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# **CINCE DE CONTRACTOR DE CONTRA**

The role of livestock in our ecosystems and economy

9<sup>th</sup> – 11<sup>th</sup> April 2024 Assembly Buildings, Belfast

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# animal - science proceedings

The international journal for conference proceedings A member of the animal family of journals

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### 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 80th Annual Conference 2024 held at the Assembly Buildings, Belfast 9th–11th April 2023.

### **Guest Editors**

Helen Ajayi, Gareth Arnott, Aurelie Aubry, Tommy Boland, Laura Boyle, Hieke Brown, Chris Browne, Judith Capper Anne Carter, Katie Dubarry, Carol-Anne Duthie, Jos Houdijk, Mark Little, Francis Lively, Elizabeth Magowan, Jean Margerison, Christina Marley, Kim Matthews, Katie McDermott, Cormac O'Shea, Jordana Rivero, Carlos Sandoval-Castro, Paul Smith, Sokratis Stergiadis, Laura Tennant, Helen Warren, Colin Whittemore, David Wilde.

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



### Welcome

The British Society of Animal Science (BSAS) aims to provide an opportunity for those with an interest in animals and animal science to exchange views, ideas and information. It is an energetic and active society with members from countries throughout the world. 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.

BSAS organises major scientific and specialist conferences on key issues facing the science related to animals. The 2024 annual conference addresses the topic of 'The role of livestock in our economy and ecosystems'.

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

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# **Proceedings**

# of the British Society of Animal Science Annual conference 2024

Presentations	Programme code	Presentations
Tuesday 9th April		Thursday 11th April
Opening Session	001	Sustainable beef production thr application of science
Advances in breeding and genetics to s sustainable livestock systems	support 002-009	Hot topics in pig production
The role of diverse pastures in future livestock sustainability Part 1	proofing 010-016	New & innovative technologies management
Poultry	017-022	Reducing the environmental im dairy systems
Controlling parasites sustainably in a owned world	changing 023-025	Precision technologies in livest
The role of diverse pastures in future livestock sustainability Part 2	proofing 026-032	The future of animal health and
The future of animal nutrition	033-035	
The role of vets and farmers in tacklir parasitic disease	<b>Ig</b> 036-040	Posters
BSAS Undergraduate Thesis of the Yea	r Winner 041	
Wednesday 10th April		
A deep dive into the role of livestock i economy and ecosystems	<b>n our</b> 042	
Climate change and endemic disease	043-049	
Advances in young stock science	050-058	
Sustainable small ruminant systems	059-065	
Dairy Cattle Production and Nutrition	066-074	
Adoption of science on farms and beha change	viour 075-082	
Sustainable Proteins: insects, co-produ	ıcts	

083-089

107

and technologies

Hammond Lecture: Livestock in a Circular Economy?

### Programme code

Sustainable beef production through application of science	090-096
Hot topics in pig production	097-103
New & innovative technologies in animal management	104-106
Reducing the environmental impact of dairy systems	108-112
Precision technologies in livestock management, health and welfare	113-121
The future of animal health and welfare when addressing environmental challenges	122-126
Posters	127-154

### Animal - Science Proceedings 15 (2024) 1-173



# Animal - Science Proceedings

journal homepage: www.elsevier.com/locate/anscip

### 1. The main output of livestock in agriculture is animal-sourced food

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The main output of livestock in agriculture is animal-sourced food. This makes a big contribution to global food and nutrition supply, particularly protein. Animal-sourced foods are sources of high-quality protein, and are also excellent sources of highly available minerals and vitamins and various bioactive components (taurine, creatine, camosine, conjugated linoleic acids). Livestock are also an important part of the agriculture sector, representing 40% of the total turnover. The value of livestock production in Europe (EU-28) in 2017 was estimated at 170 billion euros, and it employs approximately 4 million people. The wide distribution of livestock in rural areas makes livestock an important contributor to the economy and vitality of rural communities.

The output of animal-sourced foods makes livestock an essential part of the global food system. Together with crops grown for food and feed production, they give circularity to our food system, which is essential for soil health, and is helpful to optimise the use of available resources. Where this circularity is challenged or absent due to an excess of crops or animals in a region, the system can come under pressure with impacts on water quality, biodiversity or soil health.

A diversity of livestock systems is important for resilience of the livestock industries, the food they supply and the overall food system. A diversity of livestock systems allows production to be optimised to local conditions, and provides a range of animal sourced foods which are diverse in aspects such as cost to consumers, taste, nutritional value and versatility for meal preparation.

Sustainability (economic, environmental and social) is a key consideration in to-day's food systems. The contribution of livestock production to climate change receives much attention. Still, environmental sustainability has many other considerations: water quality, biodiversity, circularity (e.g. the return of animal manures to the areas where their feed is produced), soil health, the ratio of human edible food: human non-edible feed in the diet of livestock, antibiotic and pesticide usage, animal welfare. This results in a complex landscape with several interconnections, benefits and trade-offs, and livestock's contribution to environmental sustainability cannot be easily assessed using a single-dimension analysis such as a life cycle assessment of its carbon footprint. A multi-dimensional assessment is needed, and livestock can contribute positively to most of the dimensions outlined above, but the contribution can also be negative in the wrong circumstances (e.g. a manure surplus due to overstocking causing water pollution).

While food is the main output of livestock production, livestock are much more than food. Other products from livestock production include hides, wool and hair, offals and other by-products like collagen and gelatin, foods with enhanced nutritional effects, compounds used in medicine such as insulin, heparin and hyaluronic acid, manures for fertiliser and fuel (e.g. biogas), biodiversity and landscape and fire management, contributions to culture and wellness as well as being an integral part of vibrant rural communities in many regions. These products, together with food, illustrate that livestock play a very important role in our economy and our ecosystem.

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### 2. Breeding strategies to reduce ruminant methane emissions

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Genetic improvement has a significant role to play in the reduction of greenhouse gas (GHG) emissions associated with agriculture. Traditionally genetic gain has been gauged in terms of economic improvement of each successive generation of selection over its predecessor. This has been achieved through leveraging all components of "the Breeders equation". The same approach can be leveraged where carbon costs on the same traits have been successfully modelled. Hence updated national indexes will be focused on both economic and climate





sustainable goals. ICBF and Teagasc have worked collaboratively to include carbon emissions in the national dairy, beef on dairy and beef indexes in recent years. The carbon modelling undertaken by Teagasc evaluates the impact of dairy and beef production on the three main GHG gases namely methane, nitrous oxide and carbon dioxide. In addition, significant investment has been made in direct methane phenotyping with the ultimate aim of developing and including direct methane ebvs in the indexes.

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### 3. Insights into the transcriptome of sheep circulating immune cells using single-nuclei RNA-sequencing

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### Application

Improve the speed of analysing future single-cell experiments in sheep and provide a reference for digital cell sorting from bulk RNAsequencing data; reducing costs and increasing statistical power.

### Introduction

Exploring the transcriptome at cellular resolution can allow us to detect variations in gene expression across cell and tissue types with unprecedented granularity. This is particularly powerful in heterogenous tissue types where signatures from rarer cell types may be undetectable or considered background noise when using bulk RNA-sequencing, due to their relatively low level of expression. In sheep, only a few single-cell experiments have been reported in the literature, and none so far have investigated immune tissues. This experiment will investigate the heterogeneity of sheep circulating immune cells, providing new insights into their transcriptional activity, a resource for faster and improved annotation of single-cell datasets in this tissue type, and a basis for deconvolution of bulk RNA-sequencing data to cellular resolution.

### Materials and methods

We carried out snRNA-seq of peripheral blood mononuclear cells (PBMCs) sorted from whole blood obtained from adult sheep. In choosing a single-nuclei methodology over whole cells we aimed to improve recovery of all cell types after freezing; reducing the loss of delicate and rarer cells, and allowing the overall cell type proportions to be maintained. After euthanasia with pentobarbital, blood from female commercial cross-breed sheep (n = 3) was collected via cardiac puncture. PBMCs were isolated using density gradient centrifugation and stored at -155 °C. Nuclei were extracted using a modified version of Ruiz-Daniels et al. (2023) then fixed and permeabilised using the Parse Biosciences Nuclei fixation kit. Subsequently snRNA-seq libraries were produced using the Parse Biosciences Evercode WT Mini Kit targeting the capture of the transcriptomes of 13,000 nuclei with equal representation of the three samples used. Libraries were sequenced on an Illumina Nextseq 2000 P2 flowcell.

Barcoded reads were demultiplexed using the Parse Split-pipe computational pipeline v1.1.0, the latest version of the sheep genome Rambouillet V2 (Davenport et al., 2022), and STAR as the underlying genome mapping tool. The count matrix was imported into R for analysis with Seurat v4. Barcodes with <200 or >25,000 transcripts were filtered out, to remove captured ambient RNA and multiplets respectively. After filtering, 9235 nuclei successfully passed quality control criteria.

We normalised the gene expression values before performing PCA and dimensional reduction (UMAP). Clustering was carried out using a Louvain algorithm to co-locate cells based on the similarity of the gene expression. Differential expression testing between clusters allowed us to identify the genes most significantly associated with each cell cluster.

We used known marker genes to interrogate the cell clusters and produce annotations. In addition, we carried out Gene Ontology analysis to investigate the cellular pathways active within the clusters. Two large groups of clusters, thought to represent B-cell and T-cell lineages, were subset from the main dataset and re-clustered to allow finer interrogation of the cell subtypes.

### Results

14 clusters of nuclei were identified representing immune cell types and subsets. This shows that sheep PBMCs are highly heterogeneous, with groups of smaller clusters representing cell sub-types. After cluster annotation, superclusters of major immune cell types were identified as well as clusters of rarer cell types (Fig. 1).

Canonical cell marker genes were sporadic within the dataset, and some expected markers were absent entirely (CD8, TLR4). Inspecting active pathways, manually reviewing the top significant genes for each cluster (*Bonferroni adjusted p-value <0.05*), and searching for classical cell markers allowed us to identify clusters associated with B cells, T cells, monocyte-derived macrophages, NK cells, Gamma-Delta T cells, and dendritic cells.



Fig. 1. UMAP plot of single-nuclei clusters.

### Conclusions

PBMCs in sheep are a highly heterogenous and complex mixture of immune cell types and sub-types. Classical marker genes are not always helpful when annotating single-nuclei/-cell data, as they may not be captured by the snRNA-seq protocol. However, this analysis has allowed us to capture rare cell types such as dendritic cells, which might otherwise be lost in the averaged expression levels of bulk RNA-seq datasets.

The gene sets identified through this analysis can be used as a basis for cell type annotation in future snRNA-seq experiments in sheep, reducing the time required to manually classify cell types. In addition, this resource will provide a basis for deconvolution of single-cell level gene expression signatures from bulk RNA-seq data; contributing to the economical expansion of experiments from small to larger numbers of animals.

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### 4. Towards conclusive evidence that dairy cattle vary genetically in bovine tuberculosis infectivity

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### Application

Selecting cattle with low infectivity, alongside enhanced disease resistance, can help eliminate bovine tuberculosis (bTB) in the UK.

### Introduction

bTB is an infectious cattle disease that continues to impose significant financial burden to the UK. Since 2016, genetic selection of cattle with enhanced genetic bTB resistance has been contributing to UK goal to achieve an officially tuberculosis free status by 2038. Recent studies have indicated that concurrently reducing infectivity in cattle while enhancing their resistance may accelerate the process of bTB readication (Banos, 2023).

Our previous analyses of a subset of the data used in genetic evaluations for bTB resistance of dairy cattle has provided first empirical evidence that dairy cattle may vary genetically in their bTB infectivity (Madenci et al., 2023). However, the models used in that study could not properly account for genetic variation in bTB resistance, and the large extent of zero inflation and overdispersion observed in the infectivity phenotype. The main aim of the present study was to provide conclusive evidence for genetic variation in dairy cattle infectivity by applying generalized linear mixed models that can better handle the inherent complexities in the data.

### Material and methods

The dataset used was provided by Egenes and consists of the same data as used for the national genetic evaluation of bTB resistance (Banos et al., 2017), and the resulting estimates of genetic merit in bTB resistance. The dataset includes bTB records (date of first positive test) and pedigree data of dairy cattle (only Holstein Friesian) involved in all nationally recorded bTB breakdown herds in England and Wales between 2000 and 2022.

