random effect. September and mixed sward were set as the reference categories within the respective fixed effects. Fixed effects were considered significant when $p<0.05$, Tukey post-hoc test was used for multi-group comparisons.

Results

Lambs had significantly lower parasite burden in July than in August (I<0.05) and September ($P<0.05$). There was no evident difference in parasite burden between August and September ($P=0.6$). The glmm indicated lambs grazing rye grass had significantly lower nematode burden than those grazing the mixed sward ($P<0.05$). Preliminary results of Chao1 species richness estimates, and Shannon Diversity index, suggest that gut microbial alpha diversity marginally increased from July to August ($P < 0.05$) and from July to September in weaned lambs. We found no evidence that alpha diversity measures varied across pasture type or with parasite egg counts.



Conclusions

Faecal egg counts varied greatly between individual animals, and sampling occasions. Gut microbial diversity varied over time. Further analysis is necessary to explore possible relationships between parasite burden and the relative abundance of gastrointestinal bacteria.

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No effect of multispecies swards on the host response to Ostertagia ostertagi in beef steers offered multispecies swards compared with perennial ryegrass white clover swards

M. O'Rourke[1], D. Kenny[1], A. K. Kelly[2], M. McGee[1], E. O'Riordan[1], C. Machin[1], O. Keane[1]
[1]*Teagasc Animal and Bioscience Research Department, Grange, Dunsany, Co. Meath C15 PW93, Ireland*
[2]*University College Dublin, Belfield, Dublin 4, Ireland*

Presenting author
Marie O'Rourke. E-mail: MarieEKORourke@gmail.com

Key words: multispecies, herbs/forbs, gastrointestinal nematode infection, host response, beef cattle, feed intake, animal performance, condensed tannins

Introduction

Ireland's temperate climate promotes abundant grass growth and a relatively long grazing season (O'Donovan et al., 2021), thus facilitating pasture based beef cattle production systems (Drennan and McGee, 2009). However, grazing animals typically ingest larvae of parasitic nematodes which are present on the sward (O'Farrell et al., 1986). Indeed, gastrointestinal nematodes (GIN) offer the most pervasive challenge to grazing livestock globally, negatively impacting animal health, welfare and production efficiency. *Ostertagia ostertagi* is the most pathogenic of cattle nematodes, with an associated economic burden (Sharma et al., 2017). Ostertagiosis typically affects cattle in the first and second grazing seasons and can cause severe pathologies, resulting in reduced feed intake and poor thrive (Fox, 1993, Gibbs, 1998). Whilst the strategic use of anthelmintics have been an effective intervention to manage consequences of parasitic nematode infection, there is increasing evidence of anthelmintic resistance in grazing cattle (Cotter et al., 2015, O'Shaughnessy et al., 2014). The rapid and widespread development of anthelmintic resistance warrants the development of alternative GIN control strategies. Lambs grazing multispecies swards, containing grasses, legumes and herbs/forbs, had a reduced requirement for anthelmintic interventions (Grace et al., 2019, Rodriguez et al., 2019). Some studies, have reported lower faecal egg count in grazing cattle offered chicory (Pena-Espinoza et al., 2016). It has been proposed that the reduced infection level in grazing cattle offered chicory may be attributed to the reduced survival of larvae on the sward, compared to conventional grass-based pastures. Additionally, it has been suggested that there may be biochemical properties in herbs/forbs and legumes that may elicit anthelmintic benefits. Chicory contains a range of secondary metabolites with potential anthelmintic activity, including sesquiterpene lactones (Foster et al., 2006), and condensed tannins, however in low quantities (Niezen et al., 1998, Barry, 1998). White clover (Trifolium repens) contains high condensed tannins levels (Woodfield et al., 2019), and a reduction in FEC was observed in lambs grazing perennial ryegrass/white clover swards (Niezen et al., 2002). The direct impact of condensed tannins are linked to the intake of the tanniferous forages (Hoste et al., 2012) which can reduce egg excretion by reducing worm burden and/or worm fecundity (Lange et al., 2006). Moreover, there is limited existing evidence and vast ambiguity on the potential of herbs/forbs to reduce parasitic nematode infection in grazing cattle.

Materials and methods

Therefore, the objective of this study was to determine the effect of offering multispecies swards on feed intake, growth, efficiency and host response to *Ostertagia ostertagi* in beef steers. Furthermore, the aim of the study was to accurately measure feed intake of multispecies swards, as well as the feed intake of individual botanical components, in a controlled parasitic inoculation study, to accurately quantify the existence of anthelmintic benefits of these swards. The diets offered were multispecies swards containing grass (*Lolium perenne*), legumes (*Trifolium repens, Trifolium pratense*) and herbs/forbs (*Cichorium intybus, Plantago lanceolata*) or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) swards. This experiment was part of a larger study, and a cohort of forty Charolais cross steers were assigned to two dietary treatments, multispecies (MSS) (n=20) or perennial ryegrass white clover (PRGWC) (n=20). This experiment took place at the end of July, two months after the beginning of the larger trial, in situ with *O. ostertagi* occurrence in grazing cattle. In this study, fresh forage was harvested once daily (0600h) via the cut & carry method, to a target residual sward height of 5 cm using a Zero-Grazer (Model AB70 Zero Grazer, Dromone, Oldcastle, Co. Meath). Steers were offered their respective dietary herbage allowance three times daily, at 0700h, 1100h and 1600h, ad libitum. Refusals were weighed, and discarded each morning. Individual animal feed intake was recorded via Calan gates (American Calan Inc., Northwood, NH). Cattle were weighed fortnightly, before feeding. Once weekly, botanical separations were conducted on a ~200g representative sub-sample of each of the two swards offered. Ten animals per dietary treatment were orally infected with 100,000 *Ostertagia ostertagi* L3 over three days. Faecal samples were taken trans-rectally at the beginning of the experiment pre-inoculation, twice per week from weeks 3-6, and weekly thereafter for faecal egg count (FEC) and dry matter (DM). Blood samples were taken weekly for haematology, serum anti-Ostertagia antibody and serum pepsinogen analysis. Faecal egg count was conducted using the mini-FLOTAC method, with a detection limit of 5 eggs per gram.

Results and Discussion

During the experimental period, the botanical composition of MSS was 35% *Lolium perenne*, 12% *Trifolium repens*, 32% *Trifolium pratense*, 5% *Cichorium intybus*, 15% *Plantago lanceolata* and <1% weeds. The botanical composition of PRGWC was 55% *Lolium perenne*, 33% *Trifolium repens*, 12% weeds. There was no significant difference in feed intake between animals offered either pasture type (MSS vs. PRGWC) or between non-infected and infected animals. There was no significant difference in average daily gain (ADG) between animals offered either pasture type (MSS vs. PRGWC), or between non-infected and infected animals. Results of the study are shown in Figure 1. Firstly as planned there was a discernible difference (P<0.01) in FEC per g of faecal dry matter (FECDM) in non-infected vs. infected animals. However, there was no difference in FECDM between infected animals offered either pasture type. There was a difference in anti-Ostertagia antibody concentrations (P<0.05) from 24 days post infection (DPI), peaking at 31 DPI (P<0.01) between non-infected and infected animals. However, there was no differences in anti-Ostertagia antibody concentrations between cattle offered either pasture type (MSS vs. PRGWC). There was a difference (P<0.01) in absolute eosinophil count in peripheral blood, peaking at 9 DPI between non-infected and infected animals.

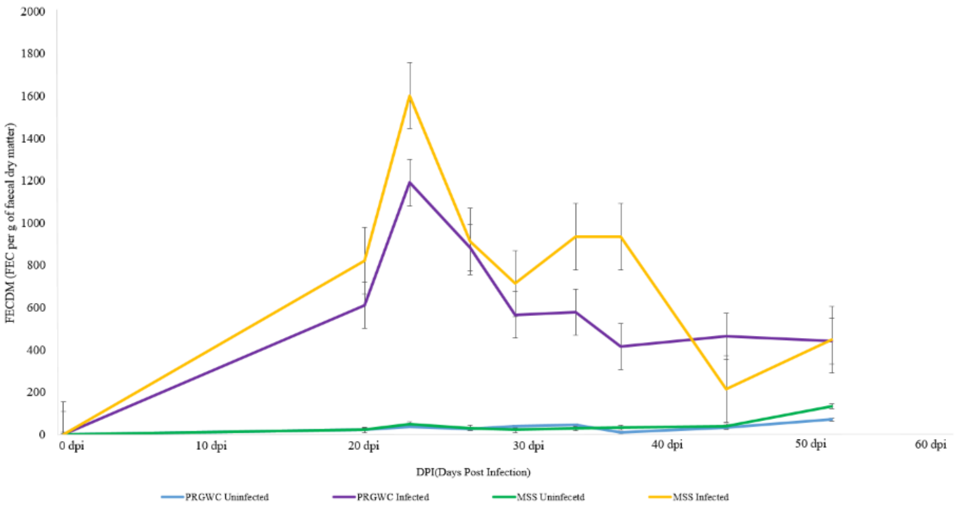

Figure 1. Faecal egg count (FEC) per g of dry matter for non-infected and infected cattle, offered MSS and PRGWC.

Conclusion and implications

In summary, there was no benefit of including additional herbs/forbs in the diet of steers on their response to inoculation with *O. ostertagi* larvae. In previous studies, the proportions of herbs/forbs were significantly higher where an anthelmintic benefit was observed. The proportion of herbs/forbs in the sward in our study was representative of an established multispecies sward, accurate feed intake of the sward and their individual components were recorded, in a controlled inoculation study.

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Understanding the drivers of community trust in livestock production

C. Bailey[1]
[1]*VoconiQ, 25 King St, Bowen Hills Q 4006, Australia*

Presenting author
Courtney Bailey. E-mail: Courtney.bailey@voconiq.com

Public trust in livestock industries is essential for sustaining their role within global society. This study, part of Voconiq's broader research programme across Australian and the United Kingdom's agricultural sectors, investigates the drivers of community trust in livestock production, focusing on environmental responsibility, industry responsiveness, and animal welfare—critical components for building social licence. Using data from over 25,000 Australians gathered through a nationally representative, longitudinal survey, the study identifies and tracks trust trends across livestock, horticulture, and other agricultural sectors, with an emphasis on developing a industry agnostic model for best practices.

Application

These findings offer actionable insights for rural industry stakeholders, particularly within the livestock sector in Australia and beyond. Emphasising proactive communication, transparency in environmental practices, and a clear commitment to animal welfare is crucial for enhancing social acceptance across livestock industries globally, where public expectations for ethical practices are rising.

Introduction

Voconiq's research programme examines the dynamics of trust between rural industries, including livestock production, and the public. As livestock plays an essential role in global food security and sustainable agricultural systems, this study provides evidence-based recommendations for addressing and managing public expectations, thereby reinforcing the livestock sector's social licence.

Materials and Methods

This research draws from an extensive survey dataset, collected annually from a representative sample of Australian adults. Survey metrics specifically targeted trust in livestock through measures of environmental responsibility, responsiveness to community concerns, and animal welfare practices. Data were analysed to understand the key factors influencing public trust and acceptance across livestock and other sectors.

Results

Results indicate that environmental responsibility remains the strongest driver of trust in livestock production, with 63% of participants emphasising the need for sustainable practices. Responsiveness to community feedback also proves critical, with 56% of respondents associating higher trust with attentiveness to public concerns. Additionally, animal welfare impacts community trust, with 72% of Australians expressing that humane treatment of livestock is essential for sector trust. These findings highlight universal principles for building trust, relevant to livestock industries worldwide.

Conclusions

For livestock industries globally, sustaining public trust necessitates a commitment to transparency in environmental and welfare practices and responsiveness to community expectations. Integrating these practices into operational strategies helps livestock sectors strengthen their societal role while addressing evolving public demands. Insights from five years of data across various sectors provide a strategic framework for fostering stronger connections between livestock industries and communities worldwide, promoting a sustainable and trusted agricultural future.

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Untapped potential: the Neglected urban interest in secondary agriculture

S. Graham[1]
[1]*Barker College, 91 Pacific Hwy, Hornsby NSW 2077, Australia*

Presenting author
Scott Graham. E-mail: SGraham@barker.nsw.edu.au

Student enrolments in agricultural courses at the secondary school level in Australia have been in decline for several decades, despite a rapidly growing population and an increasing demand for food and fibre. At the same time, university enrolments and graduations in agriculture-related degree programs have also diminished, to the extent that there are currently approximately six professional positions requiring degree-level qualifications for every agriculture graduate in Australia each year.

This decline in agricultural education and career pathways occurs within the context of Australia's highly urbanised population, with around 67% of the population residing in just five major cities. Additionally, agricultural work is often viewed by urban populations as primarily a rural pursuit. This perception may limit the recognition of the potential for significant engagement with agriculture among urban students, presenting an untapped demographic that could contribute to the future workforce of the sector.

This talk presents a case study of Barker College, a secondary school located in metropolitan Sydney, which has experienced a notable increase in urban, non-agriculture-background students enrolling in Agriculture courses from Years 9 to 12. Enrolment numbers have grown from approximately 100 students in 2010 to over 400 students in 2024. The study will explore the factors that attract metropolitan students to agriculture as a school subject, strategies for engaging academically able students in the field, and the key motivators that encourage these students to pursue further education and careers in the agricultural sector.

Autism and Agriculture – diverse people, exceptional care

K. Richards[1]
[1] *SunPork Group, 1/6 Eagleview Pl, Eagle Farm QLD 4009, Australia*

Presenting author
Kirsty Richards. E-mail: kirsty.richards@sunporkfarms.com.au

Autism is a lifelong neuro-developmental condition that affects the way a person experiences the world. Estimates suggest that more than 2% of the population are autistic. Autistic adults are often unemployed, under-employed and socially disadvantaged despite having the skills and capacity to contribute productively to the workplace. Skilled employment can be difficult to find and hard to retain. Traditional recruitment practices such as job interviews represent systematic barriers to otherwise capable employees. Workplace understanding and support are often poor.

Autism and Agriculture is a world-first initiative of SunPork, the Cooperative Research Centre for Living with Autism (Autism CRC) and Specialisterne to develop animal care career paths for people on the autism spectrum. Goals of the program include:

- To identify and employ the diverse skills and talents of autistic people in animal care.
- To build employment capacity within the agricultural sector for autistic adults.
- To develop innovative labour solutions that recognise labour force challenges in the agricultural sector, matched with the imperative for livestock industries to provide optimal animal care.

In 2017, SunPork Farms announced the commencement of their first autistic employees in Queensland and South Australia. In 2018, SunPork was honoured to receive the Autism Spectrum Australia Advancement Award. Today, some of SunPork's autistic employees are into their eighth year of fulltime employment and eleven have worked for the business for six years or more. During this time, SunPork's autistic employees have gained the satisfaction and reward of meaningful work, independence and a regular wage. Secure employment, financial independence, personal development and a sense belonging have supported individuals to transition to independent living, gain confidence, friendships and to undertake pursuits ranging from their first overseas holiday to purchasing their first home.

As a business, Autism and Agriculture has allowed SunPork to scrutinise and challenge traditional human resource management paradigms, with transformative outcomes not only for autistic employees but for their co-workers, trainers, mentors and managers. The program has fostered teamwork, engagement and has provided a sense of achievement and satisfaction across the entire business. It has also built leadership capacity, people skills and fostered business culture and pride.

Autism and Agriculture has demonstrated that shifting the traditional recruitment paradigm and removing systemic barriers to employment can have transformative outcomes for autistic jobseekers and employees. The program has also demonstrated that autistic adults absolutely have the skills, capability, commitment and motivation to work in animal care.

SunPork values and admires its autistic employees and recognises them as an asset to its business. Their achievements are truly remarkable.

The role of bush foods in future industry and ethical considerations for research

M. Watkins[1]
[1]*University of South Australia, Adelaide SA 5000, Australia*

Presenting author
Michael Watkins. E-mail: Michael.Watkins@unisa.edu.au

The Australia native foods, also known as 'bush foods', have important cultural nutritional and medicinal importance and can provide many opportunities in future industries. Australia harbours unique biodiversity where bush foods possess favourable nutrition properties compared to Western fruits. Bush foods are nutritionally dense and provide a good source and range of micronutrients and phytochemicals, particularly phenolics and antioxidants (Richmond et al., 2019). Today where climate change threatens current food systems, it is vital to explore the role versatile and functional bush foods and native plants play in the future of food consumption and environmental sustainability (Richmond et al., 2019; Williams et al., 2024). However, these foods and plants are more than just for consumption and sustenance, they play key roles in cultural knowledge and food systems which governs Aboriginal societies.

Research which involves bush foods and Aboriginal Peoples and their knowledge systems, exploring their properties and potential uses require certain ethical considerations. In an industry where Aboriginal involvement is less than two percent, researchers have a vital role to play in how they can work alongside Aboriginal communities and businesses to achieve common goals and mutually beneficial outcomes through partnerships. Historically, traditional ecological knowledge held by Aboriginal Peoples have been exploited and profited from without involvement or benefits for those who hold that knowledge. This presentation will provide insights and reflections on Michael's research collaborations and partnerships with Aboriginal communities in the bush foods space. This work outlines how two knowledge systems can interact, overlap and add value to innovative research and future industry.

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Bridging the gap from Science to Extension to Expression: Innovation only counts when it is used

C. Lewis[1]
[1]Genus PIC, London Road, Nantwich, Cheshire CW5 7JW, UK

Presenting author
Craig Lewis. E-mail: Craig.Lewis@genusplc.com

Innovation has and will continue to be at the heart of agriculture. As society demands ever more from the food production chain, the traditional scope of farmers to provide safe, affordable, abundant, and tasty food has now been expanded to include sustainable, welfare friendly, and low antimicrobial food production: food with Trust. Animal scientists continue to innovate in livestock agriculture at an accelerating rate with significant advancements in health, nutrition, management, and breeding / genetics. There are new tools developed by the scientific community to address the increasing societal demands to make very real and impactful positive contributions to animals, people, the planet, and profitability. To implement these innovations social licence is also required. An example of innovation within the agricultural space are new breeding technologies. A timely example is the gene edit to deliver resistance to Porcine Reproductive and Respiratory Syndrome (PRRS), which could be a key innovation to deliver on the expectations of multiple stakeholders within the pork chain. A key question will continue to be: Is the modern world, mostly removed from food production, truly understanding that innovation has always been core to agriculture and are we fast enough at delivering positive innovations into the hands of farmers?

European Network on Livestock Phenomics: enhancing phenotyping in animal breeding and livestock farming

L. Fontanesi[1]
[1]*University of Bologna, Via Zamboni, 33, 40126 Bologna BO, Italy*

Presenting author
Professor Luce Fontanesi. E-mail: luca.fontanesi@unibo.it

Application

Phenomics in livestock focuses on systematically describing of the animal phenome, which includes the physical and molecular traits of an animal. EU-LI-PHE is a Europe-centred multidisciplinary, interconnected and inclusive network of experts aiming to enhance scientific collaboration, catalyse developments, and transfer livestock phenomics concepts and applications to improve the sustainability of the European livestock production sector, with a global vision.

Introduction

Phenomics is emerging as a major new technical discipline in applied biology, including animal husbandry and breeding. Phenomics, when applied to the livestock production system, has one major aim: to systematically describe the phenome, which refers to the physical and molecular traits of an animal. This discipline is defined as the ensemble of methodologies and technologies for the acquisition, analysis, and exploitation of high-dimensional phenotypic data on an animal-wide scale (Houle et al., 2010). In the animal production sector, the availability of accurate and specific phenotype data can inform new breeding objectives, related breeding and selection programs and provide novel essential information for the farmer's daily activities and choices needed to improve reproduction strategies, disease control and animal welfare. Therefore, phenomics applied to animal breeding and husbandry is considered an essential innovation to support the sustainability of all animal production systems. The development and application of phenomics in livestock clearly require a multi-disciplinary and multi-actor approach to bring together different expertise, resources, and expectations. Livestock phenomics necessitates experts in many fields, with a critical mass of knowledge and technical expertise. To address this need, we developed a European-based network that has been funded by the European Union. The network is a COST Action (European Network on Livestock Phenomics – EU-LI-PHE - https://www.cost.eu/actions/CA22112/; COST – European Cooperation in Science & Technology, 2023) designed to tackle four main challenges and structured across activities in four working groups (WGs). This COST Action is funded until 2027.

Materials and Methods

The development and application of livestock phenomics tools, methods, and data analytics approaches require significant investments in time, human resources, and capital. The main challenges in livestock phenomics for research and innovation, which are largely unaddressed or only partially addressed, are grouped into four main areas that reflect the structure of the EU-LI-PHE Working Groups (WG): WG1 (Phenotyping technologies) aims to provide an overview of current phenotyping technologies and infrastructures for livestock phenomics applications and define research needs to capture high-dimensional phenotypic information on a large scale; WG2 (Genome to Phenome integration) aims to provide an overview of the links between genome/epigenome variation and phenotypic variation in livestock species, identify synergies with related initiatives on functional analyses of livestock genomes and identify knowledge gaps and research needs to pave the way for new applications; WG3 (Computational resources and methodologies for data analyses) aims to provide an overview of the computational models, methods and tools available, as well as current and future needs for the development of applications in the context of livestock phenomics and to identify the needed synergies and develop-ments required in terms of cyberinfrastructures and computational capabilities; WG4 (Economic impact, regulations, policies, and society) aims to provide an overview of the potential technological and economic impact of livestock phenomics and to summarize the regulatory frameworks around this discipline and evaluate access to information and data generated; and iii) to analyse societal perceptions of livestock phenomics. In addition to the four main technical areas that provide the scientific backbone of the project, an additional working group, WG5 (Stakeholder engagement, communication, and dissemination) is part of the founding pillars of EU-LI-PHE. WG5 links all the activities carried out in WG1-4 as follows: i) to ensure continuous engagement of stakeholders; ii) to ensure productive and efficient communications; and iii) to ensure publication of reviews, reports, surveys and the establishment of a website and an active social media presence.

Results

The specific research and coordination objectives of EU-LI-PHE are focused on: (i) advancing the state-of-the-art of high-throughput technologies and protocols required for deep phenotyping to describe phenotypic information at multiple levels in farmed an-imals; (ii) providing cross-disciplinary knowledge to develop new standards in pheno-typing technologies, phenome data descriptors, phenotype ontologies, databases, data structures, storage and sharing; (iii) evaluating available software and bioinformatic tools and defining methods for effective data mining, processing, summarising, integration and visualization of genome/epigenome to phenome data in livestock; (iv) exploring integrative dynamic responses and adaptations of animal phenomes to variable environmental factors; (v) exploring novel data integration and fusion approaches including omics and sensor data, images, videos and animal movement and sound data for generation and visualisation of complex system models of livestock populations to facilitate prediction of interventions and outcomes; (vi) investigating and proposing new applications for genomic selection and precision livestock farming; (vii) exploring the regulatory landscape around livestock phenomics, including ownership of the data, open access data policies and intellectual property rights; and (viii) analysing stakeholder opinions and societal perceptions of innovations in this field for the reduction of negative impacts on the animals and on the environment. EU-LI-PHE has specific capacity-building objectives to foster knowledge exchange by: (i) providing well-trained young researchers and professionals in livestock phenomics and related disciplines that complement and complete the background and knowledge needed for the alignment of scientific progress and industry demands; (ii) fostering the exploration and implementation of new training routes and methodologies, with the aim of widening career prospects for highly specialised researchers who can accumulate integrated skills of different disciplines around big data production and analysis, with an interdisciplinary vision; (iii) stimulating new ideas and innovative methodologies in an open innovation framework to address new opportunities generated by livestock phenomics approaches with a comprehensive strategy of communication and dissemination to attract parallel and synergistic research fields and to benefit the whole scientific community, the relevant industrial sectors and all stake-holders, including policy and decision makers; and (iv) fostering the involvement and collaboration of teams from less research-intensive countries across Europe, promoting their inclusiveness, through the sharing of new knowledge around a network of opportunities focused on livestock phenomics.

Conclusions

It is expected that EU-LI-PHE will become a reference network for animal phenotyping, not only in Europe but also worldwide. This will contribute to the development of novel solutions for the benefit of the animal production sectors. Phenotyping systems, which involves sensors, cameras, noise recorders, molecular phenotypes and artificial intelligence to extract relevant information, can change the phenotyping paradigm of animals. This will accelerate the transition towards phenomics applied in animal breeding and for various other purposes.

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Utilizing machine learning in genomic selection of Holstein dairy cattle's residual feed intake

E. J. K. Ong[1,2], M. Mooney[1], M. Shirali[2]
[1]Queen's University Belfast, University Rd, Belfast BT7 1NN, UK
[2]Agri-Food & Bioscience Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

Presenting author
Edwin Jun Kiat Ong. E-mail: eongjunkiat01@qub.ac.uk

Application

To predict dairy cows with high residual feed intake using machine learning models to increase the precision of genomic selection in Holstein dairy cattle.

Introduction

The economic viability of livestock production systems, particularly dairy operations, is significantly constrained by feed costs, which account for the majority of production expenses (Alqaisi & Schlecht, 2021). Profitability can be improved by identifying and cultivating herds with cattle that exhibit high feed efficiency (FE), allowing feed costs to be minimized without reductions in milk yield. The use of genomic selection combined with precision livestock farming (PLF) has been identified as a method to enable the detection of cattle with desirable genetic traits for FE, supporting the development of breeding programs aimed at improving FE across entire herds (Madilindi et al., 2022). However, FE is recognized as a complex trait, and its measurement within genomic selection programs is hindered by the limited availability of direct observational data. Machine learning (ML) approaches have been proposed as a means to address these challenges by facilitating the integration of large and complex genomic datasets. These techniques can enhance the identification of genetic markers associated with FE and improve the predictive accuracy of genomic selection models (Mota et al., 2024). In this study, ML algorithms were applied to genomic data from Holstein dairy cattle to predict residual feed intake (RFI), which was estimated using feed intake and milk production data

Materials and Methods

Genotypic data from 195 Holstein-Friesian dairy cattle at the Agri-Food and Biosciences Institute (AFBI) were collected using the 50K Illumina Bovine SNP panel. Over the period of 2014 to 2023, a total of 9984 weekly records of individual cattle milk production, feed consumption and weight data were used to calculate the target phenotype, RFI. RFI was regressed using a random effect model and were classified as high or low RFI cattle. The predictions of RFI were then modeled using Genomic Best Linear Unbiased Prediction (GBLUP) in addition to various ML algorithms, including logistic regression models (LogReg), support vector machines (SVM), stochastic gradient boosting (SGB), and random forests (RF) using genotype data. Feature selection was conducted to select the top 1% of SNPs for predicting RFI using random forest. Training and validation datasets were created by randomly splitting the data, with 70% used for training and 30% reserved for validation. Fisher's Least Significant difference (LSD) test of the accuracy of machine learning (ML) algorithms was performed to evaluate the performance metric between the models.

Results

The logistic regression model achieved the highest accuracy values demonstrating significantly better performance than other ML models using the LSD test. The mean accuracy scores for LogReg is 0.79, RF is 0.76, SVM is 0.74, DT is 0.58 and GBLUP is 0.51 where GBLUP achieved the lowest accuracy scores as compared to the other ML models (Figure 1). The findings demonstrated that ML-driven approaches can effectively predict complex traits such as RFI, highlighting the potential of these methodologies in genomic selection. Genetic markers identified as highly associated with RFI will be further analyzed using gene ontology to explore the biological pathways involved, offering deeper insights into the genetic mechanisms underlying FE.

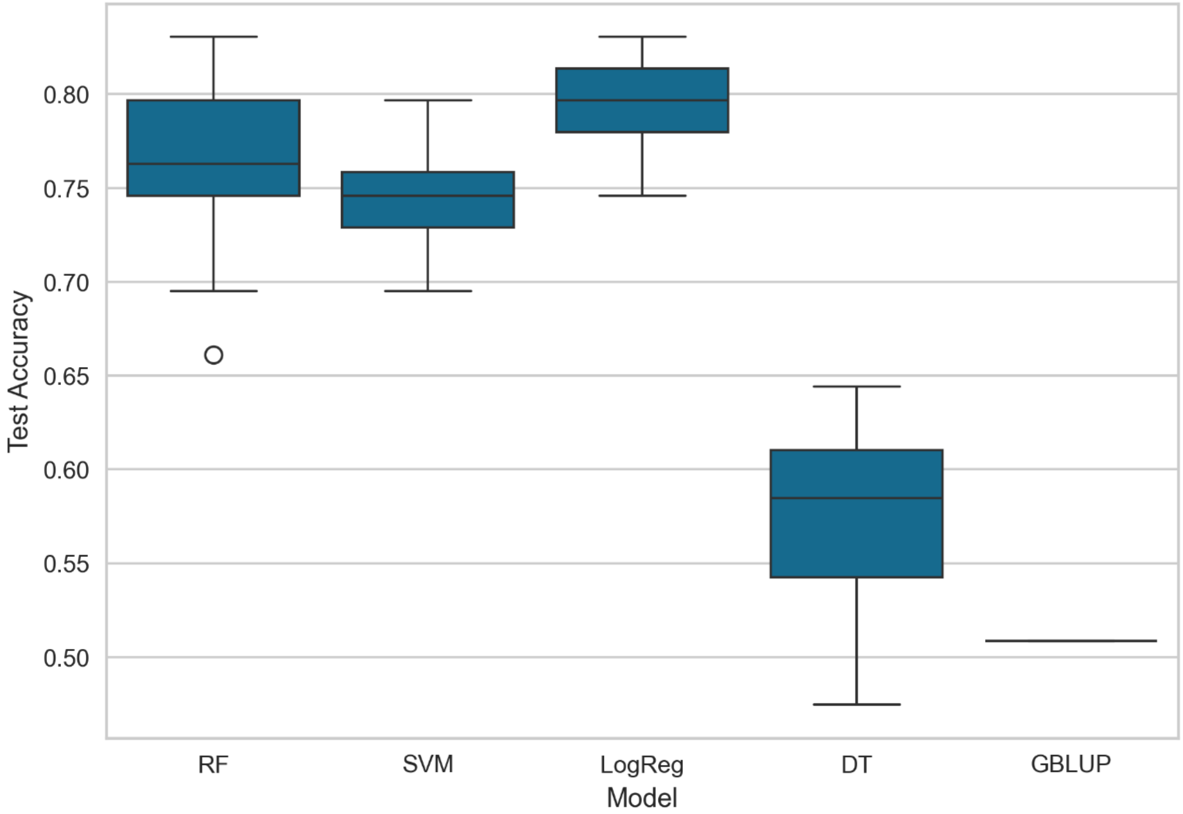

Figure 1. Boxplot of the distribution of test accuracy valyes for random forest (RF), support vector machine (SVM), logistic regression (LogReg Model), decision tree (DT) and Genomic best linear unbiased prediction (GBLUP)

Conclusions

This study demonstrates the benefit of using machine learning to enhance genomic selection for complex traits like feed efficiency in dairy cattle. The machine learning models achieved significantly higher accuracy values than traditional GBLUP model. The performance of ML algorithms, particularly logistic regression had the highest mean accuracy scores (0.79) and GBLUP (0.51). This highlights a more effective method in identifying genetic markers for breeding optimization in dairy production systems.

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Predicting feed intake of UK dairy cattle using milk mid-infrared spectra, predictor traits, and machine learning

A. Wells[1,2], P. Sofokleous[1], J. Cai[3], S. Denholm[1], E. Wall[1], G. Banos[1,2], M. Coffey[1], B. Li[1]
[1]Scotland's Rural College (Roslin Institute Building), Easter Bush Campus, Midlothian, Scotland, UK, EH25 9RG
[2]The Roslin Institute (University of Edinburgh), Easter Bush Campus, Midlothian, Scotland, UK, EH25 9RG
[3]Programme of Quantitative Genetics and Genome Analysis (University of Edinburgh), South Bridge, Edinburgh EH8 9YL, UK

Presenting author
Abbygail Wells. E-mail: abbygail.wells@sruc.ac.uk

Application

Feeding the world's growing population sustainably is a global challenge. The feed efficiency of dairy cattle is an important breeding goal for environmental and economic improvement of the dairy industry. Measuring feed intake is limited by the cost of specialist equipment to identify individual cattle and weigh the food consumed. Using machine learning to predict feed intake from other readily available data will enable us to produce more feed intake phenotypes and more effectively identify the most efficient dairy cattle.

Introduction

Producing meat and milk sustainably for a growing population can be achieved through more efficient use of feed. Feed costs also represent a large proportion of the costs on a dairy farm making feed efficiency an important breeding goal for the dairy industry (Berry *et al.*, 2014; Li *et al.*, 2021). Breeding for feed efficiency has the potential to make a permanent and cumulative, population wide improvement. Feed intake is expensive to measure requiring investment in specialist equipment which for many commercial producers is not viable. Milk sampling of dairy cattle is a non-invasive process, routinely carried out to measure milk fat and protein along with somatic cell count (an indicator for mastitis). Mid infrared (MIR) spectroscopy is a low cost method of analysing the composition of milk samples and the spectra are therefore abundantly available. MIR spectra of milk have been widely used for the prediction of expensive and difficult to measure traits such as feed intake (Denholm *et al.*, 2020).

Previous studies have indicated good potential for predicting dry matter intake (DMI) in dairy cattle from milk MIR but are limited in their dataset and computational requirements. The present study aims to (i) use an efficient ML method (XGBoost) to predict DMI from milk MIR data and (ii) assess the benefits of adding key animal traits (live weight, milk yield) in predicting DMI in dairy cattle. We used 330,336 records from 980 lactating Holstein cows, which, to our knowledge is the largest database of this kind.

Materials and Methods

The study was based on data from UK Holstein dairy cattle in the Langhill herd at SRUC Dairy Research Centre that has been running since 1973. Cows were milked twice a day and milk sampling was carried out once a week. All milk samples were analysed using MIR spectroscopy and processed according to standard procedures (Grelet *et al.*, 2015; Friedrichs *et al.*, 2015). Feed was offered ad libitum and refusal was measured using individual feed bins (HOKO-system, Insentec B.V.). Feed intake was measured for housed cattle for a period of 130 days on average for each lactation. The feed was sampled, and dry matter content of feed was analysed regularly to obtain daily DMI per cow. After the removal of spurious records from the data and aligning the feed intake and MIR spectra, the final dataset consisted of 330,336 records. Training and testing datasets were created by splitting the whole dataset by a ratio of 9:1 from random sampling which was repeated for 10 fold cross validation. The machine learning method of XGBoost was used to train the prediction model to predict DMI from MIR data and predictor traits using the training dataset. The best prediction model from training was then used to predict DMI in the testing dataset, where the prediction accuracy for DMI was measured by RMSE. The initial predictor variables for DMI were the 1060 wavepoints of the MIR spectra and this was considered the base model. Further predictor variables added to MIR data included feed group (high- and low-energy forage), genetic group (control-line and select-line), milk yield, and liveweight. The machine learning was run on an NVIDIA DGX computer running a Linux operating system

Results

DMI was predicted from milk MIR spectra with a high pearson correlation coefficient of 0.79 as seen in Table 1. The addition of other predictor variables into the model improved the correlation further to 0.82-0.88.

Table 1. Model performance for predicting dry matter intake (DMI) using different predictor traits (r=pearson correlation coefficient of predicted and actual DMI, SE=standard error, RMSE=root mean squared error)

Model variables	r	SE	RMSE
MIR (only)	0.79	0.0036	3.60
MIR + milk yield	0.82	0.0037	3.32
MIR + live weight	0.87	0.0027	2.91
MIR + milk yield +live weight	0.88	0.0027	2.77

Conclusions

We have been able to predict dairy DMI from milk MIR spectra with high prediction accuracy. Including extra predictor traits of dairy cows (feed groups, milk yield, liveweight) improved the accuracy of prediction.

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Bioinformatic investigation of the differences between traditional and modern breeds of cattle

K. O. Temiz[1,2], W. Aubrey[2], M. Hegarty[1]
[1]*Aberystwyth University Department of Life Sciences, Penglais, Aberystwyth SY23 3FL, UK*
[2]*Aberystwyth University Department of Computer Science, Penglais, Aberystwyth SY23 3FL, UK*

Presenting author
Kardelen Temiz. E-mail: kot3@aber.ac.uk

Application

The objective is to explore the feasibility of an improved test for differentiating between traditional and modern Hereford cattle populations, preventing admixture with external bloodlines, and utilizing machine learning to classify animals with missing pedigree information.

Introduction

The purebred Hereford Original Population (HOP) cattle breed has been present in the United Kingdom since 1627. It originated in Herefordshire, England, and was known for producing high-quality, rich meat despite poor grazing conditions (Willham, 1937). The Hereford breed became popular for beef production worldwide, leading to crossbreeding with outside breeds to improve productivity and toughness in local environments (Blott et al., 1998). The HOP is the progenitor of the more modern commercial North American Derived (NAD) cattle which has longer legs and larger body size. Previous studies have shown that two groups are genetically distinct due to introgression of genetic material from other breeds into the NAD (Hegarty et al., 2020).

Materials and Methods

Breed Assignment and Clustering of Genotypes

Cattle genotypes from Hereford Herdbook International (HHBI) were combined with previous data from McMahon *et al.* (2015) using the 7K BovineLD assay, to investigate genetic differences between the HOP and NAD populations. GeneSeek data was received in PLINK format and individual files were merged with data from McMahon *et al.* (2015), resulting in a final merged set of 4593 SNPs. Multidimensional Scaling (MDS) analysis was used to identify major sources of variation within the data.

Whole genome resequencing

~12-50x genome resequencing data was obtained for 20 animals (mix of HOP, NAD and those of suspect or mixed pedigree). Data for 5 animals/breed for 12 other breeds was downloaded from Sequence Read Archive (SRA). Reads were aligned to the ARS-UCD1.2 bovine reference genome via Bowtie2 and used to call SNPs (~7 million) via SAMtools/bcftools. These were then employed for Principal Component Analysis (PCA) using a custom Python script to identify relevant PC loadings.

Machine Learning

PCA was employed for dimensionality reduction. We applied non-linear classifier methods; Support Vector Machine (SVM) algorithm and trained the model with K fold (k=5) stratified cross-validation strategies.

Results

Multidimensional scaling analysis shows a clear division between Hereford (OP) and Hereford (NAD) (Figure 1). To show the strength of the division between groupings, we also plotted two non-Hereford breed groups - UK and US Simmental profiles (as examples of genetic variation due to environment/breeding practice) and Black/Red Angus (as examples of recently diverged breeds). The clear pattern of division between Hereford (OP) and Hereford (NAD) suggests that the two have been separated by a significant genetic shift, not likely to result from genetic drift or different breeding practices (Figure 1).

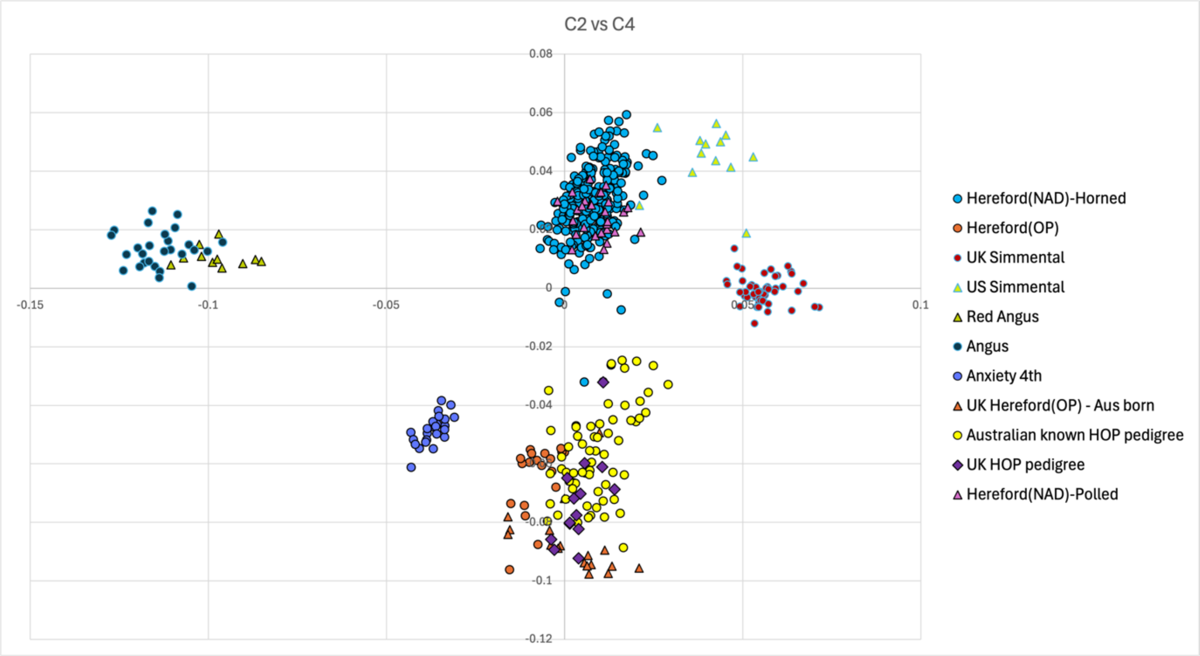

Figure 1. MDS plot of HOP vs NAD, with reference breeds

For the resequencing analysis, it was found that PC1 split the two breeds. Significant marker loadings were extracted and plotted against the reference genome to identify candidate genes near to these regions, as shown in Figure 2. Candidate genes were then subjected to literature search to identify possible function and any known role in cattle biology.

Some of the candidate genes are related to phenotypic traits involved in meat/milk production, like the MAATS1 gene linked to carcass fat in Nellore cattle, or the ARHGEF26 gene associated with milk cholesterol content in dairy cattle. Other genes are also related to phenotypic traits which indirectly affect economic performance, such as the APP gene that affects cattle temperament, or the DCBLD2 gene linked to hoof disorders and lameness in cattle.

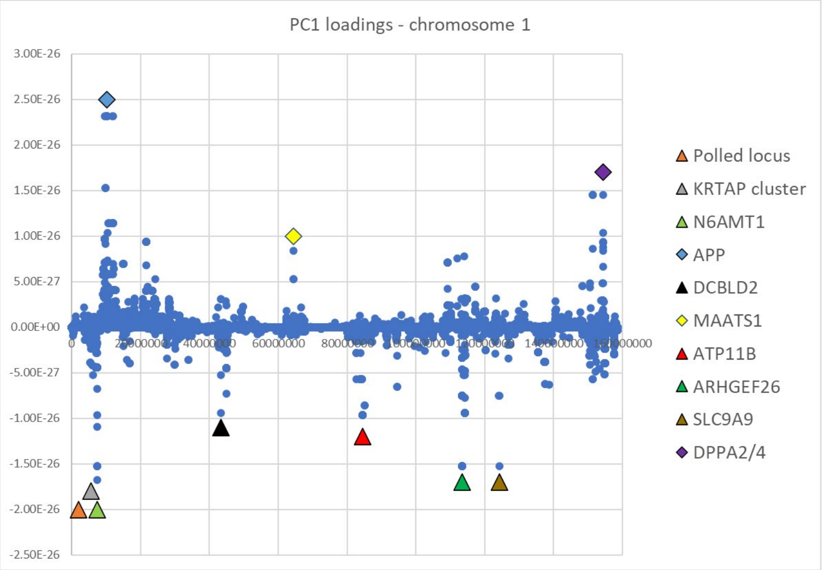

Figure 2. Results of PCA loading analysis for chromosome 1, based on WGS resequencing

Conclusions

This study identified clear genetic distinctions between the Hereford Original Population (HOP) and the North American Derived (NAD) Hereford cattle. The significant genetic shifts detected are unlikely to have occurred exclusively due to genetic drift or different breeding methods. Machine learning techniques have improved the ability to categorize breeds and manage complex datasets. Overall, the findings indicate the feasibility of developing an improved genetic test for distinguishing traditional and modern Hereford cattle populations, which is essential for maintaining the genetic integrity of the HOP and preventing undesirable admixture with external bloodlines.

Acknowledgements

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Candidate genes and pathways associated with bovine tuberculosis identified with an mRNA-miRNA regulatory network

F. Ghafouri[1,2], V. D. Reyhan[2], M. Sadeghi[2], S. R. Miraei-Ashtiani[2], J. P. Kastelic[3], H. W. Barkema[3], V. Razban[1], M. Shirali[1,4]
[1]Agri-Food & Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK
[2]Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Tehran, Iran
[3]Faculty of Veterinary Medicine, University of Calgary, 2500 University Dr NW, Calgary, AB T2N 1N4, Canada
[4]School of Biological Sciences, Queen's University Belfast, University Rd, Belfast BT7 1NN, UK

Presenting author
Farzad Ghafouri. E-mail: farzad.ghafouri@afbini.gov.uk

Application

This comparative transcriptome profiling analysis identified associated mRNAs and miRNAs, their regulatory mechanisms, and potential pathways in cows infected with bovine tuberculosis compared to healthy (H) cows.

Introduction

Bovine tuberculosis (bTB), a chronic infectious disease primarily affecting domestic dairy and beef cattle, is caused by *Mycobacterium bovis* (MB), a pathogenic bacterium within the *Mycobacterium tuberculosis* complex (MTBC) (Hall et al., 2021). The economic impact of bTB on the livestock industry is substantial, with estimated losses > $3 billion annually in global agriculture (Waters et al., 2012). Host immune responses to mycobacterial infection involve a complex interplay between innate and adaptive immune systems. Previous transcriptomic studies demonstrated that mRNA expression was significantly altered in bovine monocyte-derived macrophages (MDM) following MB infection. Thus, analyzing the host macrophage transcriptome in response to MB infection is expected to shed light on molecular mechanisms and host-pathogen interactions associated with bTB. Moreover, although non-coding RNAs (ncRNAs), e.g., microRNAs (miRNAs), have crucial roles in regulating immune system function and may offer valuable insights into the disease, their role in MB infection in cattle has not been thoroughly investigated (McLoughlin et al., 2021).

Materials and Methods

RNA-Seq and microarray datasets from blood samples, specifically MDMs of MB-infected and control Holstein dairy cattle, were retrieved from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) public database. Gene set annotation and functional enrichment analysis were conducted to explore hub mRNAs and miRNAs associated with bTB, using the DAVID and STRING databases. This analysis aimed to determine potential functions as well as metabolic and signaling pathways related to the disease. Also, interactions between the RNAs (mRNAs and miRNAs) were predicted and an mRNA-miRNA regulatory network constructed by integrating the protein-protein interaction (PPI) network with the gene regulatory network (GRN). Additionally, genes were assigned to functional categories using the Gene Ontology (GO) database, which includes biological processes (BP), molecular functions (MF) and cellular components (CC).

Table 1. Summary of the GEO accession numbers for RNA-Seq and microarray data sets associated with bTB.

No.	Data Type	GEO a Accession	Platform	Samples (MB:H)

1	Microarray	GSE33309	GPL2112 ((Bovine) Affymetrix Bovine Genome Array)	42 (21:21)
2	Microarray	GSE41401	GPL11649 (Agilent-023647 B. taurus (Bovine) Oligo Microarray v2 (Probe Name version))	12 (6:6)
3	RNA-Seq	GSE45439	GPL15750 (Illumina Genome Analyzer IIx (*Bos taurus*))	14 (7:7)
4	RNA-Seq	GSE60265	GPL15750 (Illumina Genome Analyzer IIx (*Bos taurus*))	16 (8:8)
5	RNA-Seq	GSE62506	GPL15749 (Illumina HiSeq 2000 (*Bos taurus*))	78 (39:39)

Results

Comparative transcriptomics-related analyses identified 3076 and 3229 differentially expressed genes (DEGs) between MB-infected and control Holstein dairy cattle samples for RNA-Seq and microarray datasets respectively, based on a fold change ≥ 1 or ≤ -1, and a false discovery rate < 0.05. Among these, 13 genes were common between transcriptomic profile datasets. In addition to DEGs, 9 and 39 miRNAs were simultaneously identified in the RNA-Seq datasets and literature mining, respectively. Furthermore, we identified 5 hub genes (*CCL4*, *CXCL2*, *IL12B*, *IL1A*, and *RETN*) involved in MB infection. Identified biological and regulatory networks were mainly associated with immune system signature pathways. Gene set annotation and functional enrichment of identified DEGs implicated important biological pathways. In this regard, functional enrichment analysis, based on the mRNA-miRNA regulatory network (Figure 1) revealed 6, 4 and 1 GO terms related to bTB in the biological process, molecular function, and cellular component categories, respectively. In addition, KEGG enrichment analysis identified cytokine-cytokine receptor interaction, tuberculosis, and signaling pathways for chemokine, NF-kappa B and Toll-like receptors.

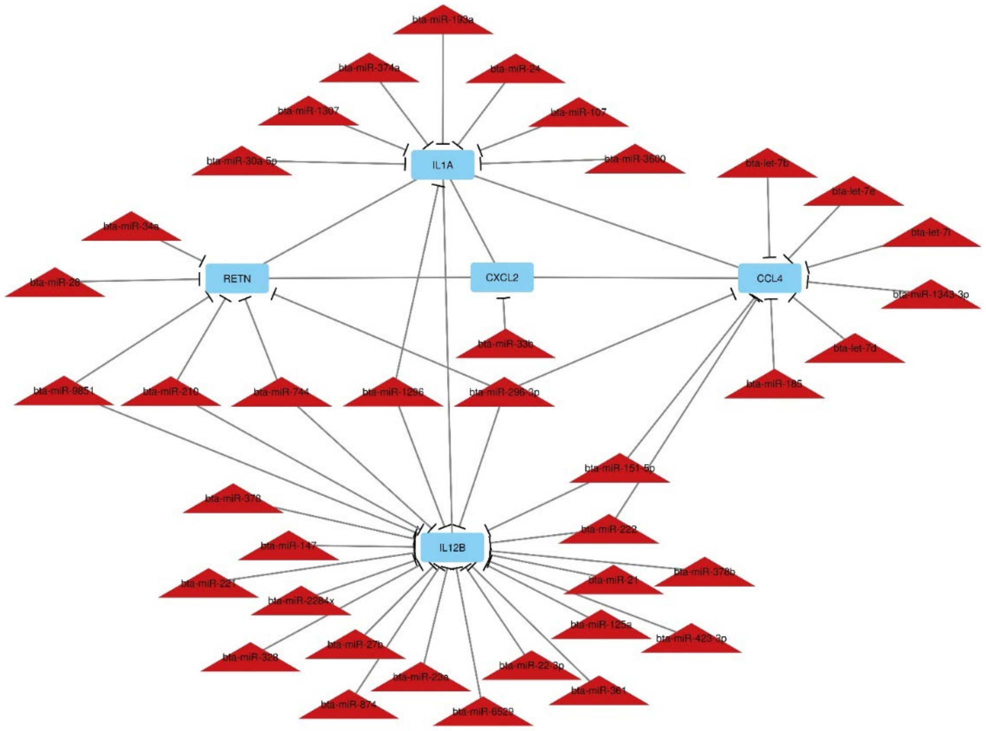

Figure 1. mRNA-miRNA regulatory network on MB infection in dairy cattle.

Conclusions

These findings offered valuable insights into the molecular evidence surrounding the regulatory mechanisms involved in transcriptome profiling of MB-infected Holstein dairy cattle, compared to noninfected controls. Additionally, they are an impetus to explore molecular networks and functions of DEGs associated with blood samples, particularly focusing on MDMs and their role in immune system function. This research provides a foundational starting point for future studies on bovine tuberculosis disease.

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Dairy cow resilience: data driven detection and quantification of perturbations using daily milk weights

F. L. Guinan[1], R. Fourdraine[2], F. Peñagaricano[1], K. Weigel[1]
[1]*University Of Wisconsin-Madison, 500 Lincoln Drive, Madison WI 53706, US*
[2]*Dairy Records Management Systems, 313 Chapanoke Rd Suite 100, Raleigh NC 27603, US*

Presenting author
Fiona Louise Guinan. E-mail: fguinan@wisc.edu

Application

Perturbation periods at the pen level can be identified using high-frequency data, such as daily milk weights, and resilience is a heritable trait, with heritability increasing as the severity and duration of the challenge intensifies.

Introduction

Resilience is defined as an animal's capacity to bounce back to normal functioning after a perturbation or maintain specific functions in the face of change or stress (Colditz & Hine, 2016; Scheffer et al., 2018). The increasing frequency of extreme weather events and persistent labor shortages highlight the need to identify and select animals that can maintain production in unpredictable environments. US dairy herds typically group cows into pens based on factors such as parity, lactation stage, reproductive status and milk production (Contreras-Govea et al., 2015). By coupling daily milk yield data with day-to-day pen location information, we can more precisely model management conditions and environmental stressors affecting groups of cows for each day of the lactation. It also allows us to detect management and environmental perturbations that may occur each day at the pen level and subsequently measure the responses of individual cows to these stressors over a particular period.

Materials and Methods

Our entire dataset included 62,580,945 daily milk weights and pen locations of 227,633 cows in parity 1, 2 and/or 3 from 204 herds representing 30 US states from 2018 to 2024. Individual lactation curves were fitted using polynomial quantile regression with a 0.5 quantile to derive expected lactation curves. Perturbation periods were identified using a data driven detection method and were based on residuals between mean expected and mean observed daily milk weights at the pen level. The initial dataset was stratified based on the severity and duration of the perturbation periods, considering 40 combinations that included severity levels from ≥ 3% to ≥ 7% and durations ranging from ≥ 3 days to ≥ 14 days. Our resilience phenotype, delta milk yield (ΔMY) was measured as the change in a cow's mean daily milk production relative to her mean expected daily milk yield during an identified perturbation period. The statistical model used to estimate variance components, heritabilities and repeatabilities for ΔMY included calving age, days in milk, parity and herd-year-season as fixed effects, along with herd-pen-milking_date, additive genetic and permanent environmental effect as random effects. Sire PTA Pearson correlations with TempVar (i.e., consistency) were calculated to assess the relationship between resilience and consistency traits. Additionally, sire PTA Pearson correlations were estimated within comparable severity thresholds to determine the genetic correlations between sire PTA during perturbations with similar characteristics.

Results

Estimated heritability of ΔMY during perturbations ranged from 0.01 (0.00) to 0.20 (0.08) depending on the severity and duration of the perturbation, while sire PTA Pearson correlations between ΔMY and TempVar ranged from -0.31 to -0.06 indicating that cows that are more consistent have lower milk loss during perturbations. Our findings suggest that animals differ in their response to perturbations at the pen level in comparison to their contemporaries within the group, and this measure of resilience using daily milk data is heritable.

Conclusions

Identifying perturbations at the pen level of varying severities and durations can more effectively capture the management and environmental conditions affecting an individual cow at a given time, and resilience can be measured by comparing how her response differs from that of her contemporaries. This enables the selection and management of more adaptable and sustainable cows capable of handling diverse challenges through a data-driven approach to detecting perturbations.

Acknowledgements

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The Signpost Farms and Signpost Advisory Programme: Farmers for Climate Action

T. O’Dwyer[1], G. Ramsbottom[2]
[1] Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[2]Teagasc, Oak Park, Carlow R93 XE12, Ireland

Presenting author
Tom O’Dwyer. E-mail: tom.odwyer@teagasc.ie

The Signpost Programme, launched by Teagasc in May 2021, is a whole-of-industry approach aimed at supporting Irish farmers in climate action. With over 60 partners, including major milk and meat processors, farm organizations, the Department of Agriculture, Food and the Marine, and Bord Bia, the programme strives to bridge the gap between researchers and farmers. Its primary goal is to translate scientific research into practical solutions resulting in the adoption of climate mitigation practices.

The programme is composed of three key components. The first is the Signpost Farms Programme, where a network of 125 farms, called ‘Signpost Farms’, play a central role in adopting climate mitigation strategies and sharing their knowledge through farmer-to-farmer learning.

The second element is the Signpost Advisory Programme, where 20 dedicated advisors have recruited approximately 17,000 farmers to participate. These advisors use the AgNav application, which combines data from Teagasc, the Irish Cattle Breeding Federation, and Bord Bia to calculate a farmer’s greenhouse gas emissions. They then help farmers create tailored sustainability action plans to reduce emissions.

The third component is the National Agricultural Soil Carbon Observatory (NASCO), an on-farm research initiative focused on understanding soil carbon sequestration. The Signpost Farms play a vital role in NASCO, contributing to the research that will shape future climate action strategies.

The Signpost Programme integrates research, demonstration, and farmer support to enable the widespread adoption of climate-smart solutions across Irish agriculture, helping the sector reduce its greenhouse gas emissions and enhance sustainability.

Anthelmintic provision to livestock farmers: there is more in common than divides prescribers

P. Crawford[1], M. Behnke[1], P. A Robinson[2]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*
[2]*Harper & Keele Veterinary School, Edgmond, Newport TF10 8NB, UK*

Presenting author
Paul Crawford. E-mail: paul@paulmrcvs.com

Application

In the United Kingdom (UK) anthelmintics can be prescribed by officially recognised, qualified individuals, known as SQPs (Suitably Qualified Persons) in addition to veterinarians. This remains controversial, with some stakeholders concerned that SQPs are not well-placed to address flock management as a veterinarian would, particularly due to the threat of anthelmintic resistance (AR). We report the fundamental similarities in hurdles faced by veterinarians and SQPs in changing farmer expectation and behaviour in relation to anthelmintic use. Addressing these hurdles should improve anthelmintic stewardship, regardless of who prescribes them.

Introduction

Endo- and ectoparasites negatively impact the productivity and health of sheep globally, and have been controlled through the widespread use of anthelmintics (Cabaret and Nicourt, 2024). Rising AR is rapidly eroding anthelmintics' power to protect sheep health and welfare resulting in sub-optimal productivity, with its economic and environmental impact. This environmental impact is compounded by the direct negative consequences profligate of anthelmintic use. Guidance has been produced with the aim of preserving and prolonging the efficacy of the available anthelmintics. The SCOPS (Sustainable Control of Parasites in Sheep) group, in the UK, have provided, and regularly updated, a technical manual with information on best-practice since 1999 (Stubbings et al., 2020). This guidance focuses on management strategies to reduce the need for treatments and optimise the efficacy of anthelmintics when indicated. All authorised medicines for sheep parasite control in the UK fall under the POM-VPS category. This should provide a control point in the supply of these medicines to farmers, as they can only be prescribed and dispensed, not simply sold, by a veterinarian, pharmacist, or an SQP trained and authorised to prescribe livestock medicines. Prescribing anthelmintics for sheep, in the UK, includes a requirement for prescribers to follow the SCOPS guidance. Despite such guidance and prescribing requirements, the research literature suggests that farmers continue to fail to optimise anthelmintic use (McIntyre et al., 2023; Williams et al., 2024). We report here on anthelmintic prescribing, from the prescribers' viewpoint, with a focus on barriers to better stewardship, and areas of common concern, and difference, between veterinarians and SQPs.