This study focuses on estimating genetic variation in the infectivity of index cases, defined as the first cows per breakdown that tested positive within first test period. Secondary cases were defined as those that tested positive in the subsequent testing period. Only breakdowns with a single index case (6,668 index cases) and pedigree data extracted for these cows spanning seven generations (2,931 sires) were considered, and infectivity was defined as the number of secondary cases per index case.

Generalized linear mixed models (implemented in MCMCglmm R-package (Hadfield, 2014)) that could handle both zero inflation and overdispersion, specifically the zero inflated poisson (ZIP), hurdle and geometric (GEO) model, were used to estimate genetic parameters for the above infectivity phenotype. All models included as fixed effects breakdown year, season (all four seasons), county, herd size, age of the index case, and the average estimated genetic bTB resistance of all herd members, and sire of the index case as random effect.

### Results

The distribution of the number of secondary cases showed that majority of the breakdowns ( $\sim$ 73%) have zero secondary cases, and large over-dispersion, justifying the choice of the models considered in this study. Table 1 presents posterior means of variance components for

### Table 1

Posterior means of variance components and the 95% highest posterior density interval l(HPD95%).

Model					
Hurdle		ZIP		Geometric	
Variance <sup>1</sup>	Binary <sup>2</sup>	Poisson	Binary <sup>2</sup>	Poisson	
$\sigma_s^2 \ \sigma_e^2$	<10 <sup>-5</sup> 1.000	0.0242 (0.0002, 0.0886) 1.2990 ( 1.131, 1.475)	<10 <sup>-5</sup> 1.000	0.0148 (0.0002, 0.0591) 3.9570 (3.556, 4.331)	0.0332 (<10 <sup>-5</sup> ,0.1177) 3.0610 (2.695,3.393)

<sup>1</sup>  $\sigma_s^2$  = sire variance,  $\sigma_e^2$  = residual variance.

<sup>2</sup> The residual variance,  $\sigma_{e}^{2}$ , for the zero-part in both ZIP and Hurdle models fixed at 1.

each model. The ZIP and Hurdle models yielded similar estimates of sire variance (Poisson part of the models), and the 95% highest posterior density (HPD) intervals for both models do not encompass zero, indicating that index cases vary genetically in their infectivity. In contrast, the geometric model estimated a slightly higher sire variance although the range of the interval was wider, and with the lower bound closer to zero. Our results also suggest that average resistance EBV does not have direct effect on infectivity phenotype, indicating that cattle may be selected for lower infectivity without impacting selection for resistance.

### Conclusion

The study provides more conclusive evidence that UK dairy cattle vary genetically in bTB infectivity, implying that genetic selection for lower cattle infectivity may be feasible to help bTB eradication. Detection of genetic variance in index case infectivity in current models is encouraging as index cases represent only a small proportion of the cattle population. Future studies that account for genetic differences in infectivity of all dairy cattle involved in bTB breakdowns are expected to uncover more genetic variation in infectivity. In addition, further investigation is ongoing to identify best model to use for future analysis which remains as a future work.

### Acknowledgements

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### 5. Potential of genetic selection for feed efficiency, carcass quality and meat quality traits in sheep: A meta-analysis

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### Application

The success of genetic selection programs depends on the heritability of the traits used as indicators in breeding programs. The study revealed that feed efficiency and carcass quality traits in sheep possess moderate to high heritability, while meat quality traits exhibit relatively lower heritability. This information could be valuable for those involved in breeding and selection programs for sheep and could help inform decisions regarding which traits to prioritize in such programs.Fig. 1



a) Heritability estimates for RFI (n=7,039)

Study	Mean SE	Weight	Mean IV, Random, 95% Cl	Me IV, Rando	an m, 95% Cl
(Mortimer et al. 2010)	0.3500 0.0612	22.6%	0.35 [0.23: 0.47]		÷ <mark>.</mark>
(Massender et al. 2019)	0.1700 0.0204	35.1%	0.17 [0.13; 0.21]		
(Mortimer et al. 2014)	0.2500 0.0408	29.1%	0.25 [0.17; 0.33]		-
(Mortimer et al. 2018)	0.3500 0.1020	13.2%	0.35 [0.15; 0.55]		
	0.2800 .	0.0%	0.28		1
Total (95% Cl)		100.0%	0.26 [0.17; 0.35]	[]	-
Heterogeneity: Tau <sup>2</sup> = 0.00	056; Chi" = 11.78,	df = 3 (P	< 0.01); l* = 75%		
				-0.4 -0.2 (	0.2 0.4
b) He	eritability estimat	tes for HO	CW (n=41,439)		



### Introduction

Over the years, genetic selection has proved to be an effective tool in enhancing the productivity and performance of livestock. Through this method, specific desirable traits are identified and selected for breeding, leading to the production of offspring with enhanced genetic makeup and improved performance in areas such as growth rate, feed efficiency (FE), carcass quality (CQ), and meat quality (MQ). With continuous improvements in genetic and genomic technologies, farmers and breeders can now use these advanced techniques to further refine their selection criteria, leading to the development of even more robust and high-performing animal breeds. To achieve successful genetic selection, trait heritability (h<sup>2</sup>) plays a crucial role (Habtegiorgis et al., 2022), with various studies reporting varying estimates of h<sup>2</sup> for these traits in sheep. This study aimed to combine the results from these individual studies in a statistical meta-analysis to obtain a deeper insight into the genetic parameters of key efficiency and production-related traits in sheep.

### Materials and methods

The search strategy for the meta-analysis was performed based on the preferred reporting items for the systematic reviews and meta-analyses (PRISMA) framework using databases namely, EBSCO, PubMed, Scopus, and Web of Science. The keyword search included ovine, sheep, lambs, ewes, or rams, and no publication date limits were enforced due to the limited availability of sheep data. Subsequently, only studies on h<sup>2</sup> of FE, CQ, and MQ traits were selected with those unrelated, such as wool characteristics and milk production, excluded. Nine published records between 2005 and 2023 were identified which analyzed  $h^2$  from a diverse range of sheep breeds, including Merino (n = 27,690), Targhee (n = 1,047), Romane (n = 951), New Zealand maternal sheep (n = 986), Canadian crossbred lambs (n = 16,565) and composite rams (½ Columbia, ¼ Hampshire, ¼ Suffolk) (n = 1,239), with a total of 48,478 sheep included. The R package meta (Borenstein et al., 2021) was utilized to perform a meta-analysis of seven FE, CQ, and MQ traits in sheep including residual feed intake (RFI), feed conversion ratio (FCR), hot carcass weight (HCW), intramuscular fat (IMF), dressing percentage (DP), pH and shear force, with a random-effects model used to account for variability in estimations. Findings were summarized, and forest plots were generated to visualize and interpret observations.

### Results

Performed meta-analysis conducted in this study indicates that RFI presents a moderate  $h^2$  estimate of 0.27 ± 0.07 (P < 0.05) as an FE trait. Similarly, CQ traits such as HCW, IMF, and DP were found to be moderately to highly heritable, with  $h^2$  estimates of 0.26 ± 0.05 (P < 0.05), 0.45 ± 0.04 (P > 0.05), and 0.23 ± 0.05 (P > 0.05) respectively. Conversely, pH and shear force, which are meat quality traits, showed less heritability, with  $h^2$  estimates of 0.09 ± 0.02 and 0.20 ± 0.08 respectively (both P > 0.05). Notably, RFI, FCR, HCW, and shear force exhibited significant heterogeneity, with  $I^2$  index values of 78, 77, 75, and 65, respectively which could be attributed to various factors such as breed, management, and diet.

### Conclusion

The results of the meta-analysis are highly informative on the genetic underpinnings of key traits such as FE and CQ, both of which have been found to have moderate to high heritability. The inclusion of these traits into the selection programs will be of substantial benefit to breeders and farmers in the production of animals with superior performance in these traits. The findings will serve as a foundation for future research on genomics applications such as genome-wide association analysis of FE, CQ and MQ traits in sheep.

### Acknowledgements

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# 6. Informativeness of interactions between abundances of Methanobrevibacter and microbial genes for microbiome-driven breeding to reduce methane emissions in beef cattle

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### Application

The use of the genomic interactions between abundances of *Methanobrevibacter* and microbial genes is recommended as further selection criteria to identify informative microbial genes included in microbiome-driven breeding to reduce methane emissions in ruminants.

### Introduction

The rumen microbiome, which includes bacteria, archaea, protozoa, fungi, and their genes, is influenced by host animal genomics while significantly affecting methane ( $CH_4$ ) emissions (Martínez-Álvaro et al., 2022). Particularly, the substantial genomic informativeness of microbial genes for microbiome-driven breeding to mitigate  $CH_4$  has been demonstrated (Roehe et al., 2016). *Methanobrevibacter* is the most abundant hydrogenotrophic methanogen in ruminants. However, to the best of our knowledge, its genomic interactions with ruminal functional microbial genes are not known. Therefore, we estimated the heritabilities ( $h^2$ ) of the abundance of *Methanobrevibacter* and functional microbial genes, the genetic correlations ( $r_g$ ) between each other and with  $CH_4$  emissions to understand functional relationships and its usefulness for microbiome-driven breeding to mitigate the potent greenhouse gas.

### Materials and methods

The experiment was conducted following the UK Animals Act 1986 and approved by the Animal Experiment Committee of SRUC. Three hundred sixty-three steers raised under the same housing conditions on the same research farm over five years were used in this project. The animals were balanced for different breeds (Aberdeen Angus, Limousin, Luing, and Charolais crosses) and basal diets (two diets of 480:520 and 80:920 concentrate:forage ratios). Blood samples for host animal and rumen samples for microbial DNA extraction were col-

lected at slaughter. Sequence reads of microbial DNA were aligned to the Kyoto Encyclopedia of Genes and Genomes database, resulting in the identification of 3361 microbial genes. To account for the compositionality of microbiome data, microbial genes abundance data were additive-log-ratio (alr) transformed, whereas *Methanobrevibacter* abundance was centred-log-ratio transformed (clr). CH<sub>4</sub> emissions were individually measured on 285 of the 363 animals during 48 h using six indirect open-circuit respiration chambers and expressed as CH<sub>4</sub> yield (CH4Y, g CH<sub>4</sub> /kg dry matter intake). Multiple genomic bivariate analyses were conducted between CH4Y and *Methanobrevibacter* clr or microbial genes alr abundances; and between those of Methanobrevibacter and microbial genes to obtain  $h^2$  and  $r_g$ .

### Results

*Methanobrevibacter* dominated the methanogen population, accounting for 93±7.36% of the total abundance of all identified methanogens. Estimated h<sup>2</sup> of the abundance of this genus was 0.30 with 95% highest posterior density interval (95%HPD) between 0.16 to 0.49; and was positively genomically correlated to CH4Y at  $r_g$  = 0.21 (95%HPD –0.23 to 0.57, and probability of being positive (Pr0) at 0.81). The h<sup>2</sup> of the abundance of microbial genes ranged from 0.16 to 0.42 (Fig. 1) with 14% greater or equal than that of *Methanobrevibacter* (0.30). Their  $r_g$  with CH4Y ranged from –0.37 (95%HPD –0.68, 0.01) to 0.43 (95%HPD 0.05, 0.73).

Of the microbial genes, we selected based on their estimated genetic parameters 500 each with highest predicted positive or negative correlated response with CH4Y to explore their genetic correlations with *Methanobrevibacter*. Some of these microbial genes exhibited strong genetic correlations with *Methanobrevibacter*, ranging from -0.49 to 0.69, with 53 microbial genes showing  $r_g \ge 0.40$  (Pr0 > 0.96) and 18 microbial genes showing  $r_g \le -0.40$  (Pr0 > 0.97) (Fig. 2).



Fig. 1. Distribution of heritabilities of 3361 functional microbial genes estimated as posterior medians.



Fig. 2. Distribution of genetic correlations between abundances of Methanobrevibacter and microbial genes using bivariate analyses.

The function microbial genes of informative magnitude negatively genomically correlated to both the abundances of *Methanobrevibacter* and CH<sub>4</sub> emissions were *pepT* (K01258), *pflD* (K00656) and *pgm* (K07126) involved in metabolism of protein, propionate, and glycolysis, respectively. Whereas those positive genomically correlated were *atpK* (K02124), *oorD* (K00176) and *bcrC* (K19302) involved in oxidative phosphorylation, carbon fixation, and lipid metabolism, respectively.

### Conclusion

Abundances of some of microbial genes and *Methanobrevibacter* were identified to be animal genomically influenced and resulted in substantial host genetic correlations with  $CH_4$  emissions and among each other. Microbial genes that correlated with both  $CH_4$  emissions and *Methanobrevibacter*, such as *pepT* (K01258), *pflD* (K00656) and *pgm* (K07126), are recommended to be selected as informative traits within the microbiome-driven breeding strategy to reduce  $CH_4$  emissions.

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# 7. Construction of the mRNA-miRNA regulatory network identifies candidate genes and pathways associated with Johne's disease in dairy cattle

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### Application

This comparative transcriptome profiling analysis allows for the identification of associated mRNAs/genes and miRNAs, their functions, and important pathways in Johne's disease and healthy samples.

### Introduction

Johne's disease (JD) is a chronic and incurable disease in ruminants that severely affects animal welfare and causes great economic losses for worldwide livestock production, especially in dairy cattle (Ariel et al., 2020). The causative agent, *Mycobacterium avium ssp. paratuberculosis* (MAP), causes chronic enteritis in ruminants, eventually leading to weight loss, diarrhea, gradual decrease in milk production, and death of infected animals. As the usual diagnostic methods to control this disease are ineffective, alternative diagnostics are urgently needed for disease control. During infection, MAP bacilli undergo phagocytosis by host macrophages, causing subclinical infections that can lead to immunopathology and disease dissemination (Casey et al., 2015). Therefore, analysis of the host macrophage transcriptome during infection is expected to elucidate molecular mechanisms and host-pathogen interactions associated with Johne's disease. In addition, although non-coding RNAs (ncRNAs; e.g., miRNAs) have an important role in regulating immune system function and may provide valuable information about the disease, their role in paratuberculosis infection in cattle has not yet been fully investigated (Marete et al., 2021).

### Materials and methods

Microarray and RNA-Seq datasets from peripheral blood mononuclear cells (PBMCs) of Johne's disease and healthy 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 applied to explore hub mRNAs/genes and miRNAs related to Johne's disease using DAVID, g:Profiler, GeneCards, and STRING database to determine potential functions, metabolic, and signaling pathways. Interactions between types of RNAs (i.e. mRNAs/genes and miRNAs) identified were predicted, and a mRNA-miRNA regulatory network was constructed by integrating the protein-protein interaction (PPI) network and gene regulatory network (GRN). Genes were assigned to functional categories using the Gene Ontology (GO) database under biological process (BP), molecular function (MF), and cellular component (CC).

### Results

Comparative transcriptomics-related analyses identified 4536 and 783 differentially expressed genes (DEGs) between Johne's disease and healthy groups for RNA-Seq and microarray datasets respectively, based on the fold change ( $\geq 1$  and  $\leq -1$ ) and false discovery rate (FDR <0.05). Among these, 23 genes were common between RNA-Seq and microarray datasets. In addition to DEGs, 19 miRNAs were also simultaneously identified in the RNA-Seq datasets. Furthermore, we identified 10 hub genes (*CCL20, CCL4, IL10, NOS2, NR4A1, PTGS2, SERPINE1, TFPI2, TNFAIP6*, and *TNFSF13B*) involved in dairy cattle Johne's disease. Identified biological and regulatory networks were mainly associated with immune system signature pathways. Gene ontology annotation and enrichment analysis, based on the mRNA-miRNA regulatory network (Figure 1) revealed 20, 1, and 1 GO terms related to Johne's disease in the biological process, molecular function, and cellular component categories, respectively. In addition, KEGG enrichment analysis identified cytokine-cytokine receptor interaction, intestinal immune network for IgA production, and signaling pathways for IL-17, TNF, HIF-1, and NF-kappa B. Gene set annotation and functional enrichment of identified DEGs implicated important biological pathways.



Figure 1. mRNA-miRNA regulatory network on Johne's disease in dairy cattle.

### Conclusions

These findings provided valuable insights into the molecular evidence for the biological mechanisms of transcriptome profiling of Johne's disease versus healthy Holstein dairy cattle. In addition, they are an impetus to elucidate molecular networks and functions of DEGs associated with peripheral blood mononuclear cells regarding immune system function and a starting point for future studies on bovine Johne's disease.

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### 8. The relationship between water efficiency feed efficiency and growth traits in beef cattle: A genetic analysis

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### Application

Estimating genetic parameters to assess the relationship between water efficiency and production traits in beef cattle is of paramount importance for implementing genetic selection as a sustainable strategy to enhance water usage efficiency while minimising any potential negative impacts on production traits.

### Introduction

Water is a vital resource for sustaining life, and concerns about quality, scarcity and accessibility pose serious challenges in many regions (United Nations World Water Development Report, 2023). The beef industry faces increasing demands to adopt production practises that minimise water usage while enhancing animal growth and feed efficiency (Pereira et al., 2021). The objective of this study was to estimate the genetic parameters of traits associated with water intake, water efficiency, feed efficiency and growth in beef cattle.

### Materials and methods

The data was collected from a group of 1249 seedstock bulls and heifers from six different breeds: Angus, Charolais, Hereford, Limousin, Simmental, and Sim-Angus. They were housed in a facility equipped with Vytelle SENSE<sup>®</sup> systems to measure their feed intake, water intake and inpen body weight, located at the West Virginia University central testing centre in Wardensville, WV, USA. The animals were part of nine performance trials conducted between November 2019 and September 2023 and were sourced from 45 different consignors, each contributing a varying number of animals to the trial. The trials were conducted either in the Summer (June to September) or in the Winter (November to February). Of the total animals tested, 624 were tested in summer and 625 in winter. The average age of the animals at the start of the test was 287 days (±21), and on average the trials lasted for 62 days in summer and 82 days in winter. The rations were formulated to achieve 1.5–1.6 kg/day of average daily gain and had a high forage inclusion (>75% corn silage and >15% dry hay, as fed). The facility consists of 5 pens and the animals were rotated through the pens every two weeks so that all animals were exposed to all pens.

This study examined the following phenotypes: Average daily water intake (DWI), Residual water intake (RWI), which is the deviation of the actual daily water intake from the expected value, Average daily dry matter intake (DMI), Residual feed intake (RFI), which is the deviation of the actual daily dry matter intake from the expected value, and Average daily gain (ADG). The traits RWI and RFI were used to measure the water and feed efficiency of the animals. The genetic parameters were estimated with multi-trait animal models using Echidna mixed model software (Gilmour, 2021). In all models, breed, sex, season, and the contemporary group (trial number and pen group) were included as fixed effects, the age at the start of the test was added as a covariate, and the consignor and animal were included as random effects. RWI and RFI were analysed by adding ADG and metabolic mid-weight as additional covariates.

### Results

The genetic parameters for water efficiency, feed efficiency and growth are presented in Table 1. The heritability of DWI, RWI, ADG and DMI were moderate (0.28–0.51), while RFI showed low heritability (0.14) in this population. Average daily water intake exhibited a positive genetic correlation with RWI (0.92), DMI (0.44) and ADG (0.64). On the other hand, the genetic correlations of RWI with DMI and RFI were not significantly different from zero.

### Table 1

Estimates of heritability (on the diagonal), genetic correlation (above the diagonal) and phenotypic correlation (below the diagonal) for water efficiency, feed efficiency and growth traits in beef cattle. SE in parenthesis.

Trait	DWI	RWI	DMI	RFI	ADG
DWI	0.51 (0.09)	0.92 (0.02)	0.44 (0.13)	-0.13 (0.24)	0.64 (0.15)
RWI	0.92 (0.01)	0.45 (0.08)	0.15 (0.16)	-0.12 (0.24)	0.36 (0.18)
DMI	0.33 (0.03)	0.07 (0.03)	0.37 (0.09)	0.57 (0.18)	0.86 (0.11)
RFI	0.08 (0.03)	0.09 (0.03)	0.72 (0.01)	0.14 (0.07)	0.51 (0.27)
ADG	0.22 (0.03)	-0.01 (0.03)	0.54 (0.02)	0.01 (0.03)	0.28 (0.09)

DWI = Average daily water intake; RWI = Residual water intake; DMI = Average daily dry matter intake; RFI = Residual feed intake; ADG = Average daily gain.

### Conclusion

The results suggest that the traits associated with water intake and efficiency in beef cattle have a sizable genetic component and show potential for genetic improvement. Selecting for enhanced water efficiency in beef cattle can effectively decrease the daily water consumption without any adverse effect on feed efficiency.

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### 9. Genome-wide association study of resilience traits in two dairy sheep breeds

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### Application

Resilience of dairy sheep to disease and environmental stressors can be improved through selective breeding. Genetic markers highlighted herein could be applied in such breeding programs, but differences among breeds should be considered.

### Introduction

Genetic selection of dairy sheep has been mainly focused on higher milk production. However, over the years, this practice increased susceptibility to diseases and environmental stressors, and adversely affected their health and welfare status and overall production efficiency (Mucha et al., 2022). Improving resilience to such stressors can enhance the ability of dairy sheep to overcome these challenges while maintaining sufficient productivity; local breeds are well-adapted to their natural environment and may have adequate genetic potential for enhanced resilience. The objective was to estimate heritability of three proxy traits for resilience (milk somatic cell count, lactation persistency and body condition score) and perform genome-wide association studies to detect single nucleotide polymorphisms and candidate genes associated with these traits in Chios and Frizarta dairy ewes.

### Material and methods

A total of 317 Chios and 346 Frizarta ewes were randomly selected from two intensive and two semi-intensive farms (one of each per breed) in Greece. Ewes were monitored monthly for two consecutive milking periods; individual records of milk yield and body condition score, and milk samples were collected. Milk samples were analysed to determine somatic cell count. Body condition score was assessed by

palpation of the lumbar region based on previously described methodology (Russel et al., 1969). Prior to further analyses, individual repeated records of body condition score were averaged over both milking periods of the study. Regression coefficient of daily milk yield (after peak of lactation) on days from lambing, corresponding to lactation persistency, and mean somatic cell count weighted over daily milk yield were calculated for each ewe per milking period. Individual lactation persistency and mean somatic cell count per milking period were averaged over both periods of the study and used for further analyses. Genomic DNA was extracted from blood samples and geno-typed with Illumina OvineSNP50 Genotyping Beadchip. Genotype quality control was performed with PLINK 1.9 software (Chang et al., 2015); thresholds for single nucleotide polymorphism and sample call rate (at 97% and 90%, respectively), minor allele frequency (at 2%) and deviations from Hardy-Weinberg equilibrium (*P*-value =  $10^{-6}$ ) were set. Breed-specific principal component analyses were performed with GEMMA version 0.98.1 (Zhou and Stephens, 2012) to explore genomic population structure. Single-trait animal linear mixed models with genomic relatedness matrices were used for variance components analyses (performed with ASReml version 4.2) and genome-wide association analyses of studied traits (performed with GEMMA version 0.98.1).

### Results

Moderate to high heritability estimates were observed for all studied traits (body condition score:  $h^2 = 0.54$  and 0.55, somatic cell count:  $h^2 = 0.25$  and 0.38 and lactation persistency:  $h^2 = 0.43$  and 0.45, for Chios and Frizarta ewes, respectively). Unlike all other estimates, heritability of somatic cell count in Chios sheep was not statistically significant ( $\alpha = 0.05$ ). In both breeds, population structure associated with farm of origin of ewes was revealed by principal component analyses. Genome-wide association studies detected seven novel single nucleotide polymorphisms associated with the studied traits. Specifically, one genome-wide (rs403061409 on chromosome 9) and two suggestive (rs424064526 and rs428540973 on chromosomes 1 and 12, respectively) significant associations with somatic cell count of Frizarta ewes were detected; one suggestive significant association with body condition score of Chios ewes (rs424834097 on chromosome 4); three suggestive significant associations with lactation persistency, two of which in Frizarta (rs193632931 and rs412648955 on chromosomes 1 and 6, respectively) and one in Chios analyses (s428128299 on chromosome 3). Single nucleotide polymorphism annotation and previously reported associations revealed a total of nineteen candidate genes: two for body condition score in Chios sheep (*POT1*, *TMEM229A*), thirteen for somatic cell count in Frizarta sheep (*NTAQ1*, *ZHX1*, *ZHX2*, *LOC101109545*, *HAS2*, *DERL1*, *FAM83A*, *ATAD2*, *RBP7*, *FSTL1*, *CD80*, *HCLS1*, *GSK3B*), three (*GRID2*, *FAIM*, *CEP70*) and one (*GRIP1*) for lactation persistency of Frizarta and Chios sheep, respectively. The above genes are involved in regulation of cell apoptosis, telomere length, reproductive hormones excretion, mammary growth and immune response.