Materials and Methods

As part of a wider PhD research programme utilising mixed methods to look at medicine stewardship in the Northern Irish (NI) sheep flock, an analysis of 52 farmers' medicine records, and interviews with veterinarians, SQPs, farmers and other stakeholders were undertaken, following granting of ethical approval. Interviews were followed by a series of discussion groups. Where possible, and with consent, interviews and discussion groups were electronically recorded and transcribed. Contemporaneous notes were made for the remaining interviews and discussions. Transcripts and notes were then analysed and thematically coded, and exemplar quotes identified as has previously been reported in detail (Crawford et al., 2024).

Results

Analysis of farmers' medicine records revealed that only 12 (23%) farmers in the sample (n=52) bought more than one litre of anthelmintic from their veterinarian, suggesting the overwhelming majority of anthelmintic was purchased from non-veterinarian sources. Records also showed that sheep farmers who did not own cattle, purchased combination flukicide/wormers (clorsulon 10mg/ml, ivermectin 100mg/ml) licensed only for cattle from their veterinarian and from farm merchants. One farm record included a substantial quantity of an abamectin-based anthelmintic which was not authorised in NI, but was authorised in the neighbouring Republic of Ireland. Anthelmintic products containing an adult flukicide were identified in the records being purchased throughout the calendar year, contrary to SCOPS guidance. Interviews and the subsequent discussion groups highlighted farmers sourcing anthelmintics from agricultural merchants, rather than veterinarians, on the basis of product cost; a viewpoint veterinarian and SQPs agreed on. Farmers reported following a calendar-based approach to the timing of anthelmintic treatments or using visual cues to initiate treatment of assumed parasite infestations in their sheep. Testing was generally only undertaken following a failure to respond to one or more anthelmintic treatment. There was a difference between veterinarians and SQPs in their perception of who had the best knowledge and experience base to advise farmers on parasite control. Veterinarians were keen to take the lead on advice (without charging the farmer), despite knowing the farmer would purchase their anthelmintic elsewhere. SQPs felt that their focus on this narrower branch of veterinary medicine left them with greater depth and experience for advising farmers. SQPs recognised the benefits of testing prior to anthelmintic treatment, highlighting differentiating lice from scab and Nematodirus from coccidia to better tailor treatment. Challenging farmers' ideas and suboptimal practices was a common theme among SQPs and veterinarians. Specific areas prescribers challenged included farmers' assumptions about the cause of observed signs in their sheep, pre-determined ideas of the product they wanted, and their desire to treat all ewes and lambs. Both prescriber groups identified some farmers had no interest in, or ignored, many of the best practice management recommendations to maintain anthelmintic efficacy: 'As soon as they hear it [faecal sample] has to be sent off to the lab, and there is this charge, they respond, 'No, just give me a bottle of something and I'll just put it down their throat.' SQP04. One veterinarian described how, even when offering free dose efficacy testing, farmers would not bring in a post-treatment sample. Similarly, SQPs found farmers uninterested in (free) dosing equipment calibration. Finally, both groups identified poor record-keeping among farmers, and their habit of sourcing medicines from multiple sources as hindering good prescribing. This they reported frequently left them lacking a clear understanding of what treatments sheep had received historically, when they were approached to prescribe subsequent anthelmintics.

Conclusions

While examples of poor prescribing were identified in both SQP and veterinarian prescribing channels, there was clear will and ambition among the prescribers participating in this study to improve anthelmintics stewardship. An ongoing, coordinated effort to achieve this improvement is needed; focused on engaging farmers to change the attitudes and behaviours that prescribers here have identified as barriers to enhanced anthelmintic stewardship.

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Exploring the Reasons for Routine Tail Docking Among Pig Farmers and the Support Needed for Change

M. Harrison[1], K. O'Driscoll[1], A. Burrell[2], C. Gomes[2], S.McCarthy[3], G. Carroll[4]
[1]*Pig Development Department, Animal & Grassland Research & Innovation Centre, Teagasc Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*Animal Health Ireland, 2 – 5 The Archways, Carrick on Shannon, Co. Leitrim N41 WN27, Ireland*
[3]*Department of Agrifood Business and Spatial Analysis, Teagasc Food Research Centre, Scribbletown, Dublin 15, Ireland*
[4]*Animal Behaviour Centre, School of Psychology, Queens University Belfast, University Rd, Belfast BT7 1NN, UK*

Presenting author
Miss Molly Harrison. E-mail: molly.harrison@teagasc.ie

Application
For progress to be made towards reducing Ireland's reliance on routine tail docking to control tail biting, farmers' perspectives and business viability must be taken into account by key industry stakeholders and policy makers.

Introduction
The routine tail docking of piglets less than a week of age is standard practice on commercial farms in Ireland despite being prohibited in EU and Irish law (D'Eath et al., 2016). Tail docking is performed to reduce tail biting later in life. However, the prevalence of tail biting is influenced by a range of on-farm risk factors, and tail docking alone does not completely eliminate the occurrence of tail biting (Nielsen et al., 2022). A free tail biting risk assessment service has been developed by the Irish government, service providers and researchers (TASAH Tail Biting Risk Factor Assessment). However, the implementation of changes at farm level that lead to a decrease in the risk has been poor. A human behavior change approach has been suggested to help address the issue of tail biting in pigs (Carroll & Groarke, 2019).

Material and methods
A qualitative study was used to investigate why farmers continue to routinely tail dock. One-on-one semi-structured interviews (n =18) were conducted with farrow to finish pig farm owners and managers between March – June 2024 until data saturation was reached. Farmers were recruited through the Animal Health Ireland database and approached at pig farming events. The interview questions were designed to encompass the components of the COM-B (Capability-Opportunity-Motivation = Behavior) and Theoretical Domains Frameworks (Michie et al., 2011). The interviews were audio recorded and then transcribed by a transcription company, checked and pseudonymised. A largely deductive framework approach was used to analyze the transcripts (Goldsmith, 2021). Using NVivo software, codes were mapped to the aforementioned frameworks and then refined. The common themes that emerged within the COM-B-based framework components were used in the Behavior Change Wheel to identify appropriate interventions.

Results and Discussion
Of the three COM-B components, a lack of physical opportunities in terms of financial, environmental and temporal resources emerged as some of the most common barriers pig farmers faced concerning stopping routine docking. Secondly, farmers lacked the motivation to cease docking; they viewed it as a necessary procedure for the pigs' welfare, saw little benefit to stopping and feared the potential negative consequences if they stopped in a system not designed for undocked pigs. Farmers' beliefs of theirs and the industry's capability to rear undocked pigs were variable. Capability was a less

frequently mentioned barrier, some farmers were confused about why the law exists and is being enforced and felt they would need more advice and training about how to successfully rear undocked pigs. The changes required to facilitate rearing pigs with undocked tails also varied between farms, but all farmers mentioned financial viability as an absolute necessity. Identifying science-backed, realistic solutions for Irish pig farms and the need for appropriate infrastructure to be in place prior to the commencement of any stronger enforcement were also major themes. Farmers’ suggestions involved changing the pigs' housing, management, feeding practices and genetics. Interventions, for example to enable and incentivize farmers, have been suggested to help overcome the barriers identified and increase compliance with legislation.

Conclusions

This study has provided an authentic insight into Irish pig farmers' perspectives around routine docking. It has highlighted the significant barriers farmers face concerning ceasing docking and the potential changes necessary to facilitate rearing undocked pigs. The results indicate that huge alterations to the current structure of the industry, the market for pig meat and stakeholders’ mindsets would be necessary for not docking to become a reality. Policy makers should use farmers’ suggestions to guide future incremental steps towards making those big changes.

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Navigating DBX (Dairy Beef Cross) Production: Motivations and Decision-Making in Integrated and Open Market Systems

J. Gordon[1], K. Glenk[1], V. Eory[1], K. Lamont[1], E. Wall[1], D. Moran[2]
[1]*SRUC, West Mains Road, Edinburgh EH9 3JG, UK*
[2]*University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL, UK*

Presenting author
Miss Jillian Gordon. E-mail: Jillian.gordon@sruc.ac.uk

Application

The production of dairy-beef crossbred (DBX) calves, bred by the dairy sector and reared by the beef sector, has increased in recent years (Berry, 2021). This study investigates the complexities of DBX production, with a focus on how breeding decisions are made in both open and integrated markets. By understanding these decision-making dynamics, the dairy and beef sectors can better align their objectives to enhance the efficiency, profitability, and sustainability of DBX systems.

Introduction

DBX production has become increasingly important in the UK cattle industry, driven by the increased use of sexed semen in dairy herds and the need to improve the economic value of dairy calves. However, bull selection decisions in DBX systems are complex, requiring a balance between the often-conflicting breeding objectives of dairy and beef producers. This study addresses the knowledge gap identified by Berry (2021) on how farmers navigate these complexities in both open and integrated markets. Specifically, it investigates the breeding objectives, information sources, and breed preferences that drive decision-making in the UK DBX sector.

Materials and Methods

Qualitative data was collected through semi-structured interviews with 47 individuals involved in DBX production in the UK, including dairy and beef farmers, advisors, and industry stakeholders. Participants were recruited through a two-stage purposeful sampling approach. The initial cohort was identified through convenience sampling at the Royal Highland Show in 2022. Further participants were identified through targeted snowball sampling. The final sample size was determined by the point of data saturation, where no new themes were emerging from the interviews. Semi-structured interviews were conducted either in-person or via telephone, depending on the participant's preference and COVID-19 regulations at the time of data collection. In-person interviews were conducted as "walking interviews," allowing the interviewer to observe the participant's farm or workplace and utilize the surroundings as prompts for questions.
The interviews were transcribed using NVivo transcription software, and the interviewer checked the accuracy of the transcriptions against the audio recordings. A theoretical framework based on the interview schedule was used to develop a set of codes for the analysis Thematic analysis was selected to identify key themes and patterns related to breeding decisions, information sources, and stakeholder interactions in DBX production.

Results

The study reveals that dairy farmers prioritize two main breeding objectives: maternal calving efficiency, focusing on traits like calving ease and short gestation, and marketable calf production, aiming for calves with strong growth potential and desirable carcass characteristics. These objectives often conflict, as traits associated with easy calving may negatively impact the calf's growth potential and carcass value. This leads to a balancing of breeding objectives as highlighted in figure 1 below.

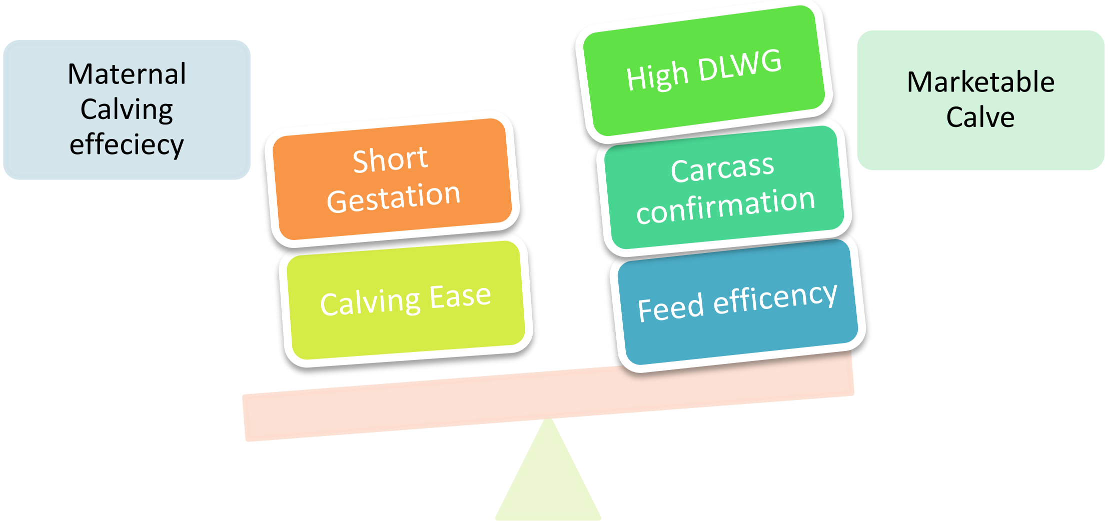

Figure 1. The balancing of the conflicting breeding objectives in combination with the associated traits.

The choice between open and integrated systems significantly influences decision-making and stakeholder roles (Crespi and Saitone, 2018). Within the two systems of the farm decision making unit (FD-MU), we can see that the actors involved are not the same. The open market system is much closer to the work of Ferreira (1998) in that the decision-making process is much smaller. However, it is important to note that the dairy farmer is still using the knowledge of a variety of trusted peers to guide the decision-making process. Integrated FD-MU systems prioritize coordination and information flow across the supply chain, potentially limiting farmer autonomy but ensuring more aligned breeding decisions roles (Crespi and Saitone, 2018). The study highlights the growing importance of artificial insemination (AI) and genomic selection tools in DBX breeding programs. However, mistrust in the reliability of genomic data for DBX systems persists.

Conclusions

This study reveals that DBX production involves navigating conflicting breeding objectives and complex decision-making processes influenced by both open and integrated market systems. The findings emphasize the need for greater collaboration and communication across the dairy and beef supply chains to optimize breeding strategies and enhance the long-term sustainability of this evolving sector. Developing standardized breeding objectives, improving information sources, and addressing mistrust in genomic data will be crucial for ensuring the efficiency and economic viability of DBX production. By understanding these complex dynamics, the DBX industry can better align its objectives and contribute to a more sustainable and profitable food production system.

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Evaluation of the impact of using internal teat sealant versus antibiotic (alone or in combination) or short versus long acting antibiotic at dry-off on SCC in the following lactation in dairy cows

P. Silva Bolona[1], C. Clabby, P. Dillon

[1]*Teagasc, Animal and Grassland Research and Innovation Programme, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork, P61 P302, Ireland*

Presenting author

Pablo Silva Bolona. E-mail: pablo.silvabolona@teagasc.ie

Application

Evaluating the impact of different dry cow treatments will contribute to design guidelines and policy to reduce antibiotic use in Irish dairy farms and to reduce the risk of antibiotic residues in milk.

Introduction

There is a need in Ireland for farmers to transition to non-antibiotic dry cow therapy (European Parliament and the Council of the European Union, 2019; Clabby et al., 2022). The common alternative is the use of internal teat sealants (ITS) to prevent infections during the dry period (Bradley and Green, 2004). Additionally, using short acting antibiotics could reduce the risk of antibiotic residues in the bulk tank due to cows calving earlier than expected for example.

The objectives of this study were to: 1- assess the impact of using ITS alone, antibiotic alone (AB), or antibiotic plus ITS (AB+ITS) at dry-off in low somatic cell count cows (SCC, <200,000 cells/mL) on somatic cell count in the following lactation; and 2- assess the impact of using a short versus a long acting antibiotic at dry-off in cows with high SCC (>200,000 cells/mL).

Materials and Methods

This study was approved by Teagasc's Animal Ethics Committee (License No. 1542017), and procedures were carried out in accordance with the Health Products Regulatory Authority (HPRA) of Ireland. The study was conducted from November 2017 (dry-off season) to the end of lactation of 2018 in three research herds. Herds were spring calving pasture-based systems. For the study related to objective 1 (**Study 1**), cows with every test day SCC below 200,000 cells/mL were blocked according to lactation, average SCC and expected week of calving in the spring 2018. Cows within blocks were sequentially assigned to receive ITS, AB or AB+ITS at dry-off. For cows in the study related to objective 2 (**Study 2**) cows which ≥1 test day SCC above 200,000 cells/mL were blocked using the same criteria as above and then were sequentially assigned to receive a short acting Cloxacillin based antibiotic + ITS (Clox+ITS) or long acting Cephalonium based antibiotic (Cep+ITS) at dry-off. Herds undertook weekly cow SCC recordings during 2018. Cow SCC was log 10-transformed (LogSCC) for analysis. The effect of dry-off treatment on LogSCC was analysed using mixed models with cow within farm as a random effect and parity (2, 3, 4 and >5), DIM and milk yield as covariates. The data was also analysed using raw SCC values to provide untransformed estimates, however p-values correspond to the analysis done on LogSCC.

Results

Study 1

Across all herds 69, 76 and 73 cows were treated with AB, ITS and AB+ITS at dry-off, respectively. In total 30 (43.4%), 22 (28.9%) and 32 (43.9%) cows had 0 high SCC (>200,000 cells/mL) in the following lactation in the AB, ITS and AB+ITS groups, respectively. Eight (11.6%), 13 (17.1%) and 19 (26.0%) cows had 1 high SCC in the following lactation in the AB, ITS and AB+ITS groups, respectively. The AB, ITS and AB+ITS groups had 31 (45.0%), 41 (53.9%) and 22 (30.1%) cows, respectively with ≥2 high SCC in the following lactation.

Dry-off treatment had a significant impact on LogSCC. Cows treated with ITS had 0.21 and 0.23 higher LogSCC compared to AB+ITS and AB cows, respectively ($P < 0.001$). The AB+ITS and AB treatments were not significantly different ($P = 0.7$).

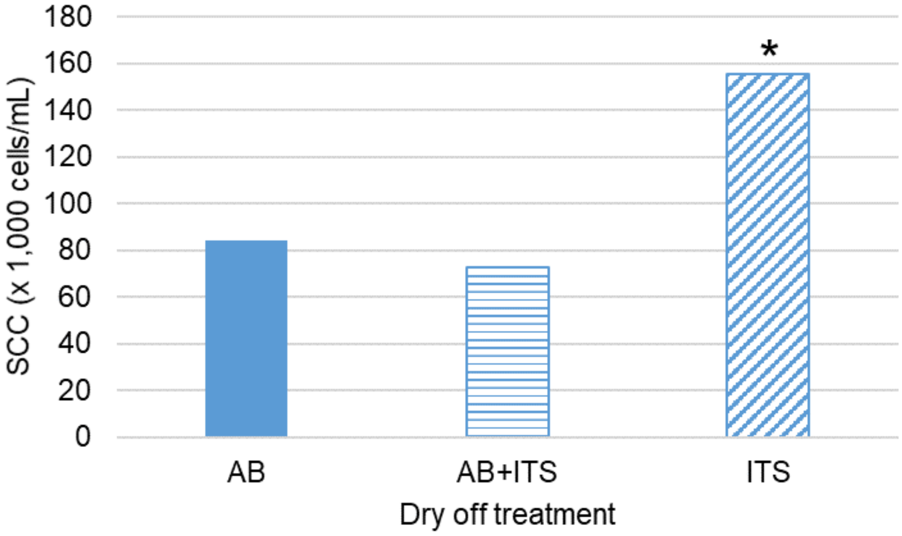

Figure 1. Raw SCC estimates for dry-off treatments applied to low SCC cows (<200,000 cells/mL). * indicates a significant difference ($p < 0.05$). Dry-off treatment: AB, antibiotic alone; AB+ITS, antibiotic plus internal teat sealant; ITS, internal teat sealant alone.

Study 2

144 and 146 cows were enrolled in the Clox+ITS and Cep+ITS treatment groups, respectively. In total, 46 (31.9%) and 44 (30.1%) cows had 0 high SCC in the following lactation for the Clox+ITS and Cep+ITS treatments, respectively. Seventeen (11.8%) and 22 (15.1%) cows had only 1 high SCC in the lactation for the Clox+ITS and Cep+ITS treatments, respectively. For the Clox+ITS and Cep+ITS treatments, 56 (38.8%) and 58 (39.7%) cows, respectively had ≥2 high SCC in the lactation.
There were no significant differences in LogSCC between these two dry-off treatments.

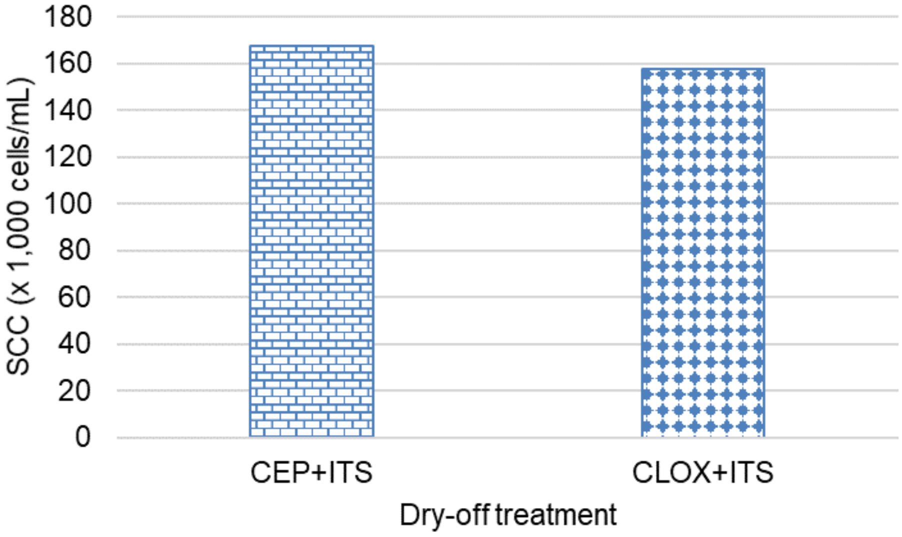

Figure 2. Raw SCC estimates for dry-off treatments applied to high SCC cows (>200,000 cells/mL). Dry-off treatment: CEP+ITS, Cephalonium based antibiotic plus internal teat sealant; CLOX+ITS, Cloxacillin based antibiotic plus internal teat sealant.

Conclusions

Using ITS alone increased SCC in the following lactation compared to antibiotic alone or combined with internal teat sealant. This is consistent with other Irish studies but different to international studies. This could be due to lack of cure of infections not detected by using a 200,000 cells/mL SCC cut-point or new infections over the dry period. In previous Irish studies, there is evidence of a high level of new infections when treating cows with ITS alone, which would suggest that more focus needs to be placed on the dry-off procedure and the dry cow management. We found no differences on SCC by treating cows with a short acting or long acting antibiotic at dry-off in high SCC cows, even if they had different active principles. This suggests that adequate capacity to cure existing infections at the end of lactation was achieved by both products and that no additional new infections occurred during the dry period as a result of using a short acting antibiotic.

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Aetiology of clinical mastitis on a large cohort of Irish dairy herds

R. Millar[1], J. Thompson[2], L. O'Grady[2], M. Green[2], T. O'Brien[1], D. Della[1], P S. Bolona[1]
[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork, P61 P302, Ireland
[2]School of Veterinary Medicine and Science, The University of Nottingham, Nottingham NG7 2RD, UK

Presenting author
Rachael Millar. E-mail: rachael.millar@teagasc.ie

Application

Identifying bacteria causing clinical mastitis on Irish dairy farms can contribute to implementing measures to prevent and control it. Determining the prevalence of the different types of mastitis causing bacteria will allow us identify risk factors associated with these bacteria and design prevention strategies.

Introduction

Mastitis is an inflammation of the mammary gland. Mastitis is classified as clinical mastitis (CM; when there's observable signs of inflammation in the milk, the gland or the cow) or sub-clinical mastitis (SCM; where no visual signs appear but there's an inflammatory process occurring) (Keane et al., 2013, Harmon, 1994). The most common cause has been observed from bacterial infection. Keane et al. (2013) collected 630 quarter milk samples from 30 milk-recording Irish dairy herds and found that *Staphylococcus Aureus* was the most prevalent (23%) pathogen causing CM followed by *Streptococcus Uberis* (17%) and *Escherichia Coli* (9%). There has been no research conducted on CM in Ireland since 2013, nor in a random sample of farms. Therefore, the aim of this study was to evaluate the aetiology of clinical mastitis on a large representative sample of Irish dairy farms.

Materials and methods

This study was conducted on a random selection of 80 Irish dairy farms, which were drawn from 276 complete responses to a previously conducted Teagasc survey (Uí Chearbhaill et al., 2024). Based on the National Farm Survey from 2022 of the number of farms located per province, we proportionally sampled farms based on geographical location: 72% of the selected were located in Munster, with the remainder spread across Leinster (14%), Connacht (7%) and Ulster (7%), as shown in figure 1.

This sample was obtained using the random number generator in Excel where each respondent was given a random number. These numbers were organised from smallest to largest, the farmers were contacted via email with information on the trial, followed by a phone call to discuss participation further. The sample size was chosen by running a simulation of herds with different CM case rates (20 to 60 cases per 100 cows/year) and a rate of cases per week was calculated. The sample size was estimated by calculating the number of farms needed to detect differences in mean CM cases per week between farms with a case rate difference of 20 to 40 cases per cow per year.

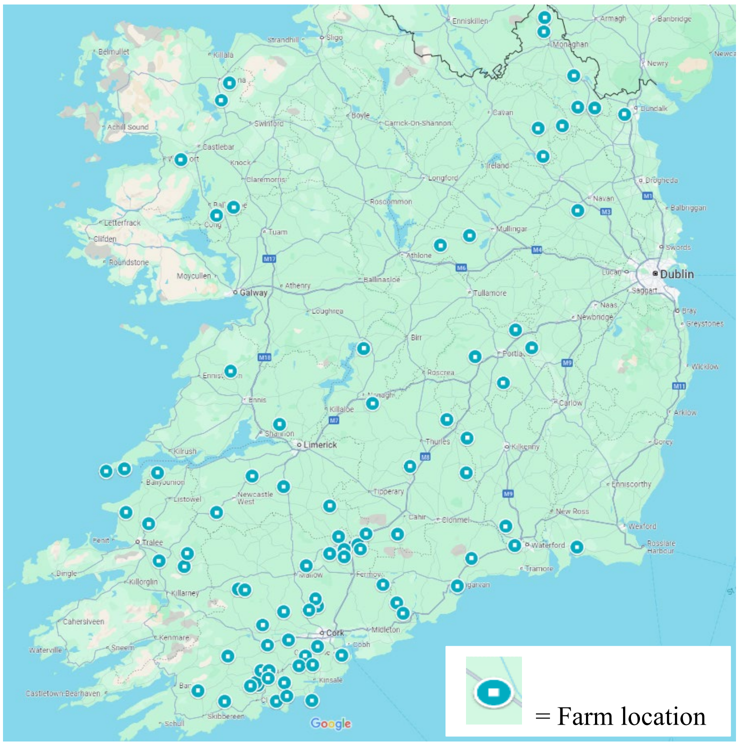
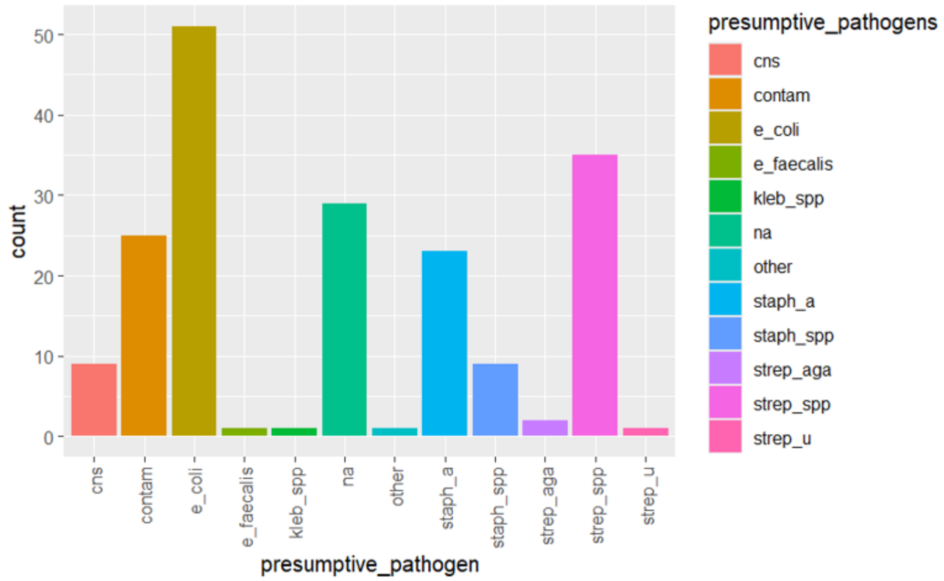


Figure . 1. Farm locations (Google maps, 2024)

We visited farms between April and May 2024. Farmers were trained to take aseptic quarter milk samples by the researchers and were asked to collect these samples from every CM case they detected throughout the lactation and freeze it immediately after. Frozen milk samples were collected bi-monthly by the researcher and transported back to Teagasc for laboratory analyses. To analyse the samples, 10 µL of milk were plated on blood agar and evaluated based on colony morphology and haemolytic activity. Samples showing fewer than three distinct colony types, with each type containing more than five colonies, were further tested on a panel of 10 agars to identify presumptive pathogens (Laboratory Handbook on Bovine Mastitis, 2019 and Farre., *et al* 2022). The further tests included, Mannitol Salt Agar, MacConkey Agar, Edward Modified Agar, Baird Parker RPF Agar, RAPID'B. cereus Agar, ALOA Agar, TBX Agar, Pseudomonas CFC Agar, HiChrome Cronobacter Agar, XLD Agar. Descriptive statistical analyses have been undertaken to visualise the preliminary data in R, using the tidyverse package.

Results

Seventy-eight farms (97.5%) operated a spring calving system (cows calving between February and April and are dried-off between November and December), with 1 farm (1.25%) operating a split calving system (where a portion of the cows are calved in autumn and spring) and 1 farm (1.25%) operating a year round calving system. Preliminary results to date are based on 281 frozen CM milk samples from 55 farms. Fifty one (18.15%) samples identified as *Escherichia coli*, thirty five (12.45%) presented as *Streptococci species* and 23 (8.2%) were identified as *Staphylococcus Aureus*.



Abbreviations:
cns = *Coagulase-negative staphylococci*
contam = Contamination
e_coli = *Escherichia Coli*
e_faecalis = *Enterococcus faecalis*
kleb_spp = *Klebsiella species*
na = Unknown

staph_a = *Staphylococcus Aureus*
staph_spp = *Staphylococcus species*
strep_aga = *Streptococcus agalactiae*
strep_spp = *Other Streptococcus species*
strep_u = *Streptococcus Uberis*

Figure. 23. Count of presumptive clinical mastitis pathogens

Conclusion

This study offers valuable insights into the aetiology of clinical mastitis in Irish dairy farms. We found E. coli as the most prevalent bacteria causing clinical mastitis. It is important that further bacteriology testing is carried out along with blood agar to ensure accurate identification of mastitis pathogens and patterns. Further research will explore current incidence rate in Irish dairy farms and factors associated with clinical mastitis to help inform prevention and control measures specific to the Irish context.

Acknowledgements

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Relationship between dairy farming system and the use of and resistance to antibiotics

N. Huda[1], C. Rymer[2]
[1]*University of Reading, Whiteknights House, Reading RG6 6UR, UK*
[2]*University of Reading, Whiteknights House, Reading RG6 6UR, UK*

Presenting author
Nazmul Huda. E-mail: nazmul.huda@pgr.reading.ac.uk

Application

Encouraging the responsible use of antibiotics as part of the sustainable intensification of dairy production in Bangladesh may reduce the irrational usage of antibiotics in Bangladeshi dairy farms, and ultimately the associated resistance to these antibiotics.

Introduction

Antibiotic resistance (AR) is a growing concern in Bangladesh because of the indiscriminate and widespread use of antimicrobials in its dairy industry (Imam et al., 2020). While no direct relationship has been demonstrated, antibiotic use in livestock production is considered one of the main causes of the evolution of antibiotic-resistant bacteria (Hölzel et al., 2010). About half the processed milk production in Bangladesh comes from the co-operative 'Milk-Vita', with average dairy herd size being 3.5 head of cattle (The Dairy Site, 2013). However, there are few data on antibiotic usage and prevalence of AR in the Bangladeshi dairy industry. The aim of this study was therefore to determine the association between sociodemographic characteristics of Bangladeshi dairy farmers, their management system, use of antibiotics and prevalence of phenotypic AR in samples of *E. coli* taken from their farms.

Materials and Methods

A total of 30 dairy farms with two contrasting dairy farming system (based on high and low input feed supplement) were chosen from the emerging milk production area (as identified by Milk-Vita, Bangladesh). High input systems also used a 'cut and carry (CCS)' whereas low input systems used a 'pastoral (PS)' system of forage provision. A cross-sectional snapshot survey was conducted from August to October 2023 to ascertain the age and education level of the farmer, their system of dairy farming, and their use of feeds and medicine for their cows. At the same time, samples of cow faeces, soil, feed and water were collected from the farmyard and surrounding environment. Isolates of *E. coli* bacteria were prepared from each sample by growing on eosin-methylene blue agar. Phenotypic resistance of these *E. coli* isolates to gentamycin, ampicillin, tetracycline and erythromycin was determined by incubating each isolate with nutrient broth, the antibiotic and resazurin dye indicator in a 96-well microtiter plate. A colour change of resazurin dye from blue to pink (indicating metabolic oxidation) was regarded as evidence of resistance to the particular antibiotic. Associations between farmer socio-demographic characteristics, antibiotic use and phenotypic AR were analysed by Pearson Chi-Square test using IBM SPSS (version: 29.0.2.0). The effect of farming system on milk production was analysed by Student's t-test.

Results

Most of the dairy farms were managed by men (CCS and PS farms: 88.8% and 83.4%, respectively), who were young (20-39 years) to middle aged (40-59 years). CCS farmers had attained a significantly (p=0.003) higher level of education than PS farmers (percentage attaining primary level or below was 83.3% for PS but only 33.3% for CCS). A larger proportion of CCS farmers identified dairying as their main occupation (44.4%) over agriculture (16.7%) and other businesses (27.8%), whereas most PS farmers chose agriculture as their main occupation (58.3%) over dairying (41.7%) and other businesses (0.0%); (effect of system, p=0.028). Annual milk production and milk yield of CCS farms was significantly higher than PS farms (production 71300 ± 23080 L/yr, 8240 ± 3467 L/yr, p=0.036; yield 3235 ± 278 L/yr, 1281 ± 417 L/yr, p<0.001 for CCS and PS, respectively). Overall frequency of antibiotic use was significantly greater (p< 0.001) by CCS farmers, who used a wider range of antibiotic classes, but between 20% and 50% of all farms used β-lactam and/or macrolide antibiotics (Figure 1). A high prevalence of resistance to ampicillin (approximately 80%) was observed in all samples, but resistance to tetracycline and erythromycin was greater on PS farms (Figure 2).

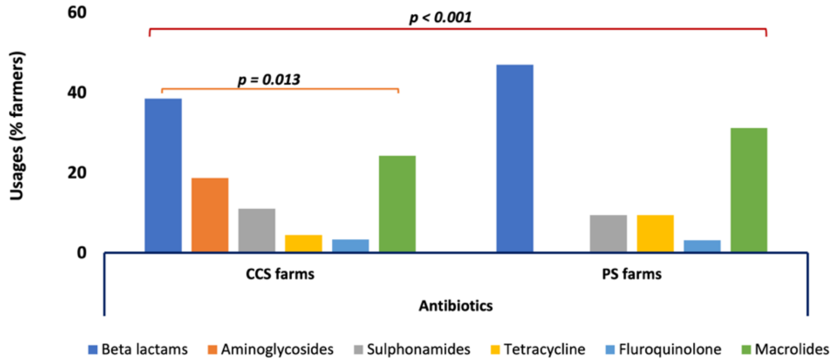

Figure 1. Frequency of usage (%) of antibiotics by different dairy farming systems

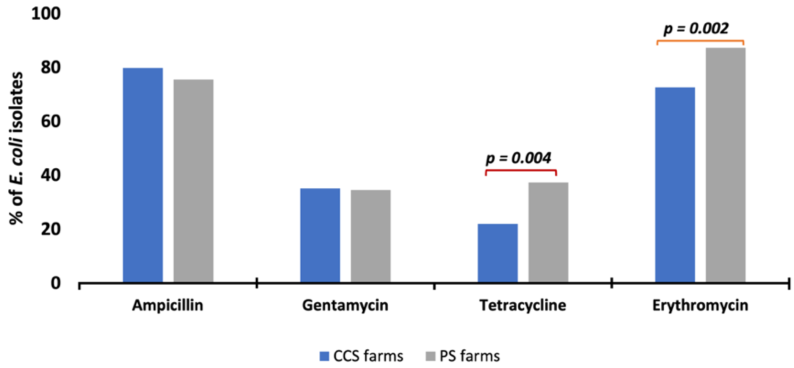

Figure 2. Effect of farming system on phenotypic resistance by E. coli to antibiotics

Conclusions

Higher input dairy systems in Bangladesh were associated with farmers with a higher level of education, and a greater frequency of administering antibiotics to their cows. However, regardless of level of input, most farms used β-lactam and many used macrolide antibiotics, and this was associated with a high prevalence of AR to these antibiotics. The higher prevalence of resistance to tetracycline and erythromycin with lower input systems may suggest that, when these antibiotics have been used on these farms, correct guidelines for their use may not have been followed.

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Exploring Factors Associated with Peripartum Subclinical Hypocalcemia in Dairy Cows Using a Multivariate Approach

E. Salgado-Hernández[1], D. Andrade-Chacon[1], D. Contreras-Caro Del Castillo[1], G. Valdez-Magaña [2], M. Espinosa-Martínez[3], V. Vilchis-Ambriz[4], J, Angeles Hernandez[1]

[1]Departamento de Medicina y Zootecnia de Rumiantes, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, CDMX, Mexico
[2]Departamento de Fisiología y Farmacología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, CDMX, Mexico
[3]CENID Fisiología y Mejoramiento Animal-Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias México, Mexico
[4]Ontrak Labs Ltd, Edinburgh, UK.

Presenting author:
J.C Ángeles-Hernández. E-mail: juanangeles@fmvz.unam.mx

Key words milk, multivariate analysis, hypocalcemia, cattle.

Application

In practical terms, identification of high-risk cows based on coat colour, temperature-humidity index (THI) level and parity can optimise herd management strategies and allow early detection and prevention of Subclinical hypocalcemia (SCH). This approach can increase productivity and improve animal welfare in dairy systems.

Introduction

Subclinical hypocalcemia (SCH) is an important metabolic disorder in dairy production because calcium plays a critical role in many physiological processes. Prolonged low serum calcium concentrations postpartum can lead to reduced milk yield, immune dysfunction, mastitis, metritis and decreased fertility (Caixeta et al 2017). Factors such as parity, metabolic alkalosis and dietary levels of Ca, Mg and P have been widely associated with disturbances in calcium homeostasis. However, other factors such as temperature-humidity index (THI), number of calves, calving month and cow coat colour have been less studied. The aim of this study was to investigate these non-conventional factors and its potential association with the incidence of SCH in dairy cows using a multivariate approach.

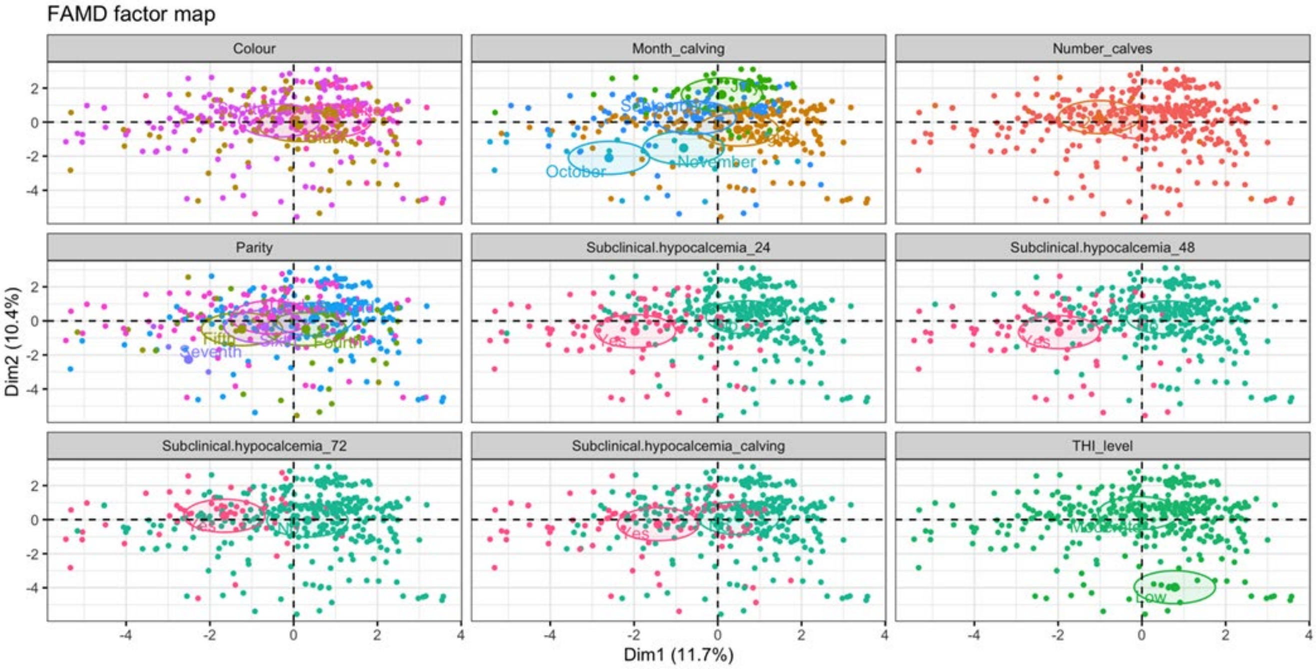
Material and methods

A total of 362 multiparous cows from a commercial farm in Jalisco, Mexico, at 21° N 163° W were studied between July 2023 and January 2024. Blood samples were collected from each animal at calving, 24, 48 and 72h postpartum. Ionised calcium levels were measured immediately after sampling using portable equipment (LAQUAtwin Ca-11C, HORIBA, Japan). Cows were classified as having or not having SCH based on their serum ionised calcium concentration, using a cut-off value of 1 mmol/L (Neves et al., 2018). In addition, the following information was recorded: coat colour (predominantly black = 121, predominantly white = 45 and spotted = 187), THI, body condition score at calving (scale 1-5), calving month, parity (1-7), number of calves (1 or 2), ambient humidity, maximum ambient temperature and calf sex. Due to the number of variables involved in the study, a multivariate analysis approach using Factor Analysis of Mixed Data (FADM) was used. This methodology analyses the similarities between individuals when mixed variables are considered; it also allows for the exploration of the association between quantitative and qualitative variables. The ellipse plot facilitates the interpretation of the FADM, which uses projections of clouds of individuals to which concentration ellipses corresponding to given characteristics were added. The FADM was carried out using the FactoMineR package (Le et al., 2008) and FactoExtra (Kassambara and Mundt, 2017) in R (R core team, 2022).

Results and Discussion

The FAMD factor map showing clustering based on coat colour, calving month, number of calves, parity and THI scores is shown in Figure 1. Our results indicate that a higher incidence of SCH at all time points was closely associated with spotted and black coat colours, whereas cows with white coats had a lower incidence. To our knowledge, this is the first work reporting these findings. Regarding the effect of THI, our results show that moderate THI levels had a detrimental effect regardless of coat colour. Conversely, lower THI levels were slightly associated with a reduced incidence of SCH across all measurements. The months of October and November showed a stronger association with the presence of SCH at all time points, whereas July and August showed a weaker association with SCH. Older cows were found to be more susceptible to SCH, as shown in Figure 1, with cows with more than five parities having a higher incidence of SCH it is in accordance with Reinhardt et. al., (2011), who found that SCH was more prevalent at higher age. Finally, cows giving birth to twins were found to have a greater predisposition to SCH.

Figure 1. Ellipse plots of the factor analysis of mixed data.



Conclusion

This study highlights the multifactorial nature of SCH in dairy cows, revealing associations between its incidence and non-conventional factors such as coat colour, THI levels, calving month, parity and calving number/size. Spotted and black coated cows, moderate THI levels and older cows were identified as being at higher risk of SCH. These results suggest that considering these factors in herd management strategies could improve the early detection and prevention of SCH, potentially improving productivity and welfare in dairy systems. Further research is warranted to validate these findings and explore targeted interventions.

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Serum analyte profiles of spring-calving, pasture-based dairy cows during the transition period

L. Horan[1,2], Á, García-Muñoz[3], C, G. McAloon[2], J, F. Mee[1], E. M. Sitko[1], A. Valldecabres[1,3]
[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland*
[3]*Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, 46115 Alfara del Patriarca, València, Spain*

Presenting author
Louise Horan. E-mail: Louise.Horan@teagasc.ie

Application

Temporal trends in blood analytes can provide a better understanding of the dairy cows' transition period, and facilitate the identification of adaptation challenges faced by spring-calving pasture-based dairy cows.

Introduction

During the transition period the dairy cow progresses from being dry and pregnant to lactating and not pregnant. This period comes with a multitude of physiological changes which challenge cows' homeorhesis and homeostasis and often evolve into mineral, energy and/or inflammatory imbalances, which decrease production and reproductive success (Hostens et al., 2012; Roche et al., 2018). Much research has focused on monitoring and preventing these imbalances in confined cows, to which blood analyte determination has been at the forefront. Less of this research has been completed with pasture-based dairy cows. Hence, the main objective of this study was to determine the temporal pattern of serum concentrations of minerals (Ca, P, Mg), energy balance markers [non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB)] and inflammatory markers (haptoglobin) during the transition period in spring-calving pasture-based dairy cows.

Materials and Methods

Five-hundred and 63 cows [mean parity ± standard deviation (SD) = 4 ± 2.3] of predominately Holstein genetics [mean % Holstein-Friesian in cows' breed (Interquartile range) = 86 (78 to 100%)] from 27 spring-calving pasture-based dairy herds in the Republic of Ireland were enrolled in this study (range = 14 – 25 cows/farm). Blood samples were collected from the coccygeal vessels 3 times within the study cows' transition period (mean ± SD days relative to calving): -8 ± 7 days, 12 ± 6 days and 25 ± 7 days. Serum concentrations of Ca, P, Mg, BHB and NEFA were determined using an automated wet chemistry analyser and haptoglobin concentration was determined using a colorimetric assay as described by Brady et al. (2019). A multivariate adaptive regression splines model (earth package in R; Milborrow et al., 2024) was used to identify breakpoints in the slope of blood analyte concentrations by day relative to calving. Slopes driven by consecutive days with ≤6 observations per day are not reported. For each blood analyte, 1,000 bootstrap observations were generated by resampling with replacement from the original data. The mean predicted blood analyte concentration at each breakpoint was calculated from the distribution of the bootstrap predictions.

Results

Regarding minerals, Ca concentration remained stable until 7 days prepartum (2.01 mmol/L), whereby it decreased reaching a nadir of 1.91 mmol/L at 2 days postpartum, increasing from there up to 2.13 mmol/L at 33 days postpartum. Phosphorus concentration remained stable until 6 days prepartum (1.88 mmol/L), decreased from there until 11 days postpartum (1.56 mmol/L), and from there it increased reaching 1.75 mmol/L by 43 days postpartum. Magnesium concentration remained stable until 8 days postpartum (0.81 mmol/L), whereby it began increasing reaching 1.08 mmol/L by

43 days postpartum. Regarding energy balance markers, concentration of NEFA remained stable until 8 days prepartum (0.27 mmol/L), and increased thereafter until 11 days postpartum (0.91 mmol/L), at which NEFA concentration began decreasing reaching 0.24 mmol/L by 43 days postpartum. Concentration of BHB remained stable until 17 days postpartum (0.66 mmol/L), whereby it began decreasing reaching 0.64 mmol/L by 43 days postpartum. Last, concentration of haptoglobin increased until 6 days postpartum (0.25 g/L), whereby it remained stable until 43 days postpartum.

Conclusions

Breakpoints were identified in the temporal concentration trends of serum minerals, energy balance markers and inflammatory markers assessed in this study. Serum Ca and P concentrations, followed similar and physiologically expected dynamics, and Mg concentration was kept within reference values. However, despite of following a physiologically expected dynamic, mean values for serum Ca concentration suggest a Ca imbalance in spring-calving pasture-based dairy cows. Serum NEFA concentration suggests body fat mobilization beginning within the first 2 weeks postpartum, while the same could not be inferred from observed serum BHB concentration. And, Haptoglobin concentration breakpoint was seen later in the days postpartum than expected.

Our findings call for a better understanding of serum Ca balance during the transition period and inflammation insults in the postpartum period in spring-calving pasture-based dairy cows.

Acknowledgements

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In vivo and in vitro investigations of host-pathogen interactions in bovine digital dermatitis

A. P. Sato[1], K. Bao[1], A. Anagnostopoulos[1], N. Siachos[1], A. Gillespie[1], N. Evans[1], D. Werling[2], G. Banos[3], A. Psifidi[2], G. Oikonomou[1]
[1]Institute of Infection, Veterinary and Ecological Science, University of Liverpool, Foundation Building, Brownlow Hill, Liverpool L69 7ZX, UK
[2]Centre for Vaccinology and Regenerative Medicine, Royal Veterinary College, 4 Royal College St, London NW1 0TU, UK
[3]Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK

Presenting author
Ana Paula Sato. E-mail: Ana.Sato@liverpool.ac.uk

Application

Our results show that breeding animals with higher genetic merit for resistance to digital dermatitis (DD) can lead to reductions in DD incidence. Our *in vitro* model allows us to study host-pathogen interactions and can lead to a better understanding of disease pathogenesis; this in turn could lead to the development of novel preventive and treatment strategies.

Introduction

Bovine digital dermatitis (DD) is a poly-bacterial disease associated with strawberry-like granulomatous foot skin lesions and lameness, which is endemic to most UK dairy farms. Spirochetes from the genus *Treponema* have been considered the main causative agent in the disease. Thickening of the epidermis, hyperkeratosis, and infiltration of inflammatory cells are found in the bovine digital skin affected by DD (Pirkkalainen et al., 2024). Among the cells of the immune system, the polymorphonuclear Neutrophil (PMN) are the most abundant cell type and provide a vital early response in host defence by migrating to sites of infection. Research indicates that resistance to DD is heritable, and selective breeding could reduce disease incidence. The UK Agriculture and Horticulture Development Board has recently launched a digital dermatitis genetic evaluation (AHDB, 2020). Here, we aimed to study the association between the DD genetic evaluations and actual DD incidence in a population of dairy heifers. Additionally, we assessed the impact of different genetic merits by developing an *in vitro* granulocyte migration assay to potentially link genotype to phenotype.

Materials and Methods

This study was conducted under the ethical approval of the University of Liverpool Research Ethics Committee (VREC1372) and ASPA-regulated procedures were operated under a Home Office License (PP8351537). A total of 361 maiden heifers were monitored over a four-month period and their hind feet were examined for presence of DD in a foot trimming crush using the M-stage scoring system. Genomic breeding value (GEBV) estimates for these heifers for DD resistance were made available from the official UK national evaluation. Our phenotypic data were not included in GEBV calculation. Associations between the GEBVs and DD incidence were investigated using logistic regression models. Animals were grouped into terciles based on their GEBVs (high, medium, or low genetic merit). An *in vitro* model of bovine foot fibroblasts to assess the impact of *Treponema phagedenis* infection was created. Fibroblasts cells were isolated and cultured from foot skin biopsies taken from 15 heifers, following the protocol by Evans et al. (2014). The heifers were divided into three groups based on their genetic merit and health status. 5 heifers with high genetic merit that were healthy (HGH), 5 heifers with low genetic merit that were infected with DD and then healed (LGH), and 5 heifers with low genetic merit that were chronically infected (LGC). Fibroblast cultures were maintained with media refreshed every 48 hours, and cell morphology and growth were monitored daily. Once cultures reached approximately 80% confluence, fibroblasts were passaged to ensure optimal growth conditions. For migration assays, blood samples were collected from the three heifers per group (HGH, LGH, and LGC). Granulocytes were then isolated from these samples using density centrifugation of EDTA-treated blood, and co-cultured with homologues primary fibroblasts cells in a transwell migration assay using the following stimulatory conditions: (1) Fibroblasts alone, (2) Fibroblasts infected with *T. phagedenis*, (3) *T. phagedenis* alone, (4) Interleukine 8 (IL-8) as positive control, and (5) medium alone as negative control. Each condition was tested in triplicate. After 16 hours, the number of granulocytes that had migrated under each condition was counted (Tombácz et al.,2019).

Results

For every 1 SD increase in the GEBVs the OR for any, active, chronic and M2 DD lesion presence were 0.66 (95%CI 0.53-0.82), 0.58 (95%CI 0.45-0.73), 0.63 (95%CI 0.51-0.79) and 0.6 (95%CI 0.42-0.84) respectively. Initially, fibroblasts from each group were seeded at a cell density varying between 200,000 and 800,000 cells per milliliter (ml). After the first passage, cell counts increased to between 2 million and 5 million cells/ml across all groups. Notably, fibroblasts from the HGH group reached 80% confluence within 8 to 10 days, while those from the LGH and LGC groups required 12 to 15 days to reach similar levels of monolayer confluence. Lower numbers of granulocytes migrated when derived from HGH heifers compared to those generated from LGH and LGC (Figure 1).

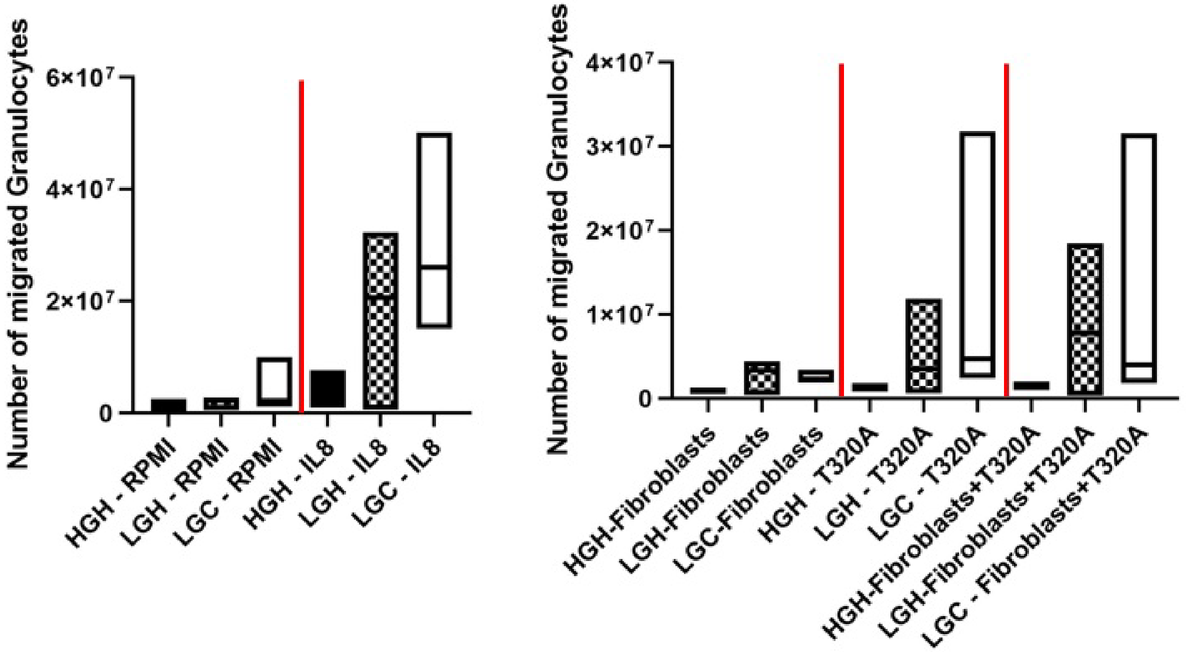

Figure 1. Number of granulocytes migration in maiden heifers with differing genetic merit for Digital Dermatitis resistance. HGH: High genetic merit healthy heifers, LGH: Low genetic merit healed heifers, LGC: Low genetic merit chronic heifers. N = 3 biological replicates, line at mean value.

Conclusions

Our preliminary results suggest that genetic merit for DD resistance is associated with DD incidence and with distinct cellular responses, as fibroblasts from heifers with high genetic merit displayed faster proliferation rates compared to those from low genetic merit. Additionally, granulocyte migration assays indicated that HGH heifers exhibited lower granulocyte migration than LGH and LGC heifers suggesting that genetic factors associated with high merit (DD resistance) may influence immune cell function, potentially leading to a more balanced immune response or altered regulation of cell migration during the inflammatory response. In contrast, the increased granulocyte migration observed in LGH and LGC heifers may reflect a less-regulated inflammatory response, which could potentially contribute to higher susceptibility to DD and related conditions. These preliminary findings provide insight into the immune mechanisms associated with the host genetics of resistance to DD. This model may prove valuable for further studies on genotype-phenotype links in DD resistance.

Acknowledgements

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Inter-herd variability in urinary metabolic profiles and milk lipidome of dairy cows

A. Ferreira[1], R. Hyde[1], M. Green[1], D. Kim[2], L. Randall[1]
[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham NG7 2RD, UK
[2]Centre for Analytical Bioscience, Advanced Materials & Healthcare Technologies Division, School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK

Presenting author

Ana Ferreira. E-mail: Ana.Ferreira1@nottingham.ac.uk

Application

We aim to provide the foundation for developing a tool farmers can use to improve management strategies to prevent lameness, by developing an unbiased method for predicting lameness, complementing mobility scoring. Early detection of lameness allows for timely treatment improving animal welfare and sustainability of the dairy herd, consequently reducing economic losses of the dairy industry.

Introduction

Previous research has shown that metabolomics and lipidomics allow for the detection of potential metabolites and lipids that discriminate between lame and healthy cows using urine (Cardoso et al., 2024; Randall et al., 2023), milk (He et al., 2022), and blood (Dervishi et al., 2020). Randall et al. (2023) used untargeted liquid chromatography-mass spectrometry (LC-MS) and machine learning (ML) algorithms to predict lameness. In that study of one herd, the best-performing ML model predicted lameness with a mean accuracy of 82% at the time of lameness event. He et al. (2022), also utilised untargeted LC-MS and ML algorithms, achieving accuracies of 100% using a different cohort from the same herd. In this cross-sectional case-control study, we aim to validate the results obtained in previous research using a larger sample size of commercial dairy herds.

Material and Methods

A total of 849 (424 urine and 425 milk) cow samples (lame vs control; @ 1:1) were collected from 10 commercial dairy herds with cows scored using 0-3 mobility scoring (AHDB, 2024). Urine samples were collected via free-flow catch and both urine and milk were snap frozen into liquid nitrogen before storage at -80 °C. Cows receiving medication in the preceding 15 days and non-lame cows treated for lameness within the previous month were excluded. A suite of 5 ML algorithms, namely Random Forest (RF), Support Vector Machine (SVM), Partial Least Squares regression (PLS), multivariate adaptive regression spline (MARS) and Elastic Net (ENET), was applied for each herd dataset (1 urine and 1 milk dataset per herd) and to all herds (1 urine and 1 milk dataset across all farms, totalling 2 datasets). Mobility score outcomes were used to train the supervised models. The correlation coefficient between milk and urine prediction accuracies was calculated alongside the Student's t-test to check for the significance between the two biofluids.