### Conclusion

The studied resilience traits were significantly heritable and hence amenable to improvement. They contribute to further unravelling the genetic architecture of somatic cell count, lactation persistency and body condition score. Absence of common single nucleotide polymorphisms or genes among breeds, suggest polygenic inheritance of the studied traits and emphasize the need for within-breed studies. Further studies will focus on the impact that different alleles of significant single nucleotide polymorphisms have on the studied traits and search for possible correlations among the studied and other production traits to allow effective incorporation into breeding programs aiming to improve resilience.

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### 10. Enhancing the sustainability of ruminant livestock systems through multispecies swards

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Ruminant livestock production systems have an undeniably important role to play in ensuring global food security and human nutrition. However, the intensification of many grassland areas, particularly in temperate regions of the world over recent decades, has given rise to increasing societal concerns regarding the environmental sustainability of these systems. These concerns and associated pressures for change, often relate to the simplification and homogenisation of more botanically diverse grasslands to monocultures of high yielding species such as perennial ryegrass (PRG; *Lolium perenne* L.). From an agronomic perspective, PRG offers many benefits, however, its performance is largely predicated on high inputs of fertiliser nitrogen (N). Such reliance on N fertilisers presents a number of significant challenges, including: 1) economic cost to farmers and 2) environmental costs, including elevated nitrous oxide emissions, increased risk of water pollution, and loss of biodiversity. Such simplification of sward composition can also result in reduced resilience to environmental perturbations such as drought.

While there is no definitive definition of what constitutes a multispecies sward (MSS), they are generally regarded as agronomically improved grasslands that have been sown with 3+ species that represent different plant functional groups i.e. grasses, legumes and forage herbs. Species are selected for inclusion based on differing but complementary traits, and commonly include, but are not restricted to: PRG, timothy (*Phleum pratense*), cocksfoot (*Dactylis glomerata*), white and red clover (*Trifolium repens and pratense*), chicory (*Cichorium intybus*) and ribwort plantain (*Plantago lanceolata*).

Based on more than a decade of research and a number of projects, including SmartGrass and SmartSward(https://www.ucd.ie/research/ impact/casestudies/smartgrassimprovingthesustainabilityoflivestockfarming/), HeartLand (https://www.heartlandproject.eu), Multi4More (https://multi4more.ie) and FaSTEN, this presentation focuses on the potential of MSS as a low-input, high-output alternative sward type, with the potential to address many concerns regarding the sustainability of ruminant production systems. In particular it will focus on MSS potential to: 1) maintain, and in many cases increase production of high nutritive value herbage dry matter, at relatively low rates of N inputs; 2) enhance animal performance and health; 3) reduce the carbon footprint of food outputs; 4) maintain high quality food production; 5) increase resilience to environmental perturbations e.g. drought; 6) enhance invertebrate biodiversity; 7) reduce economic costs incurred by farmers – all relative to high N fertiliser input PRG monocultures. In addition, barriers to MSS usage including: 1) farmer perceptions, 2) weed management, and 3) concerns regarding persistence of the forage herb component of the swards, will be considered and discussed.

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### 11. Grazing multispecies swards alters enteric methane emissions by ewes and lambs

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### Application

Grazing swards containing a mixture of grass, legumes and herbs can enhance the sustainability of pasture-based sheep meat production by reducing daily enteric methane output produced by sheep.

### Introduction

The production of enteric methane during the rumen fermentation process serves as a source of energy loss from ruminants which could otherwise be utilised for meat, milk or wool production and also contributes to environmental damage through the release of this potent greenhouse gas. Previous studies reported lambs grazing swards containing more than one plant species resulted in earlier achievement of target slaughter (Grace et al., 2019; McGrane et al., 2022) and potentially, reduced total lifetime emissions. There is a paucity of information relating to the impact of multispecies swards on enteric methane emissions, therefore this study measured daily methane output by sheep grazing a perennial ryegrass or a multispecies sward.

### Materials and methods

Two flocks were offered one of two forage treatments for the entire grazing season in 2023; A perennial ryegrass (PRG; *Lolium perenne* L.) sward receiving 163kg N/ha/year or a five species multispecies sward (MSS) composed of PRG, red clover (*Trifolium pratense* L. white clover (*Trifolium repens* L.), chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.) receiving 90kg N/ha/year. Daily methane output from sixty ewes was assessed using Portable accumulation chambers (PAC; O'Connor et al. 2021) on two occasions; during lactation (May), and during the dry (non-lactating) period (July) and from 60 weaned lambs on one occasion (July). Animals were fasted for one hour prior to entering the PAC to prevent capturing false highs of CH<sub>4</sub> measurements that can occur in the first hour after feeding (Brask et al., 2015). During this hour, animals were weighed before entering the PAC for 50 minutes. Data were analysed using a linear mixed model in PROC HPMIXED (SAS Inst. Inc., Cary, NC) with CH<sub>4</sub> (expressed as grams per day) as the dependant variable; measurement date, measurement group (time of day), sward type, breed and liveweight included as fixed effects; individual animal was included in lamb methane production analysis.

### Results

No difference was observed between forage types for daily methane output or methane intensity of lactating ewes (P > 0.05). However, forage type significantly reduced daily methane output (P < 0.001) and methane intensity (P < 0.001) of dry, non-lactating ewes with ewes grazing the MSS producing less daily methane in comparison to ewes grazing the PRG sward. Lambs grazing the MSS sward produced significantly less daily methane (P < 0.001) and had a significantly lower methane intensity (g/kg LW) (P < 0.001) than lambs grazing the PRG sward. There was a tendency for lambs grazing the multispecies sward to have a lower methane intensity (g/g ADG) than lambs grazing the perennial ryegrass (P = 0.0624) (Table 1).

### Table 1

Daily methane output and methane intensity of ewes and lambs grazing a perennial ryegrass or a multispecies sward (Least Square Means ± SEM).

	$PRG^{1}$	MSS <sup>2</sup>	SEM	Р
Methane production				
Lactating ewe (g/day)	37.97	40.25	1.522	0.1322
Dry, non-lactating ewe (g/day)	24.43 <sup>a</sup>	14.87 <sup>b</sup>	1.049	<.0001
Lambs (g/day)	17.76 <sup>a</sup>	14.85 <sup>b</sup>	0.457	<.0001
Methane intensity				
Lactating ewe (g/kg LW <sup>3</sup> )	0.508	0.537	0.0203	0.1588
Dry, non-lactating ewe (g/kg LW)	0.345 <sup>a</sup>	0.206 <sup>b</sup>	0.0153	<.0001
Lambs (g/kg LW)	0.430 <sup>a</sup>	0.384 <sup>b</sup>	0.0124	< 0.05
Lambs (g/g ADG <sup>4</sup> )	0.139	0.082	0.0212	0.0624

<sup>1</sup>PRG = Perennial ryegrass, <sup>2</sup>MSS = Multispecies sward, <sup>3</sup>LW = Liveweight, <sup>4</sup>ADG = Average daily gain, <sup>a, b</sup> Means within a row with different superscript differ (*P* < 0.05).

### Conclusion

Grazing multispecies swards reduced daily methane output in ewes when dry, but not during lactation. Multispecies swards also reduced daily methane output by lambs which can potentially contribute to an overall reduction of total greenhouse gas emissions over the lifetime of animals grazing multispecies swards.

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### 12. The effect of sward type and targeted selective treatment on lamb performance and anthelmintic usage

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### Application

This work sought to investigate the effect that multispecies sward grazing and targeted selective treatments (TST) have on anthelmintic usage and performance of finishing lambs compared to perennial ryegrass grazing. Individually, multispecies swards and TST have been found in other studies to be effective in improving performance and reducing anthelmintic usage respectively. In this study, grazing multispecies swards reduced the number of anthelmintic treatments administered to lambs compared to lambs grazing perennial ryegrass. The implementation of this strategy represents a viable approach to maintain refugia of susceptible parasites and prevent the rapid onset of anthelmintic resistance.

### Introduction

Anthelmintic resistance creates challenges for sustainable livestock farming and limits sheep productivity due to reduced anthelmintic efficacy against parasites. Instead of treating all animals in a group, which is commonly practiced, TST is a method of gastrointestinal nematode (GIN) control that treats only a proportion of the flock based on consideration of indicators such as faecal egg count (FEC), DLWG or dag score. When TST is utilised, there have been reports of reduced number of anthelmintic treatments administered while not compromising productivity of animals (Busin et al., 2014). This helps maintain a population of untreated, susceptible nematodes in refugia. Multispecies swards can reduce the need for anthelmintic treatment as they contain plant secondary metabolites that have been reported to have anthelmintic properties (Marley et al., 2002). Although the effect of TST on GINs and performance of sheep has been evaluated (Busin et al., 2014), there is a lack of research on the effects of combining TST with rotationally grazing multispecies swards on lamb performance and parasite FEC.

### Materials and methods

Weaned lambs were allocated to three grazing sward groups: (i) Perennial ryegrass with white clover (PRG/WC), (ii) a multispecies sward consisting of perennial ryegrass, timothy, white clover, red clover, ribwort plantain and chicory (MSS) or (iii) alternating between perennial ryegrass and multispecies sward (50:50) from July to October 2023 (n = 85, 91, 92). Ram lambs were slaughtered as they reached target slaughter weight (47 kg) and ewe lambs were removed from the study for breeding on 30/08/2023 when average age of ewe lambs for

PRG/WC, MSS and 50:50 were 152.2, 148.4 and 150.8 days old respectively (P = 0.26). Liveweight and application of any anthelmintic treatments were recorded at 14-day intervals during the grazing season. At this point, nematode FEC were also conducted. Dag scores were recorded at four time points and pasture larval counts (PLC) (Molento et al.,2016) were conducted at three time points. This selective treatment was applied based on DLWG and dag score. Dag score was recorded based on a 0 to 5 scale in which 0 indicated no faecal soiling and 5 indicated heavy faecal soiling (Bath and van Wyk, 2009). The threshold for treatment was a DLWG of less than 150g/day and/or a dag score over 3. The non-parametric Kruskal-Wallis test with Dunn's *post-hoc* test were used to compare means of the egg counts, dag scores, treatments and PLC between the groups. The one-way ANOVA with Tukey's HSD *post-hoc* tests were used to compare means of the liveweights and DLWG.

### Results

No significant differences were observed between the three groups in PLC, dag score, final liveweights or average proportion of the flock treated (P > 0.05). Egg counts, however, were significantly different between the groups at two timepoints. The PRG/WC group had significantly higher egg counts than MSS and 50:50 on 01/08/23 (P = 0.031, 0.026) and MSS had significantly higher egg counts than PRG/WC and 50:50 on 18/08/2023 (P = 0.039, 0.049). Average treatment per lamb was significantly greater for PRG/WC than the MSS group (P = 0.001). Liveweight gain was significantly higher for MSS compared to PRG/WC and 50:50 for ram lambs but no differences were observed for ewe lambs (see Table 1).

### Table 1

The mean, standard error of the mean (SEM) and p- value of start age, start liveweight, DLWG, FEC, proportion of flock treated, treatment per lamb and dag score of the three groups of lambs.