Results

Figure 1 shows the mean prediction accuracies by farm and for all farms for urine and milk cow samples. Overall mean prediction accuracies were higher for urine than milk, except for farm C, in which a higher accuracy was obtained for milk. When the data of all farms were included in the model, the predicted accuracy for urine was 66% and 61% for milk. At a farm level, the highest accuracy was 85% for urine and 77% for milk. Prediction accuracies using milk and urine showed a moderate positive correlation across farms (r = 0.43). A paired t-test revealed significantly higher prediction accuracies for urine compared to milk samples (t(10) = 3.68, P < 0.01).

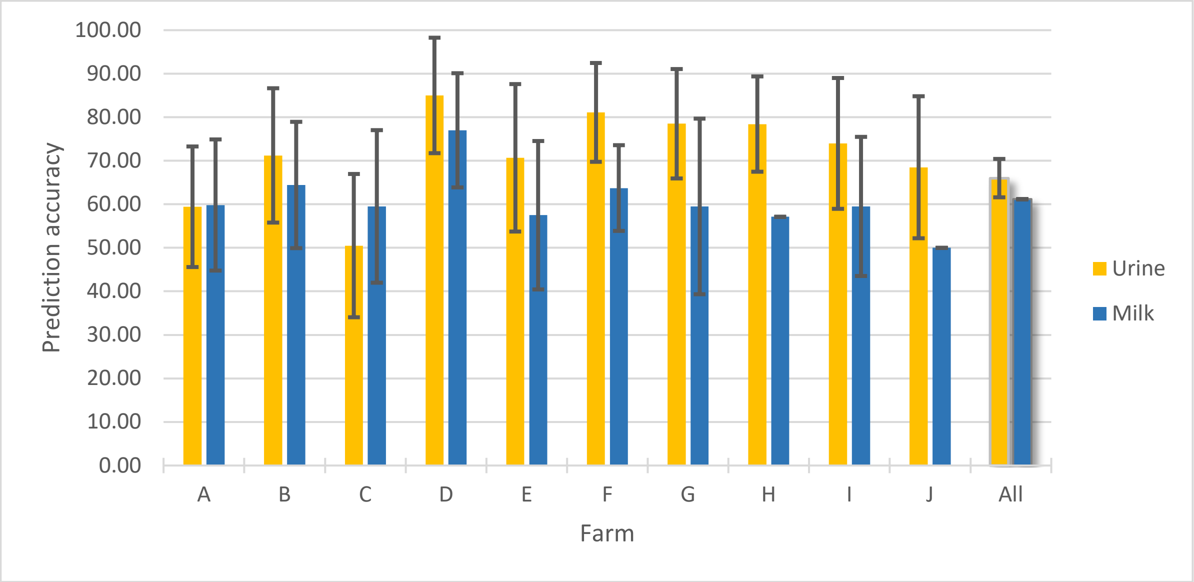

Figure 1. Mean prediction accuracies and corresponding standard deviations error bars by farm and for all farms for urine and milk samples

Conclusions

The combination of untargeted LC-MS with ML models shows promise to differentiate between lame and healthy cows based on a set of metabolites and lipids. However, a high inter-herd variability in classification accuracies was observed: given the results above, it wasn't possible to establish a correlation with farm management or rule out confounding factors. Despite urine showing approximately 10% higher prediction accuracy, further work is required to weigh in practical considerations such as the ease of sample collection and respective stability/storage, overall costs, animal welfare implications and farm management compatibility.

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Association between housing, management and environmental factors and respiratory disease prevalence in dairy calves

J. Donlon[1], J. F. Mee[2], C. G. McAloon[3]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[2]Teagasc, Oak Park, Carlow R93 XE12, Ireland
[3]University College Dublin, Belfield, Dublin D04 V1W8, Ireland

Presenting author
John Donlon. E-mail: john.donlon@teagasc.ie

Application

Currently the evidence base for calf housing design that is protective against bovine respiratory disease (BRD) is limited. The finding of this work will help farmers prioritize specific elements of calf housing design most associated with BRD to reduce its prevalence and therefore improve overall farm performance.

Introduction

Bovine respiratory disease (BRD) is detrimental to the health and welfare of dairy calves. It reduces the performance of affected dairy heifers (Buczinski et al. 2021), making its prevention a priority in dairy farming. Calf housing environment is commonly referenced as one of the major contributing factors to the prevalence of BRD on a given farm (Nordlund and Halbach, 2019). However, in a recent systematic review by Donlon et al (2023) the evidence base for the relationships between housing environment factors and BRD was found to be lacking. Given the restrictions on antimicrobial use (AMU) due to fears of antimicrobial resistance (AMR), a better understanding of the role of housing environment factors may prove useful in preventing BRD and reducing AMU and AMR. Hence, the objective of this cross sectional study was to assess the association of calf housing design, environment and management with BRD (lung consolidation and clinical signs).

Material and methods

In total, 83 dairy farms were each visited twice in total in autumn and in spring over three years. These farms were recruited via two methods, i). Random letters sent via the Irish cattle breeding federation, ii). Private veterinary practitioners who were asked to refer farms with known historic BRD problems. At the autumn visit, the calf housing was surveyed, data loggers (continuous monitoring of temperature and humidity) were installed inside the calf housing and a survey of calf management practices was conducted with the farmer. At the spring visit calves were examined to diagnose the presence of BRD and environmental and calf samples were collected. In total 1,640 calves (20 per farm) between 4 and 6 weeks were examined using thoracic ultrasound (TUS) and the Wisconsin clinical score (WCS). Bacterial air load was quantified (using blood agar versus VRGB agar) at three locations within the calf housing (alleyway, feeder, and middle of pen) using an impaction air sampler. Bedding samples were taken for dry matter analysis and nesting scores (scale of 1 to 3 (1 meaning a lying calves legs are fully visible and 3 meaning a lying calves not visible due to bedding)) were assigned. Two multivariable generalized linear regression models were constructed with prevalence of TUS lesions (score 3 or greater (at least a single patch of lobar consolidation)) and positive WCS (aggregate score ≥5 or two or more scores ≥2) as the outcome variables, respectively.

Results

The descriptive results are presented first. The calf house survey revealed that vented sheeting was the most commonly observed inlet design (17 farms), while central ridge and no outlet present were the joint most common outlet designs (29 farms each).The environmental data loggers indicated that in the week prior to calf examination in the spring the median within-calf house air temperature was 8.8°C and relative humidity 76.7%. In total 173 (10.5%) calves were classified as having complete consolidation of at least one lung lobe by TUS and 155 (9.5%) calves were considered diagnosed with BRD using the Wisconsin clinical score. The most frequently observed nesting score was 1 (on 33 farms). Results from the generalized linear regression models showed the ratio of bacterial air (BA:VRGBA) load in the middle of the calf pen was positively associated with BRD prevalence in both models ($p < 0.05$). Housing temperature exceeding 20ºC in the week prior to examination was also positively associated with BRD prevalence in both models ($p < 0.05$). In the WCS model, inlet design, feeding method (Automatic feeder vs bucket vs teat feeder), milk type (milk replacer vs whole sale able milk vs whole saleable milk & milk replacer vs other), nesting score (score 2 & 3 protective), mechanical ventilation and calf occupied area (positively associated) were all significant predictor variables for BRD ($p < 0.05$). In the TUS lesion model, the minimum temperature humidity index in the week prior to examination (negatively associated), number of calves in the housing (positively associated), outlet design, colostrum feeding method and colostrum source were significant predictors of BRD($p < 0.05$).

Conclusions

This is one of the most extensive investigations of BRD epidemiology and environmental factors that has been carried out. In this work, two models were constructed investigating the associations between these variables and on-farm WCS and TUS prevalence. These models showed that housing design factors (primarily inlet and outlet design) can play an important role in the prevalence of BRD in preweaning dairy calves. Even in the spring of a temperate climate such as Ireland both heat stress and cold stress can contribute to the prevalence of BRD in dairy calves. This work demonstrated a relationship between airborne bacteria and BRD in housed calves, however this appear to be a complex one. The characteristics of the air microbiome must be investigated further to gain a better understanding of the results demonstrated in this work.

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Factors associated with failure of passive transfer of immunity using different blood testing methods in suckler beef and dairy calves

R. R. Male Here[1,2], M. McGee[1], C. McAloon[2], A. W. Byrne[3], B. Earley[1]
[1]*Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath C15 PW93, Ireland*
[2]*School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland*
[3]*One-Health Scientific Support Unit, National Disease Control Centre (NDCC), Department of Agriculture, Food and the Marine (DAFM), Dublin 2, Ireland*

Presenting author
Rischi Robinson Male Here. E-mail: rischi.robinson@teagasc.ie

Application

Test procedures and cut-off values have a major impact on the interpretation of failure of passive transfer (FPT) of immunity and its associated factors in suckler beef and dairy beef calves.

Introduction

Calves with FPT have an increased risk of respiratory disease, diarrhoea, poor growth performance, and death. Studies describing the factors associated with passive immunity and FPT in Irish suckler beef and dairy calves are limited. The study objective was to assess dam and calf characteristics, and herd-level and calf-level management practices associated with passive immunity and FPT, determined by different blood tests and cut-offs, in Irish spring-born suckler beef and dairy calves.

Material and methods

Data obtained (face-to-face questionnaire) from herd-level (66 suckler beef farms [391 calves], 77 dairy farms [674 calves]), and calf-level (9 suckler beef farms [377 calves], 8 dairy farms [916 calves]) studies conducted in spring 2015 and 2016, respectively, were used (Todd et al., 2018). Total IgG (ELISA, ELISA-IgG), total protein (clinical analyser, TP-CA) and total solids (BRIX refractometer, TS-BRIX) were determined in calf serum collected within 1 to 14 days post-birth. Calf FPT status (yes/no) was defined using the cut-offs for classification of morbidity/mortality in Irish suckler beef and dairy calves (current cut-offs (CC); Todd et al., 2018), and published literature cut-offs (LC). The CC for ELISA-IgG was ≤ 9 and ≤ 12 mg/mL, for suckler and dairy calves, respectively, and the TP-CA and TS-BRIX were ≤ 60 g/L, and ≤ 8.4%, respectively, for both suckler and dairy calves. The LC were identical for suckler and dairy calves (ELISA-IgG < 10 mg/mL; TP-CA < 52 g/L; TS-BRIX < 8.4%). Pearson correlations between the three tests were determined. Mixed multivariable linear and logistic regression models with farm as random effect were used to assess the dam and calf characteristics, herd-level and calf-level management practices associated (P<0.05) with passive immunity and FPT, determined by different blood tests and cut-offs.

Results

There were positive correlations between the different serum passive immune measures (P<0.05) in the herd level and calf-level studies (suckler beef: ELISA-IgG *vs.* TP CA, r = 0.75 – 0.86; ELISA-IgG *vs.* TS-BRIX, r = 0.72 – 0.80; TP-CA *vs.* TS-BRIX, r = 0.91 – 0.95; dairy: ELISA-IgG *vs.* TP CA, r = 0.79 – 0.85; ELISA-IgG *vs.* TS-BRIX, r = 0.76 – 0.80; TP-CA *vs.* TS-BRIX, r = 0.92 – 0.95).

Herd-level study:

Suckler beef calves reared on farms that did not immunise the dams against diarrhoea pre-calving (*vs.* practiced vaccination) had lower passive immune measures based on ELISA-IgG and TS-BRIX but were associated with greater odds of FPT using the CC for all tests and the LC for ELISA-IgG (Table 1). Dairy calves born to primiparous dams (*vs.* multiparous) had lower serum TP-CA and TS-BRIX concentrations and were more likely to have FPT using the CC for TP-CA and TS-BRIX only. Male dairy calves (*vs.* female) had lower passive immune measures based on TP-CA and had greater odds of FPT using the CC and LC for ELISA-IgG and TP-CA, possibly reflecting differences in colostrum management practices. Length of dry period had no effect on passive immune measures in suckler beef and dairy calves (*P*>0.05); however, dairy calves born on farms with a dry period ≤ 8 weeks (*vs.* > 8 weeks) had an increased likelihood of FPT using all cut-offs except the LC for TS-BRIX.

Calf-level study:

Suckler beef calves born during the peak calving season (*vs.* early) had lower passive immune measures based on ELISA-IgG test and greater odds of FPT using the CC and LC for ELISA-IgG only (Table 1). Suckler beef calves that consumed colostrum more than 2 h post-birth (*vs.* within 2 h) had lower passive immune measures based on all tests and greater odds of FPT using all cut-offs except the CC for TS-BRIX. Suckler beef calves with perinatal problems (*vs.* no perinatal problems) had lower passive immune measures based on all tests and were more likely to have FPT using all cut-offs. In dairy calves, perinatal problems were only associated with lower serum TP-CA concentrations. Dairy calves born to primiparous (*vs.* multiparous) had lower passive immune measures based on all tests, and a greater likelihood of FPT based on the CC for ELISA-IgG and TP-CA, and the LC for ELISA-IgG. Calving supervision was not associated with any passive immune measures (*P*>0.05); however, the odds of FPT using all cut-offs were lower when calving was not supervised (*vs.* present at calving). Feeding stored colostrum (*vs.* fresh) increased the likelihood of FPT based on the LC for TP-CA and TS-BRIX.

Table 1.

Factors associated with (P<0.05) passive immune measures and failure of passive transfer (FPT) of immunity defined by different blood tests and cut-offs in suckler beef and dairy calves.

	Suckler beef			**Odds ratio** ^{1}	**Dairy**			**Odds ratio** ^{1}
	ELISA-IgG	**TP-CA**	**TS-BRIX**		**ELISA-IgG**	**TP-CA**	**TS-BRIX**	
Herd-level study								
Dam diarrhoea vaccination pre-calving (no *vs.* yes)	P, CC, LC	CC	P, CC	1.7 to 2.0	NS	NS	NS	
Dam parity (primiparous *vs.* multiparous)	NS	NS	NS		NS	P, CC	P, CC	1.7 to 1.9
Calf sex (male *vs.* female)	NS	NS	NS		CC, LC	P, CC, LC	NS	1.5 to 2.4
Length of dry period (≤ 8 *vs.* > 8 weeks)	NS	NS	NS		CC, LC	CC, LC	CC	1.7 to 2.1
Calf-level study								
Time of birth relative to calving season (peak *vs.* early)	P, CC, LC	NS	NS	2.5 to 3.8	NS	NS	NS	
Time of first colostrum feeding (> 2 h *vs.* within 2 h post-birth)	P, CC, LC	P, CC, LC	P, LC	2.3 to 10.1	NS	NS	NS	
Perinatal problems (yes *vs.* no)	P, CC, LC	P, CC, LC	P, CC, LC	3.6 to 4.5	NS	P	NS	
Dam parity (primiparous *vs.* multiparous)	NS	NS	NS		P, CC, LC	P, CC	P	1.5 to 1.6
Calving supervision (not supervised *vs.* present at calving)	NS	NS	NS		CC, LC	CC, LC	CC, LC	0.6 to 0.7
Type of colostrum feeding (stored *vs.* fresh colostrum)	N/A	N/A	N/A		NS	LC	LC	2.8 to 5.0

P – Passive immune measures; CC – Current cut-offs; LC – Literature cut-offs; NS – Not significant (*P*>0.05); N/A – Not applicable; ^{1}Odds ratio for FPT.

Conclusion

This study indicates that the factors associated with passive immunity and FPT varied depending on the tests and the cut-offs used to define FPT, possibly attributed to differences in sensitivity and specificity between tests and cut-offs. Furthermore, different factors were identified in suckler beef and dairy calves.

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Effect of a novel complementary feed on fertility and in vitro embryonic development after ovum pick-up and intracytoplasmic sperm injection in mares

E. Lorenzen[1], J. Spanner[1], A. Richardson[2], G. Beaumont[2], K. Hore[2]
[1]VetEmbryo, Hovedgaden 55, Hellevad, 6230 Rødekro, Denmark
[2]Greencoat Limited, Wonastow Road Industrial Estate, Monmouth NP25 5JA, UK

Presenting author
Kate Hore. E-mail: khore@naf-uk.com

Application

Subfertile mares are a common challenge, with both conception failure and early embryo loss frequently reported (Petroski-Rose 2018), causing stress and financial loss.
Poor fertility is often age related (Carnevale et al 2020), as horses are unusual within livestock being frequently primiparous in teenage years following an earlier, ridden, career. There is an unmet need for practical, commercial solutions to maximize mare reproductive success, particularly in older primiparous mares, and in competition mares where embryo collection needs to be timed within a competition schedule. For competition mares the convenience of a single appointment around her competition schedule, which can include outside the breeding season, makes ovum pick-up an attractive option. Assisted Reproductive Technologies are established in broodmares both to optimize success in sub-fertile females, and to offer convenient options for competition mares.

Introduction

Previous research has shown the benefit of supplementing the diet for mare fertility as a simple, practical option for owners and stud managers alike (Cantandi et al 2022). Complementary feeds specifically targeting broodmare fertility are limited, yet there is a window of opportunity for dietary interventions, from eight weeks prior to covering through to first pregnancy confirmation scan.
The project aimed to test a dietary approach to mare sub-fertility. The novel feed trialled is a unique complex of key micronutrients, including digestibility enhancers, omega fatty acids, chelated trace elements, together with vitamins recognised for oestrus and reproduction as detailed in EU and UK regulations on feed for particular nutritional purposes (EU Commission Regulation. 2020).

Materials and Methods

The trial used mares recruited to an ovum pick-up and intracytoplasmic sperm injection programme as a way to quantify oocyte numbers and successful blastocyst formation, not easily possible in the naturally covered mare. All mares (Treatment group n=28, No-treatment group n=31), were fed a standard broodmare basal diet. Treatment group also received the novel feed daily. How long they received the feed was dependent on when they entered the programme, and whether more than one three-week cycle was required. Mares were housed either at a veterinary clinic, commercial stud or in private yards. All mares were non-lactating and predominantly Warmblood breed. Ages ranged from 14 to 22 years Treatment, and 4 to 24 years No-treatment. Average number of oocytes, maturation rate, cleavage rate, blastocyst rate, percentage successful sessions (ovum pick-up $\geq$ 1 embryo) and average freeze day following sperm injection were assessed by in vitro analysis. Statistical analysis used an unpaired t-test for average oocyte numbers, ages and freeze days. Fisher's exact tests were used to analyse maturation, cleavage and blastocyst rate between groups.

Results

Data were split into three groups for analysis. Firstly, total ovum pick-up sessions in each group (Treatment n=31, No-treatment n=34). Secondly, sessions with mares over 15 years old (Treatment n=22, No-treatment n=12) to reflect the particular challenge in older mares. Finally, data from mares over 15 years old receiving the diet for a minimum of four weeks (n=15) to see effect of time on treatment. For no-treatment, the final group were sessions where age-group and a minimum of four weeks in the programme aligned, but no specific dietary change (n=10).

No significant difference ($p<0.05$) was found between groups in any parameters. However, a clearly higher proportion of successful sessions were seen in the Treatment group compared to No-treatment. This trend strengthened for mares over 15 years, and further for the group over 15 years and after four weeks of diet (Figure 1).

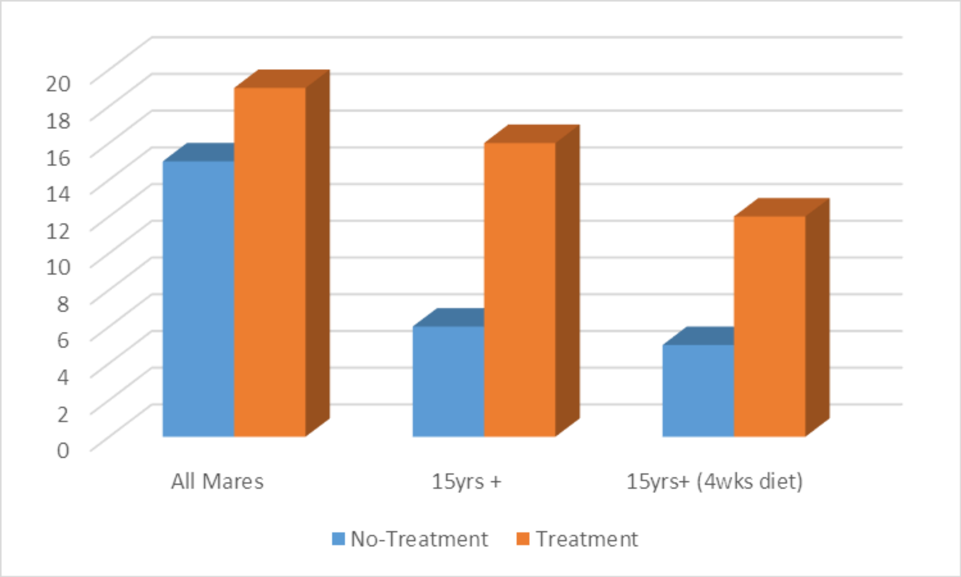

Figure 1. Successful sessions (Ovum pick-up = ≥1 embryo)

One limitation of the trial was recognised in that the two mare groups were not matched by age, weight, breed, fertility history, semen choice or, importantly, basal diet. It was not possible to implement these controls within a commercial setting, and it was recognised that the No-treatment, particularly, may have greater variation in basal diet, as they were privately housed.

Conclusions

Despite no statistical significance, clear trends for improvement in parameters were seen, and these increased with mare age and time on the diet. For proportion of successful sessions, the greatest difference was seen in mares over 15 years who had received the diet for a minimum of four weeks (80% Treatment vs 50% Non-treatment). In the study by Catandi et al (2022) trial diet was fed for 8-17 weeks, and results in this trial also support a longer feeding rate. Future research would look at feeding the novel feed for a minimum of 8 weeks, and ideally with more control over mare variables, such as age, breed and diet.

Trial results suggest that it is recommended to feed the novel feed, NAF OvuCycle, for a minimum of four weeks before ovum pick-up, and eight weeks may be preferable.

Although further research is recommended, these early results show a clear commercial application for feed supplementation, particularly for owners of older mares. Not limited to Assisted Reproductive Technologies alone, the improvement trend also has commercial relevance for the wider equine stud sector, and all broodmares, where only a single successful embryo is required, and a simple dietary approach is easily implemented.

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Centrifugation through a low density colloid is an alternative to sperm washing when preparing stallion spermatozoa for freezing

Z. Al-Kass, J. Morrell[1], T. Ntallaris
[1]*Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden*

Presenting author
Jane Morrell. E-mail: jane.morrell@slu.se

Application

Apart from thoroughbred breeding, where natural mating is the method of choice, most sporthorse breeding involves the use of artificial insemination (AI) with cooled semen. The availability of frozen semen would facilitate the use of AI in horses if post-thaw sperm quality can be improved. Cryopreservation of stallion semen is possible for some ejaculates but post-thaw sperm quality varies considerably. Using a sperm preparation protocol that is less harsh when removing some of the seminal plasma, such as reducing centrifugation force, could benefit sperm cryosurvival. This study evaluates whether centrifugation through high or low-density colloids yields better post-thaw sperm quality compared to sperm washing, which is the conventional method of preparing stallion semen for freezing.

Introduction

Cryopreserving stallion ejaculates can be challenging as not all sperm samples yield good sperm quality on thawing. One possibility is that centrifuging the semen to increase the sperm concentration prior to freezing damages the spermatozoa. Colloid centrifugation provides an alternative method for separating spermatozoa from seminal plasma which does not cause damage. Whereas centrifugation through a high density colloid selects the most robust sperm which survive cryopreservation better than the unselected samples (Hoogewijs et al., 2011), some spermatozoa are lost in the process. Centrifugation through a low density colloid was used to separate spermatozoa from seminal plasma without selecting for good quality spermatozoa (Malaluang et al, 2024) and produced sperm samples of comparable quality to sperm washing for cooled storage. Objective: to compare the effect of centrifugation through high or low density Equicoll and sperm washing prior to freezing on post-thaw sperm quality

Materials and Methods

Three ejaculates from each of 10 stallions at a commercial stud in Sweden was collected using an artificial vagina. An aliquot of each ejaculate was extended 1:1 in Kenny´s extender without antibiotics and was transported to the laboratory at the Swedish University of Agricultural Sciences in an insulated box. On arrival, the concentration was adjusted to 100 $x10^6$/mL with Kenny´s extender and each sample was split into three portions. One portion (10 mL) was centrifuged at 800x g for 10 minutes as the control (sperm washing; Malaluang et al., 2024). The other two portions (10 mL) were each layered over 15 mL high or low density Equicoll in a 50 mL tube and centrifuged at 300 x g for 20 minutes (Malaluang et al., 2024). After centrifugation the resulting sperm pellets were resuspended in EquiPlus Freeze (Minitube International, Tiefenbach, Germany) to a sperm concentration of 200 $x10^6$/mL and were used to fill 0.5 mL plastic straws. These were frozen in a programmable freezer (Cryochamber, Cryologic, Australia) and plunged into liquid nitrogen for storage. After at least 24h in liquid nitrogen, the straws were thawed in a water bath at 37 °C for 30 sec. Sperm quality was analysed using computer assisted sperm analysis and flow cytometry for membrane integrity after staining aliquots with SYBR14/propidium iodide. Chromatin integrity was assessed using the Sperm Chromatin Structure Assay. Sperm quality means were compared using a mixed model (PROC MIXED) with the SAS software (version 9.4; SAS Institute Inc., Cary, NC), followed by Scheffé adjustment for multiple comparisons.

Results

Sperm yields after centrifugation were similar for sperm washing and centrifugation through low density Equicoll but was less for centrifugation through high density Equicoll ($P<0.001$). Mean total motility and membrane integrity were similar in all treatments. The DNA fragmentation index was lower for samples prepared by Single Layer Centrifugation with high density Equicoll than for the other two treatments, indicating selection for spermatozoa with intact chromatin.

Table 1. Yield and sperm quality parameters in stallion sperm samples prepared by washing or by Single Layer Centrifugation

Variable	Sperm washing	SLC with high density colloid	SLC with low density colloid
Yield (%)	68.4 ^{b}	41.8 ^{ab}	80.2 ^{a}
Total Motility (%)	35±2.3	31±2.3	33±2.3
Membrane intact (%)	51±2.3	50±2.3	49±2.3
%DFI	16±1.8^{b}	7±1.8^{ab}	13±1.8^{a}

Notes: SLC = Single Layer Centrifugation; %DFI = DNA fragmentation index. Different superscript letters indicate statistical significance within the row.

Conclusions

Centrifugation through low density Equicoll offers an effective alternative to sperm washing for preparing stallion semen for freezing, as it maintains sperm quality while significantly increasing yield. This approach could be particularly advantageous for equine breeding programs aiming to maximize sperm recovery without compromising post-thaw motility or membrane integrity. Although SLC through high density Equioll improved DNA integrity, the reduced yield suggests this method may be better suited for specific applications where chromatin integrity is critical, such as in cases of subfertile stallions or for intracytoplasmic sperm injection (ICSI). Further research could evaluate the long-term fertility outcomes of these methods and assess their cost-effectiveness in commercial breeding programs.

Acknowledgment

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Group accommodation increases stress parameters but does not impair development of two-year old Sport Horse stallions during 12 weeks of pre-training

L. Kroschel[1], F. Pilger[1], J. Aurich[2], C. Aurich[1,2]
[1]*Graf Lehndorff-institute/ Vetmeduni Vienna, Veterinärpl. 1, 1210 Wien, Austria*
[2]*Vetmeduni Viennam Veterinärpl. 1, 1210 Wien, Austria*

Presenting author

Laura Kroschel. E-mail: Laura.Kroschel@vetmeduni.ac.at

Application

This study does not suggest an advantage of group housing of two-year-old stallions in a 12-week pre-training programme where they were regularly exercised and had daily access to outdoor paddocks.

Introduction

Sport Horse stallions preselected for breeding are often prepared for stallion licensing assessment before they are 30 months old. Although this preparation should be classified as pre-training, the initial training of young horses is a stress challenge (Schmidt et al., 2010; Kaps et al., 2022). This emphasizes the importance of a careful approach during pre-training and training of young horses. Horses are usually raised in groups but stabled individually before training. Although individual stabling allows visual and olfactory contact between animals in adjacent boxes, separation of horses previously kept in groups induced a transient stress response in some (Alexander and Irvine, 1998; Erber et al., 2013) but not all studies (Harewood and McGowan, 2005). Furthermore, horses kept in social groups were easier to handle than horses housed alone (Christensen et al., 2002). Therefore, we investigated cortisol profiles, HR, body development, presence of injuries as well as trainer scores in young stallions housed either in a group stable or in individual boxes during pretraining. We hypothesized that group housing improves physical fitness and reduces the potential stress compared to accommodation in individual boxes.

Materials and Methods

The study included 24 months-old Sport Horse stallions. They were kept as one group on pasture before they were transferred to housing in one group stable (GROUP, n = 9; 14 x 18 m) or individual boxes (BOX, n = 10; 3.6 x 4.3 m) during a 12-week pretraining programme. Horses were kept on straw and were fed concentrates and hay twice daily, water was always available. All stallions had daily access to outdoor paddocks, either individually or as a group. Using established methods, diurnal salivary cortisol (Kuhl et al., 2016) and heart rate (Schmidt et al., 2010) profiles were determined when stallions were transferred from pasture to their respective stables and once weekly for 12 weeks thereafter. Body weight (BW) and BCS, skin injuries as well as trainer scores were assessed once weekly. The trainer assessed if horses appeared to be tired, unmotivated, overstrained, stressed or showed signs of muscle strain with scores from one (not true) to five (true) for each parameter. Statistical analysis was performed with the SPSS statistics programme (Version 29). Non-scored data, after testing for normal distribution and homogeneity of variance, were analysed by repeated measures ANOVA with time as within subject and housing system as between subject factors. Scored data were analysed by Mann-Whitney-U-test between groups and Friedman-test over time.

Results

The BW of stallions decreased for two weeks after stabling ($P < 0.001$) and increased thereafter. The decrease was more pronounced in BOX versus GROUP stallions ($P < 0.05$; week 2 GROUP 75±12, BOX 68±18% of initial weight). The BCS increased over time ($P < 0.001$) but did not differ between groups. Salivary cortisol concentration increased immediately when stallions were stabled ($P < 0.001$). This increase was more pronounced in GROUP than BOX stallions ($P < 0.01$, peak concentrations GROUP

5.5 ± 1.7, BOX 3.0 ± 0.5 ng/ml, means). Thereafter, in both groups, a diurnal rhythm was established with higher cortisol concentrations in the morning and a decrease throughout the day. On individual days, cortisol concentrations were transiently elevated in GROUP but not in BOX stallions. Like cortisol, HR increased transiently at stabling ($P < 0.001$, peak values GROUP 84 ± 3, BOX 67 ± 5 beats/min). During the following 12 weeks, elevated heart rates were occasionally observed in GROUP but not in BOX stallions. Scoring of the stallions´ temperament and behaviour by the trainer was not different between groups but changed toward better scores during the 12 weeks of pretraining. Injuries and skin lesions were more evident in GROUP than in BOX stallions ($P < 0.001$, total number of lesions per animal GROUP 50 ± 16, BOX 26 ± 13) but the number of lesions decreased during the 12-week study period (time and group x time $P < 0.001$). Over 90% of the injuries were superficial skin abrasions.

Conclusions

Two-year old Sport Horse stallions presented a transient stress response when transferred from pasture to stables. This stress response was more pronounced when stallions were housed in groups compared to individual boxes. Throughout the 12-week study, occasional increases in cortisol and HR indicative of a marked arousal occurred in GROUP but not BOX stallions. Skin lesions were more frequent in GROUP than in BOX stallions. Stallion behaviour and social interactions in the stable are analysed from video recordings in additional studies. Our study suggests that both group housing and individual housing of two-year-old stallions during pretraining is acceptable when stallions are regularly exercised and have daily access to outdoor paddocks.

Acknowledgments

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Blue light and time of day of exercise impacts inflammatory markers in yearling Quarter Horses

L. Wesolowski[1], B. Williams[1], A. DiSilvestro[1], A. Boone[1], J. Isgrigg[1], B. Dickerson[1], T. Welsh, Jr.[1], B. Murphy[2], S. White-Springer[1]
[1]*Texas A&M University and Agrilife Research, 400 Bizzell St, College Station, TX 77840, US*
[2]*University College Dublin, Belfield, Dublin 4, Ireland*

Presenting author
Lauren Wesolowski. E-mail: wesolowski@tamu.edu

Application
This work is expected to improve understanding of exercise-induced inflammation and environmental conditions to optimize exercise adaptation and welfare in performance horses.

Introduction
Understanding daily fluctuations in physiological parameters can optimize livestock management by aligning practices with animals' circadian rhythms. Performance and cardiorespiratory responses (Kang et al., 2023) are known to vary based on the time of day of exercise, but the equine inflammatory response to exercise at different times of day is unknown. Additionally, the impacts of disrupted circadian rhythms due to unnatural barn lighting on inflammation is unexplored in horses. We hypothesized that exercise-induced inflammation would be greater following exercise in the morning compared to the evening regardless of barn light conditions.

Materials and Methods
Experimental protocols were reviewed and approved by the Texas A&M University Institutional Animal Care and Use Committee (2022-0268). Quarter Horses (14 fillies, 14 geldings; mean±SD 18±1mo; 360±31kg) were blocked by sex, age, and bodyweight and assigned to one of three treatments for 45 days: 1) Control (CON) – 166 lux sunlight by day, no artificial light at night; 2) Competition (COMP) – 25 lux soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with a 50-lux Equilume blue light mask by day and red-tinted visor by night to filter blue light. On d36, half of the horses performed a 2-h submaximal exercise test (SET) at 0600 h (AM) while the other half performed the same SET at 1800 h (PM). On d43, horses repeated the SET but at the opposite time of their d36 SET. Blood was collected pre-, 0, 4, 10, 22, 28, and 46h post-SET (T-2, T0, T4, T10, T22, T28, and T46, respectively) and analyzed for concentrations of anti-inflammatory cytokines, interleukin (IL)-4 and IL-10, and pro-inflammatory, IL-8 and tumor necrosis factor (TNF)α, in plasma and serum amyloid A (SAA). Data were analyzed using PROC MIXED in SASv9.4 with repeated measures (time); horse within treatment (trt) was a random effect and sex, trt, time pre/post-SET, time of exercise (AM v PM), and all interactions were fixed effects. Data presented as least squared means ± SEM.

Results
Regardless of trt or AM/PM, IL-10 increased from T-2 to T0 (92±8 pg/mL to 99±8 pg/mL, *P*=0.02) but returned to T-2 levels by T4 (91±8 pg/mL). Regardless of trt, IL-8 tended to increase from T-2 to T0 (982±109 pg/mL to 1,150±108 pg/mL) in PM SET (*P*=0.06) but decreased during that time in the AM SET (1,140±108 pg/mL to 965±110 pg/mL, *P*=0.05) resulting in IL-8 being greater at T0 in the PM compared to the AM SET (*P*=0.04). In the AM, IL-8 continued to decrease to T4 (857±109 pg/mL, *P*=0.002) but returned to T-2 levels by T10 (1,070±109 pg/mL). In the PM, IL-8 returned to T-2 levels (982±109 pg/mL) by T4 (1,025±109 pg/mL), tended to increase to T10 (1,191±109 pg/mL, *P*=0.06) to be above T-2 (*P*=0.02), then returned to T-2 levels by T22 (1,068±108 pg/mL). IL-10 and TNFα were greater in COMP during PM SET than AM SET (*P*<0.05; Table 1). For CON, IL-4 was greater (*P*<0.0001) and TNFα tended to be lesser (*P*=0.07) in the PM SET than AM SET. Conversely, TNFα tended to be

greater in CIRC horses during the PM SET than AM SET (*P*=0.09). During the PM SET, CON IL-4 was greater than CIRC (*P*=0.05) and tended to be greater than COMP (*P*=0.08) while IL-10 and TNFα tended to be greater in COMP than CON (*P*=0.09). In all horses at AM and PM, SAA increased from T-2 to T22 (*P*<0.001) and remained elevated at T28 (*P*<0.0001; Figure 1). In CON and COMP, SAA then decreased at T46 (*P*<0.05) to be similar to T4 but above T-2 (*P*<0.05); in CIRC, SAA remained elevated at T46 in AM SET (*P*<0.05) but decreased at T46 in PM SET (*P*=0.04) to levels above T-2 (*P*<0.0001).

Table 1. Mean cytokine concentrations surrounding a 2-h standardized exercise test (SET) performed at 0600 h (AM) or 1800 h (PM) in horses under three different light treatments for 45 days: 1) Control (CON) – sunlight by day, no artificial light at night; 2) Competition (COMP) – soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with an Equilume blue light mask by day and red-tinted visor by night. *Within treatment, PM differs from AM (*P*<0.05); ^{abc}Within time of SET, treatments with different letters differ (*P*<0.05)

				Pooled		***P*-Value**	
Cytokine, pg/mL	**Trt**	**AM SET**	**PM SET**	**SE**	**Trt**	**Time**	**Trt × Time**
IL-10	CON	84.9^{a}	81.1^{a}	0.23	0.3	0.001	0.02
	COMP	107.7^{a}	112.0^{a*}				
	CIRC	85.5^{a}	83.3^{a}				
IL-4	CON	508.2^{a}	605.2^{a*}	1.61	0.2	0.9	0.004
	COMP	354.2^{a}	386.3^{ab}				
	CIRC	350.1^{a}	345.3^{b}				
IL-8	CON	1155.5^{a}	1233.9^{a}	3.22	0.5	0.06	0.2
	COMP	962.1^{a}	924.0^{a}				
	CIRC	924.9^{a}	1023.9^{a}				
TNFα	CON	32.9^{a}	30.1^{a}	0.16	0.3	0.7	0.002
	COMP	47.0^{a}	51.5^{a*}				
	CIRC	45.7^{a}	48.4^{a}				

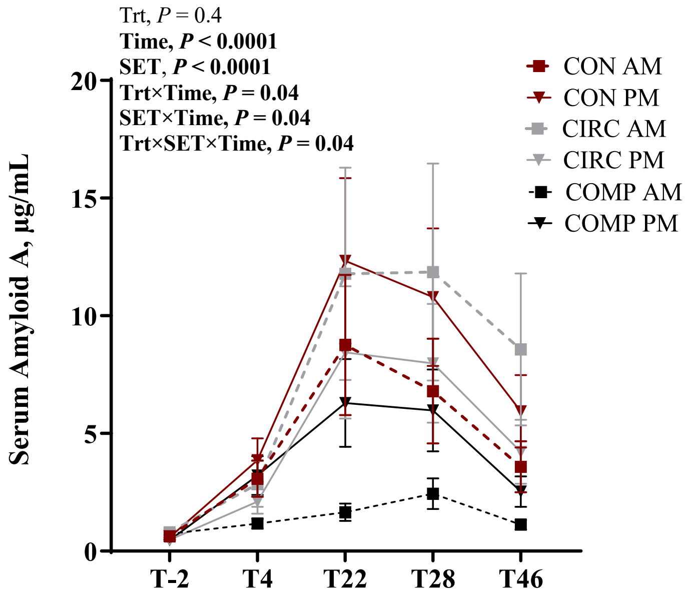

Figure 1. Serum Amyloid A concentrations pre-, 4, 22, 28, and 46h post- (T-2, T4, T22, T28, and T46, respectively) a 2-h standardized exercise test (SET) performed at 0600 h (AM) or 1800 h (PM) in horses following 45 days of one of three different light treatments: 1) Control (CON) – sunlight by day, no artificial light at night; 2) Competition (COMP) – soft white, fluorescent barn lights 24 h/d; or 3) Circadian (CIRC) – COMP lighting but fitted with an Equilume blue light mask by day and red-tinted visor by night.

Conclusions

These data suggest that PM exercise is less inflammatory than AM exercise for horses under ideal circadian lighting (blue light/sunlight by day and absence of blue light at night), but this is not consistent in horses under weak barn lights 24 h/d. Optimizing time of day of exercise and barn lighting conditions could improve exercise-induced inflammatory responses in young horses.

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Artificial neural networks improve estimation of horse body weight using morphometric measurements

X. Aguilar-Amaro[1], M. Hernández-Gil[2], A. Lizarazo Chaparro[3], E. Ramirez-Cabrera[1], J. Cervantes Bazán [4], J. Angeles HernandezJ[5]
[1]*Programa de Maestría y Doctorado en Ciencias de la Producción y de la Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autonoma de México., Mexico*
[2]*Secretaría de Vinculación y Proyectos Especiales, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Nacional de México, México*
[3]*Centro de Enseñanza Práctica e Investigación en Producción y Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México*
[4]*Departamento de Ingeniería Mecánica Agricola, Universidad Autónoma Chapingo, México,*
[5]*Departamento de Medicina y Zootecnia de Rumiantes, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma Nacional de México, México*

Presenting author
J.C Ángeles-Hernández. E-mail: juanangeles@fmvz.unam.mx

Key words: horse, body weight, morphometric measures, artificial neural networks

Application

Accurate estimation of horse body weight using morphometric measurements and artificial neural networks could provide a reliable and accessible method for equine practitioners, veterinarians and nutritionists to monitor equine health and adjust management practices. The proposed Artificial Neural Network (ANN) architecture will serve as the underpinning for a mobile application, ensuring practicality and portability. This will be particularly useful in situations where scales are not available, enabling more accurate medication dosing, nutritional planning and overall health assessment of horses.

Introduction

Accurate estimation of body weight (BW) in horses is essential for several aspects of equine management, including health monitoring, medication dosing and nutritional planning. Traditional methods based on morphometric measurements such as height at withers, chest girth and body length have been widely used, but are often inaccurate due to variations in breed, age and body composition. Recent studies have investigated more advanced approaches that integrate multiple morphometric variables and sophisticated statistical techniques to improve prediction accuracy. ANN have shown potential for improving the accuracy of BW estimation compared to conventional multiple linear regression models (Angeles-Hernandez et al., 2022). Therefore, the aim of the present study is to compare the fitting performance of traditional multiple linear regression and ANN for estimating horse BW based on a comprehensive set of morphometric measurements.

Material and methods

Data base

A database was constructed based on registers of BW and morphometric measurements of a total of 142 horses (88 females and 54 males) over 2 years of age. The horses were located in three different states of Mexico: State of Mexico, Nuevo Leon and Mexico City. BW (kg) was recorded for each animal using a digital scale (model S3, TRU-TEST) in conjunction with the following morphometric measurements: height at withers (HW), chest girth (CG), neck circumference (NC) and body length (BL). Age and body condition score (BCS) were also recorded. Morphometric measurements were obtained with the animals standing and using a flexible measuring tape.

Multiple linear regression

A multiple linear regression (MLR) model was constructed using the glm() function in R, with BW as the dependent variable. The independent variables included HW, CG, NC, BL, BCS and age.

Model training of ANN

The ANN incorporates identical inputs to those used for MLR. Data normalisation was critical due to different ranges and units. A supervised learning approach was used to train a two-layer ANN with seven hidden structures: 5-5, 5-10, 10-5, 10-10, 10-15, 15-10 and 15-15 neurons. Inputs were transferred to the hidden layer multiplied by weight W using a tangent sigmoid activation function. The data set was randomly divided into 70% for training and 30% for testing. A globally convergent algorithm based on resilient backpropagation computed the ANN with a maximum training step of 1x107 using the neuralnet package (Fritsch et al., 2019) in R software (R core team, 2022).

Goodness of fit

To compare the fitting performance of RLM and ANN, the following criteria and goodness of fit statistics were used: r, correlation between actual and predicted values; r2, coefficient of determination; MSPE, mean square prediction error; RMSPE, root mean square prediction error; AIC, Akaike's Information Criterion; BIC, Bayesian's Information Criterion. The best models were those with the lowest AIC, BIC, RMSE and the highest r and r2.

Results

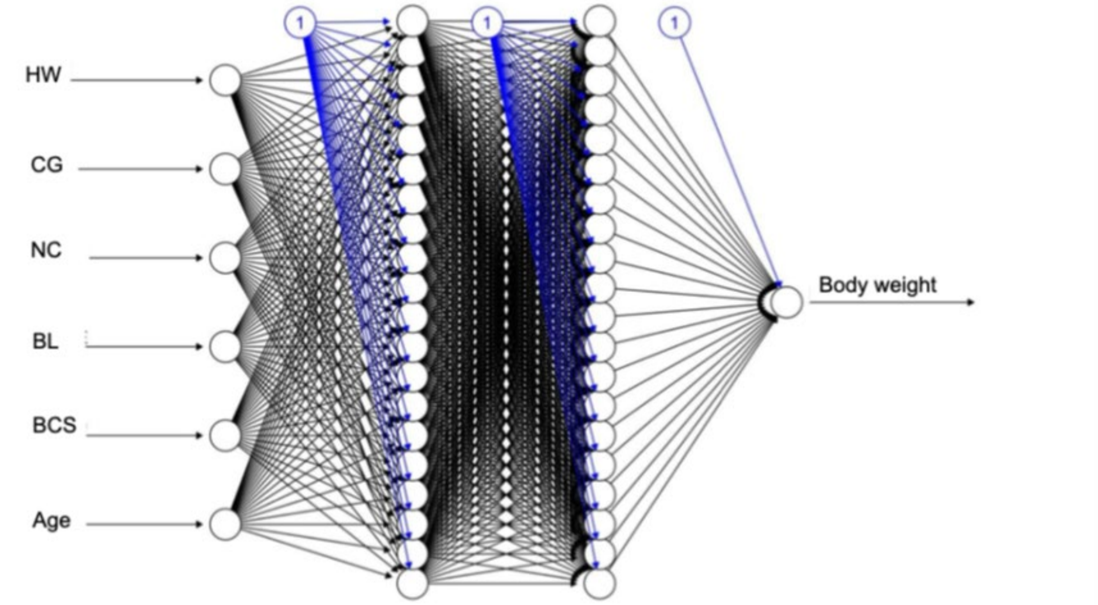
Table 1 shows a comparison between MLR and different architectures of ANN for predicting BW in horses. In general, all models show good performance, with coefficients of correlation and determination higher than 0.90 and 0.82, respectively, confirming that morphometric measurements are good predictors of BW in horses. According to the RMSPE values, MLR had a mean prediction error in BW estimation of 24.29 kg. On the other hand, ANN consistently outperformed the MLR model in all evaluated criteria. The 15-15 ANN architecture shows the best performance in all aspects (r = 0.99, r^2 = 0.97) and a mean prediction error of 9.78 kg, a reduction of 60% compared to MLR. This model also has the lowest information criteria AIC (657.62) and BIC (672.40), indicating a higher prediction accuracy and a better balance between fit and complexity. These results suggest that ANN, especially with more complex configurations, are more effective tools than MLR for predicting BW in horses.

Table 1. Evaluation of goodness of fit of full multiple linear model and artificial neural networks to predict body weight in horses.

Model	r	r^2	MSPE	RMSPE	AIC	BIC
MLR	0.91	0.82	622.55	24.29	1132.6	1356.2
Hidden*						
5-5	0.93	0.87	491.69	22.17	980.09	904.87
5-10	0.93	0.87	498.63	22.33	892.08	906.86
10-5	0.94	0.89	413.60	20.33	865.53	880.31
10-10	0.93	0.87	471.21	21.71	839.21	853.99
10-15	0.94	0.87	424.20	20.50	869.13	883.91
15-10	0.95	0.90	373.77	19.33	851.16	865.93
15-15	0.99	0.97	95.65	9.78	657.62	672.40

MLR, multiple lineal regression; -786.59 (53.79***) + 0.94 (0.43*) × HW + 1.50 (0.40) × BL + 3.61 (0.42***) × CG + 1.35 (0.33***) × NC + 0.87 (0.55) × AGE + 23.56 (7.01) × BCS. r, correlation between actual and predicted values; r^2, coefficient of determination; MSPE mean square prediction error; RMSPE, root mean square prediction error; AIC, Akaike´s Information Criterion; BIC, Bayesian´s Information Criterion.

Figure 1. Architecture of used ANN with two-layers and 15 neurons to estimate horse body weight based in morphometric measurements



Conclusion

ANN demonstrated superior accuracy and reliability compared to MLR models for predicting horse BW based on morphometric measurements. This approach provides an effective and accessible tool for equine management, particularly in the absence of scales.

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Group accommodation increases social interaction including agonistic behavior compared to traditional single stabling in two-year old Sport Horse stallions during 12 weeks of pre-training

F. Pilger[1], L. Kroschel[1], J. Aurich[2], C. Aurich[1,2]
[1]*Graf Lehndorff Institute, Vetmeduni Vienna, Veterinärpl. 1, 1210 Wien, Austria*
[2]*Vetmeduni Vienna, Veterinärpl. 1, 1210 Wien, Austria*

Presenting author
Franziska Pilger. E-mail: franziska.pilger@vetmeduni.ac.at

Application

Two-year old Sport Horse stallions can be kept in groups during pre-training. As expected, group housing allows more social interaction between horses, but this includes agonistic behaviour.

Introduction

In feral horses, non-harem stallions live in loose bachelor groups; therefore it has been suggested that domestic stallions should be kept in groups. For postpubertal stallions, group housing is, however, a rare exception. In stables preparing Sport Horses for breed registry licensing, young stallions are exclusively housed in individual boxes with limited contact to other horses. This is increasingly questioned, and in Germany Federal Animal Welfare guidelines demand that horses, including stallions less than 30 months old are kept in groups. In contrast, equestrian authorities argue that once puberty is attained, stallions should be stabled individually to avoid aggression and injuries. Therefore, we have analysed the behavior of 24 months-old stallions housed either in a group stable or in individual boxes during pre-training. We hypothesized that group housing is not associated with increased aggression among stallions.

Materials and Methods

The study included ten postpubertal, 24 months-old Sport Horse stallions. They were kept in group stables with paddock access or on pasture (depending on the season) from weaning until they were transferred to housing in a group stable (GROUP, n = 5) or individual boxes (BOX, n = 5) for a 12-week pretraining programme. The study therefore included (1) the phase of adaptation to the new housing system and (2) a comparison of the two housing systems thereafter. The pretraining included free movement in an indoor arena, showing at hand, exercise in an automated horse walker, lunging and jumping of obstacles without a rider.. Horses were kept on straw and were fed concentrates and hay twice daily, water was always available. All stallions had daily access to outdoor paddocks, either individually or as a group based on their group allocation. Continuous video observations were made in the stables with one dome IP camera per box or group stable. Data were stored on an HDD video server and for one day per week, two 30-min intervals starting at 6 a.m. (before feeding of concentrates) and 5 p.m. (no activities in the stable) were analysed. Analysis was made with the Behavioral Observation Research Interactive Software BORIS (University of Torino, Italy). The following behavior patterns were defined: Lying in sternal and lateral recumbency, standing with subcategories relaxed and alert, eating, movement in walk, trot and canter, drinking, solitary and mutual grooming, pawing, playing and social interaction. Data were analysed by repeated measures ANOVA with week and time of day as within subject factors and housing system as between subject factor (SPSS statistics programme version 29).

Results

BOX stallions spent more time in sternal and lateral recumbency than GROUP stallions ($P < 0.05$). Episodes with the stallions standing either relaxed or alert were more frequent in BOX than in GROUP stallions ($P < 0.001$), but the duration of such episodes was longer in GROUP stallions. The frequency of standing episodes was higher before feeding than when there was no activity in the stable ($P <$

0.001). GROUP stallions were seen eating hay more often than BOX stallions with episodes in both groups shorter before feeding than during observation after 5 p.m. The frequency of walk episodes did not differ between GROUP and BOX stallions but was higher at 6 a.m. than at 5 p.m. in both groups ($P < 0.001$) and decreased throughout the 12-week study ($P < 0.01$). Movement in trot or canter was close to absent in both housing systems. Drinking was hardly observed before feeding but on average once per hour after 5 p.m. ($P < 0.001$) in both groups. The frequency of solitary grooming was similar in both groups and decreased throughout the study period ($P < 0.001$). Mutual grooming was observed only in GROUP stallions but was less frequent than solitary grooming. Play behavior was observed only twice in BOX stallions and more often in GROUP stallions. Pawing the ground occurred regularly in BOX stallions before feeding ($P < 0.05$), decreased over time ($P < 0.05$), but was hardly observed in GROUP stallions. Licking the stable wall or box partition was more evident in BOX compared to GROUP stallions ($P < 0.05$). Social interaction including threatening, biting, and attempted kicking was more evident in GROUP stallions ($P < 0.01$) but - directed towards the stallion in the neighboring box - not completely absent in BOX stallions. The frequency was higher before feeding at 6 a.m. than after 5 p.m. ($P < 0.01$) and decreased throughout the 12-week study ($P < 0.001$).

Conclusions

Two-year old Sport Horse stallions housed in a group or individually showed a largely similar behaviour profile. Some agonistic behaviour, potentially causing injuries was evident in GROUP stallions whereas pawing the ground before feeding as a sign of impatience was observed in BOX stallions. None of the stallions in either housing system developed stereotypies or was injured except for minor, superficial skin lesions. Both housing systems appear to be adequate for two-year old Sport Horse stallions, given stallions are regularly exercised, allowed daily free movement and have visual, auditory, and olfactory contact to stable neighbours.

Acknowledgments

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Effect of maintaining equinox light on clinical signs of seasonal onset headshaking in horses: A preliminary study

B. Murphy[1], P. Kyriazopoulou[2], C. Mowbray[3]
[1]*University College Dublin, Belfield, Dublin 4, Ireland*
[2]*Equilume Ltd, Ladytown Business Park, Naas, Co. Kildare W91 TP22, Ireland*
[3]*Independent Researcher*

Presenting author
Barbara Murphy. E-mail: barbara.murphy@ucd.ie

Application

Headshaking Syndrome (HS) is a poorly understood facial pain condition in horses that affects 1% of all equines, with geldings generally overrepresented. Symptoms include uncontrolled violent flicking of the head, snorting, rubbing the face and striking at the nose. Therapeutic options for HS are limited, and only partially successful. This study provides a preliminary evaluation of the effectiveness of a new non-invasive treatment for the condition that has the potential to improve the welfare of affected horses globally.

Introduction

HS presents a major welfare concern for affected horses. Clinical signs include involuntary and recurring violent jerking, striking, and flicking or rubbing of the head and muzzle (Pickles et al., 2014). More than 60% of owners report an onset or exacerbation of clinical signs in spring and summer, indicating a strong seasonal component (Madigan and Bell, 2001; Pickles et al., 2014). Following elimination of common causes of the behaviour by a veterinarian, involvement of the trigeminal nerve is assumed (Pickles, 2023; Roberts, 2019). Increased firing of GnRH neurons during spring and summer months is thought to overstimulate the trigeminal nerve in seasonally affected HS horses (Pickles et al., 2011). It has been proposed that prolonged elevated levels of gonadotropins in geldings, who lack the ability for negative feedback on GnRH release by testes derived testosterone, may cause instability of the trigeminal nerve that somehow gives rise to neuropathic pain (Pickles et al., 2011). Previous attempts to alleviate symptoms by pharmacologically manipulating photoperiod signals showed only limited success. The present preliminary study aimed to ablate the seasonal photoperiodic signal using a blue light treatment that maintained exposure to the same daylength from autumn through to spring.

Materials and Methods

Seventeen owners of 17 HS horses were recruited via an online recruitment survey in Australia. Study eligibility was confined to owners of geldings with a history of moderate to severe headshaking behaviour, who had received a veterinary diagnosis of HS, and whose horses displayed seasonal onset of clinical signs during the spring/summer months. Each horse acted as their own control in a longitudinal study spanning 12 months. Horses were fitted with Equilume™ light masks that provided 15 h of blue light exposure from 08:00 to 23:00 daily for six months, starting at the autumn equinox (March 21st in Australia). Participants were instructed to only remove the light masks for exercise, grooming and to charge weekly. In addition to the pre-study recruitment questionnaire (where details were collected on peak symptomology during spring/summer), questionnaires were circulated to collect information on HS clinical signs and intensity at the start (March/April), middle (June/July), and end-of-study period (October/November). Clinical signs evaluated were vertical ticcing (flicking head towards chest); tossing head up and down; striking at head with front feet; rubbing face on objects/self and humans; and tightness of muzzle/mouth/lower lip. Clinical sign intensity was assessed on a 5-point scale: Not observed = 0; Very occasionally (i.e. observed once per week) = 1; Sometimes (i.e. observed several times per week) = 3; Often (observed at least once per day) = 4; Constantly = 5 (observed multiple times per day). The end-of-study questionnaire also asked owners to comment on perceived changes in their horse's quality of life (QoL) and assess overall changes in HS clinical sign intensity compared to the same period the previous year. Repeated measures ANOVA was conducted on normally distributed data to assess changes in the total number of clinical signs observed over time. Tukey's multiple comparison tests were used to identify differences between specific time points. Friedman tests were used to explore differences in clinical sign intensity scores reported at the start, middle and end of the study period. Analyses were carried out on GraphPad Prism (Version 10.2.3, 2024) and Microsoft Excel (Microsoft® Excel® for Microsoft 365, Version 2405, 2024).

Results

Of the 17 initial study participants, data from 10 who were fully compliant with providing survey responses, and whose horses met the study eligibility criteria were included in the data analysis. The number of reported HS clinical signs reduced over time ($P = 0.02$, Fig. 1A). There was no difference in the number of clinical signs reported between the pre-study period and the start of study ($P > 0.05$). A decrease in clinical signs from the pre-study period to the mid-study timepoint ($P = 0.03$) and from the pre-study period to the end-of-study period ($P = 0.02$, Fig. 1B) was observed. Friedman tests indicated no differences in clinical sign intensity scores for vertical ticking ($P = 0.06$), snorting ($P = 0.05$), tightness of muzzle ($P=0.44$), head swinging ($P = 0.07$) and face rubbing ($P = 0.08$). 'Striking at face' was a clinical sign reported by two participants in the pre-study questionnaire, but was not reported at the mid-study and end-of-study periods. Seven out of 10 participants reported a reduction in the overall severity of their horses' clinical signs. Of these, two participants reported complete resolution of the condition in their horse. Finally, 6/10 participants reported improvements in QoL.

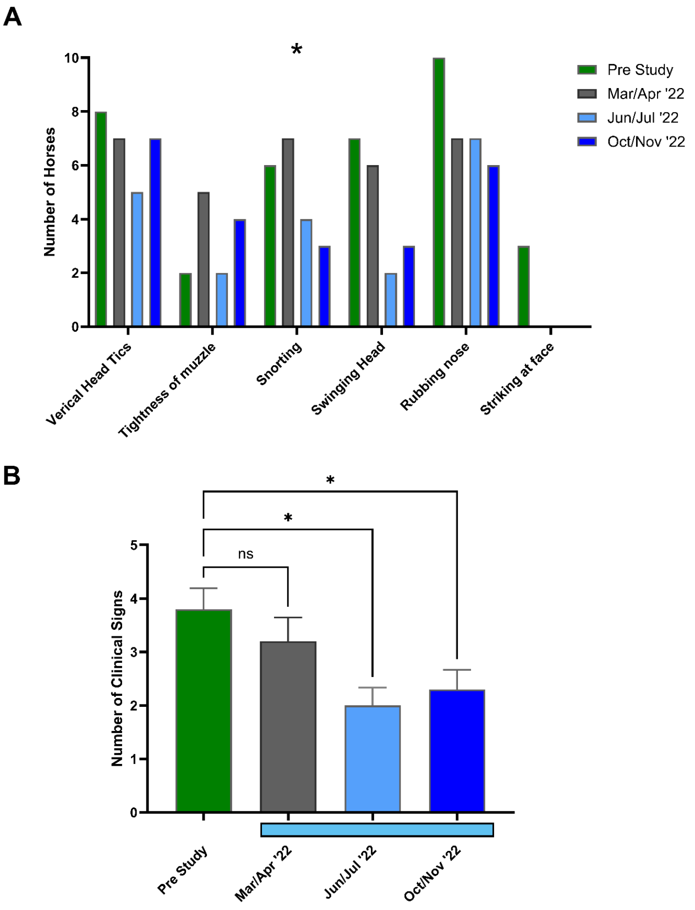

Figure 1. Changes in clinical signs of Headshaking Syndrome over time in response to blue light treatment (A). Total number of headshaking clinical signs reported by participants at the pre-study recruitment stage, the start of the study in Mar/Apr, mid-study in Jun/Jul and the end-of-study in Oct/Nov (B). The blue panel indicates the time period during which horses were exposed to blue light for 15 h/day after being fitted with an Equilume™ light mask. * indicates ($P<0.05$).

Conclusion

Results suggest that blue light treatment used to ablate perception of the shorter winter daylengths may offer a treatment option for managing HS clinical signs and warrant further studies.

Acknowledgements
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Novel predictors of fatigue in Thoroughbred horses

S. White-Springer[1], B. Williams[1], L. Wesolowski[1], A. DiSilvestro[1], M. Barshick[2], K. Mogge[2], E. Jolley[3], A. Logan[3], S. Johnson[2]
[1]Texas A&M University, 400 Bizzell St, College Station, TX 77840, USA
[2]Virginia Tech University, 925 Prices Fork Road, Blacksburg, VA 24061
[3]Middle Tennessee State University, 1301 E Main St, Murfreesboro, TN 37132, USA

Presenting author
Sarah White-Springer. E-mail: s.white@ag.tamu.edu

Application
Results from this work will provide insight into cellular changes that occur before performance horses fatigue, leading to better diagnostics to reduce incidence of fatigue-induced injury.

Introduction
Racehorses are considered amongst the elite of domesticated athletic species. Like human athletes, specific feeding, training, and management protocols should be implemented to ensure equine athlete health and welfare. A focus of these protocols should be prevention of fatigue, as fatigue enhances an individual's susceptibility to injury. However, defining objective measures of fatigue remains elusive, presenting a significant barrier in prediction of injury susceptibility. We hypothesized that gluteus medius mitochondrial metrics would decrease and production of H_2O_2, a reactive oxygen species, would increase prior to horses experiencing decreased performance, as evidenced by a decrease in run time to fatigue.

Materials and Methods
All experimental protocols were reviewed and approved by the [redacted for review] Institutional Animal Care and Use Committee prior to the start of the study (AUP 24-074). Gluteus medius samples were collected from 12 mature, unfit Thoroughbred geldings (mean ± SD 8 ± 2 yr; 510 ± 35 kg) on d 0, 2, and 4 prior to performing standardized exercise tests (SET) to exhaustion on a high-speed treadmill on d 1 (SET1), 3 (SET2), and 5 (SET3). Exercise tests began at 0700 each d. To facilitate completing SETs within a 2-h window, horses were randomly and evenly split into 2 groups

then staggered to perform SETs on alternating days (6 horses/d). Samples were not collected on d 6 as the hypothesis to be tested was that mitochondria would become impaired *before* horses demonstrated a decrease in performance. Concordantly, SET3 on d 5 was necessary to determine if horses demonstrated a decrease in run time to fatigue with repeated SETs. The SET parameters were 4 min at 4 m/s followed by elevation of the treadmill to 6% and rapid increase in speed to 10 m/s. Belt speed was then increased 1 m/s every min until the horse was unable to maintain position on the treadmill with gentle encouragement. Mitochondria were isolated from the muscle samples and analysed for oxidative phosphorylation capacities (P) and production of adenosine triphosphate (ATP) and H_2O_2 using high-resolution fluororespirometry. Data were analyzed by one-way ANOVA with repeated measures or by mixed linear models in the case of incomplete datasets with the fixed effect of day using GraphPad Prism v10.4. Significance was declared as $P \leq 0.05$ and trends declared when $0.05 < P \leq 0.1$.

Results

One horse was excused from the study due to a hoof abscess developed prior to SET3. Unexpectedly, not all horses fatigued by SET3 on d 5 (defined as decreased run time to fatigue). We, therefore, divided the horses into either responders (RESP, n = 5), those horses that had a decreased run time to fatigue by SET3 (P=0.03), or non-responders (NON, n = 6), those horses that did not decrease their run time to fatigue by SET3 (P=0.6). In RESP horses, mitochondrial leak respiration, P with complex I only (P_{CI}), maximal P with complexes I and II (P_{CI+II}), and P with complex II only (P_{CII}) decreased from d 2 to 4 (P<0.05); P_{CI+II} and P_{CII} also tended to be lower at d 4 than d 0 (P<0.1; Table 1). No P metric was impacted by d in NON horses (P>0.05; Table 1). The rate of H_2O_2 production, a reactive oxygen species, relative to O_2 flux tended to increase from d 0 to 4 during leak respiration in RESP horses (P=0.1) but H_2O_2/O_2 did not differ in any other respiratory state in RESP horses, nor in NON horses at all (P>0.05). Production of ATP during P_{CI} tended to decrease from d 2 to 4 in RESP horses (P=0.1) but ATP production did not differ in any other respiratory state in RESP horses, nor in NON horses (P>0.05).

Table 1. Mean ± SEM isolated mitochondrial respiratory capacities from the gluteus medius of unfit Thoroughbred geldings whose run time to fatigue remained unchanged through SET3 (P=0.6; NON, n = 6) or whose run time to fatigue decreased by SET3 (P=0.03; RESP, n = 5).

Variable, pmol $O_2 \cdot sec^{-1} \cdot mL^{-1}$	Treatment	d 0	d 2	d 4	P-value
Leak	NON	9.3 ± 1.8	8.2 ± 1.7	6.1 ± 1.7	0.355
	RESP	5.3 ± 0.8	6.0 ± 0.6	2.9 ± 0.3*	**0.026**
P_{CI}	NON	27.3 ± 6.5	31.9 ± 4.2	25.4 ± 4.0	0.600
	RESP	18.7 ± 3.7	25.5 ± 1.4	9.0 ± 1.8*	**0.010**
P_{CI+II}	NON	46.8 ± 9.8	44.1 ± 1.4	46.0 ± 7.3	0.880
	RESP	33.4 ± 5.9	42.1 ± 1.6	19.0 ± 2.2*†	**0.012**
P_{CII}	NON	31.2 ± 5.6	33.5 ± 3.7	31.5 ± 5.5	0.876
	RESP	21.8 ± 3.1	26.8 ± 0.8	13.4 ± 1.4*†	**0.008**

P, oxidative phosphorylation capacity; CI, complex I; CII, complex II. *Within treatment, d 4 differs from d 2 (P<0.05). †Within treatment, d 4 differs from d 0 (P<0.1).

Conclusions

With repeated exercise tests to exhaustion resulting in fatigue, mitochondria appear to become impaired, evidenced by decreased respiratory capacities, increased reactive oxygen species production, and decreased ATP production. Importantly, horses which did not decrease their run time to fatigue maintained mitochondrial health. Mitochondrial metrics may be useful predictors of susceptibility to fatigue, which may be utilized to decrease fatigue-induced injury.

Associations between gestation length and morphometric measurements of dairy foetuses which aborted or died in the perinatal period

P. Jawor[1], J. F. Mee[2]
[1]*Wrocław University of Environmental and Life Sciences, Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, ul. Norwida 25, 50-375 Wrocław, Poland*
[2]*Teagasc, Moorepark Research Centre, Animal and Bioscience Research Department, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*

Presenting author
John Mee. E-mail: John.Mee@teagasc.ie

Application
The results from this study can be used by veterinary pathologists or veterinary practitioners to estimate gestation length (GL) in aborted and stillborn dairy foetuses where the service date is unknown, e.g. natural service or no/poor records.

Introduction
In investigation of foetal mortality, knowledge of GL allows us to assign causes/s of death and associated factors to each trimester of pregnancy (Mee, 2020). However, where AI has not been used (natural service) or has been used in a herd with natural service also or where records are not available/unreliable, gestation length will need to be estimated. Individual or combinations of external indicators of maturity (e.g. pilosity, dentition) are unreliable, though, in the absence of better predictors, are commonly used. Given that foetal size increases with GL, foetal morphometrics may be used to quantify foetal size and so to estimate GL. However, the relative strength of the associations between various foetal morphometrics and GL in aborted/stillborn fetuses is unknown. Hence, the objective of this study was to use foetal morphometrics to attempt to predict gestation length (GL).

Materials and methods
Data (GL, calf breed and necropsy records) from 1,295 single birth cases of dairy abortion and perinatal mortality were examined. Gestation length varied between 128 and 316 days. The following body morphometrics were recorded at necropsy: DD - digital diameter of fetlock (measured using a calipers), Girth - behind the shoulder (measured using a tape), CRL (crown rump length)_pollc - curved from base of tail to crown (measured using a tape), CRL_eye - curved from base of tail to eye (measured using a tape), Str_CRL -straight from rump to crown (measured using a calipers) and body weight (measured using a calibrated weighing scales). Missing data were recorded for 299 GL and those records were excluded from analysis. Calf breed type was categorised as: Jersey (Je) = Je or Jex sire or dam (n=394); Non-Je = other dairy breeds or crosses (n=450); beef = beef sire on non-Je (n=142) and un = unclear (n=10). For this analysis only records from Je and Non-Je foetuses were used. In this set there was 54 - DD, 63 - Girth, 64 – CRL_pollc, 66 – CRL_eye and 73 – Str_CRL missing records. Data analysis was performed using Python 3.9.7 and linear regression was performed with statsmodels package. The final dataset (844 records) was divided into train (80%) and test/validation (20%) sets.

Results
Individual morphometric variables were highly autocorrelated (0.86-0.98), hence models with one morphometric variable and breed category were built. While breed category was statistically significant, it had a small impact on the output values. In the train set, the highest R2 with GL was for Str_CRL (0.63) while in the test set the best results were achieved using Girth and DD (0.53), (Table 1). Across all models, the R2 in the train set varied between 0.49 (body weight) and 0.63 (Str_CRL) and in the test set between 0.39 (body weight) and 0.53 (Girth and DD). The Girth and DD models in test set had also the lowest mean absolute error (MAE), (mean difference in predicted days from GL) (10.3 days). The lowest percentage of mismatch cases (more than +/-24 days from GL) were in models with

Str_CRL and Girth (9.5%). Across all models, the MAE varied from 10.3 (Girth, DD) to 11.8 days (body weight). The highest mismatch percentage of calculated GL was 13.0% (body weight).

Table 1. Statistics of foetal predictor morphometric variables for gestation length in train (n=675 cases) and test (n=169 cases) datasets.

Model variable*	R2-train	AIC	Intercept/ std. error	Model estimate/ std. error	Non_Je/std. error	R2-test	MAE-test	Cases with difference ± 24 days (%)
Str_CRL	0.63	5384	123.37/4.42	1.6763/ 0.049	-3.2948/ 1.006	0.48	10.8	9.5
Body_Wt	0.49	5606	225.09/2.02	1.65/0.065	-6,37/1.2	0.39	11.83	13.01
DD	0.61	5426	153.19/3.74	23.935/0.74	-4.90/1.04	0.53	10.3	10.0
CRL_pollc	0.57	5494	145.73/4.3	1.56/0.05	-3.3/1.09	0.48	10.7	10.6
CRl_eye	0.60	5440	129.31/4.537	1.478/0.047	-3.2869/1.049	0.52	10.43	11.2
Girth	0.61	5438	147.45/3.9	1.92/1.043	-5.95/1.043	0.53	10.3	9.5

* Body_Wt – body weight, CRL_eye - curved crown rump length from base of tail to eye, CRL_pollc - curved CRL from base of tail to crown, DD - digital diameter of fetlock, Girth - behind the shoulder and Str_CRL -straight CRL from rump to crown.

Conclusion

Bovine gestation length, from the fourth month of pregnancy onwards, could be predicted with different morphometrics with an approximate error of 10 days in dairy calves.

Acknowledgements

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Congenital defects in Irish dairy and beef cattle submitted to the Regional Veterinary Laboratories (2020-2024)

C. Ryan[1], S. Fagan[2], K. Quigley[3], J. F. Mee[4]
[1]University College Dublin School of Veterinary Medicine, Belfield, Dublin 4, Ireland
[2]Department of Agriculture, Food and the Marine Laboratories, Athlone Regional Veterinary Laboratory, Coosan, Athlone, Co Westmeath N37 N471, Ireland
[3]Irish Cattle Breeding Federation, Link Rd, Ballincollig, Co. Cork P31 D452, Ireland
[4]Teagasc, Animal & Bioscience Research Department, Moorepark, Fermoy, Co. Cork P61 P302, Ireland

Presenting author
John Mee. E-mail: John.Mee@teagasc.ie

Application

The results of this original research pilot study can be used to set up a national information infrastructure linking farmer tagging of calves at birth, private veterinary practitioners recommending clients to submit dead calves for investigation at government veterinary laboratories and these laboratories submitting animal identification and investigation outcome information to the national cattle breeding organisation for further research.

Introduction

A congenital defect is a structural or functional anomaly present at birth. The types of bovine defects recorded by Irish veterinary practitioners have recently been reported (Mee et al., 2024) but these may differ from those recorded by veterinary pathologists given the submission biases inherent in each recording system. Recently (2023), a national bovine genotyping programme was announced in Ireland by the Department of Agriculture Food and Marine (DAFM) administered by the national cattle breeding organisation, the Irish Cattle Breeding Federation (ICBF) on behalf of DAFM (Anon., 2023). The routine genotyping of all cattle at birth will play an important future role in the management of genetic diseases and lethal congenital defects in the Irish cattle population especially if the associated phenotypic information is also recorded. All calves born in Ireland must legally be tagged within 20 days of birth and registered with DAFM within 27 days of birth. If a bovine animal dies on an Irish farm, by law, the farmer must report the death to the DAFM which operates six Regional Veterinary Laboratories (RVLs) which investigate the cause/s of death. The objective of this study was to identify the most common lethal congenital defects diagnosed in dairy and beef cattle submitted to the RVLs and to describe their associated epidemiology.

Material and methods

In this retrospective observational study, data covering a five year period (2020-2024), from two organisations, DAFM and ICBF, were extracted and merged. In DAFM, the results of necropsy examinations on cattle which had a congenital defect recorded in the 'hereditary and congenital anomalies' field of the Laboratory Information Management System (LIMS) database and the location of the recording RVL were extracted. If a congenital defect was recorded in this LIMS field it indicated that this was the primary (majority of cases) or joint cause of death. Epidemiological information about the calf, dam and sire [breed, sex, age-at-death, and breeding method (AI, natural service)] was extracted from the ICBF database. In total, 202 cattle with congenital defects were recorded by DAFM of which 180 had associated data in the ICBF database.

Results

Of the six RVLs with recorded cases, the highest percentage was recorded in the Sligo RVL (36%) in the north-west of Ireland. The majority of cattle diagnosed with a congenital defect were beef (67%); the remainder were dairy (33%).The disproportionate beef calf representation probably reflects the greater value of beef compared to non-beef calves and the higher proportion of submissions to the Sligo RVL probably reflects the higher beef cattle population in its catchment area. The 3 most commonly affected body systems, the cardiovascular (127, 63%), gastrointestinal (44, 22%) and musculoskeletal (18, 9%) accounted for 94% of all cattle with a congenital defect. The majority of cattle with cardiovascular defects were beef (87%). There were a similar proportion of cattle with cardiovascular defects conceived following artificial insemination and natural service. The life-span of cattle with cardiovascular defects ranged from 0 to 1,374 days (3.75 years). There was a low sex ratio (male 45:55 female) in affected calves. The most common cardiovascular defects recorded were septal defects (atrial and ventricular) comprising 79% of all cardiac defects reported. Defects affecting the gastrointestinal system were the second most common defects recorded (22% of all cattle). Atresia was the most commonly diagnosed defect of the gastrointestinal system (20% of all cattle and 95% of cattle with a gastrointestinal defect). There was a very high sex ratio (male 63:37 female) in atretic calves. Atretic calves were most commonly conceived following AI (70%). Almost half of all atresia cases were discovered in dairy calves (47%). The average life-span of a calf diagnosed with atresia was 3 days, ranging from 0 to 14 days. Schistosomus reflexus (2% of all cattle) and dwarfism (2% of all cattle) were the two most commonly diagnosed defects of the musculoskeletal system which accounted for 9% of all deformed cattle.

Conclusion

This study achieved its objectives of identifying the hitherto undocumented most common lethal congenital defects in cattle submitted to veterinary laboratories in Ireland as cardiovascular defects and of the associated information identified beef cattle as over-represented. These findings have implications for targeting of potential genetic testing for these lethal defects in this cohort of the national cattle population.

Acknowledgements

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Determination of the apparent prevalence of Neospora caninum in bovine foetuses: a comparison of three diagnostics tests

C. Hayes[1], M. Casey-Bryars[2], J. F. Mee[3], E. Innes[4], B. Markey[2], C. McAloon[2], C. Sanchez[1]
[1]Department of Agriculture, Food and the Marine, Cork Regional Veterinary Laboratory, Model Farm Rd, Cork, T12 XD51, Ireland
[2]School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[3]Teagasc, Animal and Grassland Research Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland
[4]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 0PZ, UK

Presenting author
John Mee. E-mail: John.Mee@teagasc.ie

Application

The results from this study can be used by veterinary pathologists to inform investigatory protocols for suspected *Neospora caninum* foetopathy. To maximise sensitivity, it may be that both PCR and histopathology are necessary. Examination of foetal serum for antibodies to *N. caninum* does not appear to add sufficiently to the diagnostic yield to warrant routine testing for *N. caninum.*

Introduction

Neospora caninum is widely accepted to be one of the major causes of bovine abortion worldwide (Mee, 2023). Numerous diagnostic test can be used to diagnose exposure to the parasite (by detecting antibodies), the presence of the parasite (by detecting the antigen or parasitic cysts) and lesions associated with the presence of the parasite (by detecting tissue inflammation consistent with *N. caninum* infection). Each may produce a different result in different foetuses; positive/inconclusive/negative/non-positive. In addition, each also has advantages and disadvantages regarding sample matrix suitability, cost, labour input and test characteristics. Hence, multiple tests may be used in investigatory protocols to maximise diagnostic yield while balancing these test merits and demerits. While *N. caninum* has been diagnosed internationally as the most common cause of bovine abortion (~10-20% of cases), in Ireland, the diagnosis rate appears to be much lower than that achieved in other countries. It is not clear why. Part of the reason may be the use of different diagnostic tests in different veterinary laboratories. In order to determine whether type of diagnostic test used in Irish laboratories was contributing to this lower diagnostic rate a comparison was made between three commonly used tests. Hence, the primary objective of this study is to determine the animal-level prevalence of *Neospora caninum* determined by three diagnostic tests in a cohort of bovine foetuses submitted to a veterinary diagnostic laboratory.

Materials and methods

The foetuses enrolled in the study were voluntarily submitted to the Irish government Regional Veterinary Laboratory in Cork over a six month period during the peak abortion risk season (September to February) for seasonally calving herds. All foetuses submitted during this period were enrolled in the study (n=363). They comprised dairy, beef and crossbred foetuses. The foetuses ranged in estimated gestation length [calculated from straight crown-rump length measurements and the equation of Rexroad et al. (1974)] from 129 days to 277 days. From all foetuses, thoracic fluid or blood, a fresh swab of the midbrain and fixed brain tissue were collected. *N. caninum* foetal fluid/serum antibody ELISA testing was carried out using the IDEXX *Neospora caninum* Antibody Test Kit. A PCR assay for *N. caninum* was carried out on the brain swabs using LSI VetMAX *Neospora caninum* Detection Kit (ThermoFisher). Histopathological diagnoses were made by the individual research officer assigned to the case.

Results

The PCR results were available for all foetuses. Seventeen (4.7%) were *N. Caninum*-PCR-positive and three were inconclusive. Antibody ELISA results on blood or thoracic fluid were available for 326 foetuses. Four (1.2%) were positive and one was inconclusive. Histopathology results were available for 90 foetuses. Of these, five (5.6%) had lesions consistent with *N. caninum* infection and one was inconclusive. Twenty-one foetuses (5.8%) were categorised as positive by at least one of the three tests. Of the 17 PCR-positive foetuses, 14 had available antibody ELISA results and four had available histopathology results. Of these, one was antibody-positive and three were histopathology-positive. One of the four antibody-positive foetuses was PCR-positive. Histopathology results were available for two antibody-positive foetuses, only one of which was considered positive. Three of the five histopathology-positive foetuses were PCR-positive and one was antibody-positive. No foetus was positive in all three diagnostic tests.

Conclusion

It was concluded that differences between results from diagnostic test for *N. caninum* can contribute to differences in apparent animal-level prevalence of the parasite in bovine foetuses. Agreement between the different diagnostic tests for *N. caninum* was poor in this sample set. The 5.8% overall positivity rate was lower than an apparent prevalence of between 9 and 23% reported internationally in the existing literature over the last two decades.

Acknowledgements

The authors thank the referring veterinary practitioners and their clients for submitting the foetuses used in this study. The research was funded by The Department of Agriculture, Food and the Marine.

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Effect of inorganic nitrogen fertiliser application rates pre-grazing on rumen and blood variables of post-weaned dairy-beef calves

R. R. Male Here[1,2], C. McAloon[2], M. McGee[1], J. Donlon[1], E. O'Riordan[1], D. Kenny[1], B. Earley[1]
[1]Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath P61 P302, Ireland
[2]School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

Presenting author
Rishci Robinson Male Here. E-mail: rischi.robinson@teagasc.ie

Application

Grazing pasture with high crude protein (CP) concentrations, receiving high rate of inorganic nitrogen (N) fertiliser, increases rumen and blood ammonia concentrations but does not induce summer scour syndrome (SSS) in post-weaned dairy-beef bred calves.

Introduction

Grazing pasture with high CP concentrations has been anecdotally associated with the occurrence of SSS in post-weaned dairy-bred calves in Ireland. This syndrome is characterised by diarrhoea, weight loss, ill-thrift, and alimentary ulcerations. An on-farm clinical investigation study by Male Here et al. (2024) found that SSS-affected calves had high blood ammonia concentrations, suggesting a potential relationship with dietary N metabolism. On the farms in that study, inorganic N fertiliser (in the form of calcium ammonium nitrate or protected urea) was applied to paddocks, between one and three weeks before grazing by the calves. There is no experimental research that has evaluated the effect of grazing high CP grass on blood ammonia concentrations or on its putative association with SSS in post-weaned dairy-beef bred calves. The study objective was to investigate the effect of different inorganic N fertiliser application rates pre-grazing on rumen and blood variables, and its association, if any, with SSS occurrence in post-weaned dairy-beef bred calves.

Material and methods

Fifty-four healthy weaned male Aberdeen Angus×Holstein-Friesian calves (mean (SD) age: 79 (12) days old and body weight: 97 (16.1) kg) sourced from a commercial dairy farm were used. Calves were accommodated in straw-bedded housing units for three weeks, during which they had *ad libitum* access to water and hay, and offered 3 kg cereal-based concentrate ration/head daily. Calves were immunized against respiratory and clostridial diseases, and treated for internal parasites. Calves were then blocked by body weight, age, and lung thoracic ultrasound score (TUS) to one of three inorganic N fertiliser (calcium ammonium nitrate) treatments applied to perennial ryegrass dominant swards: low (LN), moderate (MN), and high (HN) equivalent to 15, 60, and 120 kg N/ha/grazing, respectively. Each experimental area was sub-divided into 15 equal-size sub-paddocks and N fertiliser was applied to each sub-paddock 14 days pre-grazing. Calves were moved to a new sub-paddock every two days. Post-turnout to pasture, calves were offered 1.0 kg of concentrate/head once daily which was reduced gradually to 0 kg over seven days. Pre-grazing and post-grazing sward heights of each sub-paddock were measured using a rising plate meter, and pre-grazing herbage samples were collected to determine chemical composition. Rumen fluid and blood samples were collected from each calf at turnout (day 0), daily until day 7, and thereafter every second day until day 29. Rumen fluid samples were analysed for pH and ammonia concentrations. Blood samples were analysed for ammonia, glutamate dehydrogenase (GLDH) and gamma-glutamyl transferase (GGT) concentrations. Calves were checked daily for clinical signs of SSS, and at weekly intervals, body weight was recorded, clinical examinations and lung TUS evaluations were performed, and faecal samples were collected for parasitology screening. Animal data were analysed using MIXED model in SAS 9.4, with calf as the experimental unit. The model included fixed effect of block, treatment, sampling time, and their interaction, with sampling time as a repeated measure. Herbage measurements were analysed using

PROC GLM, with sub-paddocks as the experimental unit and treatment included as a fixed effect. Calf clinical scores and TUS data were expressed as total frequency observed during the study period, and analysed using the Chi-Squared test.

Results

The HN and MN sub-paddocks had a higher pre-grazing sward height (both 9.9 cm) and herbage dry matter (DM) yield (1584 and 1588 kg/ha, respectively) than the LN (7.9 cm; 1116 kg/ha) ($P<0.05$). Post-grazing sward height and DM yield for LN (5.5 cm; 523 kg/ha) were lower than MN (6.1 cm; 677 kg/ha) and HN (6.0 cm; 646 kg/ha), which did not differ. Herbage DM concentrations were 179, 162, and 151 g/kg for LN, MN, and HN, respectively ($P<0.05$). Herbage CP concentrations were lowest for LN (190 g/kg DM), followed by MN (222 g/kg DM), and highest for HN (259 g/kg DM) ($P<0.05$). Calf daily live weight gain from day 0-29 was 0.39 kg for LN and 0.44 kg for MN and HN ($P>0.05$). There were treatment × time interactions ($P<0.05$) for rumen pH and ammonia, and blood ammonia concentrations (Table 1). There were inconsistent effects of treatment over time for rumen pH. By design, rumen ammonia concentrations did not differ between treatments on day 0, but subsequently concentrations for HN were greater than MN, which in turn were greater than LN on most, but not all, sampling days. Blood ammonia concentrations did not differ consistently between treatments up to day 11; subsequently, concentrations were higher for HN compared to MN and LN, which did not differ. Treatments had no effect ($P>0.05$) on serum GLDH and GGT concentrations, clinical scores and lung TUS. A greater proportion of faecal samples in HN (26.7%) exhibited loose consistency compared to the MN (11.2%) and LN (4.6%) treatments ($P<0.05$), possibly reflecting differences in herbage DM concentrations. No clinical signs of SSS were observed in any calves.

Table 1. Effect of inorganic nitrogen (N) fertiliser application rates on rumen and blood variables. The values are expressed as least square means.

Variables	N fertiliser treatment			SEM	Significance		
	Low N	Moderate N	High N		Treatment	Time	Treatment × Time
Rumen fluid							
pH	6.79^{a}	6.78^{a}	6.88^{b}	0.02	0.001	<0.0001	<0.0001
Ammonia (mg/L)	16.6^{a}	45.3^{b}	62.6^{c}	2.4	<0.0001	<0.0001	<0.0001
Blood							
Ammonia (µmol/L)	47.4^{a}	48.9^{a}	57.8^{b}	1.4	<0.0001	<0.0001	<0.0001
GLDH (I/U)	40.7	34.5	37.3	4.1	NS	<0.0001	NS
GGT (I/U)	16.8	15.3	16.8	1.0	NS	<0.0001	NS

Low N (15 kg N/ha/grazing); Moderate N (60 kg N/ha/grazing); High N (120 kg N/ha/grazing). $^{a,\ b,\ c}$ Columns with different superscripts indicate a statistical significance ($P<0.05$).

Conclusion

Under the conditions of this study, overall, increasing inorganic N fertiliser application rate to grazing pasture increased herbage CP, and rumen and blood ammonia concentrations, but did not adversely affect health or lead to the occurrence of SSS in post-weaned dairy-beef bred calves.

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Effects of Ischial Arch Width and Backfat Thickness on Sow Reproductive Performance

K.C. Yu[1,2], S.-P. Chen[3], P.-C. Shen[1], S.-Y. Peng[1]
[1]*National Pingtung University of Science and Technology, uefu Rd, Neipu Township, Pingtung County, Taiwan 912*
[2]*Graduate Institute of Bioresources, National Pingtung University of Science and Technology, uefu Rd, Neipu Township, Pingtung County, Taiwan 912*
[3]*Animal Industry Division, Agricultural Technology Research Institute, Miaoli 350, Taiwan*

Presenting author
Kuo-Cheng Yu. E-mail: viteight@gmail.com

Application

This study evaluates the impact of ischial arch width and P2 backfat thickness on sow reproductive performance, aiming to address a long-held belief in the pig farming industry: "the larger the sow's hips, the better the reproductive performance." The findings are expected to influence breeding strategies and improve farm profitability through better management practices.

Introduction

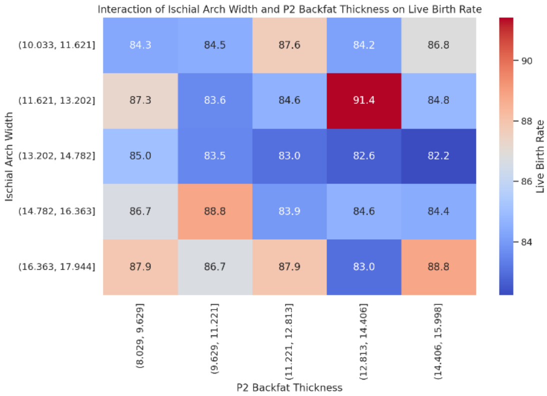
Reproductive efficiency is critical for sow productivity and farm profitability. While previous studies have independently investigated ischial arch width (Hohnholz, et al., 2019; Sawa et al., 2013) and P2 backfat thickness (Houde et al., 2010 ; Roongsitthichai and Tummaruk, 2014), their combined effects and interactions on reproductive performance remain unclear. Previous studies in cattle have identified associations between pelvic structure and reproductive traits (Lu et al., 2021), suggesting that similar relationships may exist in swine. This study aims to analyze these factors using data from Taiwanese pig farms.

Materials and Methods

This study collected data from 500 sows at Taiwanese pig farms, including records of ischial arch width, P2 backfat thickness, and reproductive performance indicators such as the number of live piglets, total piglets, and weaning intervals. Ischial arch width was measured via skeletal measurements, and P2 backfat thickness was recorded using ultrasound. Statistical analyses included simple and multiple linear regression, univariate and two-way ANOVA, and Pearson and Spearman correlation analyses.

Results

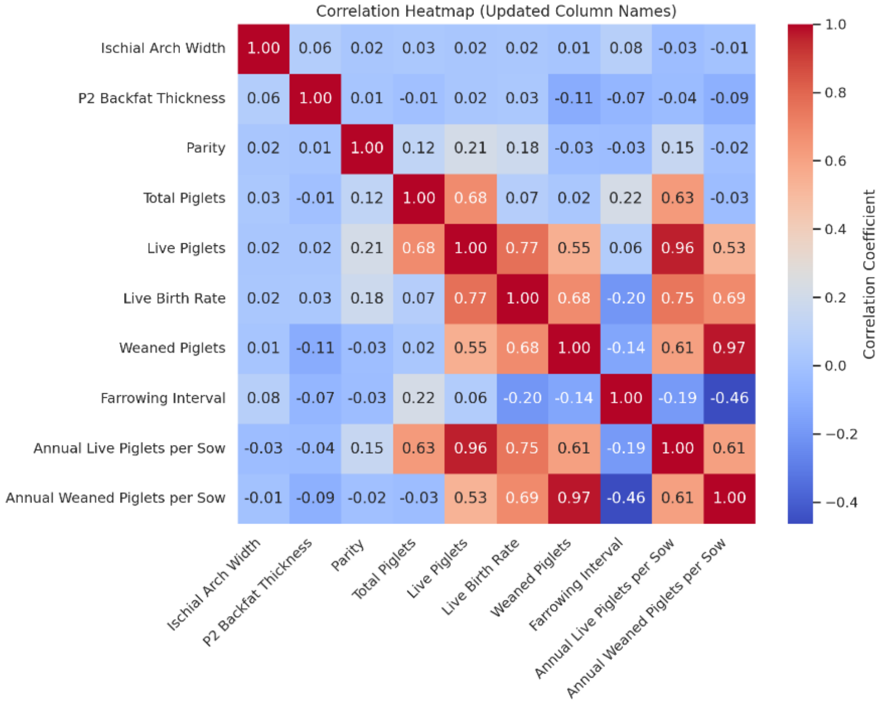
Simple Linear Regression: Ischial arch width had no significant effect on the number of live piglets (β=0.017, *P*=0.87), and P2 backfat thickness showed no significant effect on the number of weaned piglets (β=-0.012, *P*=0.90).
Multiple Regression Analysis: The explanatory power of ischial arch width and P2 backfat thickness on reproductive performance was limited (R^2=0.028, *P*>0.05).
ANOVA Analysis: Neither the main effects of ischial arch width and P2 backfat thickness nor their interaction had significant effects on reproductive performance (*P*>0.05). Figures 1 illustrates the interaction effects between ischial arch width and P2 backfat thickness on reproductive traits.



	(8.029, 9.629]	(9.629, 11.221]	(11.221, 12.813]	(12.813, 14.406]	(14.406, 15.998]
(10.033, 11.621]	84.3	84.5	87.6	84.2	86.8
(11.621, 13.202]	87.3	83.6	84.6	91.4	84.8
(13.202, 14.782]	85.0	83.5	83.0	82.6	82.2
(14.782, 16.363]	86.7	88.8	83.9	84.6	84.4
(16.363, 17.944]	87.9	86.7	87.9	83.0	88.8

Figure 1. Interaction between Ischial Arch Width and P2 Backfat Thickness on Reproductive Performance.

Correlation Analysis: Ischial arch width was weakly positively correlated with the number of live piglets (r=0.017), while P2 backfat thickness showed no significant correlation with the number of weaned piglets (r=-0.012). Figures 2 depicts the correlations between Ischial arch width and body condition traits with reproductive outcomes.



	Ischial Arch Width	P2 Backfat Thickness	Parity	Total Piglets	Live Piglets	Live Birth Rate	Weaned Piglets	Farrowing Interval	Annual Live Piglets per Sow	Annual Weaned Piglets per Sow
Ischial Arch Width	1.00	0.06	0.02	0.03	0.02	0.02	0.01	0.08	-0.03	-0.01
P2 Backfat Thickness	0.06	1.00	0.01	-0.01	0.02	0.03	-0.11	-0.07	-0.04	-0.09
Parity	0.02	0.01	1.00	0.12	0.21	0.18	-0.03	-0.03	0.15	-0.02
Total Piglets	0.03	-0.01	0.12	1.00	0.68	0.07	0.02	0.22	0.63	-0.03
Live Piglets	0.02	0.02	0.21	0.68	1.00	0.77	0.55	0.06	0.96	0.53
Live Birth Rate	0.02	0.03	0.18	0.07	0.77	1.00	0.68	-0.20	0.75	0.69
Weaned Piglets	0.01	-0.11	-0.03	0.02	0.55	0.68	1.00	-0.14	0.61	0.97
Farrowing Interval	0.08	-0.07	-0.03	0.22	0.06	-0.20	-0.14	1.00	-0.19	-0.46
Annual Live Piglets per Sow	-0.03	-0.04	0.15	0.63	0.96	0.75	0.61	-0.19	1.00	0.61
Annual Weaned Piglets per Sow	-0.01	-0.09	-0.02	-0.03	0.53	0.69	0.97	-0.46	0.61	1.00

Figure 2. Correlation between Ischial Arch Width, P2 Backfat Thickness, and Reproductive Traits.

Conclusions

This study found that ischial arch width and P2 backfat thickness had no significant effects on sow reproductive performance, challenging the traditional belief that "larger hips improve reproduction." Future studies should employ larger sample sizes and consider additional variables to further explore these factors' effects on reproductive performance.

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Prediction of Beef Sensory and Technological Quality Traits Using Vis-NIR Spectroscopy

G. Anagnostou[1,2], A. Ferragina[1,4], E. C. Crofton[1], J. M. F. Celayeta[3], R. M. Hamill[1]
[1]*Teagasc, Ashtown Food Research Centre, Scribbletown, Dublin 15, Ireland*
[2]*School of Food Science and Environmental Health, Technological University Dublin, Belfield, Dublin 4, Ireland*
[3]*Sustainability and Health Research Hub, Technological University Dublin, Belfield, Dublin 4, Ireland*
[4]*Department of Veterinary Science, University of Parma, Str. dell'Università, 12, 43121 Parma PR, Italy*

Presenting author
Georgios Anagnostou. E-mail: georgios.anagnostou@teagasc.ie

Application

This approach could facilitate rapid online prediction of meat quality traits that could be used to support quality control, process monitoring in meat processing operations and genetic improvement in breeding programmes.

Introduction

Beef is a vital source of essential amino acids, vitamins, and iron, and its eating quality greatly influences consumer repurchase decisions (Troy & Kerry, 2010). Key factors affecting quality perception of beef include colour, tenderness, juiciness, and flavour (Qiao et al., 2015). Measurement of meat eating quality is destructive and labour-intensive. To support both genetic improvement programmes for sensory quality, meat management programmes, and enhance consumer satisfaction, rapid, non-destructive, and objective methods for predicting meat quality are needed. Near-infrared spectroscopy (NIRS) operating on the principle that different chemical bonds in organic matter absorb light at specific wavelengths when irradiated (Savoia et al., 2021), offering several advantages over traditional methods for meat quality measurement, like Warner-Bratzler Shear Force (WBSF) or sensory profiling, including speed, non-destructiveness and cost (Cafferky et al., 2020).

Material and Methods

Crossbred heifers (n= 30, 17-22 months old) were slaughtered in a commercial abattoir. Following slaughter, three carcass hanging techniques (conventional achilles hung, hip tied, and hip hung (Hostetler et al., 1972) were applied to alternating carcass sides) to generate a dataset variable in tenderness. Steaks (n= 240, 2.54cm thickness) were removed from four muscles per side (*longissimus thoracis et lumborum*, *semimembranosus*, *semitendinosus* and *triceps brachii*) and blast-frozen (-19^{o}C) 13-14 days, post mortem. Meat spectral acquisition was performed on the cut surface of thawed steaks using LabSpec Vis – NIR spectrometer (Hi-Res ASD Labspec® 4, Malvern Panalytical, UK) in the range of 350-2500nm. Technological meat quality (pH, thawing loss, and colour) was measured and sensory evaluation (7-9 highly trained panellists) was carried out on adjacent steaks. Partial Least Square Regression (PLS-R), combined with different pre-treatment methods such as Standard Normal Variate, Detrend, Savitzky-Golay 1st derivative, 3rd polynomial order with 21 smoothing points and Savitzky-Golay 2nd derivative, 3rd polynomial order with 21 smoothing points, was used to optimise models for the prediction of beef sensory and technological attributes, using the package “mdatools” (R Studio Team, 2022). The samples were randomly divided by muscle in a Training (80% of samples) and a Testing (remaining 20%) sets, Ten single folds of a Venetian blind algorithm were applied to calculate the mean square error of the cross-validation ($RMSE_{cv}$) and the cross-validation determination coefficient (R^2_{cv}), used to evaluate the performance of PLS. The external validation was carried out on the Testing set and assessed using the determination coefficients of validation (R^2_{ext}).

Results

This study provides a detailed evaluation of the cross-validated coefficients of determination (R^2_{cv}) and externally validated coefficient of determination (R^2_{ext}) for both technological and sensory traits of beef meat, as presented in *Table 1*. The highest R^2_{ext} values, among the technological traits were achieved for thawing loss (R^2_{ext}= 0.774, $RMSE_{ext}$=0.013), L* (R^2_{ext}= 0.781, $RMSE_{ext}$= 1.743), and b* value (R^2_{ext}= 0.852, $RMSE_{ext}$= 0.738). For sensory traits, lower R^2_{ext} were achieved (range 0.06-0.52) with the best results obtained for overall tenderness.

Table 1. Best fitting statistics of prediction for technological and sensory traits on beef samples

Trait	Pre-treatment	nC	R^2_{cal}	$RMSE_{cal}$	R^2_{cv}	$RMSE_{cv}$	R^2_{ext}	$RMSE_{ext}$
pH	S-G 2nd derivative & SNV-D	6	0.71	0.080	0.51	0.105	0.65	0.093
Thawing loss (%)	S-G 1st derivative & SNV-D	7	0.86	0.011	0.74	0.015	0.77	0.013
Colour L*	None	4	0.79	1.499	0.76	1.594	0.78	1.743
Colour a*	None	10	0.69	0.899	0.56	1.046	0.48	1.055
Colour b*	None	6	0.83	0.753	0.80	0.814	0.85	0.738
Initial tenderness	S-G 2nd derivative	3	0.44	0.579	0.21	0.687	0.45	0.655
Chewiness	S-G 1st derivative	6	0.59	0.731	0.37	0.902	0.49	0.798
Juiciness	S-G 2nd derivative & SNV-D	3	0.30	0.469	0.042	0.548	0.06	0.609
Overall tenderness	S-G 2nd derivative & SNV-D	7	0.51	0.653	0.32	0.767	0.52	0.711

SNV-D: Standard Normal Variate – Detrend, S-G 1st derivative: Savitzky-Golay first derivative, 3rd polynomial order with 21 smoothing points, S-G 2nd derivative: Savitzky-Golay second derivative, 3rd polynomial order with 21 smoothing points, nC: number of components in the PLS-R model, R^2_{cal}= coefficient of determination for calibration, $RMSE_{cal}$= root mean square error for calibration, R^2_{cv}= coefficient of determination for cross-validation, $RMSE_{cv}$= root mean square error for cross-validation, R^2_{ext}= coefficient of determination for external validation, $RMSE_{ext}$= root mean square error for external validation

Conclusions

This study confirms the potential of Vis-NIR spectroscopy as a rapid, non-invasive tool for predicting certain beef quality traits, like pH, thawing loss and colour, while the use of different muscles and hanging methods has led to enhanced data variability, improving the models' versatility. On the other hand, the prediction of sensory traits was less accurate likely due to the narrow sensory evaluation scale used (Berri et al., 2019) or the low reference data variance (Pérez-Marín et al., 2004), indicating that while Vis-NIR spectroscopy is promising, additional refinement, such as sample set size, variability and hyperspectral imaging (HSI), is needed to better predict sensory traits of beef meat. Nevertheless, this study provides preliminary chemometric models for several sensory traits assessed by trained panels, offering insights that could support future strategies for optimizing beef sensory characteristics.

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Novel Processing Technologies for Sustainable Valorisation of Bovine Blood

S. Ghalamara[1], S. Hannon[1], B. K. Tiwari[1]
[1]*Teagasc Food Research Centre, Ashtown, Scribbletown, Dublin 15, Ireland*

Presenting author
Soudabeh Ghalamara. E-mail: soudabeh.ghalamara@teagasc.ie

Application

This study emphasizes the versatile applications of bioactive peptides and functional proteins from bovine blood in food, pharmaceuticals, and cosmetics. These hydrolysates can enhance food products as protein enrichers, emulsifiers, and foaming agents, while offering therapeutic potential in pharmaceuticals and anti-aging or moisturizing benefits in cosmetics. Moreover, this approach promotes environmental sustainability by reducing meat industry waste, aligning with circular economy goals, and unlocking innovative opportunities for diverse industries.

Introduction

The meat processing industry produces substantial by-products, including bovine blood, a rich source of proteins and bioactive compounds that remains underutilized despite its potential. Discarding blood not only contributes to environmental pollution, due to its high biological and chemical oxygen demand, but also wastes a valuable resource (Rizvi et al., 2016). Technological advancements such as enzymatic hydrolysis (Ulug et al., 2021), ultrasound-assisted extraction (Wu et al., 2020), and membrane filtration (Wang et al., 2024) have enabled the efficient valorization of blood into functional ingredients like antioxidant peptides, ACE inhibitors, and plasma proteins, supporting applications in pharmaceuticals, functional foods, and bioplastics. This study aligns with circular bioeconomy principles by utilizing enzymatic hydrolysis and ultrasonic treatment to isolate proteins and peptides from bovine blood, enhancing their functional properties (solubility, emulsifying, and foaming). While these innovations reduce environmental impact, challenges like blood perishability, contamination risks, and high processing costs remain barriers to widespread adoption.

Materials and Methods

Fresh bovine blood was collected from a local meat processing facility. The blood was immediately cooled and stored at 4°C to preserve its quality before processing.

Ultrasonic and Enzymatic Hydrolysis Process

The coagulated bovine blood was first homogenized using a blender, and the homogenized fraction was utilized for hydrolysis. The process involved an integrated ultrasonic treatment followed by enzymatic hydrolysis. In one experiment, the homogenized blood sample was hydrolyzed using combination of three enzymes—alcalase, nutrase, and flavourzyme—at a total concentration of 1.5%, under controlled conditions (pH 7, temperature 50°C, duration 3 h). Another treatment combined the same enzymatic hydrolysis process with bath ultrasonic treatment to enhance the results. For the ultrasonic treatment, a lab-scale ultrasonic bath operating at frequencies of 45 kHz, with a power output of 1000 W, was used to improve protein solubility and enzyme accessibility. The hydrolyzed sample was centrifuged at 10,000 g for 20 min to separate the soluble protein and peptide fractions from any insoluble debris. The supernatant was collected, freeze-dried, and stored for subsequent analysis.

Functional Property Analysis

The functional properties of the protein and peptide fractions, including solubility, emulsifying and foaming properties, were analyzed using standard methods. Protein solubility was measured by centrifugation of 1% (w/w) protein solutions and protein quantification using the Bradford method, while Emulsifying properties were assessed by homogenizing protein solutions (5 mg/mL) with soybean oil, measuring absorbance at 500 nm, and calculating emulsion activity and stability indices Foaming properties were determined by blending 1% protein solutions, recording foam volumes at 0, 30, and 60 min, and calculating foaming capacity and stability (Jamil et al., 2015).

Results

The combined ultrasonic-assisted enzymatic hydrolysis treatment significantly improved the functional properties of the protein hydrolysates compared to enzymatic hydrolysis alone. Protein solubility increased markedly, with the combined treatment yielding a solubility of 0.92 ± 0.02 g/g, compared to 0.75 ± 0.03 g/g for enzymatic-only treatments ($P < 0.05$). Similarly, the emulsifying activity index (EAI) and emulsion stability index (ESI) were significantly enhanced in the combined treatment group, with values of 18.5 ± 1.2 m^2/g and 16.7 ± 0.8 minutes, respectively, compared to 14.8 ± 1.0 m^2/g and lower stability in the enzymatic-only group ($P < 0.05$). Foaming capacity also demonstrated significant improvements, with initial capacity reaching 1.1 ± 0.05 mL/mL in the combined treatment versus 0.85 ± 0.04 mL/mL for enzymatic-only samples ($P < 0.05$). Foam stability remained higher in the combined treatment group, with values of 0.9 ± 0.03 and 0.8 ± 0.04 at 30 and 60 min, respectively. These findings highlight the efficacy of ultrasonic-assisted enzymatic hydrolysis in enhancing protein functionality for diverse industrial applications.

Conclusions

This study demonstrates the effectiveness of integrating ultrasonic treatment with enzymatic hydrolysis for the valorization of bovine blood into functional protein and peptide fractions. The combined process significantly enhanced solubility, emulsifying properties, and foaming capacity compared to enzymatic hydrolysis alone. These findings highlight the potential of ultrasonic-assisted enzymatic hydrolysis as a sustainable bioprocessing approach for converting an underutilized by-product of the meat industry into high-value ingredients suitable for diverse applications in food, pharmaceutical, and cosmetic industries. This method provides a promising solution for reducing waste, improving resource utilization, and advancing sustainability in the meat industry.

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In vitro evaluation of different spices for degradability, fermentation, and their ability to reduce ruminal methane production

K. Merkhan[1]
[1]*Newcastle University, Newcastle upon Tyne NE1 7RU, UK*

Presenting author
K Merkhan. E-mail: k.y.merkhan2@newcastle.ac.uk

Application

Methane from ruminants significantly impacts the environment, making it crucial to find dietary supplements that lower emissions while maintaining the fermentation and degradability of diets. This study highlights the potential of spices to reduce methane emissions, a key issue in sustainable livestock management.

Introduction

Due to the EU ban on the use of antibiotics in animal feeds in 2006 (European Commission, 2006), there is an increasing interest in using plants and plant extracts to manipulate ruminal fermentation to improve feed efficiency and animal performance (Kuralkar et al., 2021), while reducing methane generation (Faniyi et al., 2021). This study evaluated the addition of three spice powders (BS, black seeds; CS, cumin seeds; GB, garlic bulbs) in two diet types (fresh dried ryegrass=FR and a wheat straw-based mixture (WS) with 60% WS, 26% barley, and 14% peas) using rumen fluid from killed steers. The goal was to assess the effects of these spices on in vitro rumen fermentation, degradability, and methane emissions.

Materials and methods

This duplicated 4x2 factorial study evaluated the effects of either no addition (control) or the addition of 3 spices (BS, CS, GB), each at 30 g/kg DM to 2 contrasting diets (FR, WS). Each rumen fluid sample collected from two killed steers at an abattoir was duplicated to assess in vitro dry matter degradability (IVDMD), fermentation profiles, total gas production (tGP), and CH4%. Ground and dry samples of about 200 mg were incubated in 50 ml glass syringes with 20 ml of the buffered rumen solution (1:4) at 39°C for 48 hours. The methane % was determined using GC-MS, whereas the VFA profiles were determined using IC-MS. A two-way ANOVA (4x2) with GLM was performed using Minitab 21 to analyse the effects of two diet types, and 4 spices inclusion including control on the studied traits. Tukey's post hoc test assessed differences between means for significance at P≤0.05.

Results

Compared to the WS diet, the FR diet had significantly lower pH and CH4, but significantly higher IVDMD and tGP. Except for CH4, no differences were found between the spices in the study (*P*>0.05). Furthermore, including garlic bulbs in both diets significantly lowered CH4% (Table 1). The interaction between the different diet types and spices in this study did not show any differences.

Table 1. Effect of diet type and spice inclusion on IVDMD (g/kg DM), VFA (mmol/L), NH3-H (mg/L), tGP (ml/g DM), and CH4 (%) after 48h incubation.

Traits	*Feed type (F)*		*spices (S)*				*Pooled SE*	*Significance*		
	FR	**WS**	**CON**	**BS**	**CS**	**GB**		**F**	**S**	**F x S**
IVDMD	742	465	588	596	619	611	15.35	0.001	0.74	0.29
pH	6.52	6.7	6.61	6.61	6.62	6.54	0.049	0.04	0.85	0.99
NH_3-H	124.8	124.6	123.4	130.6	131.1	113.9	20.29	0.99	0.97	0.95
tVFA	32.2	27.5	19.4	44.5	31.2	24.4	5.96	0.58	0.21	0.85

Acetate	15.2	14.7	10.1	21.7	15.8	12.0	2.65	0.91	0.17	0.83
Propionate	8.5	6.8	4.6	11.8	7.2	6.8	2.65	0.91	0.17	0.83
Butyrate	6.4	4.1	3.5	7.3	5.9	4.2	1.19	0.19	0.40	0.84
tGP	173	111	147	143	139	138	5.30	0.001	0.85	0.93
CH_4	10.6	12.8	14.2^{a}	14.8^{a}	14.8^{a}	3.1^{b}	0.64	0.04	0.001	0.95

Different letters within each row for the spice inclusion factor differ significantly at $P \leq 0.05$.

FR, fresh ryegrass diet; WS, wheat straw-based diet; CON, control; BS, black seed; CS, cumin seed; GB, garlic bulb; SE, standard error of the means; IVDMD, *in vitro* dry matter degradability; NH_3-H, ammonia; tVFA, total volatile fatty acids; tGP, total gas production.

Conclusion

None of the spices influenced IVDMD, pH, NH3-H, tGP, or VFA profiles. However, adding garlic bulb powder at 30 g/kg DM reduced methane generation by 78.2% without affecting degradability or fermentation profile, suggesting it could be used as a dietary additive to suppress methanogenesis in ruminants.

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Evaluation of in vitro buffering capacity and greenhouse gas emissions from beef cattle rumen fluid supplemented with marine-based rumen buffers at half-dose compared to a conventional buffer in a high-starch diet

I. Hodge[1,2], P. Quille[2], S. O' Connell[1], B. Naughton[1]
[1]*Marigot Ltd. T/a Celtic Sea Minerals, Castletown-Bearhaven, Co. Cork, Ireland*
[2]*Munster Technological University, 46 Grand Parade, Cork City T12 VN56, Ireland*

Presenting author
Brian Naughton. E-mail: b.naughton@marigot.ie

Application

Rumen buffering and methane (CH_4) emissions are critical aspects of digestive health and environmental sustainability in intensively fed beef and dairy cattle. High-grain diets, common in intensive livestock systems, optimize energy intake but can disrupt rumen pH balance, leading to acidosis and impacting overall health and productivity. Rumen buffering strategies, including dietary interventions and feed additives, play a crucial role in stabilizing pH and promoting beneficial microbial populations that improve nutrient utilization. However, improvement in rumen fermentation processes can drive up CH_4 emissions, a potent greenhouse gas contributing to 40 % of global anthropogenic emissions, making the reduction of emissions a significant goal in sustainable animal agriculture.

Introduction

The primary objective of this study was to determine the effect of rumen buffers calcareous marine alae (CMA) alone and when fortified with magnesium oxide (CMA.MgO), compared to a conventional rumen buffer, sodium bicarbonate (SB), on pH buffering capacity, volatile fatty acid (VFA) profiles, and gas emissions under acidotic conditions induced by a high starch diet. Ruminal pH profiles were plotted to assess the stabilizing potential of rumen buffer supplements during periods of pH decline in a simulated rumen of an intensively fed animal. This study also investigated the ability of CMA buffers to reduce ruminal *in vitro* methane (CH_4) emissions compared to a conventional buffer (SB) when supplemented in a high-energy diet, with additional exploration into whether the hydrogen-sequestering buffering mechanisms of these buffers contribute to their CH_4 mitigation potential.

Materials and Methods

Six Friesian and Angus breed rumen fluid donors, with an average age of 2.5 ± 1.1 years and an average hot carcass weight of 286.5 ± 18.32 kg at the point of rumen fluid sampling, supplied the rumen inoculum samples that were assigned to three rumen buffer treatments in a 2 × 3 randomized block design. Subjects were previously fed a finishing diet consisting of approximately 50 % grass silage and 50 % concentrate on a DM basis. The experiment design consisted of six 12 h fermentations with 8 fermentation vessels assigned to a control (CON) diet containing no additional rumen buffer, and the remaining 8 fermentation vessels containing either CMA at 6.62 g/kg dry matter (DM), CMA.MgO at the same concentration, or SB supplemented at double the dose (13.15 g/kg DM). The entire set of *in vitro* fermentation data recorded was analysed using the analysis of variance (ANOVA) model through Addinsoft XLSTAT-Basic+ software. The model incorporated recorded variables such as pH values, H^+ production, gas levels, VFA profiles, and intensity of VFA/g CH_4 as dependant variables, while treatments and timepoints (in cases of pH values and gas analysis) were utilized as independent variables. All data required a compound symmetry heterogenous covariance structure, and both the CON treatment as well as the 2 h timepoint were utilised as control levels in the relevant ANOVA models.

Results

The reference CON vessels successfully induced ruminal pH conditions (pH 5.6 – 5.8) associated with a high starch diet and therefore the risk of SARA diagnosis in ruminants. The CMA.MgO (pH 5.96 ± 0.02) treatment significantly increased ($P < 0.01$) mean ruminal pH, and both CMA (pH 5.93 ± 0.03) and SB (pH 5.87 ± 0.04) treatments numerically increased mean ruminal pH values compared to the CON (pH 5.85 ± 0.02) fermentation vessels across the 4 timepoints. Corresponding increments of acid neutralisation in terms of H^+ production (mmol H+·s) above pH baseline 5.6 during the 12 h fermentation periods were attained in rumen fluid treated with CMA.MgO (0.055 ± 0.00 mmol H+·s), CMA (0.045 ± 0.01 mmol H+·s), and SB (0.040 ± 0.02 mmol H+·s) compared to the CON result (0.035 ±0.01 mmol H+·s). Headspace total gas volumes were significantly increased by CMA (10.72 ± 1.60 mL ; $P < 0.01$), and marginally by CMA.MgO (2.77 ± 1.24 mL) and SB (1.27±1.46 mL) rumen buffers in comparison to CON total gas emissions. The CH_4 production differences with rumen buffer supplementations were calculated in percentages relative to the corresponding fermentation control result (Rel % CH_4 to controls). The average of CON vessels determined the CH_4 baseline of 100 % CH_4, while CMA.MgO induced an increase of 8.62 ± 6.02 % and the CMA and SB treatments both induced significant ($P < 0.01$) average relative CH_4 increases of 30.35 ± 7.58 % and 60.57 ± 10.55 %, respectively. The CMA.MgO treatment induced a significant decrease (0.3 mmol/L fluid ; $P < 0.01$) in the acetic acid -to -propanoic acid (A:P) ratios obtained after *in vitro* fermentation compared to SB, which correlated with significant decreases in CH_4 production.

Conclusions

These findings demonstrate that CMA buffers are more effective at buffering ruminal pH compared to SB supplemented at the double the dose rate *in vitro*, supporting results from previous studies investigating CMA and SB buffer supplementation in beef cattle during the fattening period (Rossi et al., 2019). The utilisation of CMA.MgO at half the dose of SB can reduce the amount of ruminal CH_4 emissions substantially in comparison to SB supplemented diets, providing an effective rumen buffer in combatting ruminant disorders associated with SARA while allowing a reduction in enteric CH_4 emissions in contrast to the conventional rumen buffer.

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Encapsulating calcium peroxide to prolong methane inhibition effects in cow rumen fluid

A. Hall[1], P. Abberton[2], A. Bartle[1], S. O'Connor[1], V, O'Flaherty[1]
[1]University Of Galway, University Road, Galway H91 TK33, Ireland
[2]GlasPort Bio, Business Innovation Centre, Galway H91 TK33, Ireland

Presenting author
Alison Hall. E-mail: alhall@tcd.ie

Application

With Ireland's promise to reduce greenhouse gas emissions by 51% by 2030 comes a national pressure to curb our emissions in a cooperative attempt to mitigate our impact on the climate. There is growing interest in the supplementation of cattle feed with methane (CH_4)-inhibiting additives such as 3-nitrooxypropanol (3-NOP), seaweed, lipids, essential oils, and calcium peroxide (CaO_2). While these additives have so far proven effective in the short term, there is a need to develop feed additives that reduce CH_4 with long-lasting effects to reduce the rate of supplementation. This would improve their suitability for use in pasture-based systems in Ireland, and globally, where cattle predominantly graze outside and are brought inside infrequently.