	PRG/WC	SEM	MSS	SEM	50:50	SEM	P-value
Mean start age (days)	111 <sup>a</sup>	0.529	108 <sup>ab</sup>	0.972	110 <sup>b</sup>	0.635	0.0230
Mean start liveweight (kg)	36.5	0.551	34.9	0.553	35.9	0.580	0.140
Mean DLWG of ram lambs (kg/day)	0.166 <sup>a</sup>	0.00592	0.220 <sup>b</sup>	0.00629	0.180 <sup>a</sup>	0.00837	0.0200
Mean DLWG of ewe lambs (kg/day)	0.114	0.00807	0.114	0.00734	0.0988	0.00750	0.270
Mean FEC (eggs per gram)	349	53.8	364	44.5	224	22.8	0.0940
Mean proportion of flock treated (%)	28.8	13.1	26.8	13.9	23.6	13.5	0.850
Mean anthelmintic treatment per lamb	1.21 <sup>a</sup>	0.0529	1.02 <sup>b</sup>	0.0265	1.11 <sup>ab</sup>	0.0321	0.00160
Mean dag score	1.77	0.0972	1.87	0.0836	2.02	0.0907	0.130
Mean age of slaughter (days)	158	2.35	163	2.50	163	3.16	0.160

### Conclusions

Lambs that grazed multispecies swards continuously had reduced anthelmintic treatments per lamb when compared with PRG/WC. Ram lambs that grazed multispecies swards continuously had higher DLWG than PRG/WC and 50:50, although there were no differences for ewe lambs; this disparity in DLWG between rams and ewes could be attributed to the early withdrawal of ewe lambs from the study. Egg counts were higher for MSS later in the season however average FEC over the entire study did not significantly differ between the groups. This suggests that implementing TST alongside rotational grazing on multispecies swards can reduce the number of treatments issued thereby reducing anthelmintic use which helps to maintain refugia and reduce the selection pressure for anthelmintic resistance. Furthermore, it can also provide benefits for performance of lambs. This may indicate that multispecies swards provide nutritional benefits but not necessarily anthelmintic effects.

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### 13. Effects of sward species diversity on pasture productivity and botanical composition under intensive grazing with dairy cows

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### Application

Legumes and herbs incorporation in grazing swards can be an answer to reduce chemical N inputs while maintaining sward productivity.

### Introduction

As part of the management of intensive grazing systems, the focus on simple and productive forage systems has led to a limited range of plants being used in grazing swards which are predominated by *Lolium perenne* L. (perennial ryegrass – PRG) monocultures. Such swards are capable of high levels of productivity and nutritional value (Baker et al., 2023) but are reliant on high levels of mineral fertilizer application and adequate moisture availability (Grange et al., 2021). Such systems are increasingly questioned due to their negative impacts on the natural environment both in terms of agricultural greenhouse gas emissions and nutrient losses to air and water (Delaby et al., 2020). The inclusion of legumes such as *Trifolium repens* (white clover – WC) within grazing swards has received much attention in recent years to reduce dependence on chemical nitrogen (N) fertilizer application. More recently, a growing body of scientific evidence has shown that the inclusion of additional dicotyledonous such as chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.), can improve both productivity and sustainability and improve the overall resilience of grazing systems (Grange et al., 2021; Baker et al., 2023). However, much of the evidence has been derived from short-term (3–6 month) evaluations, and few of them with grazing animals. This may not reflect the longer term performance within intensively managed grazing systems. On that basis, the objective of this study was to evaluate the performance of PRG-WC and multispecies swards in a farmlet scale systems experiment with grazing herds.

### Material and methods

The experiment was a randomised block design with three sown swards: a monoculture of PRG, a classical association of PRG and WC (PRG-WC) and a multispecies sward (MSS) composed of grasses, legumes, plantain and chicory. Overall, three farmlets of 18.7 ha were created, each divided into 20 paddocks. Paddocks for each treatment were balanced for location, soil type, and soil fertility throughout the farm. During the trial, the PRG sward received 250 kg of chemical N ha<sup>-1</sup> yr<sup>-1</sup> while the PRG-WC and MSS received 125 kg. Total net herbage production, botanical composition and chemical composition were measured over the years 2021 and 2022. These data were analysed for sward, season and year effect using mixed linear models (Proc Mixed, SAS Institute, 2006).

### Results

There was no significant difference in annual herbage yield between the three swards systems during the two year study (13.3, 12.5, 13.2 t DM ha<sup>-1</sup> for PRG, PRG-WC and MSS, respectively) despite large differences in mineral N application. Botanical composition of the PRG monoculture was 995 g/kg grasses and 5 g/kg of unsown species, on average over the two years. In comparison the PRG-WC sward had, 836, 163 and 1 g/kg of grasses, white clover and unsown species respectively while MSS had 673, 151, 171 and 5 g/kg of grasses, clovers, herbs and unsown species respectively. There was no significant effect of sward system on sward nutritive parameters (CP, NDF or ADF contents of 220, 403 and 207 g/kg DM, respectively), nor was there any significant year effects, or interactions between sward system and either season or year. The effect of sward system on ash content was greater for MSS (114 g/kg DM) compared to both PRG and PRG-WC (97 and 102 g/kg DM, respectively). Relatedly, OMD content approached significance (P < 0.10) and tended to be lower for MSS (799 g/kg DM) compared to both PRG and PRG-WC (812 and 808 g/kg DM, respectively).

### Conclusion

This study confirmed the findings of previous component based evaluations (Baker et al., 2023) and indicated that the inclusion of legumes and herbs within intensively managed grazing swards can yield similar DM production and nutritive characteristics to traditional PRG swards, while substantially reducing requirements for chemical N fertilisation. Further evaluation of such swards within longer-term research platforms is required to evaluate the persistency of the species and to enhance successful adoption of both PRG-WC and MSS systems.

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### 14. A comparison of dairy cow grazing behaviour in ryegrass and ryegrass-plantain pastures

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### Application

Monitoring ingestive-related behaviours (i.e., feeding and ruminating) can yield important information about the health, productivity, and welfare of dairy cows. This study highlights how dairy cow grazing behaviour differs when plantain is included in ryegrass pastures.

### Introduction

Evidence suggests that including plants from contrasting functional groups (e.g., grasses, legumes, and forbs) in grazing pastures may be beneficial for herbage production and quality and the delivery of ecosystem services (Grace et al., 2016; Cummins et al 2021). In recent

years, plantain (*Plantago lanceolata* L.) has emerged as a potential means of improving the sustainability of dairy farms, with studies demonstrating reductions in nitrogen (N) losses when compared to perennial ryegrass pastures (Navarrete et al., 2022). It is important however to understand the effect that altering pasture species composition has on the animal. Trends in grazing and rumination behaviours and their variation can provide farmers with information about the health status, physiological state, productivity, and welfare of the animal (lqbal et al., 2022). Ingestive-related behaviours such as chewing during ruminating and eating, play a vital role in maintaining high levels of feed intake and efficient digestive function in dairy cows (Beauchemin, 2018). This study therefore aimed to determine the effect of sward plantain inclusion on grazing behaviour in high-producing dairy cows.

### Materials and methods

Twenty-one mid-lactation Holstein-Friesian cows were selected from a larger grazing experiment which comprised three pasture treatments: perennial ryegrass-only (GO); low plantain (LP, 20% sward plantain); and high plantain (HP, 49% sward plantain). Seven cows from each treatment group were fitted with a RumiWatch noseband sensor (Itin and Hoch GmbH, Liestal, Switzerland) which recorded grazing behaviours during a 7-day period, commencing 22 August 2023. The noseband sensors measured several behavioural activities including rumination, grazing and drinking. Average pre-grazing herbage mass (above ground level) was 3701 kg DM/ha for GO, 3761 kg DM/Ha for LP, and 3915 kg DM/Ha for HP. Post-grazing sward height was 6cm across all three treatments. Statistical analyses were conducted using R (version 4.2.2) and the package "rstatix" with the individual variables analysed according to their distribution and homogeneity of variances using a one-way ANOVA. Multiple comparisons of means were performed using the TukeyHSD function.

### Results

Results are outlined in Table 1. Daily grazing time was similar between treatments, however GO had more grazing bites and eating chews than both LP and HP (P < 0.05). Time spent ruminating was higher in HP than LP and GO (P < 0.05), and the overall daily number of rumination chews was also highest in HP (P < 0.05). Chews-per-minute was similar across treatments. The HP cows spent more time drinking than GO cows (P < 0.05), while number of gulps was similar between treatments. The daily number of regurgitated boli was higher in HP than LP (P < 0.05), and similarly chews-per-bolus was higher in HP than LP (P < 0.05). Activity index was lowest in HP (P < 0.05) and similar in GO and LP.

### Table 1

Effects of pasture composition on dairy cow behavioural parameters measured (mean ± SEM).

	Grass-only (GO) <sup>1</sup>		Low plantain	Low plantain (LP) <sup>2</sup>		High plantain (HP) <sup>3</sup>	
	Mean	SEM	Mean	SEM	Mean	SEM	P-value
Grazing time (min/day)	556	12.8	565	8.3	558	10.0	NS
Rumination time (min/day)	407 <sup>b</sup>	8.6	382 <sup>b</sup>	16.3	448 <sup>a</sup>	8.4	0.0003
Eating time (Min/day)	476 <sup>a</sup>	18.2	415 <sup>b</sup>	15.6	414 <sup>b</sup>	20.2	0.032
Drinking time (min/day)	4 <sup>b</sup>	0.4	5 <sup>ab</sup>	0.5	6 <sup>a</sup>	0.5	0.015
Activity index (n/day)	128 <sup>a</sup>	2.2	127 <sup>a</sup>	2.4	117 <sup>b</sup>	3.0	0.008
Rumination chews (n/day)	24,525 <sup>b</sup>	643.6	23,300 <sup>b</sup>	1170.1	27,443 <sup>a</sup>	661.8	0.002
Eating chews (n/Day)	34,927 <sup>a</sup>	1533.7	30,036 <sup>b</sup>	1130.5	30,145 <sup>b</sup>	1468.7	0.026
Grazing bites (n/day)	31,460 <sup>a</sup>	1437.7	26,249 <sup>b</sup>	1056.7	25342 <sup>b</sup>	1365.5	0.003
Chews per minute	68	0.6	69	0.5	68	0.8	NS
Regurgitated boli (n/day)	458 <sup>ab</sup>	9.5	436 <sup>b</sup>	16.2	492 <sup>a</sup>	9.7	0.004
Chews per bolus	53 <sup>ab</sup>	1.0	52 <sup>b</sup>	1.0	56 <sup>a</sup>	1.0	0.016
Gulps (n/day)	61	5.9	75	6.7	81	6.9	NS

Within rows, means with differing superscripts (a-c) differ significantly.

<sup>1</sup> Grass-only: 100% perennial ryegrass sward.

<sup>2</sup> Low plantain: 20% sward plantain content.

<sup>3</sup> High plantain: 49% sward plantain content.

### Conclusions

Results from this study indicate that the inclusion of plantain in ryegrass swards, particularly at the higher level, alters a number of ingestive-related behaviours in dairy cows. This may be caused by differences in the physical structure and nutritive properties of the swards. Further nutritional analysis of the forages will help clarify this.

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### 15. Effects of dietary plantain inclusion on milk production and nitrogen utilisation efficiency of dairy cows

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### Application

Sward plantsin inclusion rates have no effects on milk yield or nitrogen (N) utilisation in dairy cows.

### Introduction

Plantain (*Plantago lanceolata* L.) has emerged as a potential means of improving the sustainability of dairy production and several studies demonstrated that inclusion of plantain in perennial ryegrass pastures reduced N losses in dairy cows (Navarrete et al., 2022). However, this reduction might be affected by sward plantain proportions and there is little research on the impact of plantain inclusion rates on N utilisation efficiency of dairy cows, Therefore the objective of this zero-grazing study was to determine the effect of sward plantain inclusion rates on N utilisation efficiency of dairy cows.

### Materials and methods

The data used were obtained from 2 zero grazing digestibility trials which were conducted respectively at the Agri-Food and Biosciences Institute in August and September 2023. Eighteen lactating Holstein-Friesian cows (9 cows/trial × 2 trials) were selected from a main grazing experiment which comprised three sward treatments: 100% perennial ryegrass (Control), 50% perennial ryegrass + 50% plantain (LP), 25% perennial ryegrass + 75% plantain (HP). In addition, each cow received concentrate pellets at 7.1 kg DM/day. These cows had been in the main grazing study for over 2 months before selected for these 2 zero grazing trials. In each trial, cow groups were balanced by parity, bodyweight, days in milk and milk yield. Forages were harvested daily using a zero grazer and offered to cows twice daily for ad libitum consumption allowing a 5% refusal rate. Before commencing the digestibility trials, all animals were housed as a single group in cubicle accommodation and offered experimental diets for 10 d. Afterwards, they were transferred to metabolism units and housed there for 7 d with total feed intake recorded daily and faeces and urine outputs collected during the final 6 d. Milk yields were recorded daily with milk samples taken during both morning (starting at 0530 h) and afternoon (starting at 1530 h) milking during the 7 d in metabolism units. Animals had free access to water throughout the whole experimental periods. The data were analysed using GenStat (21st ed., VSNI Ltd).