Introduction

The oxidising agent CaO_2 is a promising feed additive for reducing CH_4 emissions from agriculture, including from enteric fermentation from livestock. Through its oxidising action in the rumen, the production of CH_4 by methanogens is inhibited, which has been demonstrated in slurry (Thorn et al., 2022), *in vitro* using a RUSITEC system (Graham et al, 2024; O'Donnell et al., 2024), as well as in beef cattle (Roskam et al., 2024). However, its effectiveness in pasture-based grazing systems depends on its controlled release in the rumen. As part of the Methane Abatement in Grazing Systems (MAGS) project, this study aims to identify suitable coating materials for CaO_2 to ensure stability and controlled release, with a focus on carbohydrate-based coatings, as the digestion of carbohydrates occurs in the rumen (Henderson et al., 2015).

Materials and Methods

The slow-release effect of various coating materials, including sodium alginate and cellulose derivatives such as carboxymethyl cellulose and ethyl cellulose, were evaluated by investigating their release of CaO_2 *in vitro*. Rumen fluid was obtained from Beef Cattle at Teagasc Grange, Co. Meath. CaO_2 was coated in each material, and these additive formulations were introduced to sealed bottles containing rumen fluid, maintained under strict anaerobic conditions, which simulated the rumen environment. The effect the coatings had on CH_4 inhibition was tested by measuring fluctuations in the oxidative reduction potential (ORP) together with biogas production for 48 hour durations.

Results

The results showed a sustained increase in ORP values for bottles containing the coated CaO_2 compared to uncoated, indicating enhanced oxidative conditions which correlate with CH_4 inhibition, as well as reduced CH_4 production. Of the investigated coatings, sodium alginate, ethyl cellulose, and carboxymethyl cellulose demonstrated the best slow-release performance compared to the uncoated CaO_2. Bottles which received uncoated CaO_2 maintained a positive ORP above 60 mV for 8 hours. In comparison, bottles which received CaO_2 coated with sodium alginate or ethyl cellulose maintained a positive ORP of above 100 mV for 10 hours, and bottles which received CaO_2 coated with CMC maintained a positive ORP above 150 mV for 20 hours.

Conclusion

The coatings brought about both reduced CH_4 production and an elevated ORP for a longer time compared to that of the uncoated controls. These preliminary findings suggest that carbohydrate-based coatings are promising materials for encapsulating CaO_2 to ensure the controlled release of CaO_2 in the rumen as well as sustained inhibition of CH_4 production by methanogens. Further research will focus on optimising these coatings and bringing the most promising candidates forward to further *in vitro* work in a continuous reactor system and live animal trials, with MAGS' ultimate goal to contribute to sustainable livestock management and Ireland's sustainability goals.

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Machine Learning Algorithms to explore the Relationship between Methane Emissions, Microbiome and Host Genome in Holstein Dairy Cows

S. Dzinkevicius[1,2], S. Huws[1], M. Shirali[1,2], K. Barnes[1], X. Chen[2]
[1]*Queen's University, University Rd, Belfast BT7 1NN, UK*
[2]*Agri-Food Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK*

Presenting author
Sarunas Dzinkevicius. E-mail: sdzinkevicius01@qub.ac.uk

Application

This research seeks to use genomics and Machine Learning (ML) to predict low methane emitters using the host genotype. This work will serve as a base for further refinement of genomics techniques in ruminants, as well as a potential way to produce low methane emitting cattle. Similar work focused on Linear Mixed Models(LMMs) in variously sized populations. Our work will focus on the applicability of ML with a low sample size in a Northern Irish Holstein research herd.

Introduction

Global climate change is of increasing importance, as the effects of it become visible. Farms produce a significant amount of methane, which contributes to global emissions (O'Mara, 2011). Efforts to reduce emissions from cattle have historically relied on adjusting the feed content of cattle by, for example, introducing seaweed into the feed, or by supplementing their diet with specialized mineral mixtures (Biswas et al, 2017). There is some evidence that ecological make-up of the rumen microflora is heritable, with and heritability index (h2) of more than 0.15 for 34% of the taxa found (Li et al, 2019). The above work indicates that a variety of methods were used to reduce methane emissions. ML is becoming an increasingly important consideration in the biological sciences. ML algorithms are often used to predict disease states in biological contexts (Wu et al, 2018). Our research focuses on the creation and evaluation of ML algorithms that predict the relationship between methane emissions, host genome, and microbiome in a limited sample of Holstein cows.

Materials and Methods

We used 45 samples of Holstein cow data collected from a previous, unpublished, study investigating the effect of different feeds on methane emissions. The data was converted into a .csv format using Excel, then imported to R (Version 4.4.0) and a separate subset of data containing the feed, regimen, the Oxygen(O2),Hydrogen (H2), Dry Matter Intake (DMI), and Methane (CH4) data was created, with the sample number and other non-numeric data removed. The package caret (Version 6.09-94) was used to train and test the models. Models were run with a 75:25 Training:Testing data split, then using 20 Fold cross-validation, and finally using Group-K Fold to account for repeated rows of samples. Prediction of CH4 emissions with Generalised Linear Models and Random Forests were done using the glm() and rf() functions from the caret package. Each model was run with the above-mentioned Training:Testing split, and then with 20-fold and Group-K Fold pre-processing (see above). In order to check for over-fitting and whether model results are comparable to the test set, the predict() function was used using the Testing set. Then, Spearman correlation analysis was performed of Prediction vs. Testing set using the corr.test() function, and the method="spearman". Spearman correlation was used because data was suspected to be non-normal due to low sample size. The data was then compiled into a table (Table 1).

Results

The results are shown in the table below. Generalised Linear Model with Cross-validation was the most successful model with the lowest Root Men Square Error (RMSE), the highest Coefficient of Determination (R-Squared), and the lowest Mean Average Error (MAE).

Table 1. The results of two ML model trials with, and without cross-validation, and K-fold processing. RMSE and MAE are in grams/day of methane (g/day)

Method	**RMSE (g/day)**	**R-Squared**	**Spearman Correlation (Against Testing Set)**	**P-Value**	**MAE (g/day)**
Generalised Linear Model	6.16756	0.99	0.9666667	0.0001653	4.829831
Generalised Linear Model(Cross-Validation)	4.55219	1	0.9666667	0.0001653	4.035819
Random Forests(Best available iteration)	23.67583	0.83	0.9166667	0.0001312	19.26977
Random Forests with Cross-Validation(Best available iteration)	17.37556	1	0.9	0.002028	15.9819
Generalised Linear Models with Group-K-Fold	5.037132	0.99	0.9666667	0.0001653	4.106049
Random Forests with Group-K-Fold(Best available iteration)	20.36291	0.88	0.883333	0.003075	17.23063

Conclusion

The use of machine learning in methane reduction has the potential to increase the effectiveness of current and future farm management programs. Here, we used Random forests and Generalised Linear Models to evaluate a machine learning model using existing data under low sample number conditions. The best performing models appeared to be Generalised Linear Models with 20-fold cross-Validation. The worst performing models were Random Forests, without any cross-validation. All correlation criteria were unusually high, with values approaching 1, however, the models did not appear to overfit, with the Spearman correlation between testing set and model predictions being nearly 1.

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Potential of silage microbial inoculants to mitigate methane production from the rumen. A systematic review

R. B. Afonso[1,2,3], L. N. Adivi[1,2,3], K. Linehan[1], C. Stanton[1,2,4], C. Hill[2,3], R. P. Ross[2]
[1]*Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork P61 P302, Ireland*
[2]*APC Microbiome Ireland, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*
[3]*School of Microbiology, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*
[4]*VISTAMILK SFI Centre, Teagasc, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*

Presenting author
Rebeca Bertin Afonso. E-mail: afonsomedvet@gmail.com

Application

Methane is a potent greenhouse gas, and a major byproduct of rumen fermentation in livestock, contributing significantly to agricultural greenhouse gas emissions. This systematic review provides actionable insights into sustainable livestock management by identifying specific microbial inoculants that can mitigate methane production. Additionally, incorporating effective microbial inoculants in silage production can reduce methane emissions at the source, promoting environmental sustainability while maintaining or even enhancing livestock productivity. This approach can also serve as a foundation for new *in vitro* and *in vivo* studies aimed at reducing ruminal methane emissions.

Introduction

Methane originating from enteric fermentation in ruminants is the single largest source of anthropogenic agricultural emissions and has a significant impact on global methane levels. Cattle, buffaloes, sheep and goats are the primary ruminant livestock responsible for methane emissions, collectively accounting for 96% of global emissions from enteric fermentation (Chang *et al*, 2019). Methane synthesis in the rumen is facilitated by methanogenic archaea, serving as the primary pathway for expelling excess hydrogen generated during digestion and the methane formed is predominantly expelled through eructation. According to Ban and Guan (2021), certain direct-fed microbials can effectively mitigate methane emissions while enhancing host energy utilization and improving feed efficiency. However, long-term *in vitro* and *in vivo* studies are essential to fully understand their interactions with the host and the microbiome. The addition of microbial inoculants in silage is a widespread practice globally, known to enhance the fermentative process and improve the nutritive value of feed. Such approach also shows promise as an effective strategy for mitigating methane emissions from the rumen. This systematic review aimed to investigate whether microbial silage inoculants could reduce methane formation from the rumen microbiome both ex vivo and in vivo, based on available literature.

Materials and Methods

Google Scholar was the database, searched for pertinent published studies in indexed journals with no year limitations and using keywords in English. The search keywords were based on use of silage microbial inoculants to mitigate methane production from the rumen: (1) "ruminant" AND “silage inoculants” AND "methane emissions" OR "enteric methane", (2) "microbials" AND "ruminant" AND "silage inoculants" AND "methane emissions" OR "enteric methane", (3) "microbiome" AND "ruminant" AND "silage inoculants" AND "methane emissions" OR "enteric methane", (4) "direct-fed microbials" AND "ruminant" AND "silage inoculants" AND "methane emissions" OR "enteric methane", (5) "dfm" AND "ruminant" AND "silage inoculants" AND "methane emissions" OR "enteric methane", (6) "probiotic" AND "ruminant" AND "silage inoculants" AND "methane emissions" OR "enteric methane", (7) "microbial metabolites" AND "ruminant" AND “silage inoculants” AND "methane emissions" OR "enteric methane", (8) "postbiotics" AND "ruminant" AND “silage inoculants” AND "methane emissions" OR "enteric methane". The keywords were used throughout the articles. The search ended on January 31, 2024. Two independent investigators conducted an extensive search and examined all pertinent studies for inclusion. The initial attempt to identify articles was based on the title and abstract, followed by a full-text review. In all studies, two reviewers gathered baseline data, silage preparation details, and methane gas measurements. To assess methane reduction across all treatment conditions relative to the control, each treatment's methane production percentage was expressed as a proportion of the control. Specifically, the methane production rate of the control was set as the baseline (100%), and the methane reduction in each treatment was calculated using the formula:

$$Relative\ methane\ production\ (\%) = \left(\frac{Methane\ production\ in\ treatment}{Methane\ production\ in\ control}\right) \times 100$$

The percentage reduction for each treatment condition was determined as:

$$Methane\ reduction\ (\%) = 100\% - Relative\ methane\ production\ (\%)$$

This calculation allowed for a consistent assessment of methane reduction across all the treatment conditions relative to the control.

Results

The initial database search yielded 434 records, of which 42 were identified as promising. However, 27 were duplicates, leaving 15 studies for full-text review. Based on the quality criteria, only 10 articles were selected for inclusion in this systematic review. Of these, nine studies measured methane production using *in vitro* assays, while one study reported an *in vivo* trial. The majority of the experiments (70%) were conducted using cattle rumen samples, including the *in vivo* study involving cattle. Additionally, 30% of the experiments used rumen samples obtained from sheep. Across the selected studies, ten bacterial species were used as inoculants, with *Lactobacillus plantarum* being the most prevalent, cited as an inoculant in eight experiments and in five of these cases, it was used in combination with other bacterial strains. *Lactobacillus buchneri* applied to persimmon skin silage achieved the highest methane reduction, with an 83.17% decrease. *Lactobacillus plantarum* in ryegrass silage also demonstrated a substantial methane reduction, achieving a 48.11% decrease. In the *in vivo* study, a blend of *Lactobacillus plantarum, Lactococcus lactis, and Lactobacillus buchneri* in a 10:20:70 ratio, applied to a mixture of ryegrass and timothy grass silage, resulted in a 1.98% increase in methane production.

Conclusions

The use of silage microbial inoculants is a promising approach for reducing methane emissions in livestock, with 80% of the studies reviewed showing a reduction in methane production by ruminant microorganisms. However, further research is needed to validate these findings, given the limited number of published studies in this area.

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Potential for Direct Interspecies Electron Transfer in the Rumen

S. Mills[1], A. McGrath[1], S. Timmons[1], V. O'Flaherty[1], S. Waters[1]
[1]*University of Galway, Galway H91 TK33, Ireland*

Presenting author
Simon Mills. E-mail: simon.mills@universityofgalway.ie

Application

As part of the Climate Action Plan 2023, Ireland has a legal obligation to reduce agricultural greenhouse gas (GHG) emissions by 25% before 2030, including a recommended 10% reduction in biogenic methane. These reductions must also be achieved whilst maintaining food security. Therefore, methane mitigation strategies that do not compromise food production are urgently required. However, in order to develop effective, targeted strategies, a better understanding of the routes of electron flow in the rumen microbiome is required.

Introduction

Methane production in the rumen is a result of the flow of electrons from fermentable substrates to methane. Electron flow is initiated when substrates are fermented, producing volatile fatty acids (absorbed by the animal) and CO_2. Electron flow is mediated by complex microbial communities which Transfer electrons to external terminal electron acceptors such as protons (H^+), resulting in the formation of H_2. Subsequently, H_2 acts as a reduced intermediate to transfer electrons to hydrogenotrophic methanogens, which use it to reduce CO_2 to methane in a process known as mediated interspecies electron transfer (MIET). MIET is traditionally thought of as the main pathway of methane production in the rumen. However, an alternative route of electron flow, direct interspecies electron transfer (DIET), was recently discovered in methanogenic anaerobic digesters and was subsequently demonstrated in methanogenic soils and sediments (Holmes et al., 2017). DIET facilitates the direct transfer of electrons from one species to another via outer surface bound conductive cytochrome proteins or conductive appendages known as e-pili, without the need for reduced intermediates such as H_2. There is no study to date assessing DIET in the rumen, meaning that its contribution to the production of methane emissions is unknown and the established route of electron flow may be misunderstood. DIET or extracellular electron transfer (EET) utilising an abiotic electron donor/acceptor rather than a syntrophic partner, has been utilised in several environmental biotechnologies. For example, DIET can be promoted in anaerobic digestion (AD), by stimulating electron transfer via conductive materials (Wang et al., 2021). The initial objective of this work was to utilise conductive materials to demonstrate the potential occurrence of DIET in rumen microbiomes. Additionally, we aimed to isolate electoractive microorganisms, which could potentially be carrying out DIET form the rumen microbiome. This will provide new targets for methane mitigation strategies such as feed additives or vaccines. It was hypothesised that addition of conductive materials to batch incubations of rumen fluid would stimulate methane production in the rumen, indicating the presence of DIET-capable microorganisms and that novel DIET-ers would be isolated.

Materials and Methods

Batch incubations of rumen fluid were set up in triplicate. Incubations were supplemented with either magnetite or granular activated carbon to act as a conductive material for promoting DIET. Incubations monitored temporally for pH, volatile fatty acid production, biogas volume and methane production. Both VFAs and methane were monitored using gas chromatography. Batch incubations using conductive materials were used for isolation of pure cultures using a novel isolation technique, utilising a solid medium incorporating MnO^2 which selects exoelectrogenic bacteria (Nazeer and Fernando, 2022).

Results

Powdered activated carbon increased methane production by approximately 30% in rumen fluid incubations (Fig. 1), indicating the potential presence of organisms at least capable of extracellular electron transfer. Several isolates were obtained which matched closely to several *Klebsiella pneumoniae* species. Klebsiella sp. Have previously been identified in microbial fuel cells and suggested to interact with electrodes, indicating their potential to act as alternative routes of electron flow in the rumen microbiome.

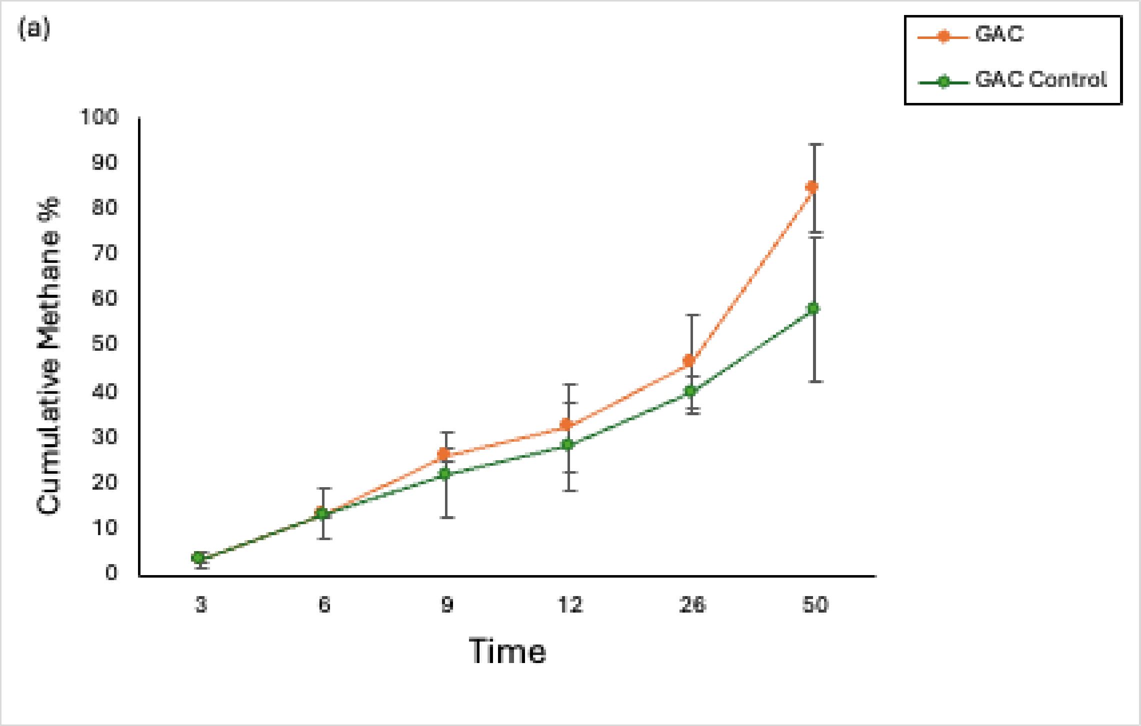

Figure 1. Methane production in control and PAC supplemented batch incubations.

Conclusions

This work indicates the potential presence of DIET-partaking microorganisms in the rumen microbiome, which could potentially re-establish the route of electron flow towards methane. This is crucial as it provides the opportunity to develop new targeted mitigation strategies, which could reduce methane emissions and help us to achieve our climate targets.

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Exploring the impact of cattle acclimation to methane measurements in GreenFeed units

S. Pérez-Márquez[1], P. Le-Grice[1], P. de Méo Filho[2], J. Rivero[1]
[1]Rothamsted Research, West Common, Harpenden AL5 2JQ, UK
[2]University of California Davis College of Agriculture and Environmental Sciences, 150 Mrak Hall Dr, Davis CA 95616, USA

Presenting author

Simon Pérez-márquez. E-mail: simon.perez-marquez@rothamsted.ac.uk

Application

Accurate methane measurement is key to mitigating livestock’s environmental impact. This study highlights the role of cattle acclimation to GreenFeed units in improving data reliability and supporting sustainable emission reduction strategies.

Introduction

Methane measurement in cattle is critical for understanding ruminant production’s environmental impact and mitigating greenhouse gas emissions. GreenFeed units reliably quantify methane flux from individual animals using periodic feed drops. While standard practices acclimate animals to diets for 15–20 days, machine acclimation is often overlooked (Della Rosa et al., 2021). This can impact data accuracy as animals may not immediately exhibit optimal visitation behavior. Over time, feeding patterns improve, enhancing efficiency. This study investigates cattle acclimation to GreenFeed units, analyzing visitation dynamics to improve methane measurement protocols.

Materials and Methods

This study analysed methane (CH_4) emissions and visit behaviour in beef cattle using GreenFeed (GF) units across five winter periods (2018/2019 to 2023/2024) at Rothamsted Research – North Wyke Farm. The herd included Stabilizer, Charolais, and Limousin cross breeds, with animals monitored during their first winter housing. Each group of 30 animals had access to one GF unit (namely 96, 97 and 98), where feed drops were used to attract them. GF units were programmed to allow a maximum of five feeding periods (FPs) per animal per day, with a minimum interval of 4 h and 40 min between each FP. During each FP, up to five feed drops were dispensed, with an average of 30 g of pellets per drop and a 35-second interval between drops (Meo-Filho et al., 2023).

The visit-to-drop ratio (v2d), per animal, was defined as the daily number of visits to the GF unit divided by the number of feed drops received during the same period. This ratio served as an indicator of efficiency, with a v2d of ≤1.0 indicating that animals received at least one feed drop per visit. The "v2d point" was identified as the day when animals achieved consecutive daily v2d ratios between 0.9 and 1.1, suggesting efficient machine use. To explore the impact of excluding pre-v2d data, CH_4 emissions were estimated both before and after the v2d point for each farmlet and year.

Results

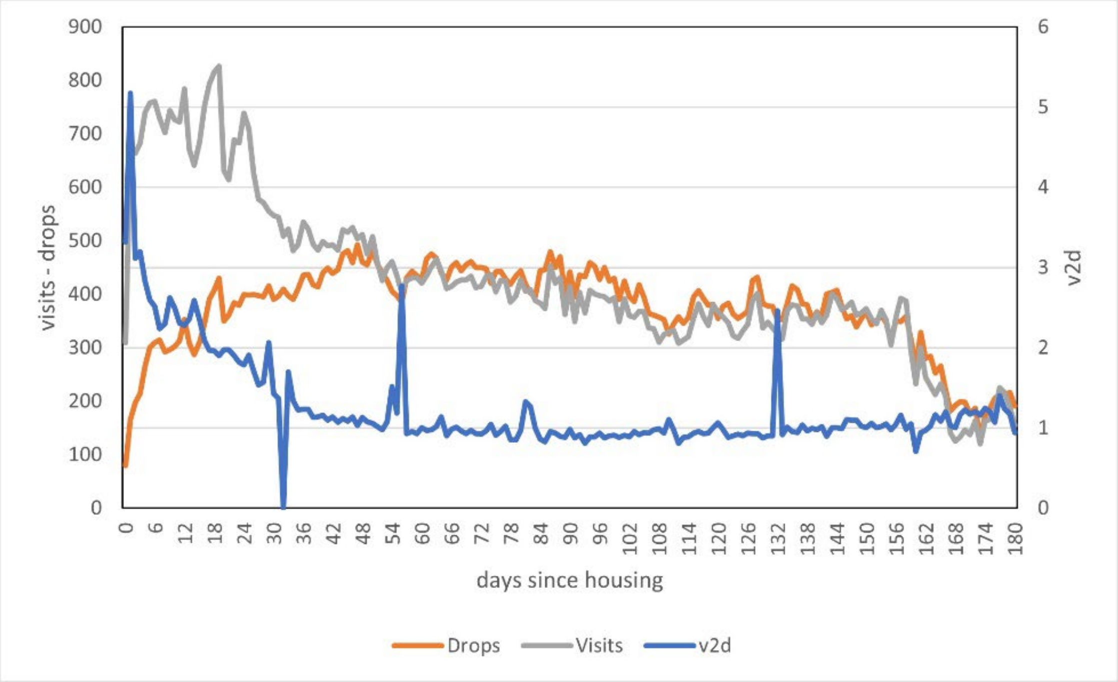

Figure 1. shows that, initially, animals visit the GreenFeed (GF) unit frequently, but with few feeds drops, likely due to shorter visits or reaching drop limits. Over time, visits decrease while drops increase, stabilizing by the v2d point. Beyond this, drops may exceed visits, as animals can receive up to five drops per visit.

The v2d point, was calculated for farmlets 96, 97, and 98 across multiple winter housing periods:

- 2019: 34, 41, and 20 days
- 2021: 38, 13, and 25 days
- 2022: 49, 35, and 20 days
- 2023: 23, 17, and 25 days
- 2024: 30 and 32 days for farmlets 97 and 98, respectively.

The variation in v2d points across farmlets and years suggests that the acclimation process to GF units is not consistent across groups and may depend on factors such as individual animal behaviour, group dynamics, or external conditions. Methane emission data were analysed before and after excluding entries recorded prior to the v2d point (table 2). On average, 1,357 entries per farmlet were excluded when using the v2d point as a starting date. After excluding these entries, daily individual methane emissions increased by approximately 3 g d^{-1}, whereas the coefficient of variation (CV) showed negligible changes, typically less than 0.1%. Given the exploratory nature of this analysis, it is not yet possible to determine whether excluding pre-v2d point data definitively improves methane emission estimates or reduces variability. Further statistical analysis and validation are required.

Table 2. Mean individual CH_4 (g d^{-1}) values of cattle in each farmlet before and after the exclusion of pre v2d point data**.**

	96			**97**			**98**		
	n	**CH_4**	**CV**	**n**	**CH_4**	**CV**	**n**	**CH_4**	**CV**
2019									
Original	7671	215.1	26.03%	7223	236.9	28.73%	8581	220.6	27.05%
After v2d	5653	219.4	26.39%	5762	244.1	28.57%	7820	219.4	27.31%
2021									
Original	6749	224.3	29.67%	7237	297.9	28.63%	9994	216.2	27.41%
After v2d	4957	232.1	29.40%	7059	299.4	28.42%	8906	216.9	27.62%
2022									
Original	9466	224.7	29.54%	13656	267.4	27.96%	9832	221.6	27.18%
After v2d	6619	224.1	29.54%	11543	274.1	27.29%	8601	224.2	27.00%
2023									

Original	11029	195.7	28.57%	14463	239.4	28.21%	10887	197.3	30.89%
After v2d	10264	196.3	28.61%	13678	242.3	27.70%	9762	199.6	30.88%
2024									
Original	-	-	-	12343	253.3	28.33%	10069	206.0	24.36%
After v2d	-	-	-	10815	259.4	27.43%	8761	207.9	24.06%

n= number of entries considered for the calculation of the methane emissions.

Conclusions

The visit-to-drop ratio (v2d) is a useful tool for identifying optimal acclimation to GreenFeed units but shouldn’t be a rigid threshold for excluding data. Instead, it should complement metrics like variability and visit efficiency. Future work will refine these methods to standardize acclimation metrics and assess their impact on methane estimates.

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Effect of Urochloa brizantha Extracts on the Reduction of Methane Emission in Sheep Evaluated by the SF_6 Technique

J. S. Silva[1], R. S. X. de Freitas, G. P. Rissi, D. F. M. Oliveira, I. C. S Bueno
[1]University Of São Paulo, R. da Reitoria, 374 - Butantã, São Paulo 05508-220, Brazil

Presenting author
Janaina S. Silva. E-mail: janaina.silveira.silva@usp.br

Application

The inclusion of hydroalcoholic extract (HE) reduced the gross energy lost in the form of CH_4 (Ym, %) more than the ethanolic extract (EE), but further investigation is needed to confirm its potential as a feed additive.

Introduction

Plant extracts with secondary compounds are widely studied for their potential in methane mitigation due to their ability to manipulate ruminal ecology. Saponins, a prominent class of secondary compounds with antimicrobial, anti-inflammatory, and anti-tumour activity properties, are particularly abundant in nature (Dhanasekaran et al., 2020). Saponins can reduce methane emissions by altering the acetate: propionate ratio and decreasing the protozoa population in the rumen (Canul-Solis et al., 2020). Many plants producing these compounds are also valued as food or medicinal sources. Forage plants provide substrates rich in secondary compounds, and the *Urochloa* genus represents approximately 90% of cultivated pastures in Brazil. *Urochloa brizantha* cv. Marandu is the main forage used as feed for ruminants and contains protodioscin, a steroidal saponin. Therefore, this study aimed to evaluate the effects of *U. brizantha* extracts on enteric methane production in sheep, using the Sulphur hexafluoride tracer gas (SF_6) technique.

Materials and Methods

Eight rumen-cannulated, castrated male sheep of industrial crossbreed [Santa Inês x Dorper] with 41.15 kg of body weight (BW) were used in this study. Methane emissions were measured using the sulphur hexafluoride (SF_6) tracer gas technique (Jonker et al., 2020). Each of the eight lambs was evaluated over five days in each experimental period, with data collected daily. They were fitted with halters and vests from days 7 to 14 to acclimatise the animals to handling and equipment. Permeation tubes (release rate 1431 ± 59 ng/min) containing SF_6 were placed in the rumen three days before the experiment to ensure stable gas flow. The lambs were equipped with gas collection halters attached to PVC sampling canisters, which filled 50% capacity over 24 hours. Gas collections began daily at 7:30 a.m., and pressure was monitored post-collection to check for blockages or leaks. Environmental samples were collected to measure background CH_4 and SF_6 concentrations. Methane emissions were expressed in several units: grams per day (CH_4 g/day), kilograms of methane per kilogram of dry matter intake (CH_4 kg/kg DMI), grams per kilogram of body weight (CH_4 g/kg BW), grams per kilogram of metabolic weight (CH_4 g/kg $BW^{0.75}$), grams per kilogram of digested dry matter (CH_4 g/kg digested DM), and methane yield (YM%) as a percentage of gross energy lost as CH_4. YM was calculated by dividing the daily CH_4 emission (converted to kg) by the energy equivalent of CH_4 (55.6 MJ/kg) and adjusted by the diet's gross energy (18.4 MJ/kg). The experimental design used was a double 4 × 4 Latin square experiment with four experimental periods of 28 days. The effects of the treatments were compared using orthogonal contrasts through the CONTRAST statement in the PROC MIXED procedure of SAS.

Results

Dry matter intake (DMI) showed a significant difference ($p = 0.0069$) between the extracts, with EE (0.59 kg) resulting in 33.7% lower intake than HE (0.89 kg). The average BW of the sheep showed no significant differences ($P > 0.05$) among treatments. Methane emissions were higher with the ethanolic extract (EE; 18.3 g CH_4/kg DMI) than with the hydroalcoholic extract (HE; 14.7 g CH_4/kg DMI) ($P < 0.02$). No significant differences ($P > 0.05$) were observed between treatments for methane emissions expressed as CH_4 g/kg $BW^{0.75}$, CH_4 g/kg BW, CH_4 g/day, and CH_4 g/DMD. However, a significant effect ($P = 0.05$) was found between the plant extract on energy loss as Ym (%), with EE (6.89 %) being higher than HE (5.67 %).

Conclusions

The results suggest that the HE of *U. brizantha* shows greater potential than EE in reducing energy losses as methane in sheep. However, further research is required to assess the effects of varying dosages and confirm the viability of HE as an additive for ruminants.

Acknowledgements

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Comparing Predicted Methane Conversion Factors to Measured Values from Beef Cattle Feeding Trials: Implications for Carbon Calculator Development

D. Edgar[1,2], D. O'Brien[2], J. Hillier[1]
[1]*Global Academy of Agricultural and Food Systems, University of Edinburgh, Easter Bush Campus, Edinburgh EH25 9RG, UK*
[2]*Soils and Environment Research Centre, Teagasc, Johnstown Castle, Co. Wexford, Ireland*

Presenting author
Dylan Edgar. E-mail: d.edgar@sms.ed.ac.uk

Application

Beef farmers require accurate tools to estimate on-farm emissions. Agricultural carbon calculators, often based on IPCC Tier 2 methodology, are widely used for this purpose. This study focuses on assessing the methane conversion factor (Ym), a critical variable in emission calculations, and highlights the implications of inaccuracies in Ym estimates on farm-level greenhouse gas (GHG) inventories. The findings aim to inform carbon calculator developers and improve emissions estimation accuracy.

Introduction

In order to respond to increasingly stringent climate targets, beef farmers need access to accurate estimates of their on-farm emissions. Many agricultural carbon calculators have been developed to allow farmers to estimate both the sources and magnitude of farm emissions. These calculators are often based on the Tier 2 calculations of the IPCC's Guidelines for National Greenhouse Gas Inventories (IPCC, 2006; 2019), and the methane conversion factor (Ym) has been identified as the most sensitive variable in these calculations for non-dairy livestock emissions (Karimi-Zindashty et al., 2012). However, there is limited empirical validation of the IPCC's Ym estimates for beef cattle. This study aims to assess the accuracy of the Ym values proposed in the IPCC Tier 2 methodology by comparing them to Ym values back-calculated from published beef cattle feeding trials.

Materials and Methods

Measured enteric methane data were sourced from 10 beef cattle trials reported in 4 peer-reviewed papers. This data was used to back-calculate the implied Ym value by rearranging the IPCC Tier 2 calculation for enteric fermentation. Statistical analysis focused on comparing these calculated Ym values to the default Ym proposed by the IPCC. To ensure robust findings, a systematic literature review was initiated using the Web of Science database, identifying over 30 papers and 70 trials from the UK and Ireland for further analysis.

Results

Preliminary findings indicate that the IPCC Tier 2 methodology underestimates Ym for housed beef cattle in the UK and Ireland. This underestimation leads to a proportionate underestimation of enteric methane emissions. Given the dominant contribution of enteric methane to beef sector emissions, even minor inaccuracies can significantly skew emission inventories. Analysis of trials sourced through the extended literature review is underway.

Conclusions

The study highlights a potential systematic underestimation of Ym in the IPCC Tier 2 methodology for beef cattle in the UK and Ireland, with implications for agricultural carbon calculators that rely on these calculations. Accurate Ym values are critical for reliably estimating emissions, this work underscores the need for region-specific estimates of Ym. Final recommendations for carbon calculator developers will follow a comprehensive analysis of Ym values across the identified trials.

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Essential oils when supplied in feed or via a slow-release rumen bolus affects the methane emissions and performance of dairy cows

C. Roxborough[1], A. Mackenzie[1], J. Bennison[2], L. Sinclair[1]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*
[2]*Agriminn Ltd, Arlanda Way, Humberside Airport, Kirmington, Ulceby, North Lincolnshire DN39 6YH, UK*

Presenting author

Charlotte Roxborough. E-mail: croxborough@live.harper.ac.uk

Application

The inclusion of essential oils either in the feed or via a slow-release rumen bolus reduces CH_4 production (g/d), CH_4 yield (g/kg DMI) and CH_4 intensity (g/kg milk yield), with no impact on DM intake or milk yield in mid-lactation dairy cows.

Introduction

The greenhouse gas CH_4 has a global warming potential 28 times that of CO_2 with CH_4 from enteric sources comprising up to 17% of the global CH_4 emissions (Knapp et al., 2014). Furthermore, CH_4 can negatively impact dairy cow production, with between 2 to12% of the gross energy intake being lost as CH_4 (Johnson and Johnson, 1995). Essential oils are naturally occurring volatile compounds consisting of plant volatiles that can beneficially manipulate rumen fermentation, reducing CH_4 emissions and increasing performance (Belanche et al., 2020). Providing essential oils in a slow-release rumen bolus is an easier means to administer to extensively grazing ruminants and may reduce the potential loss of volatiles when included in the feed. There have however been no studies undertaken to determine the effect of essential oils when included in a slow-release bolus compared with inclusion in the diet on the CH_4 emissions and performance of dairy cattle. The objective of the study was to examine the effect of an essential oil blend when supplied in a slow-release rumen bolus or in the diet on CH_4 emissions and performance in mid lactation dairy cows.

Materials and methods

Forty-Five Holstein Friesian dairy cows (12 primiparous and 33 multiparous) that were (mean ± SE) 142 ± 3.89 days in milk (DIM), yielding 41.8 ± 1.09 kg/d and weighing 663 ± 11.0 kg were blocked based on DIM and milk yield and then randomly assigned within block to one of three dietary treatments: C: basal total mixed ration (TMR; containing on a DM basis: 0.41 maize silage, 0.20 grass silage, and 0.39 concentrates), TO: basal TMR + essential oils (Agolin, Switzerland fed at 0.92 g/cow/day), or BO: basal TMR + intra-ruminal boluses containing essential oils to provide the same daily rate of release of essential oils as TO. The cows were group housed in the same shed and remained on study for 10 weeks following a two-week covariate period, with individual intakes measured daily using roughage intake control feeders, milk yield recorded daily, and milk samples collected weekly for composition analysis. Live weight (LW) and body condition score (BCS) were recorded fortnightly. Enteric CH_4 emissions were measured daily using GreenFeed units (C-Lock Inc., Rapid City, SD). The data was analysed using a mixed effect model, with time, treatment, lactation number, DIM, and week 0 data as fixed effects, and cow as a random effect using Genstat (V.23). The results are provided as the least square mean (LSM) and standard error of the difference (SED). P values ≤0.05 were considered as significant and ≤0.10 as a tendency.

Table 1. Performance and CH_4 emissions of dairy cows fed either a control diet (C), or with the addition of essential oils in the TMR (TO) or a slow-release rumen bolus (BO)

	Treatment			SED	Significance	
	C	BO	TO		Treatment	Time
DM intake (kg/d)	25.2	25.5	25.0	0.178	0.234	<0.001

Milk yield (kg/d)	39.7	39.8	39.2	0.286	0.054	<0.001
Milk fat (g/kg)	41.5^{ab}	40.1^{a}	41.8^{b}	0.695	0.015	<0.001
Milk protein (g/kg)	32.4^{ab}	32.5^{b}	32.2^{a}	0.136	<0.001	<0.001
LW (kg)	672	677	674	8.40	0.468	0.127
BCS	2.72	2.69	2.70	0.046	0.724	0.150
GreenFeed visits/cow/d	2.8	3.0	2.9	0.200	0.583	<0.001
CH_4 (g/d)	492^{b}	473^{a}	470^{a}	4.20	<0.001	<0.001
CH_4 (g/kg milk yield)	12.7^{b}	12.0^{a}	12.2^{a}	0.142	<0.001	<0.001
CH_4 (g/kg DMI)	19.6^{b}	18.8^{a}	18.9^{a}	0.191	<0.001	<0.001

$^{a\text{-}b}$ Means within a row with different superscripts differ ($p<0.05$)

Results

There was no effect of dietary treatment on DM intake, with a mean of 25.2 kg/d ($P>0.05$; Table 1). There was a tendency ($P<0.10$) for milk yield to be lower in cows fed TO compared to BO, and there was a lower ($P<0.05$) concentration of milk fat and a higher ($P<0.001$) concentration of milk protein in cows receiving BO compared to TO, with C being intermediate. There was no effect of treatment on LW or BCS ($P>0.05$). The number of GreenFeed visits per cow per day was similar ($P>0.05$) across treatments, with a mean of 2.9 visits/cow/d and the average visit duration of animals to the GreenFeed units was 5.0 minutes. The production of CH_4 (g/d) was reduced ($P<0.001$) by 19 and 22 g/d in cows on BO or TO compared to C, respectively, and was also lower when expressed as gCH_4/kg milk yield and gCH_4/kg DMI in cows fed BO or TO compared to those on C, particularly during weeks 6 to 10

Conclusions

The administration of essential oils, either by incorporation into the TMR or via a slow-release rumen bolus, decreased CH_4 emissions when expressed as g/d, g/kg milk yield or g/kg DMI, with no effect on intake, milk yield, LW or BCS. Incorporating essential oils into the feed led to an increase in milk fat content and a decrease in milk protein concentration compared with the administration in a slow-release bolus.

Acknowledgements

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Lactation curve patterns in primiparous Holstein cows

D. Kalokairinou[1], A. Soufleri[1], A. Kougioumtzis[1], G. Valergakis[1]
[1]*Department Of Animal Production, Faculty Of Veterinary Medicine, Aristotle University Of Thessaloniki, Thessaloniki 541 24, Greece*

Presenting author
Georgios Valergakis. E-mail: geval@vet.auth.gr

Application

The identification of unconventional lactation curve patterns in primiparous cows may be indicative of management problems that should be resolved to enhance productivity.

Introduction

Primiparous cows make up the largest demographic group in dairy herds; due to their special features (still growing, lower peak yields, increased lactational persistency etc), they are considered a distinct epidemiological group as opposed to older, mature cows (Wilmink 1987, Ehrlich 2013). At the same time, they are less competitive and face stressful conditions when housed together with higher parity cows. Farmers frequently report delayed peak milk yields under these conditions but large scale data, including multiple herds, is lacking. The aim of the present study was to explore whether this is a common feature in primiparous Holstein cows in Greece.

Materials and Methods

Official records kept by the Hellenic Holstein Association, from 5346 primiparous cows in 35 herds were used. Data available included age at first calving (AFC), first lactation total milk yield and 58,806 monthly milk yield records which were used to build the lactation curve for each cow. Based on AFC, cows were classified in four groups, those calving: a) at an age <23 months (AFC-1), b) between 23 and 27 months of age (AFC-2), c) between 27 and 31 months of age (AFC-3) and c) >31 months of age (AFC-4). Moreover, based on their peak milk (PM) record (1st to 7th month) cows were classified in four groups, namely: a) peak at the 1st month (PM-1), b) peak at the 2nd month (PM-2), c) peak at the 3rd month (PM-3) and d) peak at the 4th to 7th month (PM-4/7). One-way analysis of variance was used to assess the association of AFC and PM groups with total milk yield as well as that of PM with AFC (SPSS v29.0).

Results

Mean AFC was 808.2±119.2d (26.57±3.92m); AFC-1, AFC-2, AFC-3 and AFC-4 represented 7.3%, 51.7%, 29.8% and 11.2% of the total, respectively. Mean first lactation milk yield was 9,437±1,689 kg; milk yields for AFC-1, AFC-2, AFC-3 and AFC-4 were 8,693±1,704kg, 9,314±1,638kg, 9,699±1,651kg and 9,871±1,768kg, respectively. Milk yield of AFC-1 cows was significantly lower from that of all other groups ($P<0.001$); milk yield of AFC-2 cows was lower ($P<0.001$) than that of AFC-3/AFC-4 cows but there was no statistical difference between AFC-3 and AFC-4. Differences between AFC-2 and AFC-3/AFC-4 were only 4.0% and 6.0% respectively and therefore, extra heifer rearing costs appear unjustified in most cases. Distribution of PM-1, PM-2, PM-3 and PM-4/7 was 7.1%, 25.0%, 26.3% and 41.6%, respectively. No statistically significant differences ($P>0.05$) were detected among PM groups regarding AFC (807±124d, 800±112d, 810±120d and 812±122d for PM-1, PM-2, PM-3 and PM-4/7, respectively). Milk yields of PM groups were 8,671±1,730kg, 9,195±1,637kg, 9,415±1,634kg and 9,729±1,681kg, for PM-1, PM-2, PM-3 and PM-4/7, respectively.

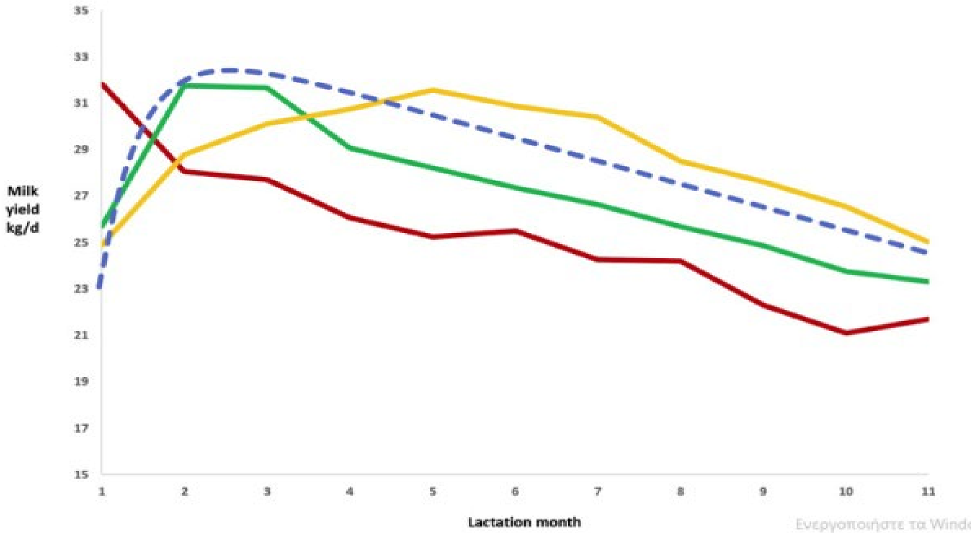

Figure 1. Lactation curves; PM-1 (red line), merged PM-2 and PM-3 (green line), PM-4/7 (yellow line) and the Wilmink curve (blue dotted line) based on actual milk production data.

Conclusions

Atypical lactation curves (PM-1 and PM-4/7) were detected for almost half of the primiparous Holstein cows this study. The former (PM-1), despite an excellent start, fail to sustain a high milk production and end up with the lowest total yield. The latter (PM-4/7), which is the most numerous group (41.6%), present a delayed peak yield while at the same time, have the highest total yield. Whether this is a genetic trait or cows with the highest yield potential suffer more stress during early lactation remains to be elucidated.

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Predicting negative energy balance in transition dairy cows using ultrasound measurements of backfat and longissimus dorsi muscle thickness

N. Siachos[1], G. Oikonomou[2], N. Panousis[3], G. Valergakis[4]
[1]SRUC School Of Veterinary Medicine, Peter Wilson Building, The King's Buildings, West Mains Road, Edinburgh EH9 3JG, UK
[2]Department of Livestock and One Health, IVES, University of Liverpool, Brownlow Hill, Liverpool L69 7ZX, UK
[3]Clinic of Farm Animals, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 541 24, Greece
[4]Laboratory of Animal Husbandry, Faculty of Veterinary Medicine, School of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki 541 24, Greece

Presenting author
Georgios Valergakis. E-mail: geval@vet.auth.gr

Application
Ultrasound-based monitoring of backfat thickness (BFT) and longissimus dorsi thickness (LDT) provided a practical, non-invasive method for assessing metabolic risk in transition dairy cows. This approach may support early management interventions to enhance cow health and productivity, without the need for blood sampling and laboratory analyses.

Introduction
Dairy cows face an increased risk of metabolic disorders during the transition period due to excessive negative energy balance (NEB). Biomarkers like non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) are commonly used to assess NEB but require blood sampling and costly laboratory analyses. This study evaluated ultrasound-measured BFT and LDT as potential biomarkers of NEB in transition dairy cows, aiming to identify accessible alternatives for metabolic health monitoring.

Materials and Methods
This study involved 238 multiparous Holstein cows from six commercial dairy farms in Greece, housing 110 to 360 lactating cows with an average milk yield of 9,000–12,000 kg per lactation. Ultrasound measurements of BFT at the sacral area and LDT at the fourth lumbar vertebra were obtained at six time points relative to calving (0d): -60d to -45d (from now on referred to as -45d), -21d, -7d, 0d, 7d and 21d. Measurements used real-time B-mode ultrasonography (ImaGo S, IMV imaging, U.K.) with a 5.0 MHz linear transducer, performed by a single trained operator to ensure consistency. For this study, we used the predicted values derived from linear mixed effects models for the repeated BFT and LDT values and their changes between time points from a previous publication (Siachos et al., 2022). Blood samples were collected at -21d, -7d, 7d, and 21d by coccygeal venipuncture. Samples were centrifuged, and serum NEFA and BHB concentrations were determined by photometry using the ADVIA 1800 Chemistry System. The binary status of NEB at each time point was defined based on elevated NEFA, defined as NEFA ≥ 0.3 mmol/L pre-calving and NEFA ≥ 0.7 mmol/L post-calving (Ospina et al., 2010), or elevated BHB values, defined as BHB ≥ 1,200 µmol/L (Leblanc et al., 2005). Thresholds for BFT and LDT parameters were determined using Receiver Operating Characteristic (ROC) curves generated from binary logistic regressions, with sensitivity (Se) and specificity (Sp) calculated to evaluate their diagnostic accuracy in predicting elevated NEFA or BHB, when the area under the ROC curve (AUC) was statistically significant. Data were analyzed using SPSS v.28.

Results

Several key thresholds of ultrasound-measured tissue reserves and their changes emerged from ROC analyses that could predict NEB status. For predicting elevated NEFA at -21d, a threshold of ≥ 17.9 mm for BFT at -45d demonstrated a moderate diagnostic capability (AUC = 0.71; 95% CI: 0.62–0.80, $P < 0.001$), with Se of 0.59, and Sp of 0.82. A threshold of ≥ 38.4 mm for LDT at -45d showed similar predictive ability (AUC = 0.70; 95% CI: 0.61–0.80, $P < 0.001$) for elevated NEFA at -21d, with Se of 0.63, and Sp of 0.78. Changes in LDT were also identified as significant predictors of NEB status pre-calving. An increase in LDT between -45d and -21d of ≥ 12.3% was less likely to result in elevated NEFA at -7d (AUC = 0.70; 95% CI: 0.62–0.77, $P < 0.001$), with high Se (0.98), and very low Sp (0.36). Regarding post-calving NEB status, a reduction of ≥ 1.5 mm in BFT between -45d and -21d could predict elevated NEFA at 7d (AUC = 0.63 (95% CI: 0.55–0.71, $P = 0.002$), with Se of 0.60, and Sp of 0.63. An LDT reduction of ≥ 10.1% between 7d and 21d was the only predictor for elevated NEFA at 21d, yielding poor diagnostic capability (AUC = 0.61; 95% CI: 0.52–0.71, $P = 0.050$), with Se of 0.64, and Sp of 0.65. For elevated BHB prediction, a ≥ 5.4% decrease in LDT between -21d and -7d was the most effective predictor of elevated BHB at 7d, with an AUC of 0.76 (95% CI: 0.65–0.87, $P < 0.001$), moderate Se (0.56), and high Sp (0.87). Finally, a decrease or a ≤ 3.3% increase in BFT between -21d and -7d was the most effective predictor (AUC = 0.71; 95% CI: 0.60–0.81, $P < 0.001$) of elevated BHB at 21d, with high Se (0.88), and moderate Sp (0.50).

Conclusions

Our findings support the use of ultrasound BFT and LDT measurements as practical, non-invasive means for identifying transition dairy cows at risk of NEB. Based on the present results, to optimise metabolic health monitoring, we recommend measuring BFT and LDT at about 45 days pre-calving to identify cows at lower risk of elevated NEFA at 21 days pre-calving. For identifying cows at risk of elevated NEFA closer to calving, changes in LDT from 45 to 21 days pre-calving may help with screening, minimising false negatives at the expense of some false positives. For post-calving NEB, BFT changes from 45 to 21 days pre-calving can assist in routine monitoring, while LDT changes from 21 to 7 days pre-calving may help confirm cows at high risk of elevated BHB at 7 days postpartum. Routine ultrasound measurements thus support decision management strategies, enhancing early intervention efforts and overall herd health and productivity.

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Utilising milk mid-infrared spectra to predict nitrogen-use-efficiency of individual dairy cows offered grass-silage based diets

A. Lavery[1], C. Grelet[2], A. Craig[1], N. Grant[1], J. Archer[1], C. Ferris[1]
[1]Agri-food And Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK
[2]Walloon Agricultural Research Centre (CRA-W), Rue Juliette Wytsmanstraat 14 1050 Brussels, Belgium

Presenting author

Anna Lavery. E-mail: anna.lavery@afbini.gov.uk

Application

The dataset used in this study did not allow NUE to be predicted using MIRS. Collaboration to combine datasets from multiple farm and countries offers real opportunity to develop improved prediction models for use dairy on-farms.

Introduction

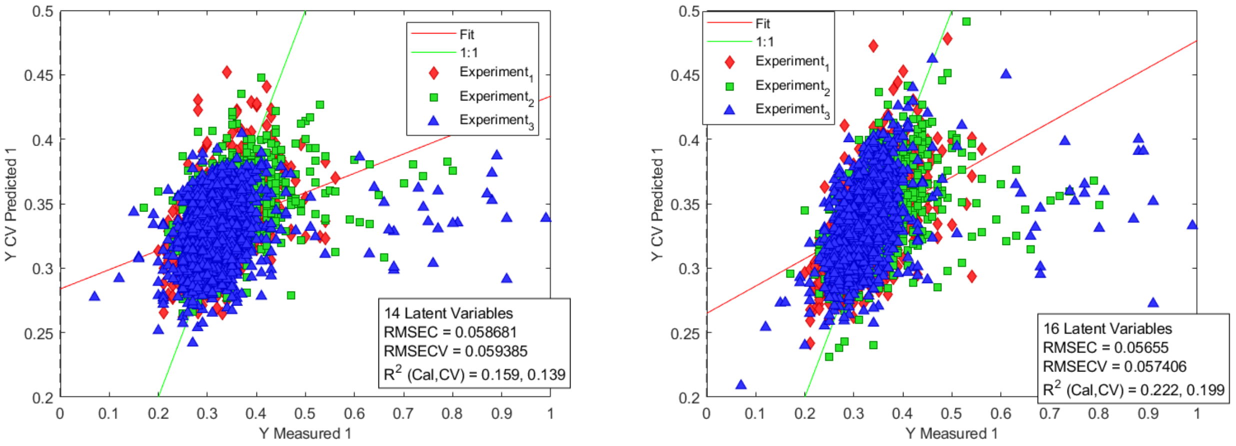
There is renewed focus on the efficiency with which dairy cows utilise dietary nitrogen (N) due to the negative environmental impact of N losses in manure. Nitrogen-use-efficency (NUE; defined as N output in milk/N consumed, %) varies greatly between individual cows and herds, ranging from 14 to 45% (Huhtanen and Hristov, 2009). While this variation demonstrates an opportunity to improve NUE by optimising diet composition and management, accurate information on NUE on farms is required if improvements are to be made. Mid-infrared spectrometry (MIRS) is a non-invasive, rapid and cost-effective method that is routinely used for analysis of milk constituents. More recently, research has examined the potential of MIRS analysis of milk to predict variables such as energy intake, methane production, metabolic profiles and NUE (Mc Parland et al., 2014; Van Gastelen et al., 2018; Luke et al., 2019). With regards the latter, Grelet et al. (2020) demonstrated that MIRS analysis of milk could be used to distinguish between cows with ‘high’ and ‘low’ NUE in early lactation (R^2= 0.74). The objective of the current study was to examine the potential of milk MIRS to predict NUE of individual cows (offered grass silage-based diets) over a full-lactation period.

Materials and Methods

Data was obtained from three ‘full-lactation’ experiments which were conducted at the Agri-Food and Biosciences Institute (AFBI), between October 2020 and September 2023. The three studies all involved comparisons of diets that differed in crude protein content. Cows were enrolled on the studies from calving until approximately 35 weeks in milk, with data from a total of 265 Holstein cows included in the analysis. Milk samples were collected during two consecutive milkings (pm and am) on a fortnightly basis and each individual sample analysed using MIRS (Milkoscan Combifoss TM7). Spectra samples from pm and am samples were combined into a daily spectrum by weighting the value at each wavenumber by the appropriate pm and am milk yield values. Spectra were standardised following the procedure outlined in Grelet et al. (2015). NUE data aligning with each 24 h milk sampling period (spectra data) was determined as the mean NUE over the 7-day period around the sampling day (day of sampling ± 3 days). Models were developed using partial least squares or support vector machine regressions. Computations and models were carried out with programs developed in MatLab and the PLS toolbox. Cross-validation data sets were an internal sub-set of data randomly constituted, with records from the same cow but with different dry matter intakes could be in both the calibration and validation data sets.

Results

Individual NUE values ranged from 7.9 to 73.2%, with a mean of 32.8% (standard deviation, 5.6%). The cross-validation of the partial least squares (PLS) model was performed with 10 subsets. The model with milk MIR spectra as the sole predictor of NUE (Figure 1a), had a relatively good (low) error of cross validation (RSMEcv = 0.059) but a low R^2 of cross-validation (R^2 CV = 0.139). The inclusion of both parity and milk yield improved the predictive ability of the model but overall R^2 was still low (R_2 CV = 0.199) with similar RSME (RSMEcv = 0.057, Figure 1b). The low predictive ability of the models is likely due to the homogenous nature of the data set, coming from a single farm with cows offered relatively similar diets.



(a) (b)

Figure 1. Plots of measured individual NUE (x axis) of dairy cows compared with NUE values predicted (y axis) by the PLS models using mid-infrared spectra (a), or mid-infrared spectra, parity and milk yield (b) as predictors in cross validation with 10 subsets. RMSEc = root mean square error of calibration; RMSEcv = root mean square error of cross-validation with 10 subsets; R2cal =coefficient of determination of calibration; R2cv = coefficient of determination of cross-validation with 10 subsets.,

Conclusions

In this study NUE was poorly predicted from milk MIRS spectra data, with this likely due to the use of a dataset collected from a single research herd offered a relatively controlled diet under one environment. Improved accuracy in developing MIR predictions has been obtained by combining datasets from multiple farms and countries, thus highlighting the need for collaboration to develop predictions with broader ‘on-farm’ applications.

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Supplementation of high-producing dairy cows with a standardised rumen-protected grape extract supports milk production in early lactation during heat stress conditions

P. Engler[1,2], A. Roques[1], N. Ollivier[1], M. El Amine Benarbia[1,2]
[1]Nor-feed, 3 Rue Amédéo Avogadro, 49070 Beaucouzé, France
[2]FeedInTech LabCom, 3 Rue Amédéo Avogadro, 49070 Beaucouzé, France

Presenting author
Paul Engler. E-mail: paul.engler@norfeed.net

Application
The supplementation with a standardised rumen-protected grape extract allowed dairy cows to produce more milk in a situation of heat stress, where the milk output should be negatively impacted, thus improving the profitability of the farm in summer conditions.

Introduction
With the intensification of global warming come negative consequences for animal production, the most visible one being heat stress, which is the struggle of an animal to evacuate the excess heat due to high environmental temperature and/or humidity. This leads to physiological stress and negative impacts on health and production. When combined with other intrinsic stressors such as the transition period in peri-parturient cows, it can be detrimental to milk production. The aim of the present study was to evaluate the potential for a standardised rumen-protected grape extract (RPGE, Nor-Grape® BP-O, Nor-Feed, France) to alleviate the burden of heat stress in dairy cows during the start of lactation.

Materials and Methods
30 high-producing Prim-Holstein cows from a commercial dairy farm in Western France were selected, and allocated, in the same barn, to a control group (CTL, n=15) or a supplemented group (BPO, n=15, receiving the same diet with an extra 500mg RPGE/head/day through an automatic dispenser located at the milking robot), to have balanced groups (average parity in both groups: 1.95, 50% primiparous, 50% multiparous cows in each group). The study took place over a summer period of 66 days and lasted from lactation D1 to D40 for every cow enrolled. Cows' milk production was recorded daily using the data from the two milking robots of the farm. Temperature and humidity data were recorded using 2 dataloggers (FI84ED, Francaise d'Instrumentation, France) placed at cows' level in 2 locations of the barn and computed to calculate the daily THI value (Vitali *et al.,* 2009) throughout the trial. Average milk output after 20 days of lactation and after 40 days of lactation, as well as their fat and protein content were compared between groups using Welsh t-test.

Results
Overall, BPO cows' milk output tended to be significantly higher than CTL ones, both on L20 (CTL: 33.7kg vs. BPO: 37.5kg, *P*=0,096) and on L40 (CTL: 40.8kg vs. BPO: 44.7kg, *P*=0.097). No difference was observed in terms of fat or protein content of the milk between groups at both timepoints of the study. THI calculations showed that the intensity of heat stress was overall moderate, with only 13 days of 'moderate heat stress' (72<THI<78) and 4 days of 'intense heat stress' (THI>78). The milk production of BPO cows was significantly higher than CTL ones during moderate heat stress days (42.3kg vs. 37.7kg respectively, *P*<0.001) and tended to also be higher during intense heat stress days

(41.9kg vs. 38.1kg, respectively, *P*=0.059). These results confirm previous findings evidencing that RGPE supported the metabolism of dairy cows during heat stress events (Amato *et al*, 2024).

Conclusions

The results show that efficient dietary intervention with RPGE to support dairy cows in their early lactation helps them face heat stress challenges and results in higher milk output when heat stress intensifies, thus benefiting for the farm productivity, even in challenging conditions.

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Application of near infrared reflectance spectroscopy to quantify total purines in equine faeces

É. Murphy[1], B. A. Younge[1]
[1]University of Limerick, Limerick, V94 T9PX, Ireland

Presenting author
Étaoin Murphy. E-mail: etaoin.murphy@ul.ie

Application
Faecal near infrared spectroscopy (NIRS) has been routinely implemented to monitor the nutritional status of animals in a rapid and non-invasive manner. It can potentially be used to optimise management practices; ensure feed efficiency is maintained and promote improved animal wellbeing.

Introduction
Accurate and efficient methods to determine dietary nutrient composition, digestibility and feed efficiency of grazing animals are becoming increasingly important as production requirements increase and a greater emphasis is placed on reducing environmental strain in agriculture. Despite extensive research being conducted on the application of NIRS in ruminant nutrition, few studies have investigated its capabilities in equine nutrition. Various dietary attributes have been investigated via faecal NIRS including dietary composition, intake and digestibility as well as physiological attributes such as sex, age, pregnancy and lactation status. Microbial nitrogen has previously been reported as an index of microbe synthesis. Ruminant nutrition recognises the contribution of microbial nitrogen for protein synthesis and availability (Zinn and Owens 1986). However, few studies have investigated the contribution of microbial nitrogen to the amino acid supply available to equines (Santos *et al.* 2013). The quantification of total purines has previously been applied to estimate microbial nitrogen present in digesta and faecal material of ruminant and non-ruminant animals. While some studies have demonstrated the ability of faecal NIRS to estimate microbial nitrogen through purine quantification in ruminant faeces, this has not been explored in equines (Atanassova *et al.* 1998; Tigabu and Felton 2018). The objective of this investigation was to quantify total purines in equine faeces to estimate microbial nitrogen and to develop calibration models and evaluate the ability of NIRS to predict total purine concentration.

Materials and Methods
Spectral data was collected from dried and milled equine faeces (n=93) which were scanned in reflectance mode at 0.5 nm intervals in the visual near infrared region of the electromagnetic spectrum, 400 nm to 2500 nm. Calibration models were developed using modified partial least squares regression and spectral data were refined using a combination scatter corrections and derivative mathematical treatments. Selection of the optimum calibration equation was based on a low standard error of cross-validation (SECV), a high coefficient of determination of calibration (R^2_{cal}) and a high one minus variance ration (1-VR). Reference data was acquired following wet chemistry analysis, faecal nitrogen was quantified with the Kjeldahl method, while purines were quantified using an acid digestion method. Faecal samples were hydrolysed with 2M perchloric acid and free purines precipitated with the addition of silver nitrate (Makkar and Becker 1999). Spectrophotometric quantification of purines was conducted at 260 nm. Yeast RNA was used to construct a standard curve and absorbance values were then converted to mg RNA equivalent. External validation was conducted to monitor calibration performance using spectra of samples which were excluded from the calibration development phase. Performance of the models was assessed according to the following criteria, a high coefficient of determination of validation (R^2_{val}), a high ratio to performance deviation (RPD) and a low standard error of prediction (SEP).

Results

Calibration statistics for the best performing equation for faecal purines were desirable, $R^2_{cal} = 0.82$ and SECV = 1.34.

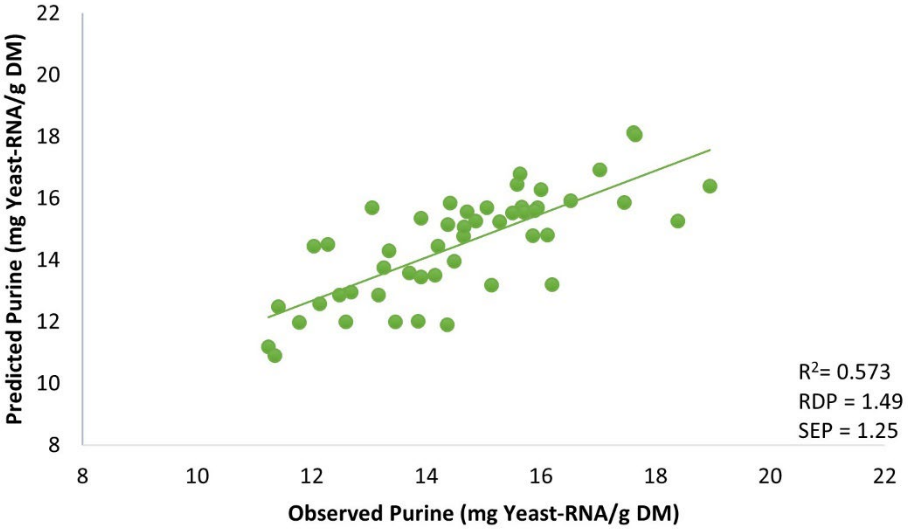

Figure 1. Comparison of observed and NIRS predicted values of purines in equine faeces, where purines are expressed as mg RNA equivalents.

Validation statistics for the prediction of purines (mg Yeast-RNA/g DM) in equine faeces showed moderate predictive ability, $R^2_{val} = 0.57$ and RPD = 1.49. Figure 1 demonstrates this correlation between observed values using wet chemistry and those predicted by NIRS. An RPD > 1.4 was considered acceptable, with the possibility of improving the model with additional chemometric techniques and/or the addition of more samples. Data on faecal nitrogen calibrations and purine:nitrogen ratios will be included in final presentation.

Conclusions

The findings of this study indicate that NIRS is suitable for the prediction of purines in equine faeces. The inclusion of additional unique faecal spectra and further chemometric modelling should be evaluated to improve the predictive ability of the model. Further research should also be conducted to evaluate the ability of NIRS to estimate faecal microbial nitrogen.

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Effect of a novel complementary feed on presales radiographic grades in Thoroughbred weanling foals

K. Hore[1], A. Richardson[1], G. Beaumont[1]
[1]Greencoat Limited, Wonastow Road Industrial Estate, Monmouth NP25 5JA, UK

Presenting author
Kate Hore. E-mail: khore@naf-uk.com

Application

Developmental Orthopaedic Disease is a common disorder of horses with prevalence of over 60% at weanling radiographic scans (Lepeule et al 2008). Of particular concern in young Thoroughbreds is prevalence of osteochondritis dissecans and bone cysts, two commonly encountered forms of bone disorder. Evidence of disorder on presales radiographs reduces sales potential and future racing performance (Preston et al 2010).

With industry results suggesting over two thirds of Thoroughbred breeding in the United Kingdom is unprofitable (Mouncey et al 2024), there is an unmet need commercially to ensure foals are presented with healthy radiographs to optimize market potential. Additionally, considering the social license to operate around equestrianism, a simple dietary application supporting healthy growth is potentially beneficial to both equine welfare and the future of horse sport.

Introduction

Previous studies show specific nutritional combinations in the mare’s diet contribute to minimizing the occurrence of developmental disorders in the foal (Harris et al 2005). Supplementation is commonplace, with products used ranging from straight calcium carbonate to novel formulae. However, published data in horses is lacking, and incorrect ratios of nutrients could further unbalance nutrient uptake, thus having a detrimental rather than positive outcome.

The novel feed trialled is a unique complex of key micronutrients, prebiotics and probiotic yeast at levels designed to provide optimal nutritional ratios, whilst also allowing for typical intake from other feed sources.

Materials and Methods

Mares (n=22) were housed on commercial stud farms, and received a balanced basal diet suitable for broodmares. All mares foaled in both years.

A radiographic set typically consisted of a minimum of thirty-six views covering the front and hind fetlocks, carpi, tarsi and stifles.

Presales radiographs were taken ahead of the November 2021 sales from Thoroughbred weanlings (n=22) from previously un-supplemented mares. Those mares were then fed the novel feed from month nine of their 2021 gestation through to weaning. Following weaning, the 2022 foals (n=22) received the feed until presales radiographs were taken ahead of the November 2022 sales. Two experienced equine reproduction veterinary surgeons curated a grading system marked on disorder severity, and likely impact on sales income and racing performance (Table 1). The radiographs of all weanlings were reviewed before assigning each a grade. The results were split into control group (2021) and treatment group (2022), with the mare acting as her own longitudinal control.

Table 1. Radiographic grading system

1	Either no radiographic abnormalities OR abnormalities of little or no significance, unlikely to interfere with sales revenue or racing potential.
2	Mild radiographic abnormalities. Vets may comment at the sales, however considered low risk.

3	Moderate radiographic abnormalities. Vets will discuss with prospective purchaser and may impact sales revenue. Considered medium risk for racing.
4	Marked radiographic abnormalities, likely to be significantly detrimental to sales revenue. Considered high risk for racing.

A two-tailed, two-sample t-test, assuming unequal variance was chosen to compare the groups to minimise risk of bias.

Results

The p value of 0.04 (3 s.f.) shows a significant ($P<0.05$) difference between control and treatment groups in presales radiograph grades. The 2021 cohort had an average grade of 2.09, compared to 1.55 in 2022. In the mares, seven bred foals with grades considered a risk to racing and sales value (grade 3-4) in 2021, compared to three in 2022.

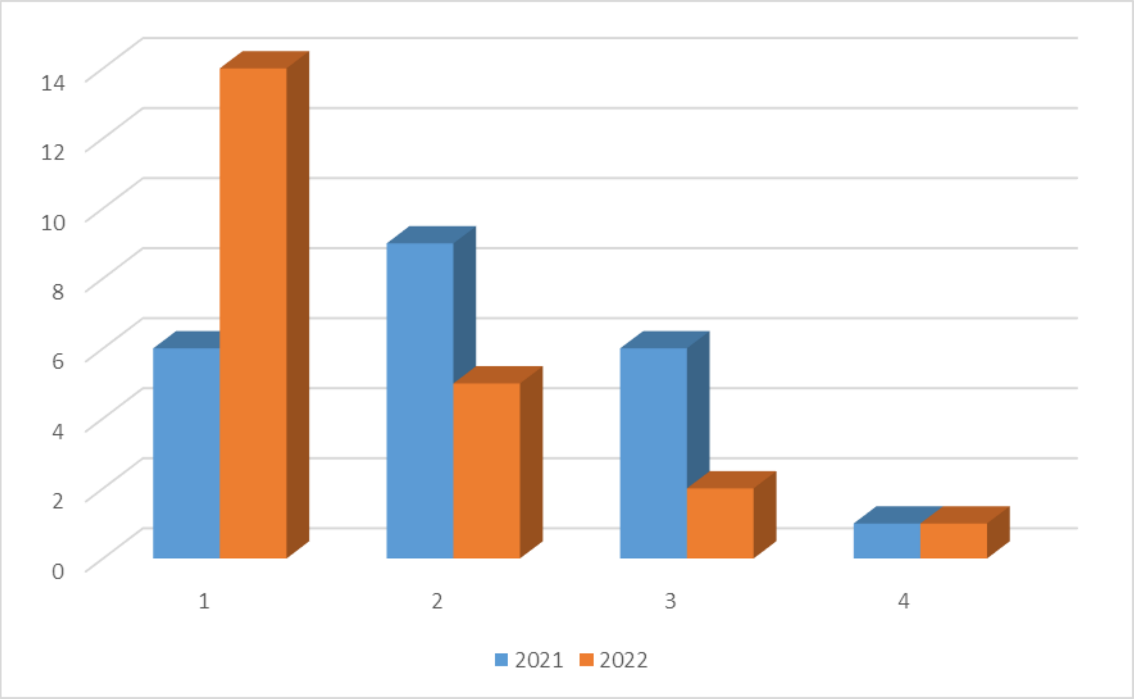

Figure 1. Radiographic grade results

Conclusions

Results demonstrated a role for the novel feed, NAF OsteoSure, on bone growth in young Thoroughbreds. Results support recommending this functional feed to supplement the diet of Thoroughbred broodmares and weanlings. There is a clear commercial application for this novel feed, with the potential to improve sales income and racing performance. High radiographic grades would be expected to be clinically observed in the gait of weanlings. By significantly reducing grades, use of the novel feed in mares and weanlings supports the social license of racing by maintaining soundness in young Thoroughbreds. Study limitations include that stallion choice could not be standardized each year. However, heritability of bone disorders is considered low, with the dam's environment and nutrition potentially more significant (Russell et al 2017). Sales income and racing results were also outside the scope of this trial; a follow up study of these parameters would make an interesting area of future research.

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A preliminary study investigating the effect of rug-wearing on measured stride length in horses

M. Troup[1,2], S. Charlton[1], N. Routledge[1], J. Selfridge[3], A. Hunnisett[1]
[1]Mctimoney College of Chiropractic, 2 New York Street, Manchester M1 4HJ, UK
[2]McTimoney Animal Association/Private Practice
[3]McTimoney Animal Association/Private Practice

Presenting author
Melissa Troup. E-mail: melissatroup@aol.com

Application

When using rugs on horses, consideration should be given not only to the effects of one rug on physical parameters such as stride length, but also the combined effect of multiple rugs. Layering of rugs appears to affect horses' stride length over time.

Introduction

The use of equine rugs is common practise in colder climates to assist thermoregulation and prevent the horse from reaching the lower critical limit, where the horse's core temperature requires use of energy resources for maintenance. Layering rugs in the stable for warmth is anecdotally common practise (Clayton et al, 2010). Layering of clothing has shown to produce restrictions in human movement, limiting joint angles and causing participants to alter motion strategy (Rahmatalla et al, 2005). This study investigated the effect on the equine musculoskeletal system of the number of rugs worn, as measured by stride length.

Materials and Methods

Riding School horses(n=12), stabled or loose housed from the same yard were selected; 8 geldings and 4 mares, mean age ± s.d. (range) 10.9 +/- 2.91 (5-15)years, mean height ± s.d. (range) 152.5 +/- 16.15 (127-175)cm with no known existing conditions. Horses were divided into two evenly matched groups. Group 1(n=6) wore 1 x 200g rug for four weeks; group 2(n=6) wore 2 x 100g rugs for four weeks. Rugs were taken off only for ridden or walker exercise, and stabled horses wore a different rug for paddock turnout of one hour per day. A digital video camera recorded each horse walk a straight 10m length track in an indoor school at the start and end of a 28-day period. Each walk consisted of 4 passes (2 from right, 2 from left) under three conditions: no rug, 1x 200g rug and 2 x 100g rugs. Markers were placed laterally on the coronet band of each hoof, hair clipped 1cm square for repeatability. Kinovea software measured 5 stride lengths for each limb per condition per horse (n=20). Data was tested for normality (skewness test) and variance, ANOVA-2 factor without replication with post-hoc paired T-Tests Statistical significance $P < 0.05$.

Results

When comparing stride length of different walk conditions for all horses on Day 0 and Day 28 there were significant differences in mean stride length(cm) between walk conditions in all except one instance (table 1). Day 0 (n=12); no rug with 1 rug ($P = 0.0002$); 1 rug with 2 rugs ($P < 0.0001$); no rug with 2 rugs ($P < 0.0001$). On Day 28 (n=12); no rug with 1 rug ($P = 0.0004$); no rug with 2 rugs ($P < 0.0001$) but no significant difference between 1 rug with 2 rugs ($P > 0.05$).

Table 1. Horse stride length (cm) (mean ± s.d.) (n=12) for all horses at each walk condition at Day 0 and Day 28

Walk condition all horses (n-12)		No rug	1 rug	2 rugs
Time period	Day 0	204 ± 29.7^{AC}	202 ± 28.8^{AB}	199 ± 28.7^{ABC}
	Day 28	200 ± 26.0^{AB}	198 ± 26.7^{A}	197 ± 26.5^{B}

Significant differences between mean values within each row are indicated $^{A\ B\ C}$ *(P < 0.001)*

When comparing stride length of the same walk condition for each Group at 0 and 28 days, the results varied between groups. For Group 1 (1 x 200g rug) horses (n=6), there was no significant difference (*P* > 0.05) in stride length for each walk condition at 0 and 28 days. For Group 2 (2 x 100g rug) horses (n=6), there were significant differences in stride length (*P* < 0.0001) for each walk condition at 0 and 28 days (table 2).

Table 2. Horse stride length (cm) (mean ± s.d.) for each Group (n=6) at each walk condition at day 0 and day 28

	Walk condition					
Groups (n=6)	No rug		1 rug		2 rugs	
Group 1 (1 x 200g rug for 28 days)	day 0: 199 ± 29.2 day 28:199 ± 26.7	*P* > 0.05	day 0: 197 ± 27.0 day 28:196 ± 26.6	*P* > 0.05	day 0: 194 ± 26.4 day 28:195 ± 27.2	*P*>0.05
Group 2 (2 x 100g rugs for 28 days)	day 0: 209 ± 29.9 day 28:201 ± 26.0	*P* < 0.0001	day 0: 207 ± 30.2 day 28:200 ± 27.3	*P* < 0.0001	day 0: 204 ± 30.4 day 28:199 ± 26.2	*P*<0.0001

Conclusions

The physical number of rugs worn significantly affects walk stride length (*P<0.001*). Stride length decreases as the number of rugs increases. Wearing one rug decreases stride length compared with not wearing a rug. Wearing two rugs further decreases stride length in comparison to wearing only one rug. Wearing one rug(1x200g) daily over a 28-day period, does not significantly reduce stride length compared to day 0 *(P>0.05*). However, wearing two rugs(2x100g) over a 28-day period significantly reduces stride length *(P<0.0001)* whether walking with no rug, one rug or two rugs. Wearing rugs and the number of rugs worn has an effect on walk stride length indicating a potential effect on horses' musculoskeletal system. The number of rugs worn daily over a 28-day period, (two rugs compared to one rug) has a negative effect on walk stride length. Further research is warranted to evaluate further the effects of layering rugs and types of rugs on horses' musculoskeletal system which could potentially affect performance and welfare.

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Influence of lighting on sleep behaviour, circadian rhythm and spontaneous blink rates in stabled riding school horses (Equus caballus)

L. Greening[1], E. Harkin[2], P. Kyriazopoulou[3], Z. Hepplethwaite[4], F. Aragona[5], J. Browne[2], A. Hemmings[4], J. Williams[1], B. Murphy[2]
[1]*Hartpury University, Hartpury House, Gloucester GL19 3BE, UK*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland*
[3]*Equilume, Ladytown Business Park, Naas, Kildare, Ireland*
[4]*Royal Agricultural University, Cirencester, Gloucestershire GL7 6JS, UK*
[5]*University of Messina, Piazza Pugliatti, 1, 98122 Messina ME, Italy*

Presenting author
Barbara Murphy. E-mail: barbara.murphy@ucd.ie

Application

The study supports the use of a red/white light system to standardize the light/dark cycle without negatively influencing sleep behavior or circadian synchrony of stabled horses. It also supports the importance of distinct and routine light/dark periods to promote wellbeing of stabled horses.

Introduction

Modern horse husbandry can involve significant time spent indoors, often in suboptimal lighting conditions and with frequent night-time disturbances by humans for management purposes (Lesimple et al. 2016). The aim of this study was to investigate the influence of a customised light-emitting diode (LED) lighting system and a standard fluorescent lighting fixture on equine sleep behaviours, circadian rhythmicity, and spontaneous blink rates in horses.

Materials and Methods

Ten riding school horses experienced two stable lighting conditions for four weeks each in a cross-over study running from January to March 2023 (Figure 1) at an Equestrian Centre in Gloucestershire, UK. The treatment lighting consisted of an LED system that provided timed, blue-enriched white polychromatic light by day (peak wavelength 460 nm and mean light intensity 487.8±41.5 lux) and dim red light (peak wavelength 620 nm and mean light intensity 9.7±0.2 lux) at night. Lights transitioned gradually over 20 min from red to white at dawn (07:20) and white to red at dusk (20:20) to give approximately 13 h of daytime light when in use. Control lighting was a fluorescent tube (peak wavelength 560nm and mean light intensity of 227.8±7.5 lux) that was turned on and off manually morning and evening.

Figure 1. Images of the stable lighting for A) Treatment nighttime (Equilume® Stable Light), B) Treatment daytime (Equilume® Stable Light), and C) Control daytime (fluorescent strip light)

During week 4 of each experimental period, spontaneous blink rate was recorded twice for 30 min by securely attaching Go-Pro Hero 10 cameras (7 x 5 x 4 cm Go-Pro Incorporated) to the headcollars of each horse individually. Analysis via Shapiro-Wilk's test indicated that the data sets were normally distributed and Paired t-tests were used to compare mean values of full blinks under both lighting systems. Behaviour of horses in their stables was recorded using Hikivision CCTV equipment comprising a 16 channel 4K Power over Ethernet (PoE) Network Video Recorder (NVR) against a pre-defined ethogram (Greening et al. 2021) and focal continuous sampling for 72 consecutive hours. Statistical differences between lighting conditions (Treatment versus Control) were assessed using paired t-tests or Wilcoxon tests where data were shown to be normally or not normally distributed, respectively. One-way repeated measures (RM) ANOVA or a Friedman's test was conducted to determine the impact of the lighting period (Day or Night) on the horses' behaviour depending on data normality. Mane hair samples complete with follicles were collected at 4-h intervals for 52 consecutive hours, following which samples were analysed in the lab to identify expression of circadian clock genes. RM two-way ANOVA was used to assess the effect of time, treatment, and time x treatment interaction on gene expression in all horses. All data were analysed using GraphPad Prism version 10.3.1 for Windows, presented as means (± S.E.M.) and considered statistically significant if $P<0.05$.

Results

No differences were detected for total sleep, lateral or sternal recumbency, wakefulness, standing, standing sleep, or spontaneous blink rate ($P>0.05$), between lighting conditions (Table 1). The lighting period (Day versus Night) had an effect on total sleep ($P<0.01$) (Figure 1), total recumbency ($P<0.01$), wakefulness ($P<0.01$), and standing sleep ($P<0.05$) in both conditions. For the treatment condition only, higher wakefulness was recorded during Day ($P<0.05$) (Table 1). An overall effect of time for clock genes *PER2* and *DBP* was detected ($P<0.01$), but there was no effect of treatment, or time by treatment interaction. Cosinor analysis detected significant 24-h rhythmicity for *PER2* and *DBP* ($P<0.01$) in both lighting conditions.

Table 1 Comparison of behaviour patterns for all horses (n=10) between Treatment and Control lighting conditions and between Day and Night.

	LED Lighting Treatment				**Lighting Period**		
	Control (n=10)	Treatment (n=10)	P-value		Day (n=10)	Night (n=10)	P-value
Behavioural parameter	Duration of behaviours in hours Mean ± SEM				Duration of behaviours in hours Mean ± SEM		
Total Sleep[a]	3.04±0.57	3.02±0.53	0.94		0.43±0.12	2.62±0.42	<0.01
Total Recumbency[b]	2.62±0.39	2.57±0.38	0.77		0.06±0.01	2.42±0.37	<0.01
Sternal Recumbency[c]	2.04±0.28	2.02±0.28	0.70		0.05±0.01h	1.89±0.26	<0.01
Lateral Recumbency	0.58±0.2	0.55±0.18	0.76		0.005±0.003	0.53±0.18	0.02
Wakefulness	12.41±0.58	11.93±0.37	0.25		6.86±0.28	5.05±0.31	<0.01
Standing	3.46±0.59	4.29±0.64	0.11		2.01±0.39	2.05±0.24	0.87
Standing sleep	1.34±0.45	1.23±0.43	0.79		0.40±0.12	0.98±0.27	<0.01
Out of stable	1.68±0.14	1.94±0.11	0.15		1.80±0.11	0±0h	N/A
Unknown	2.09±0.68	1.63±0.61	0.47		0.64±0.24	1.24±0.48	0.06

P-value of <0.05 was considered significant
LED: Light emitting diode, SEM: standard error of means;
[a]Total Sleep includes the following behaviours: Sternal Recumbency (Sleep), Lateral Recumbency and Standing (Sleep)
[b]Total Recumbency includes the following behaviours: Sternal Recumbency (Sleep), Sternal Recumbency (Awake) and Lateral Recumbency
[c]Sternal Recumbency includes the following behaviours: Sternal Recumbency (Sleep), Sternal Recumbency (Awake)
*Not Applicable (N/A); The horses were not out of the stable during the night.

Conclusions

Results imply that dim red light at night does not negatively impact levels of arousal, normal sleep patterns, or circadian rhythmicity. Blue-enriched LED light may promote increased wakefulness during daytime in stabled horses. These results contribute to our understanding of how stable lighting can promote healthy sleep behaviour patterns and optimise circadian health in horses and provide a foundation to inform future research and practice in this important area.

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Genomic study of digital dermatitis progression in dairy heifers

E. Tarsani[1], B. Li[1], A. Anagnostopoulos[2], A. Sato[2], A. Gillespie[2], K. Bao[2], N. Siachos[2], M. Coffey[1], A. Psifidi[3], G. Oikonomou[2], G. Banos[1]
[1]Scotland's Rural College (SRUC), Department of Animal and Veterinary Sciences, West Mains Road, Edinburgh EH9 3JG, UK
[2]University of Liverpool, Department of Livestock and One Health, Institute of Infection, Veterinary and Ecological Sciences, Liverpool L69 3BX, UK
[3]Royal Veterinary College, 4 Royal College St, London NW1 0TU, UK

Presenting author
Eirini Tarsani. E-mail: Eirini.Tarsani@sruc.ac.uk

Application

A novel phenotype for Digital Dermatitis (DD) progression over time in dairy heifers is assessed. Results reveal a heritable trait that has the potential to be amenable to selection, thereby contributing to disease control.

Introduction

Digital Dermatitis (DD) is an infectious, polymicrobial claw disease. The condition primarily affects the skin of heels and causes pain, discomfort and lameness in cattle compromising animal welfare and incurring substantial financial losses (Schöpke et al., 2015). Key control measures include early diagnosis and topical treatment (Schöpke et al., 2015). Tools for genetic selection for enhanced resistance to DD occurrence are also available (AHDB, 2024). Most genetic studies to-date have focused on DD status at given time-points but the progression of the disease over time in individual animals has not been addressed. The objectives of the present study were to (i) develop and assess a DD progression phenotype based on longitudinal data and (ii) examine the genetic profile of the new trait.

Materials and methods

A prospective study was conducted on 921 Holstein heifers that were clinically examined individually four times, approximately every 3 weeks, by two veterinarians. The study ran from March 2021 to June 2024. During examination, the hind legs of the animals were lifted and assigned a DD score according to the M-stage system. Scores were M0 (healthy foot), M1 (active lesions <2 cm in diameter), M2 (active lesions $\geq$2 cm in diameter); M3 (healing M2 covered with a scab), M4 (chronic lesions), and M4.1 (M4 with new M1 within perimeter). In the first instance, we focused on 670 heifers that were healthy (M0) in the first examination. DD progression across the four examinations was defined in three phenotypic classes: (1) heifers that remained healthy (57% of all animals); (2) heifers that developed DD lesions but recovered to M0 by the last examination (14%); (3) heifers that developed DD lesions and did not recover by the last examination (29%). Pedigree records and genome-wide genotypes were available from the UK national dairy genetic evaluation. Animals were genotyped with multiple density genome-wide arrays. After quality control (call rate=0.90, Minor Allele Frequency=0.05 and Linkage Disequilibrium pruning) 39,816 autosomal Single Nucleotide Polymorphisms (SNPs) were retained. A single-step genome-wide association analysis was conducted on the new DD progression phenotype (1-3). The model included the fixed effects of group and scorer as well as the combined genomic and pedigree relationship matrix among individual animals. The BLUPF90 software (Misztal et al., 2014) was used.

Results

The genomic inflation factor was nearly unity (λ=0.99) suggesting absence of systematic bias attributed to population structure or analytical approach. The heritability estimate for DD progression was 0.27 ($\pm$0.09). No single SNP attained genome-wide or suggestive significance after multiple-test Bonferroni correction (Figure 1). The highest percentage of trait genetic variance (1.1%) was explained by a 1-Mb window on chromosome 11 (85,075,162-86,061,595 bp), illustrated in Figure 1. A protein coding gene (*TRIB2: tribbles pseudokinase 2*) was detected within the region. *TRIB2* reportedly plays diverse roles in neurological disorders, metabolic diseases, autoimmune and inflammatory diseases, arthritis, and cancer types in humans (Fang et al., 2021). In cattle, *TRIB2* has been reported as a potential regulator of target genes during ovarian follicular development (Warma et al., 2021).

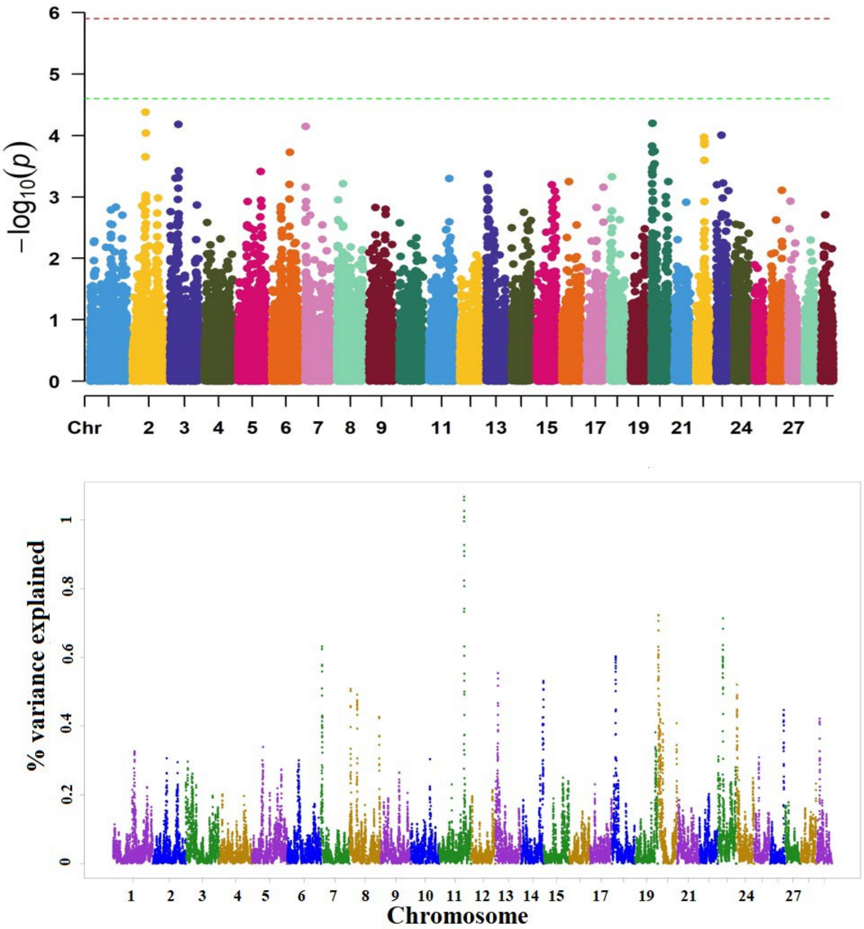

Figure 1. Manhattan plot showing the $-\log_{10}$(P-values) of SNPs across the 29 autosomes for the examined trait (top). Proportion of trait genetic variance explained by 1-Mb windows on bovine chromosome 11 (bottom).

Conclusion

Our study presents a novel phenotype of DD progression in Holstein heifers that is heritable and largely polygenic and can be improved with genetic selection. Future research to independently validate results, refine phenotype definition, and evaluate the correlation of disease progression with other animal traits is warranted.

Acknowledgments

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Selection of discriminant markers in sheep breeds for breed assignment and traceability purposes

A. Tsoureki[1], E. Pavlou[1], M. Kyritsi[1], A. Argiriou[1,2], S. Michailidou[1]
[1]*Centre For Research And Technology Hellas - Institute Of Applied Biosciences, Charilaou-Thermi Rd, 57001 Thermi, Thessaloniki, Greece*
[2]*University of the Aegean - Department of Food Science and Nutrition, Mitilini 811 00, Greece*

Presenting author
Antiopi Tsoureki. E-mail: adatsoureki@certh.gr

Application

The selected set of genetic markers can be applied in breeding schemes for breed assignment and identification of origin. Moreover, it can be applied throughout the dairy production chain for adulteration detection.

Introduction

The breeding of cosmopolitan, high-yield sheep breeds worldwide has led to the admixing of national flocks in many countries, resulting in genetically mixed populations. This raises concerns about the accuracy of genetic improvement programs of indigenous breeds and on the protection of Protected Designation of Origin and Protected Geographical Indication products, which in many cases are tied to specific breeds. While pedigree data can offer valuable information into the origin of individuals, they are often incomplete or inaccurate, necessitating the use of alternative methods for breed assignment. Genetic markers serve as the best alternative, and can be used both in the field and across the food production chain. In this context, we analysed the most numerous Greek sheep breeds and two cosmopolitan dairy breeds that are frequently reared in Greece in large flocks, to identify genetic markers that can discriminate them.

Materials and Methods

We genotyped 362 sheep samples originating from nine Greek sheep breeds (Boutsko, Chios, Kalarritiko, Karagkouniko, Katsika, Lesvou, Pelagonias, Serres, Thrakis) and two cosmopolitan dairy breeds (Assaf, Lacaune) using different versions of Illumina's OvineSNP50K bead arrays (v1, v2, and v3). After retrieving only the SNPs common among all versions (N=53,137 SNPs), quality filtering was performed in PLINK v1.90 (Chang et al., 2015), by excluding SNPs with call rate <98%, minor allele frequency <1%, those deviating from Hardy-Weinberg equilibrium (p-value $1x10^{-6}$), those lacking genomic location, and mitochondrial SNPs. Moreover, animals with genotype missingness >10% were excluded. In order to identify discriminatory markers, we applied two different methodologies i) calculation of FST value for each SNP with the "--fst-within" command in PLINK v1.90; SNPs at a threshold corresponding to the 0.995 percentile of the total distribution were acquired, and ii) identification of discriminatory SNPs using the Toolbox for Ranking and Evaluation of SNPs (TRES) software (Kavakiotis et al., 2015); evaluation of SNPs was performed using the Informativeness for Assignment method. Evaluation of discriminatory SNPs was conducted in GeneClass2 software (Piry et al., 2004) by assigning or excluding populations as origin of individuals based on the discriminatory SNPs using frequency-based criteria, enabling Monte Carlo resampling with a number of simulated individuals of 1,000 and a type I error (alpha) threshold of 0.001. Population structure based on the two SNP sets was examined through principal component analysis (PCA). Moreover, discriminant analysis of principal components (DAPC) was employed, to further examine the genetic structure of the breeds and the power of the two SNP sets to discriminate between them, using the "adegenet" R package (Jombart and Ahmed, 2011). For DAPC, the number of clusters was set to the number of breeds and the optimal number of PCs was selected by performing cross-validation, as that achieving the lowest mean squared error.

Results

Based on FST and the Informativeness for Assignment method, 249 and 200 SNPs, respectively, were selected for breed discrimination. Both sets performed comparably in terms of assignment of individuals, with both of them assigning correctly >96% of individuals, irrespective of the criteria applied. Four and 6 individuals were identified as admixed animals based on the 249 and 200 SNP set, respectively. In both cases, these individuals belonged to Serres and Laucane breeds, while for the 200 SNP set one animal belonged to Karagkouniko breed. PCA captured in the first three components 50.04% and 51.23% of the breeds' genetic variation based on the 200 and 249 SNP sets, respectively. Further evaluation of the genetic structure of the breeds revealed that both sets were able to clearly discriminate Chios, Pelagonias, and Assaf breeds. In addition, Boutsko and Kalarritiko breeds formed a separate, partially overlapping cluster, as expected according to historical records and their geographic distribution. The rest of the breeds formed a relatively tight cluster, at the edges of which were located Lesvou and Laucane breeds, presenting little overlap with the rest of the cluster's breeds and showing hints of differentiation. For DAPC, the first 40 and 100 components for the 200 and 249 SNP sets, respectively, were retained after cross-validation, along with 10 discriminant functions, conserving 48.5% and 84.7% of the variation in the two sets, respectively. DAPC confirmed PCA's clustering of the breeds. However, based on the 249 SNP set, DAPC revealed a clearer clustering of the previously overlapping breeds. Specifically, breeds Katsika, Lesvou, and Serres formed more distinct, albeit closely located to the main cluster, groups, possibly due to the higher degree of variation captured by DAPC, compared to PCA. Additionally, based on the discriminant functions retained during DAPC for each SNP set, all individuals were correctly assigned to their respective breed for both SNP sets, with individual membership probabilities >0.995, with the exception of one sample belonging to Serres breed for the 200-SNP set, suggesting its admixed genetic background.

Conclusions

In this work we identified two reduced SNP sets tailored to differentiate Greek sheep breeds from one another as well as from two cosmopolitan dairy breeds (Assaf and Lacaune). These SNP sets can be utilized in breeding schemes for genetic improvement and conservation strategies of indigenous breeds. In addition, results can be integrated into routine laboratory analysis in the food industry for traceability purposes, leveraging innovative molecular technologies, such as amplicon-based sequencing or the development of a custom SNP panel.

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The influence of genetic merit and country of origins on feed intake and digestibility in lambs post-weaning

P. McCarron[1]
[1]Teagasc, Oak Park, Carlow R93 XE12, Ireland

Presenting author
Patrick McCarron. E-mail: Patrick.McCarron@teagasc.ie

Application

To enhance the understanding of genetic merit and country of origin may have an influence on feed intake and digestibility in lambs post-weaning.

Introduction

In Irish sheep systems a large proportion of lambs are dependent on perennial ryegrass (*Lolium perenne* L.; PRG) based swards during the post-weaning period. Selection of lambs that are genetically pre-disposed to have a higher dry matter intake (DMI) and more efficient digestion of this diet would lead to improved lamb growth performance during this time. Optimal lamb growth performance on a PRG based diet has the potential to improve live-weight gain relative to DMI and digestibility of the diet. The objective of this study was to determine if animal genetic merit and country of origin influence DMI and digestibility of PRG.

Materials and Methods

Twenty-four entire male lambs from the Teagasc, Ireland New Zealand across breed animal comparison study (INZAC flock; Fetherstone *et al.,* 2023) were selected. Animals represented three genetically diverse groups selected on maternal breeding objectives consisting of: elite genetic merit Irish (High Irish), low genetic merit Irish (Low Irish), elite genetic merit New Zealand (NZ) animals, representing two breed types namely, Suffolk and Texel. Animals were approximately 155 days of age at the beginning of the study. Following selection, animals were housed in metabolism crates for 10 days following an initial 2 day adaption period in individual loose pens. There was an in-crate adaption period of 4 days and a measurement period of 6 days. Individual DMI and faeces output data were recorded daily on individual animals. The animals were provided with zero grazed PRG *ad libitum*. Fresh grass was cut each morning and offered to sheep across two feeds. Herbage refusals were weighed back each morning prior to feeding out. Samples of fresh feed and refusals were collected daily and sward quality analysis was carried out on these samples. Data were analysed using a linear mixed model, PROC MIXED (SAS 9.4. Inst. Inc., Cary, NC).

Results

The average live-weight of animals across all genetic groups was 43.84 kg, with DMI ranging from 1.00 (SD=0.067) to 1.09 (SD=0.067) kg DM/animal per day. The average dry matter of the diet over the recording period was 13.32%. Neutral detergent fibre (NDF) was 401.48 g/kg, acid detergent fibre (ADF) was 240.96 g/kg and crude protein was 171.70 g/kg, on average, over the recording period. Dry matter intake differed by genetic group ($P<0.05$), with New Zealand Texel lambs having a greater DMI of 1.22 ± 0.085 kg DM when compared with High Irish Suffolk (0.95 ± 0.094kg; $P<0.05$) and Low Irish Texel lambs (0.93 ± 0.120 kg; $P<0.05$). Average dry matter digestibility (DMD) of the diet across all three groups for the measurement period was 86%, with Low Irish lambs digesting a greater amount of the feed offered when compared to NZ lambs ($P< 0.05$). Organic matter digestibility (OMD) of the diet averaged 83% across all three groups, with a similar pattern observed to that of DMD. Average digestible organic matter intake per kg LW (DOMI g/kgLW) was 16.88 ± 1.17g/kg LW with no differences observed between the groups ($P>0.05$). Average diet refusal across all groups for the measurement period was 14.48% ± 1.505% with no differences observed between groups ($P>0.05$). Given that the lambs included in this study were of a similar live-weight and age, and also had a similar DMI, the results obtained from this study do not determine vast differences between groups measured. If this study was to be repeated, it should be done over multiple time periods to increase the sample size and duration from which the data is collected. Selecting a group with greater divergence in traits currently recorded on the replacement index such as days to slaughter, may lead to greater differences being observed.

Conclusions

Post weaning lambs from groups of varying genetic merit for maternal traits and country of origin did not display differences in DMI or DMD when provided with a uniform diet and conditions. Variation in these traits could possibly be determined by using a larger sample size and an extended measurement period.

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Candidate genes and pathways associated with bovine tuberculosis through the mRNA-miRNA regulatory network

F. Ghafouri[1,2], V. D. Reyhan[2], M. Sadeghi[2], S. R. Miraei-Ashtiani[2], J. P. Kastelic[3], H. W. Barkema[3], V. Razban[1], M. Shirali[1,4]

[1]Agri-Food & Biosciences Institute, 18a Newforge Ln, Belfast BT9 5PX, UK

[2]Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, 16th Azar St., Enghelab Sq., Tehran, Iran

[3]Faculty of Veterinary Medicine, University of Calgary, 2500 University Dr NW, Calgary, AB T2N 1N4, Canada

[4]School of Biological Sciences, Queen's University Belfast, University Rd, Belfast BT7 1NN

Presenting author

Farzad Ghafouri. E-mail: farzad.ghafouri@afbini.gov.uk

Application

This comparative transcriptome profiling analysis identified associated mRNAs and miRNAs, their regulatory mechanisms, and potential pathways in cows infected with bovine tuberculosis compared to healthy (H) cows.

Introduction

Bovine tuberculosis (bTB), a chronic infectious disease primarily affecting domestic dairy and beef cattle, is caused by *Mycobacterium bovis* (MB), a pathogenic bacterium within the *Mycobacterium tuberculosis* complex (MTBC) (Hall et al., 2021). The economic impact of bTB on the livestock industry is substantial, with estimated losses > $3 billion annually in global agriculture (Waters et al., 2012). Host immune responses to mycobacterial infection involve a complex interplay between innate and adaptive immune systems. Previous transcriptomic studies demonstrated that mRNA expression was significantly altered in bovine monocyte-derived macrophages (MDM) following MB infection. Thus, analyzing the host macrophage transcriptome in response to MB infection is expected to shed light on molecular mechanisms and host-pathogen interactions associated with bTB. Moreover, although non-coding RNAs (ncRNAs), e.g., microRNAs (miRNAs), have crucial roles in regulating immune system function and may offer valuable insights into the disease, their role in MB infection in cattle has not been thoroughly investigated (McLoughlin et al., 2021).

Materials and Methods

RNA-Seq and microarray datasets from blood samples, specifically MDMs of MB-infected and control Holstein dairy cattle, were retrieved from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) public database. Gene set annotation and functional enrichment analysis were conducted to explore hub mRNAs and miRNAs associated with bTB, using the DAVID and STRING databases. This analysis aimed to determine potential functions as well as metabolic and signaling pathways related to the disease. Also, interactions between the RNAs (mRNAs and miRNAs) were predicted and an mRNA-miRNA regulatory network constructed by integrating the protein-protein interaction (PPI) network with the gene regulatory network (GRN). Additionally, genes were assigned to functional categories using the Gene Ontology (GO) database, which includes biological processes (BP), molecular functions (MF) and cellular components (CC).

Table 1. Summary of the GEO accession numbers for RNA-Seq and microarray data sets associated with bTB.

No.	Data Type	GEO a Accession	Platform	Samples (MB:H)
1	Microarray	GSE33309	GPL2112 ((Bovine) Affymetrix Bovine Genome Array)	42 (21:21)

2	Microarray	GSE41401	GPL11649 (Agilent-023647 B. taurus (Bovine) Oligo Microarray v2 (Probe Name version))	12 (6:6)
3	RNA-Seq	GSE45439	GPL15750 (Illumina Genome Analyzer IIx (*Bos taurus*))	14 (7:7)
4	RNA-Seq	GSE60265	GPL15750 (Illumina Genome Analyzer IIx (*Bos taurus*))	16 (8:8)
5	RNA-Seq	GSE62506	GPL15749 (Illumina HiSeq 2000 (*Bos taurus*))	78 (39:39)

Results

Comparative transcriptomics-related analyses identified 3076 and 3229 differentially expressed genes (DEGs) between MB-infected and control Holstein dairy cattle samples for RNA-Seq and microarray datasets respectively, based on a fold change ≥ 1 or ≤ -1, and a false discovery rate < 0.05. Among these, 13 genes were common between transcriptomic profile datasets. In addition to DEGs, 9 and 39 miRNAs were simultaneously identified in the RNA-Seq datasets and literature mining, respectively. Furthermore, we identified 5 hub genes (*CCL4*, *CXCL2*, *IL12B*, *IL1A*, and *RETN*) involved in MB infection. Identified biological and regulatory networks were mainly associated with immune system signature pathways. Gene set annotation and functional enrichment of identified DEGs implicated important biological pathways. In this regard, functional enrichment analysis, based on the mRNA-miRNA regulatory network (Figure 1) revealed 6, 4 and 1 GO terms related to bTB in the biological process, molecular function, and cellular component categories, respectively. In addition, KEGG enrichment analysis identified cytokine-cytokine receptor interaction, tuberculosis, and signaling pathways for chemokine, NF-kappa B and Toll-like receptors.

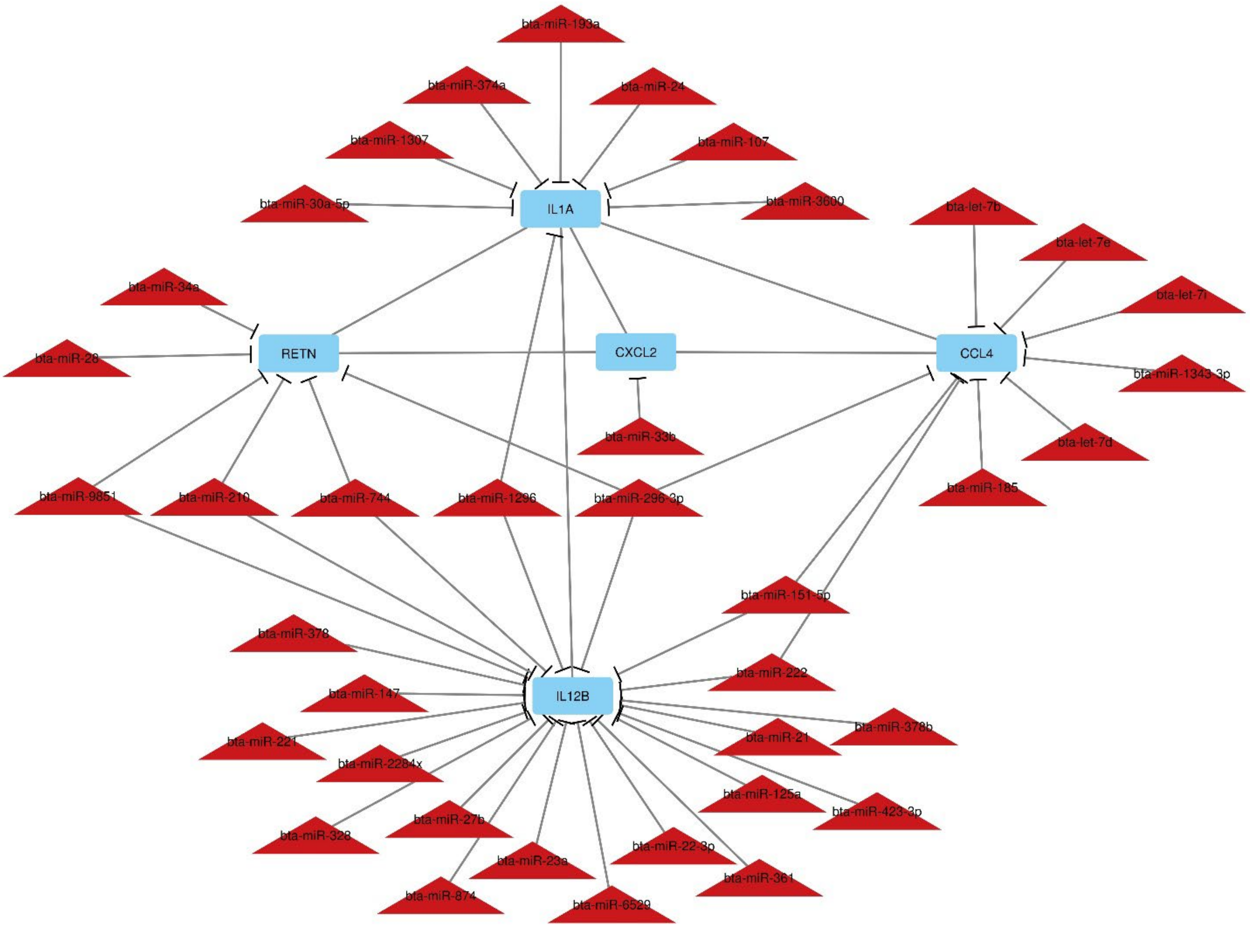

Figure 1. mRNA-miRNA regulatory network on MB infection in dairy cattle.

Conclusions

These findings offered valuable insights into the molecular evidence surrounding the regulatory mechanisms involved in transcriptome profiling of MB-infected Holstein dairy cattle, compared to noninfected controls. Additionally, they are an impetus to explore molecular networks and functions of DEGs associated with blood samples, particularly focusing on MDMs and their role in immune system function. This research provides a foundational starting point for future studies on bovine tuberculosis disease.

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To examine a range of autumn closing dates and initial spring defoliation strategy in a perennial ryegrass and white clover sward to increase overall sward clover content and herbage mass

F. Beamish[1,2], F. Buckley[2], W. Burchill[2], M. Egan[1]
[1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork P61 C996, Ireland*
[2]*School of Biological, Earth and Environmental Sciences, University College Cork, College Rd, University College, Cork, Ireland*

Presenting author
Fiona Beamish. E-mail: fiona.beamish@teagasc.ie

Application

This study documents the impacts of a range of autumn closing dates and initial spring defoliation strategy to inform farmers, advisors and the wider grassland industry on optimal grazing management for grass/clover swards in autumn and spring. The findings offer practical guidance enabling them to make informed decisions to optimize clover persistence and increase herbage mass. By applying these results, stakeholders can improve pasture productivity, clover persistence and enhance the sustainability of grassland systems.

Introduction

The inclusion of *Trifolium repens* (White clover; WC) in pasture-based milk production systems in Ireland continues to be a valuable component enhancing grazed pastures through improved herbage quality, increasing milk production (Dineen *et al*., 2018), and providing a sustainable alternative to nitrogen (N) fertilizer through N fixation (Egan *et al*., 2018). To achieve these benefits, a minimum clover content of 20% in the sward is required (Egan *et al*., 2018). Grazing management is the one of the main determining factors in determining clover content, due to its influence on light availability to the base of the sward, which is required for stolon production (Phelan *et al*., 2014). Autumn closing date/spring opening farm cover (OFC) can have a significant impact on subsequent sward clover content. Murray *et al.* (2022) reported that swards with an OFC > 900 kg DM/ha reduced sward clover content compared to paddocks with a lower OFC (<700 kg DM/ha). Limited research has explored optimal over-winter management practices for perennial ryegrass–WC (PRG-WC) swards, including whether earlier defoliation could help manage heavier over winter covers and support greater clover persistence. The aim of the present study focuses on the effects of varying autumn closing dates and initial spring defoliation strategies on herbage mass and clover persistence.

Materials and Methods

The experiment was conducted at the Teagasc Animal and Grassland Research and Innovation Centre in Moorepark, Fermoy, Ireland, from September 2023 to October 2024. A split-plot design with a 5x2 factorial design, with five autumn closing dates (CD); September 29, very high (VH), October 16 high (H), November 1 medium (M), November 14 low (L) and November 24 very low (VL). With two initial defoliation strategies: February 14 (early) and March 19 (late) resulting in 40 plots across four replicates within a paddock with an initial clover content of 41% prior to the study commencement. Prior to each defoliation strategy, sward clover content was measured by taking three random grab samples across the plot; a 70 g sub-sample was then separated into grass and clover fractions and dried at 90°C for 16 hours to determine DM proportions of each. All plots were defoliated by stimulated grazing. Herbage mass was measured by harvesting the entire plot using an Etesia mower (Etesia Hydro 124D; Etesia UK Ltd.), the sample was weighed and a random grab sample of 100 g was taken dried at 90°C for 16 hours to determine DM content. All data was analysed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002).

Results and Discussion

Closing date had a significant effect on the average DM yield (*P*<0.05; table 1). The mean herbage mass across five closing dates were 1,518, 1,461, 1,377, 1404 and 1393 kg DM ha^{-1} for VH, H, M, L and VL, respectively. Looney *et al.* (2022) reported a similar impact of closing date with later closing reducing herbage availability the following spring.

Table 1. Mean herbage yields (t DM ha^{-1}) and clover contents (%) as affected by autumn treatment.

	VH	H	M	L	VL	S.E	Autumn Treatment	Spring Treatment	AutumnXRotation
Dry Matter Yield (Kg DM/ha)	1,518^{b}	1,461^{ab}	1,377^{a}	1,404^{a}	1,393^{a}	34.6	<0.05	N.S	<.0001
Clover Content (%)	17.4	16.1	20.6	21.6	21.5	0.016	N.S	N.S	<0.05

Note: Values followed by the same letters are not significantly different (*p* >0.05).

Spring grazing date, early or later, had no significant effect on mean herbage yield (1433 kg DM/ha) or clover content (19%). In contrast, Murray *et al.* (2022) found that higher herbage mass negatively impacted clover persistence. There was a significant interaction for autumn closing date and rotation (*P*<0.05) on sward clover content (Figure 1). The VH and H treatments had the lowest average clover content (17.4% and 16.1%) across all seven rotations, M was intermediate (20.6%), while L and VL had the highest (21.6% and 21.5%). Similar to, Phelan *et al.* (2014), who reported that higher herbage mass in the summer-to-winter period reduces light penetration to the sward base, thereby limiting clover growth.

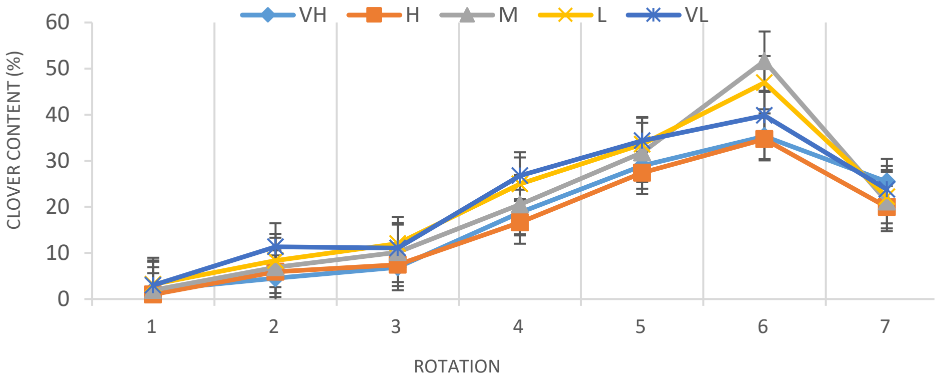

Figure 1. Mean clover content of plots from cut 1 to cut 7 (February 2024-October 2024) with VH (September 29, very high), H (October 16 high), M (November 1 medium), L (November 14 low) and VL (November 24 very low) closing dates.

Conclusion.

Autumn closing date significantly influences DM yield for the following year, with earlier closing promoting greater herbage DM accumulation over winter and higher DM yield in spring, while also negatively impacting sward clover content and persistence throughout the year. Delaying the closing date of high-clover paddocks can help minimize these negative effects on clover persistence while still achieving satisfactory herbage mass.

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The effect of red clover variety on herbage production and persistence in silage swards

S. Kearney[1], T. Tubritt[1], Z. McKay[2], M. Dineen[1], M. Egan[1]
[1]*Teagasc, Moorepark Research & Development Centre, Moorepark, Fermoy, Co. Cork P61 P302, Ireland*
[2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland*

Presenting author

Sinead Kearney. E-mail: sinead.kearney@teagasc.ie

Application

Red clover variety has a significant impact on yield stability and sward red clover content in a mixed perennial ryegrass – red clover silage sward in Ireland. Red clover variety selection is critical for sward persistency and yield.

Key words: Cultivar, perennial ryegrass-red clover, legumes, forage

Introduction

Red clover (*Trifolium pratense* L.; RC) inclusion in perennial ryegrass silage swards can maintain herbage production and feed quality under lower rates of nitrogen (N) fertilisation (Clavin et al., 2016) however, dry matter yield (DMY) and clover content persistency is a key management challenge for RC swards. Although not previously investigated in Ireland, there has been reports that different RC varieties can have a significant impact on sward clover proportion and yield stability across multiple harvest years (Tucak et al., 2009, Marshall et al., 2017). Significant cost savings (Doyle et al., 2024) transpire from RC inclusion however high costs associated with reseeding (O'Donovan et al., 2011), prove cultivar selection for longer term persistence to be critical. The objective of this experiment was to determine the effect of RC variety on herbage production and persistency, in an Irish context.