### Results

Plantain inclusion rates didn't affect feed intake or milk yield (Table 1). The plantain inclusion significantly reduced N intake but had no significant effect on N excretion in faeces, urine or milk or N retention. Nitrogen losses from urine and manure, when expressed as a proportion of N intake, were not affected by plantain inclusions, whereas plantain inclusions tended to increase faeces N/N intake (P = 0.086) and milk N/N intake (P = 0.073).

### Table 1

The effect of dietary inclusion of plantain on feed intake, milk yield and nitrogen utilization of dairy cows.

	Control	Low Plantain	High Plantain	SED	P-value
Animal and feed intake data					
Milk yield, kg	25.4	25.9	26.2	1.02	0.722
Forage DM intake, kg/d	15.7	14.8	15.5	0.58	0.280
Total DM intake, kg/d	22.8	21.9	22.6	0.58	0.280
N intake and output, g/d					
N intake	584 <sup>b</sup>	540 <sup>a</sup>	541 <sup>a</sup>	12.8	0.005
Faecal N output	171	168	175	8.2	0.697
Urinary N output	168	132	146	20.9	0.262
Manure N output	339	300	322	17.8	0.130
Milk N output	151	154	156	6.08	0.722
Retained N	94.6	85.6	63.5	16.88	0.202
N utilization efficiency, g/g					
Faecal N : N intake	0.293	0.311	0.323	0.0089	0.086
Urinary N : N intake	0.286	0.245	0.273	0.0368	0.537
Manure N : N intake	0.579	0.556	0.596	0.0299	0.423
Milk N : N intake	0.259	0.285	0.288	0.0130	0.073
Urinary N : Manure N	0.486	0.440	0.453	0.0413	0.542

### Conclusions

Inclusion of plantain in ryegrass swards significantly reduced N intake, but had no effects on feed intake, milk yield or N partitioning rates in faeces, urine or milk in dairy cows.

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### 16. Performance of dairy cows offered grass silages produced within either a three- or five-harvest system

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### Application

Cows offered silage produced within a five-harvest system produced an additional 1.8 kg energy corrected milk (ECM) yield compared to cows offered silage produced within a three-harvest system.

### Introduction

In order to improve grass silage quality, more producers are moving towards multi-harvest silage systems (considered as four or more harvests). However, there is little research evidence to quantify the effects of multi-harvest silage systems on herbage yield or cow performance. Previous work indicated that increasing harvesting frequency from three to four harvests improved silage quality, silage dry matter intake (DMI), milk yield and milk fat plus protein yield (Craig et al., 2023). The current study investigated the effect of offering grass silages produced within either a three- or five-harvest system on cow performance.

### Material and methods

Two treatments, comprising perennial ryegrass silages produced within either a five-harvest (5H) or three-harvest system (3H), were examined. Cutting dates for 5H were 30 April, 1 June, 29 June, 10 August and 7 September 2021, while cutting dates for 3H were 17 May, 28 June, and 23 August 2021. Silages were offered in a 21 wk study involving 34 mid-lactation (av. 147 days in milk) Holstien cows (30 multiparous and 4 primiparous). Within each treatment cows were offered silage from each harvest in consecutive order (harvest 1, followed by harvest 2, etc.) for a pre-determined number of days (in proportion to the herbage dry matter (DM) yield for each harvest). Silages were offered *ad lib*, with a single concentrate type offered at a flat rate (mean of 9.5 kg/cow/d) through an out-of-parlour feeding system. Rumen fluid samples were obtained from each cow during the final week when silage from each harvest was offered and analysed for volatile fatty acids (VFA), ammonia and pH. Treatment effects on DMI, milk yield and composition, body weight (BW) and body condition (BCS) were examined. Mean weekly data were analysed using REML, with cow as the experimental unit and week as the repeated measure. Rumen data was weighted according to the amount of time the cows were offered each silage and results analysed using ANOVA (GenStat 21st ed., VSNI Ltd.).

### Results

Total herbage DM yield was 11.2 and 12.6 t/ha for the 5H and 3H systems, respectively. Mean crude protein, metabolizable energy and neutral detergent fiber concentrations of the silages produced were 152 g/kg DM, 11.5 MJ/kg DM and 341 g/kg DM respectively within the 5H treatment, and 131 g/kg DM, 10.9 MJ/kg DM and 413 g/kg DM respectively within the 3H treatment. Silage DMI and total DMI was greater in 5H than 3H (P < 0.001; Table 1). Milk yield, milk fat plus protein yield and ECM were also greater with 5H (P = 0.037, P = 0.010 and P < 0.001 respectively), while milk fat and protein content were unaffected. Treatment had no effect on BW or BCS. All parameters changed over time (P < 0.001), while there were significant Treatment × Week interactions for all parameters (P < 0.05) except milk fat content. Rumen pH was unaffected by treatment, while rumen ammonia concentration was greater (7.9 vs. 6.2 mg/dL; P = 0.009) within the 3H treatment. Concentrations of VFA differed between the two treatments with 3H exhibiting higher acetate (66 vs. 61 mMol/L; P = 0.040) and total butyrate concentrations (17.1 vs 13.7 mMol/L; P < 0.001) compared to 5H.

### Table 1

Effect of silage harvesting frequency on cow performance.

				P-Values:		
	5H	3H	SED	Treatment	Week	Interaction
Silage DMI (kg/d)	14.1	11.7	0.40	<0.001	<0.001	<0.001
Total DMI (kg/d)	23.4	21.1	0.44	< 0.001	< 0.001	< 0.001
Milk yield (kg/d)	33.5	31.9	1.02	0.037	< 0.001	< 0.001
Fat (g/kg)	47.7	48.0	1.14	0.851	< 0.001	0.619
Protein (g/kg)	35.9	35.1	0.75	0.359	< 0.001	< 0.001
Fat + protein yield (kg/d)	2.78	2.65	0.072	0.010	< 0.001	0.001
Energy corrected milk yield (kg/d)	37.4	35.6	0.94	0.007	<0.001	<0.001

### Conclusion

Increasing harvesting frequency from three to five harvests improved silage nutritive value, but reduced herbage DM yield (t/ha). Silage DMI, milk yield, milk fat plus protein yield and ECM were increased within the five-harvest silage system. The increase in rumen ammonia in 3H may indicate an imbalance between availability of nitrogen and energy for microbial protein synthesis.

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# 17. Age influenced the apparent metabolisable energy and true metabolisable energy of some selected cereal grains in commercial turkey

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### Application

Young turkeys metabolise cereal grains more efficiently than older turkeys. Applying this knowledge can improve how we feed turkeys and benefit the poultry industry and the environment.

### Introduction

Dietary energy is the main factor influencing feed intake in poultry birds (Classen, 2017). Dietary energy is conventionally measured using metabolisable energy values derived from adult cockerels as a standard. Studies have documented variations in metabolisable energy values across various poultry species and age groups. Consequently, relying on metabolisable energy values from adult cockerels for formulating turkey diets at different ages may introduce inaccuracies. Inaccurate metabolisable energy matrices can increase feed costs and nutrient waste in the environment. Cereal grains have been used to feed animals for centuries and they constitute the major source of energy in poultry (Poutanen et al., 2022). This study aims to provide information on the metabolisable energy values of acha, wheat, maize, and sorghum and to determine the effect of the age of turkeys on these values.Table 1.

Table 1

Influence of turkey age on the apparent metabolisable energy and true metabolisable energy of acha, wheat, maize and sorghum.

Parameters (kcal/kg)	8 weeks	16 weeks	24 weeks	SEM	<i>P</i> -value
ACHA					
AME	3459 <sup>a</sup>	3345 <sup>b</sup>	3367 <sup>ab</sup>	34.84	0.050
AMEn	3406	3327	3337	24.63	0.116
TME	3577	3487	3476	32.14	0.154
TMEn	3505	3458	3414	26.45	0.182
WHEAT					
AME	3312 <sup>a</sup>	3196 <sup>b</sup>	3249 <sup>b</sup>	33.70	0.000
AMEn	3226 <sup>a</sup>	3171 <sup>b</sup>	3175 <sup>b</sup>	17.56	0.006
TME	3431 <sup>a</sup>	3338 <sup>b</sup>	3358 <sup>b</sup>	28.30	0.009
TMEn	3325 <sup>a</sup>	3302 <sup>ab</sup>	3252 <sup>b</sup>	21.60	0.025
MAIZE					
AME	3451	3370	3370	27.10	0.286
AMEn	3377	3333	3324	16.35	0.612
TME	3570	3512	3479	26.71	0.364
TMEn	3476	3454	3409	19.69	0.552
SORGHUM					
AME	3406	3282	3308	37.83	0.100
AMEn	3327	3275	3262	20.04	0.455
TME	3525	3424	3417	34.81	0.197
TMEn	3427	3406	3338	26.74	0.341

 $^{a.b.c}$  Means with different superscripts on the same row are significantly different (P < 0.05).

AME = Apparent Metabolisable Energy.

AMEn = Apparent Metabolisable Energy corrected for zero nitrogen retention.

TME = True Metabolisable Energy.TMEn = True Metabolisable Energy corrected for zero nitrogen retention.

### Materials and methods

One-day-old (n = 360) starter; nine-week-old (n = 300) grower and 17-week-old (n = 240) finisher Nicholas turkeys were randomly allotted to five treatments (acha, wheat, maize, sorghum and dextrose (control)) in a completely randomised design. Each treatment had six replicates and each phase lasted eight weeks. Commercial turkey diets (starter: ME = 2900.00 kcal/kg, CP = 26%; grower: ME = 3100.00 kcal/kg, CP = 24.05%; finisher: ME = 3250.00 kcal/kg, CP = 18.5%) were supplied *ad libitum* for the first seven weeks of respective phase. At week 8 of each phase, four turkeys per replicate were randomly allotted to metabolic cages and intubated with respective feedstuff to determine

Apparent Metabolisable Energy, nitrogen-corrected Apparent Metabolisable Energy, True Metabolisable Energy and nitrogen-corrected True Metabolisable Energy. Data were analysed using descriptive statistics and ANOVA (SAS, 2013) and means were separated using Duncan's Multiple Range Test  $\alpha 0.05$ .

### Results

Apparent metabolisable energy for acha decreased from 3459.15 Kcal/kg at 8 weeks to 3345.24 Kcal/kg at 16 weeks, slightly increasing to 3367.62 Kcal/kg at 24 weeks. The age of turkeys did not influence the nitrogen-corrected apparent metabolisable energy, True Metabolisable Energy and nitrogen-corrected true metabolisable energy of acha. All the metabolisable energy values of wheat were influenced by turkey age; apparent metabolisable energy decreased from 3312.93 Kcal/kg at 8 weeks to 3196.33 Kcal/kg at 16 weeks, with a slight increase to 3249.40 Kcal/kg at 24 weeks; nitrogen-corrected apparent metabolisable energy, True Metabolisable Energy and nitrogen-corrected true metabolisable energy followed the same trend as AME. The age of turkeys did not influence the metabolisable energy values of maize and sorghum.

### Conclusion

Turkey age significantly influenced wheat's metabolisable energy values, decreasing from 8-16 weeks with a minor rebound at 24 weeks. Notably, acha's AME was also influenced by the age of turkeys while other metabolisabe energy values remained unaffected by age. In contrast, for maize and sorghum, age had no significant effect on these metabolisable energy parameters. This study emphasises the crucial role of age in wheat-based feed formulations and the results provide valuable reference metabolisabe energy values for these grains, enabling precise feeding strategies for turkeys across their life stages.

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### 18. Liveability and growth of turkey poults fed honey early post hatch

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### Application

Liveability of turkey poults was enhanced when 5% honey solution was fed early post hatch.

### Introduction

Excessive depletion of glycogen during hatching has been shown to negatively impact growth and may increase early mortality in young turkeys (Lingens et al., 2021). Studies have indicated that higher blood glucose levels are associated with increased glycogen concentrations and accelerated growth in turkey embryos and hatchlings (Wang et al., 2023). The young hatchlings' ability to survive in the first several days after hatching when they are adjusting to a high-energy diet, relies on glycolysis. Karabagias et al. (2014) states that carbohydrates make up the majority of honey's dry weight, accounting for approximately 95%, while water and various minor components are present in smaller quantities. Therefore, effects of early post hatch feeding of honey on liveability and water consumption of turkey poults were investigated.