Materials and Methods

The experiment took place at Teagasc, Moorepark, Co. Cork, Ireland (520 9' N; 80 16' W) over 2 years; 2023 and 2024 (Year 1 & 2, respectively). Field plots (7 x 1.5 m) were established in May 2022; eight RC varieties (Aberclaret, Fearga, Sinope, Spurt, Bonus, Garant, Pastour, and Amos (T)) were sown (10 kg/ha) with two perennial ryegrass varieties Gracehill (12 kg/ha) and Astonconqueror (12 kg/ha). Nitrogen, phosphorus and potassium was applied at a rate of 75 kg N ha^{-1}, 35 kg P ha^{-1} and 375 kg K ha^{-1} per year, respectively, split across each defoliation. Plots were defoliated on three occasions (May, July and August/September) using an Agria 3600 BM mower to a post-cut height of 6 cm. Dry matter herbage yield was determined by weighing fresh harvested material and drying a 100 g subsample at 90ºC for 16 h to determine DM content. Red clover content was determined at each silage cut by separating a 200 g subsample into grass and RC proportions and drying at 90ºC for 16 h to determine DM content. Data analysed using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002); variety, cut number and associated interactions were included as fixed effects, repetition was included as a random effect and plot as subject.

Results and discussion

Variety had a significant ($P < 0.001$) effect on annual DMY, similar to Marshall et al. (2017) who reported significant differences between varieties. Aberclaret and Fearga (15543 kg DM ha^{-1}, SE ± 322.8) had the greatest DMY followed by Spurt, Sinope, Amos, Pastour, Garant and Bonus (14372, 13873, 13727, 13724, 13005 and 12965 kg DM ha^{-1}, respectively). There was a significant ($P < 0.001$) interaction between variety and year on annual DMY (Figure 1). Despite a significant ($P < 0.001$) yield reduction across all varieties from an average 16500 kg DM ha^{-1} in year 1 to 11500 kg DM ha^{-1} in year 2, Fearga, Aberclaret and Pastour declined the least at 19, 24 and 25%, respectively indicating greater yield stability while, Spurt, Amos, Sinope, Garant and Bonus reduced by 34, 36, 35, 32 and 42%, respectively.

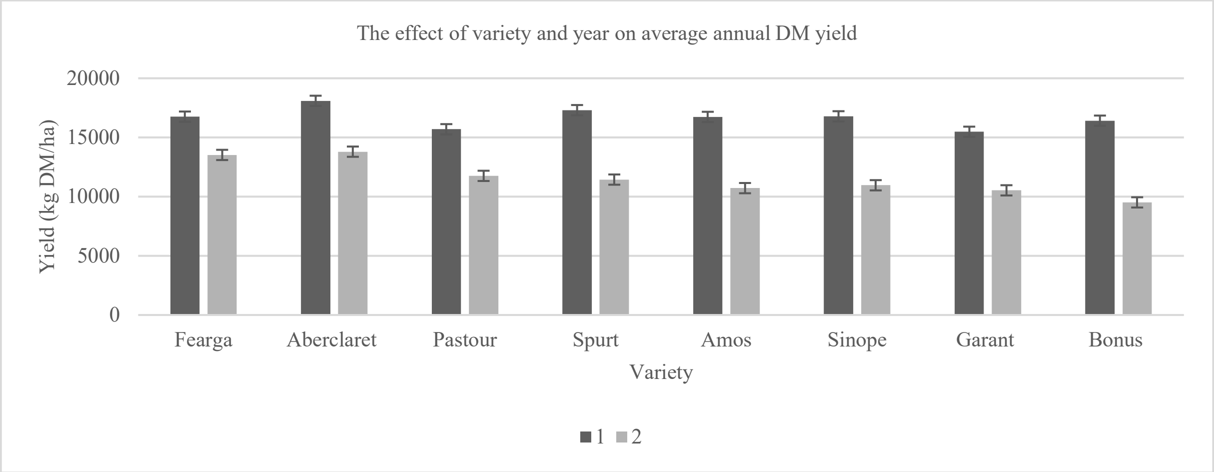

Figure 1. The effect of variety and year on average annual DM yield.

Variety had a significant effect (*P* < 0.001) on average clover content; 749, 734, 630, 618, 617, 596, 521 and 442 g/kg DM ± 26.1 for Aberclaret, Fearga, Spurt, Pastour, Amos, Sinope, Garant and Bonus, respectively. There was a significant interaction (*P* < 0.001) between variety and year on average annual clover proportions (Table 1). Fearga, Aberclaret and Pastour had the same clover content in Year 1 and 2, while all other varieties reduced significantly (by 26, 26, 29, 41 and 68% for Spurt, Amos, Sinope, Garant and Bonus, respectively). Similar to Tucak et al. (2009) and Marshall et al. (2017) who reported varietal effects on persistence over multiple production years.

Table 2. Average annual red clover content.

Variety	Clover Content (g/kg DM)	
	Year 1	Year 2
Aberclaret	739	759
Fearga	684	783
Pastour	659	576
Amos	709	526
Bonus	669	216
Garant	653	389
Sinope	697	494
Spurt	724	535

Standard error in year 1 and year 2 ± 32.7

Conclusion

Red clover variety has a significant effect on yield stability and sward red clover content of a mixed perennial ryegrass – red clover silage sward in Ireland. To ensure long term persistence of red clover in a commercial setting, varieties must be evaluated over a number of years. In the current study Aberclaret and Fearga were consistently the highest performing varieties in terms of dry matter production and sward red clover content.

Acknowledgements

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Short- and long-term consequences of epizootic haemorrhagic disease on dairy cattle health, production, and reproduction

I. C. Gómez[1], J. Lozano[2], L. Muñoz-Fernández[3], D. Cano-Terriza[4], M. Á. Risalde[3], J. M. Sánchez[5]
[1]Department of Animal Reproduction, INIA-CSIC, Avda. Puerta de Hierro km 5.9, 28040 Madrid, Spain
[2]Livestock operation "Las Rozuelas del Valle", Calle el guijo 10 14410,Torrecampo, Córdoba Spain
[3]Department of Comparative Anatomy, Pathology, and Toxicology, GISAZ-ENZOEM, University of Cordoba, Avenida Medina Azahara, 5, 14071, Córdoba, Spain
[4]Department of Animal Health, GISAZ-ENZOEM, University of Cordoba, Avenida Medina Azahara, 5, 14071, Córdoba, Spain
[5]Department of Agricultural Engineering and Food Technology, IFAPA

Presenting author
Inmaculada Cuevas Gómez. E-mail: inmaculada.cuevas@inia.csic.es

Application
This study highlights the potential losses in dairy cattle health, production, and reproduction caused by epizootic haemorrhagic disease, an emerging vector-borne disease in Europe. Understanding its impact and possible long-term effects is essential for developing effective strategies to monitor, control, and prevent future outbreaks, thereby enhancing farm efficiency and sustainability.

Introduction
Epizootic haemorrhagic disease (EHD) is a reportable, vector-borne disease caused by an *Orbivirus* related to the bluetongue virus. This uncommon disease in Europe first emerged in Italy and Spain in the autumn of 2022 with the little-known serotype 8 (EHDV-8), primarily affecting cattle and deer with nervous, respiratory, reproductive, and vascular disorders. Since then, infection has spread across Spain and reached Portugal and France in 2023. In Europe, the emergence of this poorly understood disease is concerning, as climate change is contributing to the northward expansion of *Culicoides* vector habitats, increasing the risk of disease transmission in new regions. Additionally, this is the first time that EHDV-8 has been associated with reproductive disorders but its short- and long-term effects remain largely unquantified. Thus, this study aimed to evaluate the impact of EHDV-8 infection on health, reproduction, and milk production parameters in dairy cattle.

Material and methods
From July to October 2023, an EHDV-8 outbreak was detected by PCR on a commercial dairy farm with 520 milking cows in southern Spain. Clinical sings observed included fever, nasal petechiae, seropurulent discharge, reproductive failure, and mortality. Blood samples were collected from 233 animals (≥13 months old) in February 2024, outside the main activity season of the *Culicoides* vector. Serum samples were analysed for VP7 protein of EHD specific antibodies using a commercial competitive sandwich ELISA kit (ID Screen® EHDV Competition, IDvet, Francia). Additionally, we collected health, milk production and reproduction data from the farm databases spanning from 2018 to 2024. Milk production parameters included daily milk yield per cow, daily milk fat percentage per cow, and daily milk protein percentage per cow. Health parameters included the percentage of cows treated for respiratory (nasal discharge, petechiae, tachypnoea), digestive (diarrhoea, indigestion, rumen atony) and other symptoms (lameness, acute milk drop production), as well as the involuntary loss rate of cows. Reproductive parameters included the percentage of cows with retained placenta, and pregnancy loss (PL) rate and preterm birth (PB) rate (≤270 days gestation length) for both cows and heifers. Two key periods were established for analysis: (i) July-October, the period when the EHDV-8 outbreak was reported, where data from July-October 2023 were compared with the same period in the previous five years (2018-2022) to assess short-term impact, and with July-October 2024 to assess long-term impact; (ii) November-June, to compare data from the five years preceding the outbreak (2018-2023) with the period immediately after (2023-2024), evaluating

long-term consequences of EHDV-8. Statistical comparisons were made across the different time periods using SAS software. For continuous variables, non-parametric Wilcoxon-rank test was applied since assumptions of normality and/or homoscedasticity were not met. Categorical variables were analysed through logistic regression.

Results and Discussion

Out of the 233 animals tested, 226 (97%) were positive for EHDV-8 antibodies, 2 were inconclusive, and 5 were negative. Comparisons of milk production, health, and reproductive parameters before, during, and after the EHDV-8 outbreak are summarized in Table 1.

Table 1. Milk production, health and reproductive parameters before, during, and after EHDV-8 outbreak in a commercial dairy cattle farm.

Production, health, and reproductive variables	**July to October period**				**November to June period**		
	Pre-EHD 2018-23	**Outbreak 2023**	**Post-EHD 2024**	***P-value***	**Pre-EHD 2018-2023**	**Post-EHD 2023-2024**	***P-value***
Daily milk yield per cow (L)	35.3 ± 0.89^{a}	32.4 ± 2.37^{b}	35.1 ± 2.62^{a}	***<0.001***	37.2 ± 1.17	37.3 ± 1.00	*>0.05*
Daily % milk fat per cow	3.53 ± 0.16^{a}	3.47 ± 0.24^{b}	3.73 ± 0.07^{a}	***<0.001***	3.7 ± 0.14	3.8 ± 0.09	*>0.05*
Daily % milk protein per cow	3.24 ± 0.06	3.20 ± 0.13	3.27 ± 0.07	*>0.05*	3.3 ± 0.11	3.3 ± 0.10	*>0.05*
% cows treated (resp. symptoms)	9.5^{a}	47.7^{b}	6.4^{c}	***<0.001***	12.0^{a}	5.1^{b}	***<0.01***
% cows treated (digestive symptoms)	8.8^{a}	15.3^{b}	4.8^{c}	***<0.001***	19.7^{a}	11.8^{b}	***<0.01***
% cows treated (other symptoms)	2.5^{a}	5.2^{b}	1.1^{a}	***<0.001***	3.0	2.4	*>0.05*
Involuntary loss rate of cows (%)	2.0^{a}	6.9^{b}	2.4^{a}	***<0.001***	3.8	2.6	*>0.05*
% cows with retained placenta	8.9^{a}	14.4^{b}	6.8^{a}	***<0.05***	8.5	7.3	*>0.05*
Pregnancy loss rate in cows (%)	30.2^{a}	50.0^{b}	26.0^{a}	***<0.01***	18.4	17.2	*>0.05*
Pregnancy loss rate in heifers (%)	7.5^{a}	21.3^{b}	4.0^{a}	***<0.01***	7.8	7.9	*>0.05*
Preterm birth rate (≤270 d gestation, %)	15.3^{a}	32.1^{b}	13.1^{a}	***<0.001***	11.8	8.5	*>0.05*

^{a,b}Within a row, medians/percentages with different superscripts differ significantly ($P< 0.05$). Milk parameters are expressed as median ± IQR. PP, postpartum; EHD, epizootic haemorrhagic disease.

This study provides the first description of EHDV-8 impact on milk production, health, and reproduction in dairy cattle. During the outbreak, milk production and milk fat percentage decreased, and health issues and reproductive problems increased compared to pre-outbreak levels, but these parameters returned to pre-outbreak levels by 2024. While no persistent long-term effects were observed (with November-June and July-October values post-outbreak comparable to the previous five years), some subtle impact may have been masked as cows that did not recover after the outbreak, as well as those with remaining reproductive issues, were among the first to be replaced, potentially introducing a bias in the assessment of long-term EHDV-8 consequences. Moreover, it is important to consider that the progressive improvement in the herd's genetic index, including health, reproductive, and production traits, may also mask potential long-term effects of EHDV-8.

Conclusion

The EHDV-8 outbreak caused short-term losses in milk yield, health, and reproductive performance, previously unreported for this serotype. Long-term recovery of production, health, and reproductive parameters was observed relative to pre-outbreak levels. However, potential biases from selective replacement of affected animals during the outbreak and genetic progress may underestimate the outbreak’s enduring effects. Continued monitoring of production and reproductive performance is recommended to better understand the long-term impact of EHD.

Financial support statement

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Epizootic haemorrhagic disease in beef bulls: evaluation of seminal transmission, RNAemia persistence, and impact on sperm quality

J. A. Bravo[1], I. C. Gómez[2], C. Arrobas[3], A. González[4], M. .L. Aranda[1], A. Domingo[1], J. M. Sánchez[5]
[1]Animal Selection and Reproduction Centre, Censyra, Spain
[2]Department of Animal Reproduction, INIA-CSIC, Avda. Puerta de Hierro km 5.9, 28040 Madrid, Spain
[3]ADVACAS Extremadura S.L, Calle santa maria de la cabeza, 4 - piso 3 k 06004, Badajoz
[4]Department of Animal Health Junta Extremadura, Regional Laboratory, Avenida de la Universidad, s/n. 10003-Cáceres, Spain
[5]Department of Agricultural Engineering and Food Technology, IFAPA

Presenting author
Inmaculada Cuevas Gómez. E-mail: inmaculada.cuevas@inia.csic.es

Application
This study highlights the impact of epizootic haemorrhagic disease, an emerging vector-borne disease in Europe, on sperm quality in beef bulls. Understanding its impact and transmission risk is essential for developing effective control strategies, thereby supporting economic efficiency and sustainability.

Introduction
Global warming contributes to the expansion of *Culicoides* vector habitats into northern regions, increasing the risk of vector-borne disease emergence in new areas. A recent example is the spread of epizootic haemorrhagic disease (EHD) in Italy, Spain, France, and Portugal since 2022, where the little-known serotype 8 (EHDV-8) has caused nervous, respiratory, reproductive, and vascular disorders in cattle and deer. To date, no studies have reported reproductive consequences of EHDV-8 in males. Additionally, seminal transmission of EHDV-8 is unknown; however, other *Orbiviruses*, specifically bluetongue virus serotype 8, have been isolated from bovine semen samples. Therefore, understanding the risk of EHDV-8 transmission through semen, as well as its impact on sperm quality, is essential for implementing effective control measures against this emerging virus. This study aimed to: (i) assess the prevalence of EHDV-8 infection in beef bulls; (ii) determine the persistence of EHDV-8 viral RNA circulation in whole blood (RNAemia); (iii) evaluate the risk of EHDV-8 seminal transmission in bulls with concurrent RNAemia; and (iv) assess the impact of EHDV-8 on the sperm quality of beef bulls.

Material and methods
In this study, beef bulls (n=281; 1.5-10 years old; Limousine, Charolais, and Retinta breeds) from 46 free-range farms in Extremadura, Spain, were evaluated. To assess EHDV-8 prevalence and risk of seminal transmission, blood and semen samples were collected from 144 bulls between October and December 2023, prior to the breeding season and following the EHDV-8 outbreak notification in the area. EHDV-8 RNA detection was performed by PCR on both blood and semen samples. To evaluate RNAemia persistence, monthly blood samples from eight bulls were tested by PCR from July 2023—when all bulls were confirmed EHD-negative—to April 2024. To assess the impact of EHDV-8 on sperm quality, sperm analyses in bulls from October-December 2023 were compared to that of bulls analyzed in the same period in 2021 (n=55) and 2022 (n=74). Following a standardized physical examination, semen samples from bulls that met physical criteria were collected by electroejaculation. Semen analyses included macroscopic and microscopic parameters using Computer-Assisted Sperm Analysis (CASA; ISAS® system). Bulls were classified as satisfactory potential breeders (SPB) if they passed the physical examination and met criteria of ≥ 30% progressive motility and ≥ 70% morphologically normal sperm. Comparisons of sperm parameters were made between pre-EHD bulls (2021-22) and EHDV-8 positive bulls with and without EHD-compatible symptoms (fever, lethargy, redness of nasal and oral mucosa, oral lesions, lameness) during the outbreak. Statistical analyses (SAS software) included t-tests or ANOVA for continuous variables meeting normality and homoscedasticity, with non-parametric

Wilcoxon-rank tests as needed, and logistic regression for categorical variables. Farm was included as a random factor.

Results and Discussion

This study provides the first description of EHDV-8 prevalence, RNAemia persistence, and its impact on sperm quality in beef bulls. Of the 144 bulls tested in 2023, 142 (98.6%) were positive for EHDV-8 in whole blood samples, while only one (0.7%) tested positive in semen samples. Of the 142 EHDV-8 positive bulls, 13 (9.1%) exhibited EHD-compatible symptoms, while the rest were asymptomatic. From the eight bulls evaluated monthly, seven showed prolonged RNAemia (5-6 months) following initial detection, while one bull remained positive for 2 months. Pre-EHD bulls were considered EHD-negative, as no cases were reported in the study area prior to 2023.

Table 1. Comparison of sperm parameters pre-EHD bulls and EHDV-8 positive bulls.

Variable	**Pre-EHD (2021-22)**	**EHDV-8 + No symptoms (2023)**	**EHDV-8 + Symptoms (2023)**	***P-value***	**Pre-EHD (2021-22)**	**EHDV-8 + (2023)**	***P-value***
n	129	129	13	-	129	142	-
Seminal volume (VOL, mL)	5 ± 3.0^{a}	4 ± 2.0^{b}	5 ± 2.5^{ab}	***0.004***	5 ± 3.0^{a}	4 ± 2.0^{b}	***0.002***
Sperm concentration (CONC, spz/µl)	528 ± 451.0^{a}	652 ± 640.0^{b}	449 ± 248.0^{ab}	***0.232***	528 ± 451.0^{a}	634 ± 602.0^{b}	***0.014***
Subjective individual motility	75 ± 20.0	70 ± 30.0	70 ± 30.0	*0.336*	75 ± 20.0	70 ± 30.0	*0.251*
Subjective movement quality (SMQ, 0-5)	3 ± 1.0	3 ± 0.7	3 ± 0.5	*0.455*	3 ± 1.0	3 ± 0.7	*0.744*
Curvilinear velocity (VCL, µm/s)	132 ± 30.9^{a}	124 ± 43.1^{ab}	113 ± 40.2^{b}	***0.038***	132 ± 30.9^{a}	124 ± 43.6^{b}	***0.026***
Straightness index (STR, %)	81 ± 10.4	83 ± 8.5	84 ± 10.5	*0.122*	81 ± 10.4^{a}	83 ± 8.5b	***0.042***
Linearity index (LIN, %)	55 ± 1.1^{a}	60 ± 1.1^{b}	57 ± 2.9^{ab}	***0.007***	55 ± 1.1^{a}	60 ± 0.1^{b}	***0.002***
Wobble index (WOB, %)	69 ± 0.8^{a}	72 ± 0.8^{b}	69 ± 1.9^{ab}	***0.022***	69 ± 0.8^{a}	72 ± 0.7^{b}	***0.010***
Amplitude lateral head displacement (ALH, µm)	4 ± 0.1	4 ± 0.1	3 ± 0.2	*0.114*	3.8 ± 0.07	3.6 ± 0.08^{b}	***0.043***
Beat-cross frequency (BCF, Hz)	10.3 ± 1.90^{a}	9.4 ± 1.70^{b}	10.5 ± 1.00^{ac}	***<0.001***	10.3 ± 1.90^{a}	9.5 ± 1.80^{b}	***<0.001***
Normal sperm (NS, %)	78 ± 15.0^{a}	75 ± 16.0^{a}	60 ± 24.0^{b}	***0.005***	78 ± 15.0^{a}	74 ± 18.0^{b}	***0.029***
Static sperm (EST, %)	18 ± 20.1	16 ± 22.3	23 ± 29.4	*0.222*	18 ± 20.1	17 ± 21.9	*0.901*
Rapid sperm (RAP, %)	69 ± 26.3^{a}	70 ± 26.0^{a}	61 ± 30.0^{b}	***0.071***	69 ± 26.3	68 ± 27.0	*0.785*
Medium sperm (MED, %)	7 ± 5.8^{ab}	6 ± 5.0^{a}	9 ± 6.0^{b}	***0.073***	7 ± 5.8	6 ± 5.0	*0.375*
Slow sperm (SL, %)	5 ± 4.0^{a}	4 ± 4.6^{a}	8 ± 6.3^{b}	***0.010***	5 ± 4.0	4 ± 4.9	*0.158*
Progressive movement (PM, %)	52 ± 20.4	53 ± 18.0	52 ± 30.8	*0.647*	52 ± 20.4	53 ± 18.0	*0.582*
Satisfactory potential breeder (SPB, %)	65.9 (85/129)^{a}	58.9 (76/129)^{ab}	30.8 (4/13)^{b}	***0.049***	65.9 (85/129)	55.6 (79/142)	***0.084***

^{a,b}Within a row, means/medians/percentages with different superscripts differ significantly (P < 0.05) or show a tendency (P = 0.05-0.10). All variables are expressed as median ± IQR, except for LIN, WOB, ALH, expressed as mean ± SEM.

Comparisons of sperm parameters (Table 1) indicated a lower proportion of NS and SPB among bulls during the EHDV-8 outbreak, especially in symptomatic bulls. Additionally, symptomatic bulls exhibited higher proportions of MED and SL sperm. This study focused only on sperm quality rather than complete breeding soundness evaluation, which is important because bulls with severe EHD symptoms likely did not meet the physical examination criteria and may have been excluded from semen collection. Consequently, the impact of EHDV-8 on bull breeding soundness could be even more significant than reported here. This study is the first to identify semen as a potential route for EHDV-8 excretion. However, the presence of the virus in semen is minimal compared to blood, where RNAemia persists for extended periods. Of note, study constraints limited assessing whether viral shedding in semen could be higher in early infection stages, as the infection onset, especially in asymptomatic animals, was unknown. This finding is significant, as current regulations prohibit semen collection, storage, and transport from bulls testing positive for EHDV-8 by PCR in blood, causing delays and economic losses for reproductive centers. Given the lower viral presence in semen compared to blood, revising regulations to prioritize PCR testing in semen over blood samples could help avoid unnecessary disruptions in reproductive activities.

Conclusion

EHDV-8 adversely affects sperm quality in beef bulls, particularly in symptomatic animals. Semen is a potential route for EHDV-8 transmission though viral presence in semen is minimal compared to blood, where RNAemia can persist for 2–6 months.

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Evaluating Rumen Fluid Viability and Adaptation in a Dual-Flow Fermentor: Microbial Stability and Digestibility Over 30 Days

M. Mcdonagh[1], S. Mills[1], C. Thorn[1], D. Hughes[1], V. O'Flaherty[1]
[1]University Of Galway, Galway H91 TK33, Ireland

Presenting author
Michael Mcdonagh. E-mail: michael@glasportbio.com

Application
The rumen is a complex microbial ecosystem where fermentation processes are essential for nutrient breakdown and absorption in ruminant animals. Research into the rumen microbiome and fermentation dynamics is crucial to advancing our understanding of ruminant nutrition, efficiency, and methane emissions. In vitro systems, such as the Dual-Flow Fermentor (DFF), offer controlled environments that allow researchers to study the rumen microbiome and fermentation processes without the variability of live animal models. However, one limitation of in vitro systems is the potential for rumen fluid to lose microbial diversity and function over time. As rumen fluid is continuously removed and replenished with artificial saliva and feed, microbial populations may gradually shift, impacting the physiological representativeness of the model.

Introduction
To ensure that DFF systems provide reliable data in extended trials, it is critical to determine the duration for which rumen fluid maintains stability in terms of microbial activity and physiochemical parameters. This study aims to evaluate the viability of rumen fluid over a 30-day period in a DFF system, providing insights into the point at which microbial composition and fermentation products deviate from those observed in vivo. Additionally, an important objective of this study is to determine the optimal adaptation phase needed to establish stability within the DFF. Traditionally, a seven-day adaptation period is allowed for rumen fluid stabilization before sample collection begins, yet the actual adaptation time required for full microbial and physiochemical stabilization may vary. By assessing microbial activity and physiochemical changes from the start of the experiment, we aim to identify whether a shorter or longer adaptation period might enhance the accuracy and reliability of rumen fluid studies in DFF systems.

Materials and Methods
To achieve the study objectives, three DFF systems were set up, each containing an initial 800 mL of fresh rumen fluid collected from fistulated cows. The donor cows were maintained on a diet similar to that used in the fermentors to ensure consistency. The DFF systems were fed a 50:50 forage-to-concentrate diet, reflecting a balanced diet commonly fed to dairy cows. Daily feeding was conducted twice at 9:00 AM and 4:00 PM, with each fermentor receiving 10g of dry matter (DM) at each feeding time and a total of 20 grams of DM per day. The forage was pre-processed by drying at 60°C for 72 hours and then ground to a uniform particle size using a 2 mm screen. This preparation aimed to standardize the feed particle size and improve consistency across experimental runs. To replicate physiological conditions in the rumen, each fermentor was maintained at 39°C with constant agitation at 150 rpm. Anaerobic buffer was used to maintain pH stability, and artificial saliva was infused at a rate of approximately 122 mL per hour to simulate natural saliva flow. Liquid outflow and solid overflow were set at 88 mL and 44 mL per hour, respectively, corresponding to the 11% and 5.5% passage rates typical of dairy cows. These flow rates aimed to create a realistic simulation of rumen fluid dynamics and nutrient retention time, which are essential for microbial stability and activity. Samples for microbial analysis, physiochemical measurements, and digestibility assessments were collected consistently throughout the 30-day trial, including during the initial

adaptation phase. Microbial samples were analyzed for community composition, tracking bacterial, archaeal, and fungal populations to detect any shifts over time. Physiochemical parameters, including VFA profiles, biogas composition, and pH levels, were measured to monitor temporal changes in rumen fluid stability. Effluent samples were collected daily, freeze-dried, and later analyzed for dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) to assess nutrient digestibility and examine how feed breakdown may change throughout the trial.

Conclusion

This study is designed to simulate rumen conditions as closely as possible in vitro, aiming to align microbial activity, VFA production, and nutrient digestibility with the digestive dynamics observed in live dairy cows. By carefully controlling the liquid and solid flow rates, the DFF system mimics natural passage rates in the rumen, allowing for an accurate representation of microbial and physiochemical changes over time. Ensuring the microbial and physiochemical stability of rumen fluid is essential for generating reliable data in long-term studies. Significant deviations in microbial composition, VFA levels, pH stability, or nutrient breakdown would indicate that the rumen fluid has lost its representational accuracy, limiting the validity of the model beyond a certain time point. The findings from this study are expected to identify both the optimal adaptation phase and the duration over which rumen fluid maintains its viability as a model for in vivo rumen conditions. By evaluating the timeline of microbial and physiochemical changes from the start of the trial this research will support the development of more reliable, long-term in vitro methods for studying rumen microbiology, digestibility, and fermentation processes.

Unveiling the rumen as a reservoir of novel cyclodextrin glycosyltransferases: A versatile enzyme for biotechnology and industry applications

I. de Carvalho Batista Muniz[1,2], O. S. Gonçalves[3], F. G. Santos[2], R. F. Santana[1,2], N. Dimonaco[2], E. Fuertes[2], J. Pickup[2], L. Oyama[2], S. Huws[2], R. C. F. Bonomo[1]
[1]*Universidade Estadual do Sudoeste da Bahia, Brazil*
[2]*School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, University Rd, Belfast BT7 1NN, UK*
[3]*Universidade de São Paulo, 374 - Butantã, São Paulo - SP, 05508-220, Brazil*

Presenting author
Esperanza Fuertes. E-mail: e.fuertes@qub.ac.uk

Application
CGTases are enzymes that catalyze the production of cyclodextrins, which are widely used as encapsulating agents for nonpolar molecules. This unique property enhances the solubility, stability, and controlled release of compounds, making cyclodextrins highly valuable in various industries such as food, pharmaceuticals, and cosmetics.

Introduction
The cyclization mechanism, an unusual pathway in carbohydrate metabolism, is exclusively catalyzed by glycoside hydrolase enzymes from the GH13 family, known as cyclodextrin glycosyltransferases (CGTases). These enzymes convert starch and other polysaccharides into cyclodextrins (CDs), amphiphilic compounds capable of forming inclusion complexes with a broader range of compounds, including toxic or inhibitory substances (Astray et al., 2020; Saini et al., 2022). This pathway enhances CGTases producer microorganisms to dominate starch-rich environments, offering a significant competitive advantage in complex environments where diverse microorganisms compete for nutrients. Furthermore, it enhances microbial survival, facilitating symbiotic relationships between the microorganisms in the environment. Despite the recognized potential of the ruminal microbiota for enhanced amylolytic capacities, CGTase mechanisms are still underexplored in the rumen. Thus, the present study performed a bioprospection in the rumen microbiota to investigate the occurrence of the CGTase gene in ruminal bacteria through genome-centric approach.

Materials and Methods
An *in silico* mining using sixteen amino acid sequences of experimentally characterized CGTases was used as reference to BLASTp search against 488 ruminal bacteria genomes available in the Hungate1000 collection. The retrieved putative CGTase sequences were extracted and aligned, and subsequent phylogenetic trees were performed in Clustal Omega and visualized with the Interactive Tree of Life (iTOL). Conserved regions of CGTase amino acid sequences extracted from the Hungate 1000 database were used to design specific and degenerate primers, which were further used to amplify the putative CGTase gene from *Streptococcus bovis*. The amplified CGTase gene was inserted into an expression vector and then heterologously expressed in *E.coli*. The enzymatic activity of the recombinant strain was tested after five days of fermentation in a starch-enriched medium. Cyclization activity was assessed using the β-CD-PHP complexation method.

Results
The results suggested a widespread presence and conservation of CGTase genes within the rumen microbiome, revealing insights about their functional and evolutionary significance. 811 homology sequences, with percentage identity ranging from 21.68 to 58.42 %, were identified in the rumen bacterial genomes. The highest occurrences of hits were found in genus *Butyrivibrio* (197), *Lachnospiraceae* (108), *Streptococcus* (46), *Ruminococcus* (46), *Pseudobutyrivibrio* (45), *Prevotella* (35),

Eubacterium (24), *Selenomonas* (22) and *Lachnobacterium* (21). Unexpectedly, minor hits were detected in *Bacillus* species (8), with low identity percentages (< 30%). Found sequences showed high similarity among different bacterial ruminal genomes. Although the CGTase gene has been widely distributed across the ruminal genomes, the *Streptococcus* genus presents the highest percentage identity (> 40%), emerging as a promising candidate. The alignment of the putative CGTase protein sequence from ruminal *Streptococcus* with the references sequences from soil *Bacillus* demonstrates a relative amount of well-conserved residues in the region corresponding to the C, D, and E domains. The nucleotide sequence alignment of CGTase genes between different genera, specifically *Bacillus* and *Streptococcus*, exhibited relatively high identity within these critical domains, suggesting that CGTase genes must have maintained a significant level of conservation across these genera and also across distinct niches. The homology protein sequences of *Streptococcus* showed highly conserved fragments, such as the region 409-427, indicating the putative CGTase genes have preserved a significant portion of the identity within the genomes. The presence of conserved CGTase sequences may suggest an adaptation or a specific functional role for this enzyme in the metabolic processes of *Streptococcus* within the rumen microbiome. The heterologous expression of CGTase in *E. coli* resulted in high enzymatic activity, ranging from 90.31 to 111.35 U/mL.

Conclusions

These findings support the hypothesis that rumen microbiota could be a promising reservoir of CGTase isoforms, adding a new dimension to understanding CGTase expression in this microbiome. These findings pave the way for further exploration and development of novel CGTases for sustainable and innovative applications.

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Effect of microalgae cells as dietary supplementation for finishing lambs: A microbiome-based approach

F. Santos[1], F. Rubino[1], O. Cristobal-Carballo[2], J. Brans[1], A. Aubry[2], T. Yan[2], S. Morrison[2], E. Lewis[3], C. Creevey[2], S. Huws[2]
[1]Queen's University of Belfast, University Rd, Belfast BT7 1NN, UK
[2]Agri-Food and Biosciences Institute, 18a Newforge Lane, Belfast, Co Antrim BT9 5PX, UK
[3]Devenish, 33-37 Finaghy Rd N, Belfast BT10 0JB, UK

Presenting author
Fernanda Santos. E-mail: f.godoysantos@qub.ac.uk

Application
This study explores the potential of Schizochytrium sp. cells supplementation to improve ruminal fermentation, reduce methane emissions, and enhance meat quality by increasing omega-3 fatty acid content. The findings could inform sustainable livestock management practices, reducing environmental impact while improving feed efficiency and meat nutritional value. Understanding the effects on the gastrointestinal microbiome will also help optimize dietary strategies for ruminants.

Introduction
Modulating ruminal fermentation through dietary supplementation is a widely employed strategy to enhance feed efficiency in ruminants. Schizochytrium sp., a microalgae rich in docosahexaenoic acid (DHA), represents a promising dietary supplement that may influence ruminal fermentation via fatty acid biohydrogenation pathways, potentially reducing methane emissions while increasing omega-3 fatty acid content in the meat. In this study, we investigated to evaluate the effects of varying supplementation levels of Schizochytrium sp. cells on ruminal fermentation, methane emissions, lipid composition in meat and GIT samples, and the gastrointestinal microbiome.

Materials and Methods
Fifty-six male finishing Texel/Scottish Blackface lambs were randomly assigned to four groups based on age and body weight. Each group received one of four treatments, with the following levels of microalgae (on a dry matter basis): 0% (Control), 1.2% (Low), 2.4% (Medium), and 3.6% (High) for a 35-day period before slaughter. All diets were isoenergetic and consisted of a 50:50 mixture of grass silage and concentrate. During the intervention, individual dry matter intake and body weight were recorded daily and weekly, respectively. Methane emissions were estimated using respiration chambers. Oral swabs and ruminal fluid samples were collected both before and after diet intervention, while fecal samples were collected only after the intervention. During slaughter, the gastrointestinal tract (including rumen, small intestine, large intestine, and cecum) was harvested and dissected for sample collection. Lamb's carcasses were collected and stored following the regular meat processing procedures (48h at 2°C). Feed, meat and GIT samples were submitted for fatty acid analyses using the GC method, and the GIT microbiome was characterized by shotgun metagenomics sequencing. Gastrointestinal samples were submitted to DNA extraction using a commercial kit (Power Pro soil kit, Qiagen). Libraries were generated on the Illumina Miseq using the Illumina XT library prep kit at the Genomics Core Technology Unit, QUB, following shotgun sequencing using Illumina Nova seq 6000 S4 300 flow cell (150bp PEat ~>6.2GB/ sample). MetaPhlAn was used to functionally annotate and generate the taxonomic profiles of microbial communities from metagenomic shotgun sequencing data. Alpha and beta diversity were calculated using the 'vegan' package in R. The effects of each parameter (Treatment, Time, and Sample), as well as their interactions on microbiome diversity, were assessed through permutational multivariate analysis of variance (PERMANOVA). This analysis, based on Bray-Curtis dissimilarity, was performed using the 'adonis2' function in the vegan package with 1000 permutations. Additionally, Spearman correlation analysis was used to explore relationships between microbiome diversity and other variables.

Results

The high level of microalgae supplementation significantly affected DMI and total VFAs, but methane emissions were not significantly different, although a reduction of 6.3% in methane (g/d) was observed. The content of DHA, the main PUFA found in Schizochytrium sp., increased in loin samples corresponding to the microalgae diet supplementation levels. Firmicutes and Bacteroidetes were the most abundant across all groups, with notable shifts observed between time points (before, after, slaughter). At genus level, preliminary analyses of alpha diversity indices (Shannon, Simpson, and Chao1) across treatments (Control, High, Low, Medium) indicate significant variations. Notably, significant differences were observed between the Control and High treatments in ruminal fluid after ($p = 0.003$) and in Simpson diversity in the large intestine ($p = 0.021$). PERMANOVA revealed significant effects of Treatment, Sample, and Time on microbiome composition, with Treatment showing significant impacts at "after" ($p = 0.002$) and "slaughter" ($p = 0.029$) time points. Preliminary post-hoc analyses indicate differences in microbiome composition, especially between the Control and High treatments. Functional genomic analyses are currently underway.

Conclusions

Microalgae supplementation represents a promising dietary strategy that can improve the health of ruminants, humans, and the environment, potentially reducing methane emissions. However, additional studies are necessary to gain a more comprehensive understanding of how the gastrointestinal microbiome influences the fatty acid profile of lamb meat.

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In vitro assessment of different herbs for degradability, fermentation, and methane generation in two contrasting forage diets

K. Merkhan[1]
[1]*Newcastle University, Newcastle upon Tyne NE1 7RU, UK*

Presenting author
K. Merkhan. E-mail: k.y.merkhan2@newcastle.ac.uk

Application

Ruminant production is perceived to be a major contributor to global greenhouse gas emissions. Improving diet quality is one of the most promising methods for lowering ruminant methane output. Incorporating herbs into animal pastures might increase sustainability by mixing them with grass. Since herbs are a readily available by-product feed, incorporating them into ruminant diets offers a practical strategy to reduce the environmental impact of the animal industry.

Introduction

Enhancing the nutritional content of the grazed forage is essential in pasture-based systems as supplementation may be less practical in these conditions (Wilkinson et al., 2020). Moreover, herbal leys have been supported by the UK agricultural-environment schemes due to their potential to offer more agronomic and environmental advantages than traditional grass pastures (Jordon et al., 2022). Therefore, this study aimed to evaluate the inclusion of three herb leaf powders (GTL, green tea; GBL, great burnet, OL, oregano) in two contrasting forage diets (fresh ryegrass=FR and a wheat straw-based mixture (WS) with 60% WS, 26% barley, and 14% peas) using rumen fluid from killed steers. The goal was to assess the effects of these herbs on in vitro rumen fermentation, degradability, and methane emissions of 2 forage types.

Material and methods

This duplicated 2x4 factorial trial employed two contrasting diets (FR, WS) to investigate the effects of either no addition (control) or the addition of three herbs (GTL, GBL, OL), each at 50 g/kg DM. Rumen fluid from two killed steers at an abattoir was duplicated before their use to assess in vitro dry matter degradability (IVDMD), fermentation profiles, total gas production (tGP), and CH4%. Ground and dried samples of about 200 mg were incubated in 50 ml glass syringes with 20 ml of the buffered rumen solution (1:4) at 39°C for 48 hours. The methane production was determined using GC-MS, whereas the VFA profiles were determined using IC-MS. A two-way ANOVA (2x4) with GLM was performed using Minitab 21 to analyse the effects of two diet types, and 4 herbs inclusion on the studied traits. Tukey's post hoc test assessed differences between means for significance at P≤0.05.

Results

The FR diet had significantly higher IVDMD, propionate, butyrate, and tGP values than the WS diet (Table 1). There were no differences found between the herbs and the interaction between diet types and herbs in this investigation.

Table 1. Effect of diet type and herb inclusion on IVDMD (g/kg DM), VFA (mmol/L), NH3-H (mg/L), tGP (ml/g DM), and CH4 (%) after 48h incubation.

Traits	*Feed type (F)*		*Herbs (H)*				*Pooled SE*	*Significance*		
	FR	**WS**	**CON**	**GTL**	**GBL**	**OL**		**F**	**H**	**F x H**
IVDMD	702	473	588	586	588	589	16.55	0.001	1.0	0.13
pH	6.53	6.67	6.61	6.60	6.60	6.62	0.052	0.06	0.99	0.99
NH_3-H	121	126	123	122	125	124	20.47	0.86	1.0	0.93

tVFA	38.8	20.0	19.4	35.4	26.2	36.7	7.35	0.09	0.63	0.94
Acetate	17.9	11.0	10.2	17.3	12.7	17.7	3.43	0.17	0.66	0.94
Propionate	10.4	4.3	4.6	8.9	6.7	9.1	1.97	0.04	0.66	0.88
Butyrate	7.4	3.3	3.5	6.2	4.7	6.9	1.32	0.04	0.59	0.98
tGP	166	111	147	140	141	127	4.89	0.001	0.25	0.62
CH_4	12.9	14.2	14.2	15.1	12.7	12.2	0.81	0.30	0.32	0.45

FR, fresh ryegrass diet; WS, wheat straw diet; CON, control; GTL, green tea leaves; GBL, great burnet leaves; OL, oregano leaves; SE, standard error of the means; IVDMD, in vitro dry matter degradability; NH3-H, Ammonia; tVFA, total volatile fatty acids; tGP, total gas production; CH4, methane.

Conclusion

The addition of various herbs did not affect the in vitro degradability or fermentation of either the FR or WS diets. However, the FR diet exhibited superior degradability and fermentation than the WS diet, but it resulted in higher tGP and slightly lower methane percentages.

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Effects of replacing corn silage and soybean meal with Oat-vetch mixed silage and cottonseed meal on milk production, rumen fermentation, and nutrient digestibility in high producing dairy cows

Z. Luo[1]
[1]University College Dublin, Belfield, Dublin 4, Ireland

Presenting author

Zhijie Lou. E-mail: zhijieluo@yeah.net

Application

Mixed silage made of oats and vetch, and cottonseed meal are a good source of forage and protein feeds, and can be used as a substitute for more expensive diets comprising corn silage and soya bean meal.

Introduction

A mixture of oat and vetch has gained interest in western China and in high alpine regions due to its local production and poentially high feed value. Dry matter (DM) solubility and degradability of forages are related to concentrations of NDF and CP, and are also closely correlated with the intake and digestibility of forages (Nsahlai and Umunna, 1996). To displace expensive soya bean meal (SBM), cottonseed meal (CSM) is considered a possibility due to its high production in western China, its relatively low cost, and its high protein content (Wang et al., 2017). The inclusion of 15% CSM promotes the improvement of feed intake, but reduced milk production compared to 30% CSM (Imaizumi et al., 2016). The objective of the study was to investigate whether locally cultivated mixed silage (MS), comprising legumes and oats, could partially replace corn silage, and if CSM could replace SMB in the diets of high-yielding dairy cows. The specific aim of this experiment was to investigate the effect of forage type (corn silage versus mixed silage) and protein concentrate type (SBM versus CSM) on production parameters and N efficiency in lactating dairy cows.

Materials and Methods

Nine multiparous Holstein dairy cows (DIM: 94.1±9d, milk yield: 41.0±2.5 kg) were each fed three experimental diets in a replicated Latin square design. The experimental diets contained corn silage, alfalfa hay and mixed silage in 3 different proportions (Diet 1, 40:10:0; Diet 2, 30:5:15, and Diet 3, 20:0:30; DM basis), with SBM (18.2%, 10.6%, and 0%) and CSM (0%, 7.3%, and 17.1%) as the major protein supplements. The MS was made from oats and vetch grown in a 50:50 ratio and all diets were formulated to contain 16% CP, 1.6 Mcal /kg NE_L and 50:50 forage-to-concentrate ratio (DM basis). Daily feed intake and milk yields were averaged over the last 7d of each experimental period and used in statistical analysis. Milk composition data were weighted averages based on the milk production at each collection. Data were analyzed as a replicated 3×3 Latin square using the MIXED procedure of SAS (Statistical Analysis System, version 9.3) with animal being fed each diet for 22 days. The statistical model included treatment and period as fixed effects, and square and cow within the square as random effects. Statistical significance was considered when *P* ≤0.05 and tendencies as 0.05＜ *P*≤ 0.10 for all variables.

Table 1. Feed intake and milk performance of dairy cows (n=9 per diet) fed diets with varying corn silage, mixed silage, soybean meal and cottonseed meal ratios.

	Diet (main ingredients)				
	Diet 1: (corn silage, soybean meal)	Diet 2: (corn & mixed silage, soybean & cottonseed meal)	Diet 3: (corn & mixed silage, cottonseed meal)	SEM	P Value

DMI[1], kg/d	24.1a	23.0ab	21.9b	0.39	0.0063
Milk yield, kg/d	31.2a	28.7b	25.3c	0.48	<0.0001
Milk Protein, %	3.95ab	4.04a	3.81b	0.05	0.008
Milk Fat, %	4.52	4.18	4.70	0.19	0.18
Milk Lactose, %	4.33	4.45	4.34	0.05	0.23
Milk Protein, kg/d	1.23a	1.15a	0.96b	0.02	<0.0001
Milk Fat, kg/d	1.40a	1.20b	1.18b	0.05	0.02
Milk Lactose, kg/d	1.35a	1.27a	1.10b	0.03	<0.0001
FCR[2]	1.53a	1.43ab	1.39b	0.04	0.04
Cost of feed[3], ¥/kg MY,	3.03a	2.91ab	2.89b	0.04	0.03

[a-c] Means within a row with different letters differ (*P* < 0.05)

[1]DMI=Dry Matter Intake

[2]FCR (Feed Conversion Ratio) =Energy-corrected milk/DMI

[2]Cost of feed per Milk Yield (MY) = feed cost for 1 kg milk (feed costs / MY) (DM, %)

Table 2. Nutrient intake and apparent digestibility (%) of dairy cows (n=9 per diet) fed diets with varying corn silage, mixed silage, soybean meal and cottonseed meal ratios.

	Diet (main ingredients)			SEM	P Value
	Diet 1: (corn silage, soybean meal)	Diet 2: (corn & mixed silage, soybean & cottonseed meal)	Diet 3: (corn & mixed silage, cottonseed meal)		
Intake, kg/d					
NDF	10.9a	10.5a	9.6b	0.21	0.02
ADF	4.91b	5.30a	5.30a	0.11	0.047
CP	3.97	3.95	3.82	0.08	0.40
Starch	4.79a	3.99b	3.46c	0.08	<0.0001
Digestibility, %					
DMI	77.9a	74.3ab	71.3b	0.95	0.0014
NDF	65.8a	68.5a	60.6b	1.37	0.0046
ADF	61.5a	60.9ab	56.6b	1.31	0.04
CP	79.6a	76.5ab	74.3b	1.07	0.01
Starch	94.3a	91.5b	91.7b	0.55	0.0065

a-c Means within a row with different letters differ (*P* < 0.05)

Table 3. Nitrogen intake, excretion, and utilization of dairy cows (n=9 per diet) fed diets with varying corn silage, mixed silage, soybean meal and cottonseed meal ratios (Intake N was calculated by feed samples from 21d and 22d)

	Diet (main ingredients)			SEM	P Value
	Diet 1: (corn silage, soybean meal)	Diet 2: (corn & mixed silage, soybean & cottonseed meal)	Diet 3: (corn & mixed silage, cottonseed meal)		
Intake N, g/d	635	632	611	13.05	0.40
Fecal N					
g/d	128	140	149	7.67	0.19
% of N intake	20.0a	22.3ab	24.6b	1.03	0.03
Urinary creatinine, mM	3.4a	3.2ab	2.9b	0.09	0.006
Urinary N					
g/d	265a	232ab	212b	12.70	0.03

% of N intake	41.7a	36.4ab	34.2b	1.96	0.05
Total N excretion[1]					
g/d	393.2	372.0	360.8	12.39	0.21
% of N intake	61.7	58.7	58.8	1.69	0.39
Milk N					
g/d	187a	171a	130b	5.89	<0.0001
% of N intake	29.4a	27.1a	21.1b	0.69	<0.0001

[a-c] Means within a row with different letters differ (*P* < 0.05)

[1] Total N excretion = fecal N + urinary N

Results

Cows fed diet 3 decreased 9 % of the DMI (*P*<0.01) compared to Diets 1. Associated with the reduction in DMI, cows fed Diet 3 also had reduced (*P*<0.05) yield of milk protein (22.0 %), fat (15.7 %) and lactose (18.5 %) compared to cows fed Diet 1 (Table 1). The replacement of corn silage and SMB with mixed silage and CSM reduced the cost of the diets (Diet 1, ¥3.88; Diet 2, ¥3.59; Diet 3, ¥3.32 per kg DM) and while milk production was reduced, the feed cost per milk yield was lowest for cows fed Diet 3 (¥2.89) and highest for cows fed Diet 1 (¥3.03). Cows fed Diet 3 also had reduced (P<0.05) total-tract apparent digestibility (%) of DM (6.6 %), NDF (5.2 %), ADF (4.9 %), CP (5.3%) and starch (2.6 %) compared to cows fed Diet 1 (Table 2). Nitrogen intake was not different among cows fed the three diets, but cows fed Diet 3 excreted less (*P*<0.05) urinary nitrogen than cows fed Diet 1 (Table 3).

Conclusions

We conclude that cows fed diets where corn silage and SBM was replaced with Oat-vetch mixed silage and CSM, consumed slightly less feed, that may be due to slightly lower palatability of this diet, had a lower starch intake and produced less milk. None-the-less cows fed these mixed silage diets produced less urinary nitrogen (total per day and as a proportion of intake) and the cost of feed for milk production (¥/kg) was lower than cows fed corn silage diets. This demonstrated that partial displacement of expensive corn silage and SMB with mixed silage and CSM can be achieved, but it did compromise milk production. The challenge now is to produce high quality mixed silage that ensures high DMI and milk production.

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Understanding the relationship between consumer attitudes and behaviours towards dairy products: a cross-sectional survey of UK adults

H. Bracey[1], M. Barnard[1], S. Mendowski[2], J. Renaud[3], S. R. Gray[4], E. Combet[1], R. Pullar[5]
[1]*Department of Human Nutrition, University Of Glasgow, Alexandra Parade, Glasgow G31 2ER, UK*
[2]*Valorex, La Messayais, 35210 Combourtillé, France*
[3]*Bleu-Blanc-Cœur, 35210 Combourtillé, France*
[4]*BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Alexandra Parade, Glasgow G31 2ER, UK*
[5]*ForFarmers N.V., Horizon House, Rougham Industrial Estate, Bury St Edmunds IP30 9ND, UK*

Presenting author
Robert Pullar. E-mail: robert.pullar@forfarmers.eu

Application
Balancing the nutritional importance of cows' milk with the environmental consequences and ethical concerns of its production is necessary for planetary and human health. These parameters can be improved through altering production methods. To assess whether there is a market for such products, however, it is necessary to ascertain people's attitudes and perceptions of dairy products and the industry underpinning current consumer behaviours.

Introduction
Dairy products are nutritionally rich foods; however, their production is associated with ethical and environmental concerns. This survey explores consumers' knowledge and attitudes toward dairy products and the industry and how these are linked with consumption and purchasing behaviours. We specifically investigated i) what UK dairy consumers know about dairy production and the nutritional qualities of milk, ii) if there is a difference in how different consumer groups perceive dairy farmers and the industry, iii) how knowledge and attitudes influence dairy purchasing behaviours, iv) willingness amongst UK consumers to buy milk with improved nutritional qualities and sustainability.

Materials and Methods
An online survey of UK adults was conducted from November 2021 to March 2022, covering knowledge, attitudes towards dairy, dairy consumption, likelihood to purchase improved milk, and socio-demographics. To assess knowledge, answers to multiple choice questions (n=12) were scored 1 if correct or 0 if incorrect (or "don't know"). Cumulative scores were tallied and transformed into percentages. Attitude questions were categorised into four domains (food security (n=4), animal welfare (n=7), nutrition (n=5), and sustainability (n=4)) and assessed using 5-point Likert scales ('strongly agree' to 'strongly disagree'). Responses were scored from 0 to 4 points, then transformed into percentages. The intention of purchasing an improved milk was evaluated by presenting participants with a table describing packaging information for four different milk products, asking about the likelihood of purchase for each product (6-point Likert: 'definitely' to 'definitely not') and the ranking of seven factors affecting the purchase decisions (7-point Likert: 'most important' to 'least important'). Consumers were classified as high (≥2 portions dairy per day) or low (<2 portions) based on responses to a short dairy-specific food frequency questionnaire. Statistical analysis was performed using SPSS version 29.0 and RStudio. For continuous variables, a t-test or ANOVA was used to examine differences between consumption groups; for categorical data, Chi-squared was used, and non-parametric tests were used where appropriate. Qualitative analysis of free text answers was conducted using NVivo 14.

Results

The survey received responses from 706 dairy consumers, of whom 42% reported consuming two or more portions of dairy products daily. High consumers indicated greater knowledge of dairy production than low consumers (74% vs. 69% average, $P<0.001$), whereas there was no difference in nutrition knowledge (mean score 59%, $P=0.224$). Participants generally indicated a positive attitude towards dairy farmers, particularly their role in ensuring UK food security (91% agreement) and maintaining high animal welfare standards (84% agreement). The strength of agreement was greater for high consumers, particularly regarding animal welfare ($P<0.001$). Conversely, low consumers tended to show more agreement with statements relating to the negative environmental implications of dairy production ($P<0.001$). Qualitative analysis found that many respondents commented on differential environmental and ethical outcomes arising from different production systems, with an overwhelming favour for smaller-scale farms. Several respondents commented on the spread of misinformation or conflicting information by different media outlets, whilst others recognised a personal lack of knowledge on the subject. We identified a strong sense of patriotism for British dairy farmers, with many respondents stating greater welfare standards and quality compared to other countries. Several respondents denied or rationalised the environmental consequences of dairy production, drawing comparisons with other industries, e.g. aviation. Sentiment analysis showed that dairy products were viewed positively (n=1983 words, summed AFINN score: 506); the most frequently used descriptors for dairy products were 'nutritious' and 'healthy', whilst the industry was described as ‘hardworking’, ‘underpaid’ and ‘undervalued’. Supermarket own-brand milk dominated purchasing, with price having the greatest influence on behaviour. However, when presented with descriptions of milk with differing production standards, 50% of participants favoured the product that displayed fair trade standards, improved welfare and sustainability, nutritional enrichment through cows' diet, production location, and a novel quality assurance brand, regardless that this product had the highest price per litre. Binomial logistic regression found that low consumers were more than twice as likely to choose this product than high consumers.

Conclusions

Although many respondents commented on animal welfare concerns and an intention to purchase products with improved ethical and trade standards, in practice, most consumers purchase milk products that seldom carry information regarding these traits. These findings indicate that an intention-behaviour gap exists between consumer values and behaviours. Possible explanations for this may be misperceptions about the industry, such as a lack of knowledge about the environmental impact of dairy production, resulting in cognitive dissonance. Additionally, our findings indicate that consumers may rationalise their behaviours due to the belief that personal actions have little broader impact; thus, the personal cost of changing behaviours is too great. Choice architecture and product availability are key factors of purchasing behaviour. In UK supermarkets, own-brand milk seldom carries more information than a basic quality guarantee and nutritional information. This can make it more difficult for consumers to make informed purchasing decisions. Increasing awareness of production means by providing more information on packaging could aid in bridging the intention-behaviour gap and enable consumers to purchase products aligning with their values.

Seasonal variations in meat quality of (Large white x Landrace) x Hampshire pigs reared in agroforestry and grassland systems

O. Amponsah[1,4], S. A. F. Buijs[2], R. Muns[2], R. Olave[3], J. P. Kerry[4], C. Alvarez[1], R. M. Hamill[1]
[1]*Department of Food Quality and Sensory Science, Teagasc Food Research Centre Ashtown, Scribbletown, Dublin 15, Ireland*
[2]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[3]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[4]*School of Food and Nutritional Sciences, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*

Presenting author
Oscar Amponsah. E-mail: oscar.amponsah@teagasc.ie

Application

Limited information is available on meat quality of commercial pig lines reared in outdoor production systems at different times of the year. Colour parameters and cook loss were influenced by season in both agroforestry and grassland systems, while texture appeared to be more consistent with season in agroforestry than in grassland plots. These findings are relevant to outdoor pig producers who lack information on the seasonal variability of meat quality attributes in such systems.

Introduction

Outdoor pig rearing is increasingly favoured in Europe and North America due to its alignment with environmental sustainability and enhanced animal welfare standards. (Sato, Hötzel and von Keyserlingk, 2017). Holistic outdoor pig rearing in agroforestry systems are well-established in Mediterranean countries, notably, the Dehesa system in Spain. Iberian pigs finished in this system with access to acorns with grass produced redder and more yellow meat, higher intramuscular fat and monounsaturated fatty acid content compared to intensively raised pigs (Tejerina et al., 2012). The meat quality of commercial breeds reared in agroforestry systems, particularly in oceanic climate regions remains under-researched. Moreover, the current population of outdoor-reared pigs in Ireland is relatively small (Boyle et al., 2022). This study aimed to assess meat quality of a controlled pig production in agroforestry and grassland environments in Ireland and examine the effect of seasonality.

Material and methods

Ninety-six boars (Large white x landrace) x Hampshire, half reared during spring/summer and the other half during the autumn/winter, were divided across two rearing systems: grassland and agroforestry. Within each batch, six pigs each in four plots were used per system. Each plot was further divided into three strips, with pigs staying on each strip for approximately a third of the rearing period. Along with commercial pelleted diet provided throughout the study, the outdoor pigs could forage. Agro-forestry pigs had access to acorns in the winter. Pigs were slaughtered at 22 weeks of age. Pork samples were obtained from the *Longissimus thoracis et lumborum* muscle of pigs across two seasonal studies. In the spring/summer study, samples were collected from twenty-four pigs (n = 12 grassland and n = 12 agroforestry) balanced by treatment and plot. In the autumn/winter study, samples were collected from thirty-three pigs (n = 17 grassland and n = 16 agroforestry). Loins were aged under refrigerated conditions until day eight post-mortem. After trimming, 2.54 cm sections were bloomed for 60 minutes, and colour was measured at three random points on the *Longissimus thoracis et lumborum* using a Hunterlab UltraScan (Hunter Associates Laboratory, Inc., Reston, VA, USA). Steaks were cooked in a water bath to an internal temperature of 75°C, cooking loss was subsequently recorded. Shear force was recorded with a Warner-Bratzler blade on an Instron Universal testing machine (Model 3342, Instron, UK). Statistical analysis employed the general linear model (GLM) in Minitab 17.1.0, with production system and season as fixed effects, plot as a covariate, and interaction of fixed effects, treating individual pigs as the experimental unit. Tukey's post-hoc test was applied for pairwise comparisons when significance ($P<0.05$) was detected

Results

The study revealed significant seasonal variations in lightness, redness, and significant interactive effects of season and rearing condition on cooking loss ($p<0.05$) and a significant interactive effect on shear force (Table 1). Shear force and cooking loss in meat from grassland-reared pigs were higher in the winter compared to the summer. In contrast, meat from pigs reared in agroforestry showed similar shear force and cook loss across seasons.