### Materials and methods

Two hundred and forty (n = 240), one-day old turkey poults were randomly assigned to: untreated water (control), 5% glucose, 1% honey and 5% honey solutions for three days early post hatch and raised for four weeks in six replicates of 10 eggs each. Liveability, water consumption (mL/bird), Average Daily Feed Intake-ADFI (g/poult/day), Average Daily Weight Gain-ADWG (g/poult/day) and Feed Conversion Ratio-FCR were calculated. Data were analysed using descriptive statistics and ANOVA (SAS, 2013) and means were separated using Duncan's Multiple Range Test  $\alpha_{0.05}$ .

### Results

The result of feeding honey early post hatch on performance of turkey poults at week 0 to 4 was as presented in Table 1. The final weight, average daily feed intake and average weight gain showed no significant difference from the control when honey solution was fed. Feed conversion ratio was significantly (P < 0.05) reduced (2.55) in birds that received 5% honey early post hatch. Liveability was also significantly (P < 0.05) increased (93.80%) when 5% honey solution was fed early post hatch although the value was similar to that of poults that received 5% glucose and 1% honey. The result of feeding honey early post hatch on water consumption of turkey poults in the first three days early post hatch was as presented in Table 2. At day 1, values obtained for water consumption were significantly higher (P < 0.05) and similar to the control except poults on 5% glucose (75 mLs) which had the highest value. Significantly higher (P < 0.05) values were also

## Table 1 Effects of feeding honey early post hatch on performance of turkey poults (week 0-4).

Treatment	Control	5% Glucose	1% Honey	5% Honey	SEM	P-value
Initial weight (gbird)	50.82	51.53	51.78	52.14	0.17	0.033
Final weight (g/bird)	270.93	282.17	284.14	314.00	8.61	0.351
ADFI (g/bird/day)	26.72	28.53	24.41	23.84	0.91	0.240
ADWG (g/bird/day)	7.86	8.24	8.30	9.35	0.31	0.377
FCR	3.48 <sup>a</sup>	3.51 <sup>ª</sup>	3.03 <sup>ab</sup>	2.55 <sup>b</sup>	0.15	0.071
Liveability (%)	76.91 <sup>b</sup>	91.79 <sup>ªb</sup>	89.64 <sup>ab</sup>	93.80 <sup>a</sup>	2.78	0.124

<sup>a,b</sup>Means on the same row with different superscripts are significantly (P < 0.05) different. ADFI-Average Daily Feed Intake, ADWG-Average Daily Weight Gain, FCR- Feed Conversion Ratio.

### Table 2

Effects of feeding honey early post hatch on water intake of turkey poults.

Day/mL Consumed	Control	5% Glucose	1% Honey	5% Honey	SEM	P-value
Day one (mL/bird)	27.00 <sup>c</sup>	$75.00^{a}$	51.00 <sup>b</sup>	50.00 <sup>b</sup>	6.31	0.048
Day two (mL/bird)	42.00 <sup>c</sup>	140.00 <sup>ab</sup>	108.00 <sup>b</sup>	182.00 <sup>a</sup>	14.90	0.001
Day three (mL/bird)	156.00 <sup>b</sup>	233.00 <sup>a</sup>	171.00 <sup>b</sup>	264.00 <sup>a</sup>	12.68	0.001

 $\overline{a^{a,b,c}}$  Means on the same row with different superscripts are significant  $P \le 0.05$ .

recorded for water consumption on day two with the highest value recorded in poults that received 5% honey (182 mLs). At day 3, poults that received 5% glucose (233 mLs) and 5% honey (264 mLs) had the higher (P < 0.05) values for water consumption than poults that received 1% honey which was similar to the control.

### Conclusion

Early post hatch feeding of 5% honey enhanced liveability and utilisation of nutrients. Finding a perfect alternative way to augment or maintain a glucose balance during the early post hatch phase has potential of producing a very healthy poults with good liveability.

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### 19. Effect of phytogenic feed additive blends on haemato – Biochemical indices and carcass yield of broiler chickens

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### Application

Phytogenic feed additives can be used as enhancers of growth performance in broilers with no residual side effect on the

### Introduction

Phytogenic feed additives (PFA) have emerged as alternatives to antibiotics due to increased public and scientific concern regarding residual effects on birds, drug resistance in human and environmental issues. This study investigated the effect of PFA (Lemon peel – LP, Curry leaf – CL, Lemon grass – LG and Orange peel – OP) on haemato – biochemical indices and carcass yield of broiler chickens.

### Materials and methods

Two hundred arbor acre broiler chickens was used for the experiments. Birds were allotted 5 treatments (40 birds/treatments) in 4 replicates of 10 birds each using Completely Randomized Design. The treatment groups include: Treatment 1 (T1) = Control (with antibiotics), Treatment 2(T2) = Blend A (LP: 35.0% + OP: 30.5% + LG: 22.5% + CL: 12.0%), Treatment 3 (T3) = Blend B (LP: 32.5% + OP: 33.0% + LG: 23.5%

+ CL: 11.0%), Treatment 4 (T4) = Blend C (LP: 30.0% + OP: 35.5% + LG: 24.5% + CL: 10.0%), Treatment 5 (T5) = Blend D (LP: 27.5% + OP: 38.0% + LG: 25.5% + CL: 9.0%). Haematological parameters (Red Blood Cell, White Blood Cell, Packed Cell Volume, haemaglobin concentration and White blood cell differentia) were determined while serum biochemical analysis (Total Protein, Albumin, Aspartate aminotransferase and Alanine aminotransferase) were also evaluated. All data obtained were subjected to one-way ANOVA.

### Results

Heterophils and Mean corpuscular haemoglobin concentration were not significantly (P > 0.05) influenced by PFA. Birds fed T2 had the highest value 6.83 g/dl and 4.25 g/dl for total protein and albumin. However, alkaline phosphate 34.75u/l, triglyceride 168.33 mg/dl, glutathione peroxidase 13.68u/l and very low-density lipoprotein 33.68mg/dl were significantly higher in birds fed diet T1 except superoxide dismutase. Antibiotics and PFA did not have any significant effect (P > 0.05) on the carcass parameters.

### Conclusion

This study has been able to show that the inclusion of phytogenic feed additives should be encouraged in order to enhance the health of the broiler chicken. PFA can also be used as replacement for tetracycline antibiotics because it is generally safe with no residual side effect on the birds.

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### 20. Microbial and oocyst count in feacal material of broiler birds administered phytochemicals (naringin and hesperidin)

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### Application

The phytochemicals – Hesperidin and Narigin can be used as potential compounds in preventing coccidiosis as well as reduce microbial load in poultry in order to enhance growth performance.

### Introduction

Avian coccidiosis affect poultry health and performance, leading to significant economic losses in many developing countries, including Nigeria. Due to the adverse side effects of antibiotics and vaccinations used to manage these diseases and the increasing demand for organic and healthy proteins from meat consumption, there is a need to explore alternative natural compounds. Hesperidin and naringin



**Fig. 1.** Average weight of broiler birds in different treatment groups for six weeks of treatment. Key: T1 = Control (uninfected) T2 = Hesperidin (uninfected) T3 = Narigin only (uninfected) T4 Narigin and Hesperidin (uninfected) T5 = Control (infected) T6 = Hesperidin (infected) T7 = Narigin (infected) T8 = Narigin and Hesperidin (infected).

are flavonoids derived from citrus fruits. This study aims to investigate the preventive effects of hesperidin and/or naringin on coccidiosis – induced toxicities as well its implication on microbial load in broilers.

### Materials and methods

A total of 320 day – old broiler chicks were randomly divided into eight groups, with forty birds per group and in four replicates. Four groups were not inoculated with Eimeria oocysts but administered coccidiostat (T1A – D), hesperidin alone (T2A – D), naringin alone (T3A – D) and a combination of naringin and hesperidin (T4A – D) from day eight (8) to day fourteen (14) while four other groups (T5A – D to T8A – D) were inoculated with  $2 \times 10^4$  oocysts per 0.5 ml of *Eimeria tenella* on the 16th and 19th day of age after they were administered conventional antibiotics and coccidiostat, naringin (50 mg/body weight), hesperidin (50 mg/body weight) and a combination respectively from day 8 to 14. McMaster counting technique was used to count the oocysts while pour plate technique was used to determine the bacterial load.

### Results

The results as observed in Fig. 1 showed a steady increase in the growth performance of all the birds with an average weight ranging from  $1.55 \text{kg} \pm 0.02 - 2.00 \text{ kg} \pm 0.04$ . The microbial load showed the presence of *Enterobacteriacae* in the population range of  $3.5 \times 10^4 - 4.5 \times 10^4 \text{ CFU/ml}$ . The study also found that the administration of naringin and hesperidin to broiler birds inoculated with coccidia oocysts significantly reduced the feacal oocyst counts, with the lowest count in combined treatment (T8) (10%) and indicating a lower degree of coccidiosis infection in the treated groups whereas control group (T5) had the highest oocyst count (35%). Mortality and Morbidity rate was 0% as none of the bird showed signs and symptoms.

### Conclusion

The result of this study showed a promising approach in poultry which would improve the organic production of antibiotic-free chickens and reduce the excretion of antibiotic residues into the environment, thereby improving environmental sustainability. The reduction in oocyst counts could help to strengthen the immune system of broiler birds and limit the severity of coccidiosis infection which could be an effective strategy for improving performance, immune function, and mitigating the impact of coccidiosis infection in broiler birds.

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### 21. Precision nutrition for laying hens

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### Application

Although several advances have been made in the commercial laying sector, there are still nutritional techniques that need to be revalidated to improve production performance, together with better nutrient utilization and lower production costs.

### Introduction

The commercial laying sector has stood out for its high production rates, and this scenario is the result of technological innovations, automation, and changes in the areas of genetics, nutrition, health, and ambience. One of the main factors in achieving maximum production is the supply of a balanced diet. However, for balanced rations to be properly formulated, it is necessary to know the nutritional requirements of the animals, the content and availability of nutrients, and to consider the cost of the ingredients, as they account for around 60 to 70 percent. In this sense, precision nutrition has become a fundamental strategy for improving sustainable improvements in the production process. The concept of precision nutrition is not new, but it can be defined as an approach that involves altering dietary constituents to favor body composition, health, and environment. The success of its implementation depends on ingredient characterization, determination of nutritional requirements, and careful management to ensure that the two previous requirements are met so that feed management is flexible throughout the production cycle to meet the bird's daily needs (Moss, et al. 2021). The aim of the study was to evaluate the concept of precision nutrition on the performance of poultry.

### Materials and methods

A total of 288 Hisex Brown layers, from 90 to 96 weeks of age, were used in a completely randomized design (DIC), in a  $2 \times 2$  factorial (2 dietary compositions of maize and soybean meal: tabulated or analyzed by NIRS and 2 diets formulations according to the recommendation of Rostagno et al. (2011) or the strain) with nine replicates of eight birds per pen. Hens were allocated in cages and were given water and feed ad libitum, and the light schedule was 16 hours per day. Diets were maize and soybean meal-based, following the nutrient levels used by the industry according to recommendations of Rostagno et al. (2011). Two evaluation periods were considered, both with two cycles of 21 days each (from 90 to 93 and from 94 to 96 weeks of age). At the end of each cycle, performance was evaluated: egg production, feed consumption, egg mass, and feed conversions per dozen eggs produced and per egg mass. Egg quality was assessed on the last two days of each cycle (i.e. days 20 and 21), when the eggs produced were collected, identified, and analyzed using the Digital Egg Tester (DET6000). Shell strength (Kgf), egg weight (g), albumen height (mm), Haugh unit (HU), yolk color, and shell thickness (mm) were ana-

lyzed. All mortalities were recorded daily. Performance and egg quality data were analyzed using the Statistical Analysis System (SAS, 2015), using the ANOVA procedure, and the homogeneity of the data and normality of the residuals were checked. Data that did not meet the assumptions were transformed or excluded and finally subjected to analysis of variance and Tukey's test at 5% significance.