Table 1. The effect of season and outdoor pig production systems on meat quality of (*Longissimus thoracis lumborum*)

	Season							
	Summer		Winter		RSD	P-value		
Trait	Grass N=12	Forest N=12	Grass N=17	Forest N=16		System	Season	System x Season
L*	56.92	58.53	59.38	59.18	2.77	0.33	0.03	0.21
a*	5.14	5.62	4.79	4.79	0.95	0.34	0.03	0.35
b*	12.39	13.26	12.96	13.10	1.03	0.08	0.47	0.20
CL (%)	24.54^{B}	26.12^{A,B}	27.79^{A}	26.96^{A}	2.09	0.51	<0.001	0.04
WBFS (N)	28.45^{B}	31.70^{A,B}	34.47^{A}	30.67^{A,B}	5.38	0.85	0.09	0.02

$^{A-B}$ Values with different superscripts in the same row differ significantly ($P<0.05$).WBSF= Warner-Bratzler Shear Force, RSD = Residual standard deviation, System x Season= Interaction between treatment and season, CL= Cook Loss

Conclusion

Meat lightness, redness and cook loss were influenced by season, but were not associated with production system. Shear force values and cook loss of meat from pigs reared in agroforestry remained relatively consistent across seasons, compared to their counterparts reared in grassland, which produced tougher meat with greater cook loss in winter. These findings suggest that factors influential on meat quality may exhibit less seasonal variation within agroforestry, relative to grassland system, but this requires further elucidation.

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Relative influence of production system and post mortem ageing on meat quality of pigs from agro-forestry, grassland and conventional indoor production systems

O. Amponsah[1,4], R. Muns[2], R. Olave[3], J. P. Kerry[4], S, Novaković[1], E, M. Cabral[1], R, M. Hamill[1]
[1]*Department of Food Quality and Sensory Science, Teagasc Food Research Centre Ashtown, Scribbletown, Dublin 15, Ireland*
[2]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[3]*Agri-Food and Biosciences Institute (AFBI), 18a Newforge Ln, Belfast BT9 5PX, UK*
[4]*School of Food and Nutritional Sciences, University College Cork, Distillery Fields, North Mall Campus T23 N73K, Ireland*

Presenting author
Oscar Amponsah. E-mail: oscar.amponsah@teagasc.ie

Application
Important meat quality parameters, such as colour and shear force, from pigs reared under two outdoor production systems, i.e. grassland and agroforestry, were similar to each other and to pork from a conventional indoor production system, but meat ageing resulted in a significant improvement in texture in all production systems.

Introduction
The growing demand for higher-welfare pork has driven the expansion of alternative production systems (Pietrosemoli and Tang, 2020). While the meat quality of heritage breeds, such as Iberian in holistic outdoor systems like the Dehesa and modern pig lines reared outdoors on pasture or dirt lots has been extensively studied (Lebret, 2008), the meat quality of commercial breeds reared in agroforestry systems remains under-explored. *Post-mortem* ageing influences key meat quality traits, such as tenderness, which may be affected by factors linked to production system (Pandey et al., 2024). This study aimed to compare technological meat quality traits of pigs reared in three production systems—agroforestry, grassland, and conventional—at two time-points post-mortem.

Material and methods
(Large white x landrace) x Hampshire litters were allocated to indoor (one slatted pen), grassland or agroforestry systems (four plots of six pigs per system). Pigs were fed a commercial pelleted diet; outdoor pigs foraged, with agroforestry pigs accessing acorns. At 22 weeks, pigs were slaughtered. After trimming, 2.54cm sections were bloomed for 60 minutes, and colour was measured in triplicates using a Hunterlab UltraScan Pro (Hunter Associates Laboratory, Inc., USA). Steaks were cooked in a water bath to an internal temperature of 75°C, cooking loss was recorded afterwards. Shear force was determined on cooled steaks using a Warner-Bratzler blade on an Instron Universal testing machine (Model 3342, Instron, UK). Moisture, fat, protein, and ash contents were determined using Smart 6, Oracle NMR, LECO FP628, and ISO 936, respectively, all in triplicate. Data were analysed with a general linear model in Minitab 17.1.0, with production system and days aged as fixed effects, plot as a covariate, and interaction of fixed effects, treating individual pigs as the experimental unit. One-way ANOVA was performed on composition data. Tukey's post-hoc test was applied when ($p<0.05$).

Results
Colour parameters (L*, a*, b*), cook loss and WBSF measurements (Table 1) were altered over meat ageing ($p<0.05$) but were not significantly influenced by the production system. After eight days of ageing, meat from pigs reared in the agroforestry system exhibited a significant reduction in cook loss. All production systems exhibited marked improvements in tenderness, as indicated by a significant reduction in shear force between Day 1 and Day 8 post-mortem. However, at both time points shear force was similar across all production systems.

Table 1. The impact of production system on meat quality of pork aged for 1 and 8 days

	Day 1			**Day 8**			RSD	P-value		
Trait	Grass N=10	Forest N=11	Indoor N=10	Grass N=17	Forest N=16	Indoor N=10		System	Days aged	Sys x Age
L*	$56.74^{B,C}$	56.25^{C}	$57.59^{A,B,C}$	59.38^{A}	$59.18^{A,B}$	60.39^{A}	2.18	0.142	<0.001	0.972
a*	$3.45^{B,C}$	$3.60^{B,C}$	3.18^{C}	4.79^{A}	4.79^{A}	$4.52^{A,B}$	0.98	0.477	<0.001	0.954
b*	10.69^{B}	10.58^{B}	10.57^{B}	12.96^{A}	13.10^{A}	13.13^{A}	0.92	0.996	<0.001	0.833
CL(%)	29.59^{A}	29.27^{A}	$28.90^{A,B}$	$27.79^{A,B}$	26.96^{B}	$29.03^{A,B}$	1.89	0.292	0.004	0.085
WBSF(N)	56.35^{A}	51.31^{A}	55.03^{A}	34.47^{B}	30.67^{B}	31.54^{B}	9.61	0.265	<0.001	0.884

$^{A-B}$ Values with different superscripts in the same row differ significantly ($P<0.05$).WBSF= Warner-Bratzler Shear force, RSD = Residual standard deviation, Sys x Age= Interaction between system and ageing, CL=Cook loss

Although moisture and protein content (Table 2) remained consistent across the production systems, intramuscular fat tended to be lower in outdoor-reared pigs. Notably, ash content was significantly higher in the meat of both groups of outdoor-reared pigs, compared to the indoor group.

Table 2.
The impact of production system on composition of pork aged for 8 days

	Production system			**RSD**	**P-value**
Trait	Grass N=17	Forest N=16	Indoor N=10		
Moisture (%)	73.88	74.26	73.44	0.93	0.10
IMF(%)	1.49	1.13	1.92	0.81	0.06
Protein(%)	23.42	23.64	23.72	0.77	0.60
Ash(%)	1.25^{B}	1.23^{B}	1.16^{A}	0.07	0.01

Conclusion

A commercial pig line exhibited similar meat quality development and outcomes across indoor and outdoor production systems. Outdoor rearing did have some specific effects on meat quality, for example cook loss was lower at typical point of consumption (Day 8) for the agroforestry system, while the compositional data also suggests potentially increased mineral acquisition by outdoor-reared pigs, which warrants further investigation.

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Delivering and evaluating a motivational interviewing training programme for veterinarians

L. Gribben[1,2], A. Burrell[3], M. Dean[1], B. Hartzler[4], Á. Regan[2]
[1]*Queen's University Belfast, University Rd, Belfast BT7 1NN, UK*
[2]*Teagasc, Oak Park, Carlow R93 XE12, Ireland*
[3]*Animal Health Ireland, Bridge St, Townparks, Carrick-On-Shannon, Co. Leitrim, Ireland*
[4]*University of Washington, 1410 NE Campus Pkwy, Seattle, WA 98195, US*

Presenting author
Laura Gribben. E-mail: lgribben04@qub.ac.uk

Application

Delivering motivational interviewing training to livestock veterinarians improves their communication knowledge and skills, which can be used to support farmer-veterinarian interactions about animal health and welfare, and behaviour change on-farm.

Introduction

Livestock veterinarians play a key role assisting farmers to safeguard animal health and welfare. However, the communication style of livestock veterinarians has been shown to influence the nature of the farmer-veterinarian relationship and the enactment of veterinary advice (Bard et al., 2017; Grant et al., 2023). Veterinary communication styles focused on understanding farmers' individual circumstances, collaborating to achieve a shared goal, and upholding autonomy, are valued by farmers and increase the likelihood of on-farm behaviour change (Bard et al., 2019; Grant et al., 2023). Yet, it is increasingly recognised that veterinarians must be supported to develop the necessary communication competencies through veterinary education and professional training (Mossop et al., 2015). Motivational interviewing, an evidence-based collaborative communication methodology aiming to help a person resolve their ambivalence towards change, has shown promise as a means of providing veterinarians with useful communication knowledge and skills (Svensson et al., 2020). Accordingly, this study aims to deliver and evaluate the feasibility of a brief motivational interviewing training programme for livestock veterinarians.

Materials and Methods

A mixed-methods case study was conducted. Ethical approval was obtained from the Faculty of Medicine, Health and Life Sciences Research Ethics Committee at Queen's University Belfast. The programme consisted of a 2-hour online module, two full day face-to-face workshops and a reflective practice session. Twelve veterinarians completed the online module and workshops, while five completed the reflective practice session. The programme was facilitated by a certified motivational interviewing trainer based in the animal health setting, along with a private veterinary practitioner trained in motivational interviewing. Evaluation consisted of assessing veterinarian satisfaction post-workshop via questionnaire, along with motivational interviewing skills using an adapted version of VASE-R (Video Assessment Simulated Encounters Revised). Additionally, to qualitatively assess programme delivery, learning and application of learning, trainers completed reflective logs and veterinarians participated in focus groups/interviews five months after the workshop. The quantitative data (satisfaction and motivational interviewing skills) was descriptively analysed. While qualitative data including trainer logs and focus group/interview data was thematically analysed.

Results

Descriptive analysis found veterinarians were highly satisfied with the programme (mean = 4.4 out of 5-point Likert scale). Thematic analysis determined three themes: valuable activities, takeaways, and programme evolution. Scenario-based skills practice and open discussions were identified as important to the learning process. Veterinarians noted developing a greater awareness of their own personal communication patterns and increased knowledge of communication practices which promote successful conversations about change. Veterinarians also reported learning how to use motivational interviewing skills, such as reflections, which promote deeper listening and better understanding of others. This finding was supported by at least half of participants achieving beginner proficiency in VASE-R 'Reflective Listening' and 'Summary' sub-scale scores. Finally, incorporating additional skills practice and reflective activities into the curriculum was deemed beneficial. However, given the workload and seasonality associated with the role of the veterinarian, the timing and frequency of any additional activities must be carefully considered.

Conclusions

Brief motivational interviewing training can help veterinarians become more aware of their own communication patterns and promote positive changes in communication skills. The findings are of relevance to those involved in veterinary continuing professional development, as a way of enhancing the skills of veterinarians to support farmers to enact behaviour change on-farm.

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Estimation of straw bedding intakes in growing pigs

C. Graham[1], S. Mansbridge[1], V. Pirgozliev[1], A. Stewart[1]
[1]*Harper Adams University, Edgmond, Newport TF10 8NB, UK*

Presenting author
Clorinda Graham. E-mail: 15040000@live.harper.ac.uk

Application

Acid Detergent Lignin (ADL) is suitable for use as an independent marker to estimate straw intakes in pigs, however, there is no benefit of including Acid Insoluble Ash (AIA) to complement ADL as dual marker method to estimate straw bedding intakes in growing pigs.

Introduction

In the UK, it is a legal requirement to provide pigs with some form of enrichment and for many producers, straw is the most effective and accessible option. The benefits of providing straw enrichment are multifactorial, and include pig cleanliness and improved welfare. However, straw often contains antinutritional factors and contaminants such as mycotoxins, and due to its high fibre content, can reduce nutrient digestibility by speeding up the rate of passage of digesta through the gut. There has been limited research to quantify straw bedding intakes in commercial pigs and past research has yielded variable results (0-235g/day in growing pigs) (Mansbridge and Stewart, 2012; Staals et al., 2007). Historically, single feed markers such as Cr_2O_3 have been used in pig studies, however recently, using multiple markers has been suggested to improve accuracy of results. Due to regulatory and safety concerns, these classic markers can make methodology unsuitable for use in commercial pigs due to the required authorisation for animals entering the food chain. The aim of this study was to refine marker methodology to estimate straw intakes in housed pigs under commercial conditions and to identify factors that may contribute to levels of straw bedding consumption.

Material and methods

The study was approved by Harper Adams University Research Ethics Committee (reference: 0251-202303-PGMPHD). The study was comprised of two trials conducted on the same research farm but in different accommodations. All pigs used were JSR 9T x JSR Tempo. ***Trial one (methods development)*** – Forty-two pigs (approx. 35 kg) housed on slatted floors and in groups of six were fed one of seven rations containing differing quantities of straw (0, 5, 10, 15, 20 and 25%) and a fixed quantity of diatomaceous earth (20 g/kg). Two of the seven diets had the same straw content (15%), one with and one without the inclusion of AIA, allowing this diet to act as validation for the dual marker method (AIA/ADL). Faecal samples were taken from each pig from the pen floor immediately after defecation, on d 5 and d 7. Both feed and faecal samples were analysed for ADL and AIA content. One-way ANOVA followed by Tukey test and liner regression were applied in Genstat (23rd edition) to determine the relationship between straw content of the diet and either ADL alone or AIA and ADL (dual marker method) excreted, as a predictive marker of straw intake for use in trial two. ***Trial two (straw intake estimation)*** – Pigs were housed on straw bedding under commercial conditions on proprietary feed rations. Pigs were weighed at d 0 and d 35 post-weaning and freshly voided faecal samples (two per pen pooled from different depositions) were taken from five pens at d 7, 21 and 35 post-weaning. Feed, straw and faecal samples were analysed for ADL content. Using the regression methodology developed in Trial one based on ADL and adjusting for predicted feed intake (from the unit feed intake curves), estimated daily straw intakes were calculated.

Results

Excreta recovery of AIA between diets in trial one was significantly different ($P<0.05$) reducing the reliability of using an AIA/ADL dual marker regression to estimate straw intakes ($P<0.001$) ($y = -0.372x + 25.101$, $R^2 = 0.7588$). There was a significant positive relationship ($P<0.001$) ($y = 0.7136x + 1.0155$ R^2

= 0.9352) between straw content of ration and ADL excretion in faeces when adjusted for feed intake (Figure 1). Pigs were estimated to consume 137 g/d, 96 g/d and 65 g/day of straw bedding at 7-, 21- and 35-days post-weaning, respectively (trial two). Age of pig post-weaning had a significant impact (*P*=0.002) on estimated daily straw consumption with pigs 7- and 21-days post-weaning consuming more straw bedding than pigs at 35 days post-weaning. The regression model tested on the 15% straw inclusion group (not used to train the model) estimated consumption for this group to be 15.11 % straw inclusion, validating the model (Figure 1).

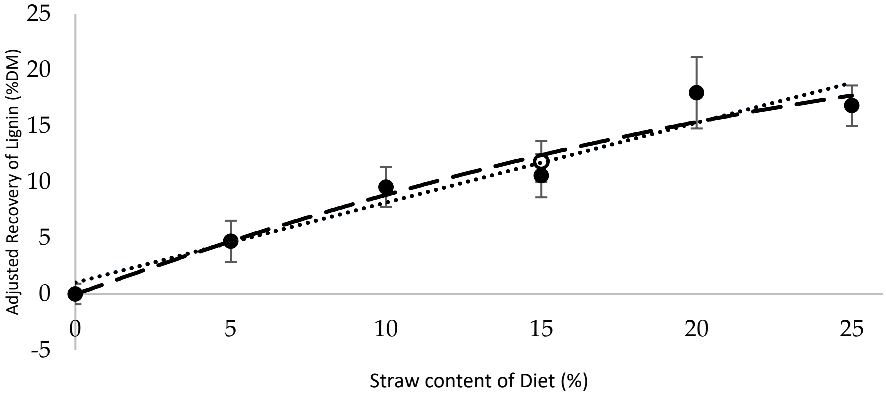

Figure 1. Adjusted faecal recovery of lignin for pigs fed different quantities of straw (solid fill circular markers). White marker (no fill) represents 15% straw inclusion validation sample. Dashed line represents polynomial regression model. Dotted line represents linear regression model. Error bars represent standard deviation.

Conclusion

Increasing the straw content of diets results in increased ADL excretion which can be used as a single marker method to estimate straw consumption of pigs in commercial settings. However, AIA could not be used reliably as a dual marker to improve straw intake estimations. Relative straw bedding consumption may be higher in young pigs (7 – 21 days post-weaning) compared to growing pigs (35 days post-weaning). Younger pigs have been found to interact more with enrichment materials than older pigs (Docking et al., 2008) which may explain the higher consumption.

Acknowledgements

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Effects of adding different concentrations of deer antler stem cell secretions to semen diluent on black pig semen quality

S.-Y. Peng[1], Z.-Q. Xiao[1], W.-H. Tsai[1], P.-C. Shen[1], K.-C. Yu[1]
[1]*National Pingtung University of Science And Technology, Neipu Township, Pingtung County, Taiwan 912*

Presenting author
Shao-Yu Peng. E-mail: simon052273@gmail.com

Application

Deer antler stem cell secretions hold the potential to elevate the black pig semen quality for increasing the reproductive efficacy and economic value.

Introduction

In modern pig farming, after collecting boar semen, it is mixed with diluent at a ratio of 1:1 and stored in a constant temperature refrigerator at 15-17°C for use in breeding. The storage time of refrigerated semen in the industry is about three days. Usually, the sperm motility and survival rate are greatly reduced on the third day. During the refrigerated storage process of semen, it is often due to problems such as transportation, improper operation, and bacterial infection lead to reduced fertilization rates. Deer antlers grow during deer development and regenerate each year and rich in active ingredients, including growth factors, stem cells and various proteins. It was revealed that stem cells secretion is capable of holding the therapeutic effects (Wei-Je T., 2020). Hence, in this research, we are about to explore the effect of deer antler stem cell secretions to black pig semen quality.

Material and Methods

The sixth-generation deer antler stem cells were taken out of the incubator. After discarding the culture medium, and washed three times with DPBS. Then 9ml of DPBS was added to the culture dish. Place it in the incubator for 2 hours, take it out, and aspirate out the DPBS. Place it in a centrifuge tube, centrifuge it at 650g for 10 minutes, take out 8ml of the supernatant (K-DPBS), and store it in a -80 degree refrigerator for use. The survival rate, viability, Straightness (STR), curvilinear velocity (VCL), Velocity straight line (VSL) and Linearly (LIN) of black pig sperm were measured within 72 hours when K-DPBS, the secretion fluid of Antler Stem Cells (ASCs), is added to the sperm diluent.

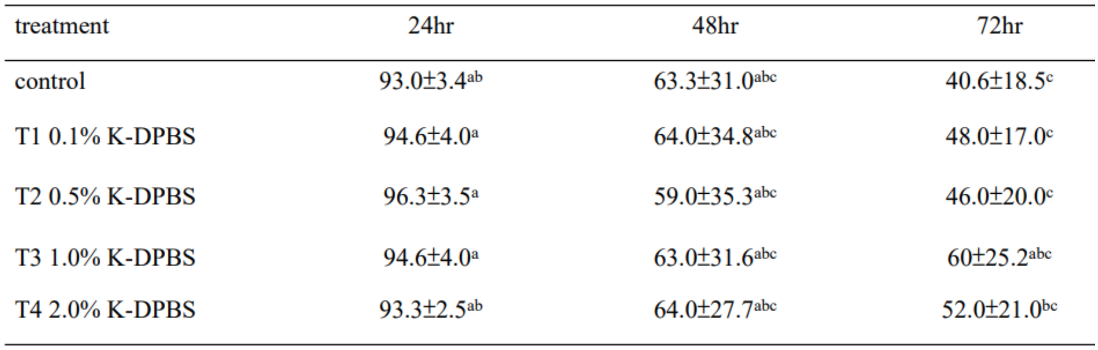
Results

The research results show that adding 1% and 2% K-DPBS to the sperm diluent can significantly improve the survival rate of black pig sperm for 72 hours (P<0.05). Whereas, there are no significance differences in terms of index of viability, Straightness (STR), curvilinear velocity (VCL), Velocity straight line (VSL) and Linearly (LIN).

Table 1. Effects of adding different concentrations of K-DPBS to boar semen on sperm survival rate after 24, 48 and 72 hours

treatment	24hr	48hr	72hr
control	88.0±3.0^{ab}	86.0±2.6^{abc}	74.0±6.0^{d}
T1 0.1% K-DPBS	90.0±0.5^{a}	88±1.7^{ab}	80.0±5.0^{abcd}
T2 0.5% K-DPBS	90.3±2.0^{a}	87.6±2.5^{ab}	79.0±9.0^{cd}
T3 1.0% K-DPBS	91.3±0.5^{a}	90.0±1.7^{a}	82.6±7.6^{abc}
T4 2.0% K-DPBS	90±0.0^{a}	89.0±1.0^{a}	82.8±7.7^{abc}

Table 2. Effects of adding different concentrations of K-DPBS to boar semen on sperm viability after 24, 48 and 72 hours



treatment	24hr	48hr	72hr
control	93.0±3.4^{ab}	63.3±31.0^{abc}	40.6±18.5^{c}
T1 0.1% K-DPBS	94.6±4.0^{a}	64.0±34.8^{abc}	48.0±17.0^{c}
T2 0.5% K-DPBS	96.3±3.5^{a}	59.0±35.3^{abc}	46.0±20.0^{c}
T3 1.0% K-DPBS	94.6±4.0^{a}	63.0±31.6^{abc}	60±25.2^{abc}
T4 2.0% K-DPBS	93.3±2.5^{ab}	64.0±27.7^{abc}	52.0±21.0^{bc}

Conclusions

Taken together, deer antler stem cell secretions show the possibility to elevate the sperm survival rate for optimizing the pig reproductive efficacy.

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Monitoring drinking in cows inside a barn with a simple camera setup and machine learning

D. Tousios[1], O. Koskela[1], N. Narra[1], I. Kunttu[1]
[1]Häme University Of Applied Sciences, Vankanlähde 9, 13100 Hämeenlinna, Finland

Presenting author
Nathaniel Narra. E-mail: nathaniel.narra@hamk.fi

Application

Using an off-the-shelf camera and customization of existing machine learning models showed potential in detecting drinking times at a water trough in a cow barn. Such a low-investment and non-disruptive setup can be used to monitor water equipment usage and herd behaviour.

Introduction

Traditional methods of animal monitoring in livestock agriculture have relied on manual observation and simple mechanical devices. Precision livestock farming is an advanced agricultural practice that integrates technology and data-driven decision-making to enhance the management of livestock. It involves some combination of sensors, data analytics, and automation systems to monitor and manage individual animals in real-time, ensuring optimal health, productivity, and welfare.

In livestock agriculture, computer vision (CV) can be used to monitor and track animal behaviour, assess health and body conditions, and optimize resource allocation in real-time and with precision. At the same time, it does it with minimal animal intrusion and with a good sensor-to-animal ratio, being scalable to work with low and high number of animals. For instance, tools have been developed to detect early signs of disease in dairy cows by monitoring behaviours such as standing time to identify hoof diseases or feed intake to detect mastitis (Yu et al., 2024). Similarly, monitoring respiratory responses helps assess heat-stress adaptation (Wu et al., 2023). However, integrating CV into animal monitoring comes with several challenges which hinders its wider use and applicability. These include the need of high-quality annotated livestock data, the significant computational resources for training deep learning models for accurate predictions, and the variability of natural phenomena such as animal behaviour and environmental factors such as lighting and weather conditions. Addressing these challenges is crucial for the effective application of computer vision in agriculture. The goal of this study was to assess the behaviour of cows near water troughs for a herd in a loose housed barn. The approach relied on monitoring traffic around the water troughs through CV based animal tracking and aimed to understand the use of this water resource.

Materials and Methods

The data in this study was a subset from a two-month continuous image acquisition of cows in front of an automatic milking station inside a teaching campus barn, using a commercially available security camera. The data consisted of 253 videos (25 fps) averaging 4 minutes each as described by Koskela et al. (2022). Python language scripts were developed to extract and filter frames from the video footage at fixed frame intervals of 1250 and 3000 frames, resulting in a dataset of 1140 unique images, providing sample images from almost every video file. Images with very low visibility (less than 10% brightness in total image) were filtered out to ensure a higher-quality dataset. For computer vision processes, the Ultralytics YOLOv8x model was employed, while DeepSORT was used as a tracker. Additional libraries such as OpenCV (cv2) for handling videos, Pandas for data manipulation and analysis, Matplotlib for plotting, and NumPy for numerical operations were utilized.

Results

A cow was considered drinking, when trough and cow head were “interacting”, i.e., the distances of the bounding box centres on the image were below a set threshold. Interactions occurred in 18.8% of all frames (Figure 1), with no interactions observed in the remaining 81.2% (15.2 hours). This suggests that cows were not interacting with the troughs for most of the time that they were detected being nearby. Among the interaction events, 12.1% (2.3 hours) involved single-cow drinking, 2.2% (0.4 hours) involved 2 cows drinking, 2.5% (0.5 hours) involved 3 cows drinking, and 2% (0.4 hours) involved more than 3 cows drinking (Figure 2).

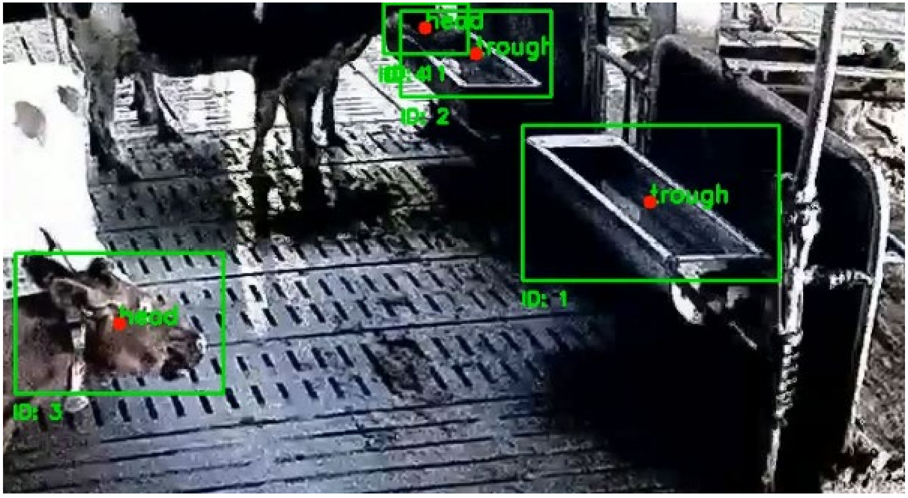

Figure 1. During drinking sessions, when two or more unique cow have a confirmed interaction with the same trough it is considered a multi-cow drinking interaction.

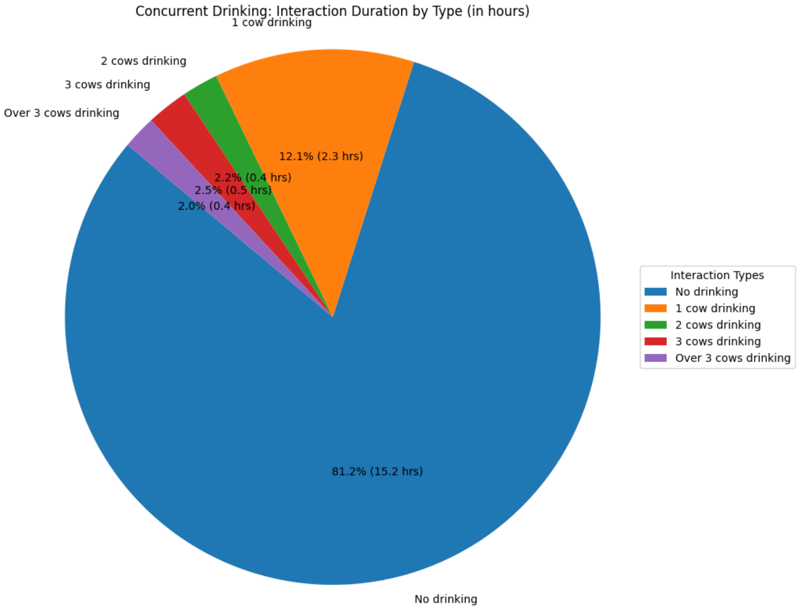

Figure 2. Concurrent drinking interaction (which tracks the number of cows at a single trough engaged in drinking) by type.

Conclusions

This case study demonstrates the potential of leveraging a simple off-the-shelf camera and computer vision workflow to monitor drinking interactions of cows in a barn to assess water trough availability. The results of this workflow, using established machine learning model structures, did not require significant investment in hardware setup or machine learning model development. Mere customization of existing learning model structures returned promising results. The potential of such an approach would be enhanced by increasing accuracy through refinement of the setup and increasing model training data. Further development could be pursued in expanding number of cameras to cover greater fields of view, integration of other sensors (e.g. environment sensors, barn equipment sensors, RFID tags) and advanced data analyses towards animal identification for tracking individuals. It should be noted, however, that seasonal factors such as temperature, light changes, and feeding habits (e.g., pasturing during summer but not during winter) may influence drinking habits and impact the generalizability of these findings. Thus, any management decisions based on such a workflow need to take these factors into account.

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Innovative Livestock Production Systems (ILPS): EU case studies from the Horizon Europe STEP-UP project

Á.Macken-Walsh[1], D. Neumeister, V. Dalle, D. Kenny, W. Osawe
[1]Teagasc, Mellows Campus, Athenry, Co. Galway H65 R718, Ireland

Presenting author
Áine Macken-Walsh. E-mail: aine.mackenwalsh@teagasc.ie

Application

Assessment of Innovative Livestock Production Systems (ILPS) case-studies to accommodate experimental processes for future evidence-based sustainability of livestock project systems.

Introduction

Innovative Livestock Production Systems (ILPS) are operating at various scales EU-wide, offering evidence-based guidance for charting future sustainability pathways for livestock farming. Case-studies of ILPS offer potential to identify characteristics of these systems that are transferable to mainstream European Livestock Production Systems (ELPS) as they transition to have greater sustainability. This poster outlines the process of selection and assessment of ILPS case-studies EU-wide, which was carried out using both a 'top down' and 'bottom-up' process. 'Top down' knowledge within the STEP-UP consortium of established approaches to EU data collection/sustainability measurement; and the needs of experimental processes in developing and testing new indicators and sustainability metrics influenced the selection and assessment approach. However, the approach was also shaped by the knowledge and experiences within the ILPS themselves, which are experimenting 'on the ground' with new approaches and ways of assessing the impacts of these new approaches. The combined 'top-down', 'bottom-up' approach is presented as a framework to achieve a balance between the continued need for standardised data collection while also accommodating diversity among and innovation within ILPS themselves, offering capacity for developing new indicators and metrics for sustainability.

Materials and Methods

Criteria for the selection of ILPS were identified through a multidisciplinary assessment of current sustainability indicators and metrics, which were thematically grouped. ILPS were purposively selected by members of the multi-actor STEP-UP consortium and associations representing livestock farming interests EU-wide on the basis of their innovativeness; and how they intersect with and offer guidance for the sustainable transformation of mainstream ELPS. A data collection template was developed to assess ILPS on the basis of standard sustainability indicators; new and experimental 'pipeline' sustainability indicators; and features of the ILPS. The ILPS case-study assessments were designed for implementation using a multi-actor, multi-disciplinary approach, involving mutual knowledge exchange between case-study actors and project scientists to scope potential for developing more holistic and comprehensive data capture for livestock farming sustainability.

Results

Classification of primary characteristics revealed that ILPS case-studies encompassing environment & health and economy attributes were most readily identified; while ILPS encompassing attributes pertaining to human nutrition & public health, animal health & One Welfare; biodiversity; and social sustainability attributes were less easily identified. However, on assessment of primary attributes of case-study ILPS, secondary functions revealed multi-functionality and cross-cutting relevance to multiple attributes. An intrinsic feature of all ILPS is that they are experimental in nature; and extension, innovation support and linkages with wider AKIS structures are vital in their operation. This indicates the needs for sustainability indicators oriented to governance, human capital and knowledge flows.

Conclusions

From a wide-ranging search for ILPS and a purposive selection and assessment process, this poster presents preliminary findings regarding the nature of the data available and the process of data collection as a multi-actor dialogue between ILPS actors on the ground and STEP-UP partners. This paper suggests that this dialogic and interactive process is necessary due to the highly dynamic nature of the concept of sustainability and associated challenges & opportunities in developing new indicators and metrics for sustainability in diverse EU regions.

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Overcoming challenges to enable the practical implementation of genomic evaluations in sheep

E. Smith[1], S. I. Lahoucine[2], M Coffey[2], J. Yates[1]
[1]British Texel Sheep Society, Stoneleigh Park, Unit 74, 4th Street, Kenilworth, Warwickshire CV8 2LG, UK
[2]Scotland's Rural College, West Mains Road, Edinburgh EH9 3JG, UK

Presenting author

Ed Smith. E-mail: edsmith@texel.co.uk

Application

The Texel is the leading terminal sire sheep breed in the UK and has significant influence on the national maternal flock. The implementation of genomic evaluations for this breed will help to predict future performance more accurately than using simple genetic pedigree information. This allows breeders to optimise the profitability of their flocks by informing animal selection and supporting breeding decisions. There are benefits for individual animals and at the breed-level from genomic evaluations. All animals benefit from the genomic evaluation whether they have been genotyped or not, though those that have been genotyped will benefit more. In addition, genomic information can confirm an animal's parentage where genomic data for the offspring and at least one parent is available.

Introduction

The expanding utilisation of DNA technologies in the livestock sector presents numerous opportunities to enhance genetic programs and accelerate genetic gain. However, in sheep breeding programs, the high relative cost of genotyping limits the adoption of genomic technologies to a subset of animals within a population. One effective method to address this challenge is the Single-Step Genomic evaluation (SSGBLUP), which integrates genomic data with genealogical information to assess the genetic potential of genotyped and non-genotyped animals. This work aimed to assess the predicted and realised animal and population-level benefits of the implementation of genomics in a large commercial pedigree sheep breed.

Materials and Methods

A total of 19,494 animals were genotyped with different SNP arrays (Ovine_600K, GGP_Ovine_50K, OvineSNP50 and Ovine_15K) and imputed to 44,065 SNPs using findhap.f90 V3 software (VanRaden et al. 2011). A quality control (QC) procedure was initiated to identify genotypes that were not suitable for inclusion, based on the approach of Kaseja et al. (2022). Parentage verification was based on 8,130 SNPs, using the 'opposing homozygote' method (Hayes, 2011) and parent-offspring pairs considered to fail if the proportion of inconsistencies were greater than 1%. The prediction ability of SSGBLUP and pedigree-based genetic evaluation (PEDBLUP) were compared using a cross-validation method (Linear Regression (LR); Legarra and Reverter, 2018) prior to commercial release. The evaluated dataset included 1,900,000 Texel animals. SSGBLUP and PEDBLUP evaluations were conducted using a multi-trait model with nine traits: eight-week weight (8WW), scan weight (SW), muscle depth (MD), fat depth (FD), computed tomography (CT) scan lean (CTLean), CT muscularity (CTMusc), CT fat (CTFat), CT eye muscle area (EMA) and CT-predicted intramuscular fat percentage (IMF). Post commercial release, the realised benefits of the implementation of SSGBLUP were assessed by summarising the published breeding values, economic indices and their accuracies for animals born 2019-2023 (n=293,168).

Results

Out of the 19,494 genotypes analysed, the QC procedure identified 145 instances (0.7%) where the same animal ID was associated with a different genotype and 32 instances (0.2%) where two identical genotypes were associated with different IDs. The <1% overall error rate was acceptable. Reasons included poor genotype quality and sample mislabelling, and confirmed the importance of the QC procedure, as both technical and human errors are impossible to eradicate completely. In total, 20,954 genotyped parent-offspring relationships were present in the data and 1,256 inconsistencies detected, suggesting a parentage error rate of 6.4%, similar to a preliminary analysis on the same population and rates observed in other UK livestock species (Kaseja et al. 2022). Pre-release analyses were performed on complete and partial datasets (excluding phenotypes for animals born after 2021). The H-inverse was constructed with Quaas-Pollak transformation using HGINV V1.03 and the MIX99 V22.0228 package (Vuori et al. 2006). For the focal [validation] dataset (~1,350 genotypes) the prediction accuracy increased from 0.27 to 0.45 for individual traits when comparing SSGBLUP with PEDBLUP. The accuracy of IMF increased from 0.43 to 0.59, and SW accuracy increased from 0.41 to 0.59 when estimated using PEDBLUP and SSGBLUP, respectively. Post-release, the population-level Terminal Index accuracy increase based on SSGBLUP was negligible (0.005) compared to the PEDBLUP evaluation. However, genotyping increased the average accuracy of the Terminal Index by more than 0.3 for individual animals and phenotyping specific traits increased this by a further 0.08.

Conclusions

SSGBLUP is a promising method for integrating genomic information into commercial sheep evaluations. The integration of this disruptive technology into a viable business model, supporting genetic progress and enhancing Breed Society services, represents a ‘science into practice’ success. The initiative, drive and financial investment from the Breed Society to adopt the genomic service is unprecedented in the UK sheep industry and provides a valuable tool to support breeders in the breed’s development. This provides significant value to the industry through improved rates of genetic improvement in traits of commercial value in an influential breed. The ability to identify and correct pedigree errors offers integrity benefits to the commercial delivery of this technology in a Breed Society registry. This improves flock book authenticity and contributes to improved prediction accuracies by correcting parentage assignment. The high relative costs of genotyping limits the uptake of the technology, however it has been demonstrated that genotyping offers significant benefits for individuals, particularly unproven animals [lambs] as their genetic potential can be determined, with a high level of accuracy, from birth. These benefits can be exploited by breeders to enhance genetic gain and produce animals better suited to the commercial market.

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Rumen microbiome development in lambs supplemented with prebiotic Mannan Rich Fraction (MRF)

A. Corrigan[1], S. Stockdale[2], H. Warren[3], J. Taylor-Pickard[3], R. Murphy[1]
[1]Alltech Biotechnology, Summerhill Rd, Sarney, Dunboyne, Co. Meath, Ireland
[2]BioFigR, 10 Plunkett Terrace, Cobh, Cork P24 N524, Ireland
[3]Alltech UK, Ryhall Rd, Stamford PE9 1TZ, UK

Presenting author
Aoife Corrigan. E-mail: acorrigan@alltech.com

Application

This study investigated the rumen microbiome of lambs who received MRF supplementation through four approaches: direct feeding only, maternal provision only, a combination of both, and a control group. These data can be used to enhance the current knowledge on the impact of maternal nutrition on neonates, as well as prebiotic-driven shifts in the microbiome that may inform future feeding practices on farm.

Introduction

The rumen microbiome is a complex community of microorganisms that play a critical role in the digestion and overall health of ruminant animals by facilitating the breakdown of fibrous plant materials into essential nutrients (Qi et al., 2024). Recent research has highlighted the potential of dietary supplements to modulate this microbial environment (Belanche et al., 2021). The present study aimed to evaluate the impact of dietary supplementation with MRF on the rumen microbiome in lambs, provided either directly, through maternal supplementation, or via a combination of both.

Materials and Methods

Animal experiment: The experiment was conducted at Harper Adams University between January and April 2021 in the Agriculture and Environment Department animal project buildings. Forty-eight twin bearing Suffolk x Mule ewes were housed individually on sawdust from day 105 (week -6) of gestation to week +4 of lactation and allocated one of two treatments (Control or Control + 1g/kg MRF) in a randomised block design with 24 replicates per treatment (Figure 1).

From week +4 of lactation, ewes and lambs on each treatment were allocated by block into two groups (12 ewes per group), group housed and maintained on their experimental diets until week +8. From week +4, suckling lambs were offered control creep feed or creep supplemented with 1.0 g/kg MRF. Following weaning at week +8 one lamb from each set of twins was selected and rumen samples were collected. The remaining 48 lambs were individually penned and offered their respective creep feeds with a small amount of straw in racks. At approximately 42 kg liveweight rumen samples were collected from the remaining twin.

Metagenomic sequencing: DNA extracted from the ruminal content was sequenced with Illumina NovaSeq technology with paired-end 150 bp reads, to 5 Gb sequencing depth. Kraken2 and Bracken were used to taxonomically classify the metagenomic reads and abundances. The R packages vegan and phyloseq were used to assess diversity changes, such as the Shannon index for alpha-diversity and Bray-Curtis metric for beta-diversity. Alpha-diversity comparisons were made using the Wilcox test with Benjamini-Hochberg false discovery rate applied ($p<0.05$). Beta-diversity significance was measure with PERMANOVA and ANOSIM tests ($p<0.05$).

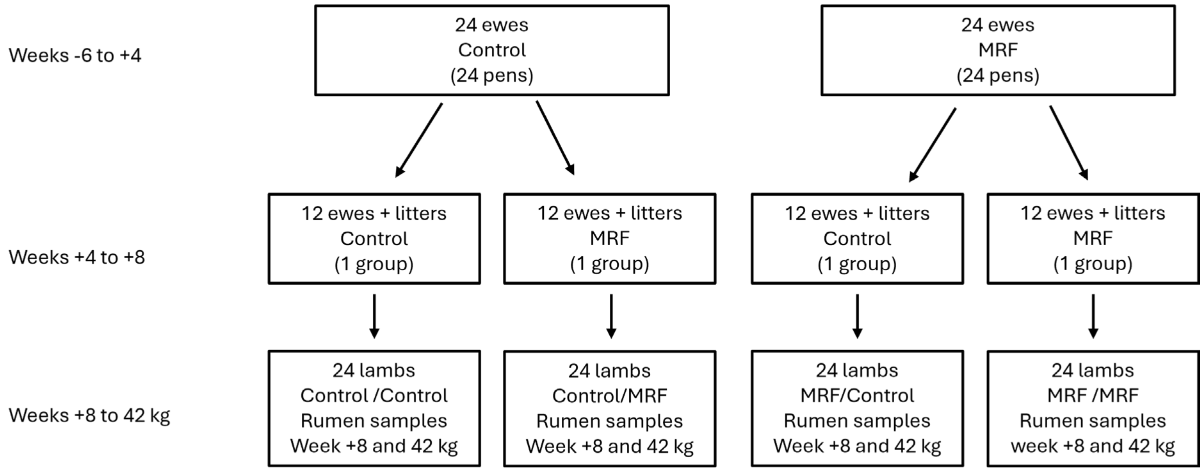

Figure 1. Trial design for study investigating the effect of MRF supplementation in ewes and/or lambs on the lamb rumen microbiome.

Results

Alpha-diversity median measurements using Shannon's index was shown to be greater in all three lamb groups that received MRF either directly, with maternal supplementation, or both. The lambs that received MRF directly, with or without the maternal supplementation, had significantly greater alpha diversities (*P*<0.05) compared with the lambs in the control group (Figure 2a).

MRF supplementation explained a significant proportion of the inter-sample beta-diversity changes across lamb microbiomes (PERMANOVA R2=0.046, *P*=0.02, Figure 2b). Focused comparisons between lambs on a Control or MRF-supplemented diet, but differing due to the maternal MRF supplementation, indicated maternal diet also influenced the beta-diversity of lamb microbiomes (Control ANOSIM: R2=0.057, p=0.041; MRF ANOSIM: R2=0.045, *P*=0.048).

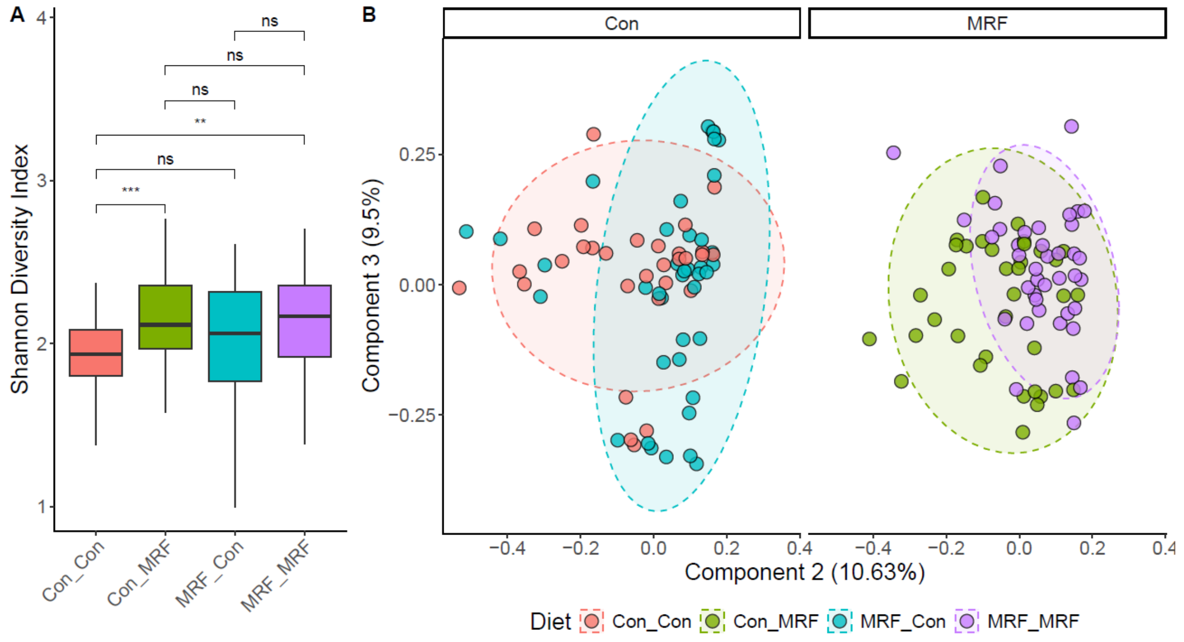

Figure 2. a&b: Alpha-(2a) and Beta-diversity (2b) composition in lambs, showing alterations induced by MRF supplementation through direct feeding, maternal provision, or a combination of both.

Conclusions

By elucidating the relationship between prebiotic supplementation and rumen microbiome dynamics, this study contributes valuable insights into optimizing lamb nutrition and health through targeted microbiome manipulation. These findings lay the groundwork for future research into sustainable, microbiome-focused approaches to livestock management.

Acknowledgments

Robert Wilkinson and Harper Adams University Agriculture and Environment Department who carried out the trial and aided in trial design.

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Distribution, preservation and people´s awareness about the Fallow deer

T. Ntallaris[1], E. N. Kantidenou[1], J. Morrell[1]
[1]Swedish University of Agricultural Sciences, Almas Allé 8, 750 07 Uppsala, Sweden

Presenting author
Theodoros Ntallaris. E-mail: theodoros.ntallaris@slu.se

Application
The study on the Fallow deer (*Dama dama*) on Rhodes, Greece, highlights its critical importance as an endangered species unique to the Mediterranean and genetically distinct from other populations. The deer's conservation holds significant environmental value, as it plays a vital role in maintaining ecological balance, and also offers potential economic benefits through eco-tourism and education-driven initiatives. The findings show that local knowledge about the deer varies, with older individuals and those living closer to deer habitats being more aware of the species. This suggests the need for educational programs to raise awareness, bridging knowledge gaps and fostering stronger community involvement in conservation efforts. By increasing public understanding and engagement, this work supports the preservation of a culturally and ecologically valuable species while promoting sustainable development for Rhodes.

Introduction
The only (and oldest) wild deer population in the Mediterranean is the Fallow deer (*Dama dama*) found on the island of Rhodes. The population known and as Platoni, is endangered due to climatic factors, fires, agricultural activities, and tourism, among others. A better understanding about the species´ biology by the local population would play a major role in conservation efforts. The purpose of this study was to map the level of knowledge about Fallow deer among the inhabitants of Rhodes, to gain an understanding of what measures are required to protect the species and how measures for their survival could be carried out.

Materials and Methods
An anonymous questionnaire consisting of 15 questions was sent to vets and local inhabitants. A total of 117 responses were included in the statistical analysis. Data was analysed by mixed model (PROC MIXED) with the SAS software (version 9.4; SAS Institute Inc., Cary, NC), followed by Scheffé adjustment for multiple comparisons

Results
The results showed an association ($P<0.05$) between the age, gender and origin of the respondent and the level of knowledge about Fallow deer. Men above 40 years old, living in villages had greater knowledge about Fallow deer biology than young women living in the city. The distribution of knowledge about the species clearly followed the geographical distribution of the species. Men above 40 years old knew more about the Fallow deer compared to any other participants. This can be explained by the fact that in the past it was customary for people to stay in the villages where they were born. Therefore, older people grew up in villages where fallow deer live and often come in contact with the local inhabitants often. In addition, it was evident that men knew more about the deer compared to women. This can be explained by the fact that men meet Fallow deer more often and therefore possibly have more knowledge about them compared to women.

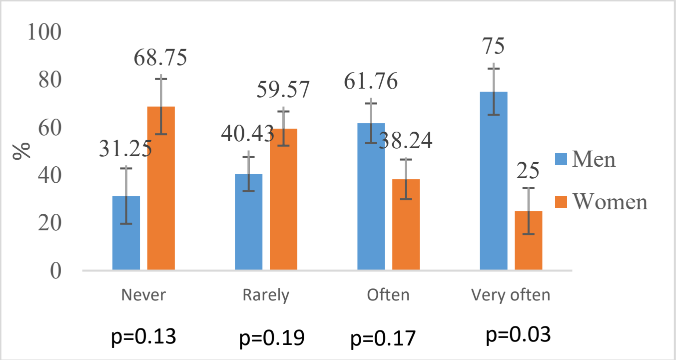

Figure 1. How often do you see a Fallow deer?

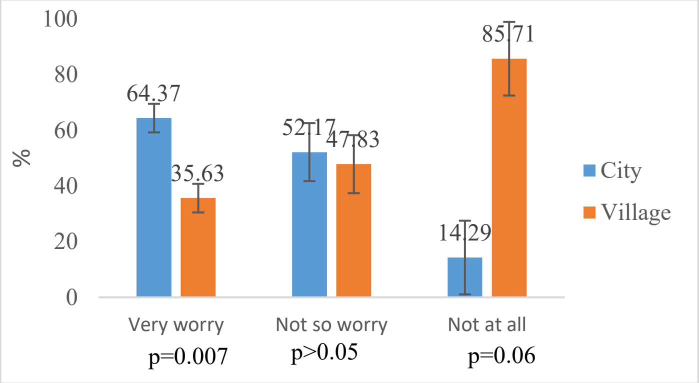

Figure 2. How worrying is it for you if Fallow deer disappears from the island of Rhodes?

Conclusions

In summary, the study shows that people who often meet Fallow deer have a higher level of knowledge about the species than those who do not. Therefore, the level of knowledge of the local population has a similar geographical distribution to the species. The recommendation of the study is for institutions to facilitate educational programs in which people will hear about the species often, as this will lead to a higher knowledge level and therefore a better protection of this indigenous species.

Financial support statement

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Estimation of Phenolic, Flavonoid, and Antioxidant Properties of Stingless Bees Honey Based on Colour and Chemical Characteristics Using Artificial Neural Networks (ANN)

A. Zaldivar-Ortega[1], E. Flores-Santiago[2], F. Castro-Espinoza[3], P. Vázquez-Landaverde [4], M. Hernández-Gil [5], A. Garduño García[6], E. Hernández-Rojas[7], J. Angeles HernandezJ[7]

[1]Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, México, Mexico,
[2]Unidad Regional Universitaria Sursureste - Universidad Autónoma Chapingo, México,
[3]Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Mexico,
[4]Centro de Investigación en Ciencias Aplicadas y Tecnología Avanzada, Unidad Querétaro, Instituto Politécnico Nacional, Mexico,
[5]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico,
[6]Departamento de Ingeniería Mecánica Agrícola, Universidad Autónoma Chapingo, Mexico,
[7]Departamento de Medicina y Zootecnia de Rumiantes, Facultad de Medicina Veterinaria y Zootecnia. Universidad Nacional Autónoma de México, Mexico

Presenting author
J.C Ángeles-Hernández. E-mail: juanangeles@fmvz.unam.mx

Key words antioxidant, honey, machine learning, artificial intelligence

Application

In practical terms, the identification of honey with high content of phenols and flavonoids could predict the antioxidant activity as a biological function in honey. This approach could be useful for producers and consumers for a better product.

Introduction

Honey from stingless bees is gaining attention for its high phenolic content and strong antioxidant properties, which are superior to those found in honey from Apis mellifera. Phenolic compounds such as flavonoids and phenolic acids are transferred from nectar, pollen and propolis, enhancing the nutraceutical value of stingless honey. These compounds are crucial for the antioxidant activity of honey, which plays an important role in reducing oxidative reactions beneficial to human health. Therefore, the aim of the current study is to estimate the phenolic compound content and antioxidant activity of stingless honey, based on colour and chemical properties, using conventional and artificial intelligence approaches.

Material and methods

Samples

Honey samples were collected in April 2024, directly from hives managed by meliponicultors from eight locations in the warm humid forest of Puebla, México

Psychochemical analyses

Moisture and pH analyses were performed according to AOAC (2005). Colour was measured using a colorimeter (Chroma meter, CR-400,2008, Konica Minolta, Japan) (Habib et al., 2014). Total phenolic compounds were determined according to the Folin-Ciocalteu method described by Gonzalez-Montiel et al. (2024) and the absorbance was measured at 765 nm. The total flavonoid content was determined by the method described by Gonzalez-Montiel et al. (2024) and the absorbance was measured at 415 nm. The ABTS (2,2-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assay was performed as described by Gonzalez-Montiel et al. (2024) with modifications. A stock solution of 7 mM ABTS (Sigma-Aldrich) was prepared and reacted with 10 mL of 2.45 mM $K2S2O8$ for 16 h in the dark. The absorbance was adjusted with 20% ethanol to a value of 0.7 ± 0.1 at 734 nm. Aliquots of 0,2 mL honey in ethanol/water (80 % w/v) were mixed with 3 mL ABTS methanol solution.

Data base

A dataset comprising information from a total of 297 stingless bees honey samples was used, containing the following response variables: phenolic and flavonoid concentrations and ABTS assay, measured according to established procedures. Colour parameters (a, b, L), pH and moisture content were considered as predictor variables. In a first step, the data were randomly split into a training set (75%) and a test set (25%). The data were then normalised before training a neural network, using the min-max method to scale the values within the interval [0,1].

Mathematical modelling

Supervised learning was employed to train and teach the network using a two-layer artificial neural network (ANN) with 10 neurons in each layer (Fig. 1). The calculation of the ANN was conducted in R software using the neuralnet package [3], applying a globally convergent algorithm based on resilient backpropagation. To compare the predictive performance of the ANN with conventional methods, a multiple linear regression model was fitted using the same response variables with the glm() function in R. The goodness of fit for the ANN and linear regression was assessed using the mean square prediction error (MSPE), the root of MSPE (rMSPE), the correlation between observed and predicted values (r), and accuracy.

Results and Discussion

Table 1 presents a comparison of goodness of fit metrics for Multiple Linear Regression (MLR) and Artificial Neural Network (ANN) models in estimating phenolic, flavonoid and antioxidant properties of stingless honeys. Figure 1 illustrates the ANN architecture used to predict phenolic content based on colour parameters (L*, a*, b*) and chemical properties of the honey. The results show that ANN estimated phenolic content more accurately, achieving an rMSPE of 10.36 units compared to 20.65 units for MLR, with a higher correlation (r = 0.89) and accuracy (0.91). Similarly, ANN performed better in estimating flavonoid content with an rMSPE of 1.25 units, r = 0.76 and an accuracy of 0.85. For ABTS antioxidant activity, ANN also outperformed MLR, with higher correlation (r = 0.79 vs. 0.34) and accuracy (0.87 vs. 0.64). These results are consistent with the understanding that honey colour is a primary quality attribute perceived by consumers and influences their preference. The parameter L* indicates lightness, while a* and b* represent reddish and yellowish tones respectively. Honey colour is influenced by factors such as floral origin, mineral content, phenolic compounds and storage conditions (Kek et al., 2014). Darker honeys tend to have higher mineral and antioxidant capacities compared to lighter honeys (Chua et al., 2012).

Table 1. Comparison of goodness of fit for multiple regression model and artificial neural networks.

	Linear regression model (LRM)						Artificial Neural Network (10-10)				
	Actual	MSPE	rMSPE	R	Accuracy	Predicted	MSPE	rMSPE	r	Accuracy	Predicted
Phenols (mgAGE/g)	56.89	426.75	20.65	0.45	0.74	57.88	117.84	10.36	0.89	0.91	57.0
Flavonoids (mgEQ/g)	3.53	2.69	1.63	0.41	0.63	3.90	1.56	1.25	0.76	0.85	3.56
ABTS (mgAGE/g)	58.93	656.04	25.61	0.34	0.64	62.74	291.09	17.06	0.79	0.87	58.83

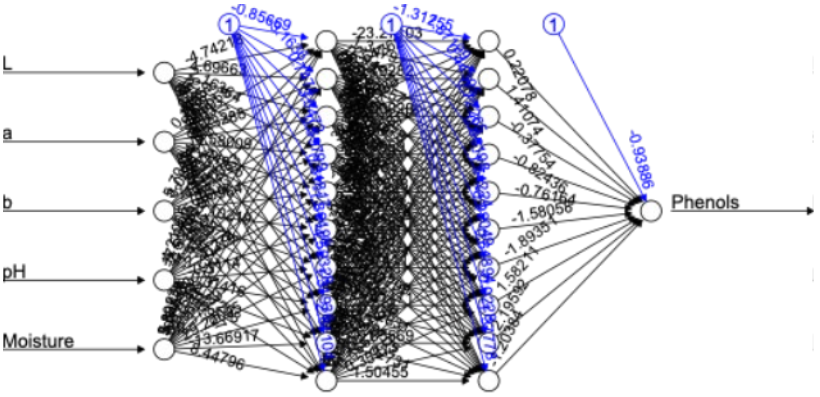

Figure 1. Architecture of used ANN with two-layers and 10 neurons to estimate phenolic, flavonoid, and antioxidant properties of stingless bee honey

Conclusion and implications

The colour could be related to the amount of bioactive compounds (phenols and flavonoids) and these in turn to the antioxidant activity; the higher the content of bioactive compounds, the higher the antioxidant activity. The results suggest that colour could be a quality parameter indicating the benefits of honey for consumers and producers.

Acknowledgements

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