### Results

There was no significant interaction between the factors studied for the performance traits analyzed. Feed consumption was significantly influenced by the individual factors, but there was an improvement in egg production and mass, feed conversion per dozen, and egg mass for birds fed diets formulated according to Rostagno when maize and soybean meal were analyzed by NIRS (P < 0.05). Feed conversion per dozen and egg mass were proportional indices of feed consumption, egg mass, and egg production. By analyzing the ingredients of the feed beforehand, it is possible to meet the birds' needs more accurately than by using the nutrient values given in the various tables of recommendations. This is because a fixed composition value doesn't always correspond to what the cereals provide. Also, the inputs are subject to the effects of climatic, soil type, cultivation, and storage, among others, which influence their nutritional composition. Regarding egg quality, there was no interaction between the factors studied nor isolated for albumen height, Haugh unit, and shell strength (P>0.05). However, there was a significant interaction between the factors studied for the yolk color and shell thickness. For yolk color, it was observed that when using the Rostagno dietary recommendation, the tabulated value had a higher color than the analyzed one, contrary to the recommendation proposed by the strain. Maize is an ingredient rich in carotenoids, which are the pigments responsible for the color of egg yolks. Therefore, the higher the maize content the better the egg yolk color. Regarding shell thickness, it was observed that when the Rostagno diet was used, the tabulated value resulted in better shell thickness. Similarly, to maize, there was a greater incorporation of calcitic limestone in the diet formulated based on the nutritional composition of the tabulated ingredients. It is well known that calcitic limestone is one of the most used sources of calcium in layer diets and is more responsible for shell formation. The use of the concept of precision nutrition, through meeting the nutritional needs established by Rostagno, reduces the cost of production and improves the profitability of egg production.

### Conclusions

Diets formulated by analyzing the ingredients according to Rostagno's recommendations improved the performance. The analyzed composition of the ingredients used to formulate the feed allows the bird's needs to be met more efficiently, improving nutrient utilization.

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# 22. Optimal conditions for the extraction of keratin from chicken feathers with sulphites and its structural and thermal characteristics

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### Application

Keratin extraction can generate revenue and mitigate the environmental and health problems caused by the disposal of feathers in landfills. This research provides evidence that high keratin yields can be achieved using cheap non-toxic solvents in relatively short durations.

### Introduction

Every week ~22 million chickens are slaughtered in the UK with an average weight at slaughter of 2.3 kg (Department for Environment Food & Rural Affairs, 2023). Feathers represent ~5.5% of chicken weight and they are currently underutilized (McGauran et al., 2021). Keratin accounts for >90% of feathers dry weight (Zhang et al., 2021). It has several applications in the biomedical and cosmetic industries, and it can be used to produce a biodegradable polymer for packaging, wound dressing, and tissue regeneration (Senthilkumar et al., 2022). Keratin is insoluble in polar and non-polar solvents due to the cross-linking by disulphide bonds, which must be broken to solubilize keratin (Dabrowska et al., 2022). This is usually achieved through chemical or enzymatic treatment. The study aims to optimize keratin extraction, using sulphites as reduction agents, by investigating the effects of varying the extraction parameters on the dissolution rate.

### Materials and methods

Feathers were obtained from Moy Park, Armagh, UK. They were milled, washed with water and detergents, and dried in an oven at 37.5 °C before extraction. The composition of raw feathers was analysed using proximate analysis. All chemicals used were of analytical grade. Ground feathers ( $m_1$ ) were mixed with a solution containing reduction agent, urea, and sodium dodecyl sulphate and heated in a water bath with frequent agitation using a vortex mixer. The mixture was then centrifuged, and the undissolved feathers were washed thoroughly and dried in an oven at 105 °C until a constant mass was achieved ( $m_2$ ). The soluble keratin was dialyzed against distilled water for two days using a dialysis tubing (molecular weight cut-off of 3.5 kilo Dalton) and the water was changed twice a day. Keratin was then dried for three days at 60 °C and weighed ( $m_3$ ). The dissolution rate and yield were calculated using Equations (1) & (2).

(1)

Dissolutionrate (%) = 
$$100 - \left(\frac{m_2}{m_1 \times \% Drymatter} \times 100\right)$$

$$Yield \ (\%) = \frac{m_3}{m_1 \times \% Drymatter} \times 100$$

The parameters investigated and their ranges were, temperature (40-100 °C), time (1-10 h), urea concentration (2-8 M), reduction agent (sodium sulphite, sodium bisulphite, and sodium metabisulphite), reduction agent concentration (0-0.7 M), sodium dodecyl sulphate concentration (0-1 g/g feathers), and solid-to-liquid ratio (1:25-1:10).

Keratin secondary structure, thermal stability, crystallinity, and surface morphology were characterised using Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), X-ray diffraction analysis (XRD), and scanning electron microscopy (SEM).

All analyses were carried out in triplicate and results are expressed as means with standard deviations ( $\pm$  SD). Differences among means, using the t-Test statistical method, were considered significant at *P*-value < 0.05.

### Results

The parameters with the most significant influence on the dissolution rate were temperature, urea concentration, and time. The dissolution rate increased linearly from 61.2% to 98.3% when temperature increased from 40 °C to 100 °C and from 72.5% to 92.3% when urea concentration increased from 2 M to 8 M. It also increased with time up to 6 h (92.3%) with no significant increase at longer durations. The conditions resulting in the highest dissolution rate were:

- Temperature: 100 °C.
- Time: six hours.
- Urea concentration: 8 M.
- Reduction agent: sodium sulphite.
- Reduction agent concentration: 0.5 M.
- sodium dodecyl sulphate concentration: 1 g/g feathers.
- Solid-to-liquid ratio: 1:15.

The dissolution rate and yield were  $98.3 \pm 0.3\%$  and  $73.3 \pm 5.4\%$  respectively.

FTIR spectra of keratin show all the distinct protein absorption peaks (amide A, amide I, amide II, and amide III) which confirms the preservation of protein secondary structure. The disulphide bridge peak in the spectra of raw feathers at 531 cm<sup>-1</sup> disappeared in the keratin spectra which confirms the breakdown of disulphide bonds. TGA results show that the highest mass loss for keratin occurred at a temperature of 250 °C compared to 325 °C for raw feathers, indicating a reduction in the thermal stability because of the breakdown of disulphide bonds. Nevertheless, keratin has a relatively high thermal stability that allows for thermal processing. XRD results show that keratin has a higher degree of crystallinity evident by the sharp peak at 22.6° compared to the broad peak at 18° in raw feathers. While the shift to a higher angle indicates a reduction of  $\beta$ -sheet structure, because of disulphide bond breakdown, and an increase of random coil structure. SEM images show that keratin had a smooth flake-like structure while raw feathers have hollow fibrous structure.

### Conclusion

Keratin can be extracted efficiently using sulphites. Under the optimal extraction conditions >98% of the feathers were dissolved and the yield was  $73.3 \pm 5.4\%$ . The secondary structure of keratin was preserved during extraction. The thermal stability of keratin was lower than raw feathers, but high enough for thermal processing.

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# 23. Many developed countries are going to have to reduce the emissions from ruminant agriculture in order to meet their mitigation commitments

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Many developed countries are going to have to reduce the emissions from ruminant agriculture in order to meet their mitigation commitments. Evidence suggests that improving ruminant health could lead to significant reductions in carbon footprints. However, there is also evidence of growing resistance amongst some important parasites, which could render current treatments less effective. In this talk the evidence on trends in anthelmintic resistance in gastro-intestinal nematodes (GINs) and liver fluke (LF) are reviewed. The future trends in resistance are predicted and their potential impact on cattle and sheep performance, production and GHG emissions estimated.

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### 24. Managing parasites in the face of resistance

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Roundworm infections are ubiquitous in grazing ruminants, threatening the health, welfare and environmental impact of livestock. Infections are typically controlled using anthelmintics but resistance to the few available compounds is increasing, limiting control options on-farm. A recent study involving 77 efficacy tests on commercial sheep farms in the UK, identified reduced efficacy to all four drug classes tested. An egg reduction of >90% was observed in only  $\sim$ 30% of tests, highlighting the scale of the problem facing sheep producers. In cattle, anthelmintic resistance is at an earlier stage; estimates suggest  $\sim$ 50% of *Cooperia* worms are resistant to the most used drug class (macrocyclic lactones). Benzimidazole resistance has only recently been identified in cattle roundworms but a genotyping study found  $\sim$ 4% of roundworms carrying the mutations coding for resistance, including multiple worm species.

Slowing the development of anthelmintic resistance is important to safeguard control options for the future, and this can be achieved through targeted treatment. Diagnostic tools have been developed to assist farmers in assessing which animals require treatment and when. Faecal egg counts are the primary tool available, providing a snapshot of the parasite population within infected animals, these can be used in several ways to identify strategic times to treat and to test the efficacy of interventions. Alternatively, weight gain and movement sensors can be useful indicators of productivity, identifying individual animals which are not performing optimally. Decision support tools also play a role in supporting stakeholders in understanding and implementing recommended on-farm practices.

Sustainable control of roundworms can best be achieved using a combination of diagnostic tools, targeted anthelmintic treatment and alternative control strategies, maintaining production efficiency and reducing greenhouse gas emissions from grazing livestock. Despite the availability tools, uptake remains relatively low. Understanding the barriers to uptake of recommendations and developing effective communication methods will therefore be key in promoting sustainable practices on-farm to meet ambitious industry goals.

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### 25. How can the sheep industry achieve sustainable parasite control?

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Gastrointestinal nematodes, liver fluke and ectoparasites are a major source of losses in sheep production, causing mortality and compromising health and performance. Historically, control has relied heavily on the use of effective parasiticides to suppress the negative impacts of these parasites. However, with this strategy came the inevitable development of resistance to these medicines, and the need for us to reexamine our approach to parasite control.

The number of reports of resistance on farms through the 1980s and 1990s increased, but there was a dearth of potential solutions and mitigating actions to what was developing into a threat to the very existence of the UK sheep industry. It was in response to an urgent need for action that industry brought together the expertise needed to produce evidence-based guidelines, designed to slow the development of resistance, and in 2004 the Sustainable Control of Parasites in Sheep (SCOPS) group was born. The group's remit was to develop sustainable, practical strategies for parasite control in sheep, which ensured the responsible use of parasiticides, in conjunction with other means of control.

SCOPS is a pan-industry group consisting of organisations and individuals with an active interest in the sustainable control of parasites. It includes industry bodies, UK levy bodies, sheep farmers, vets, animal health advisers, parasitologists, researchers and others. The group facilitates and oversees the delivery of these recommendations to the industry, advises on areas of research required and ensures that new research and developments are incorporated to refine and improve advice to the sheep industry. Over time, evidence to support those initial guidelines has been gathered and this has underlined both their importance and credibility.

SCOPS remains very proactive, highlighting upcoming problems and initiating action to mitigate new threats. For example, the group started work on the implications of resistance in sheep scab mites to endectocides in 2018, and has been key to the development of new approaches to scab control. More recently, we have broadened our 'sustainable' credentials to include more direct consideration of the environmental impacts of parasiticide use. Using our mantra of 'use as little as possible but as much as necessary' with regard to parasiticides, we are able to directly apply our principles to help reduce environmental exposure, while maintaining animal health and welfare.

The key to the success of SCOPS has been the wide buy-in and commitment to the SCOPS principles and approach across industry, and the group's ability to build a reputation as not only a trusted source of information, but principles that must be taken into account when any

new products or options are proposed. This approach has subsequently been adopted by the cattle industry (COWS) and, very recently, the equine sector (CANTER).

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### 26. Pasture based agriculture is facing significant challenges

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Pasture based agriculture is facing significant challenges, including achieving reductions in environmental emissions, reducing fertiliser nitrogen use, improving water quality and maintaining food security. Grassland continues to provide farmers in the UK and Ireland with a low-cost competitive advantage for milk and meat production. In order to achieve ambitious, but necessary sectoral targets for 2030 farmers are dependent on robust science to ensure the correct strategies are implemented to achieve the reductions required. As well as adapting technologies at farm level, such as low emission slurry spreading, earlier age to slaughter, protected urea and applying lime, farmers are increasingly interested in using legumes to reduce the need for artificial fertiliser without compromising on animal performance. Recent research has demonstrated the role of clovers and herbs in multispecies grazing systems.

Germinal is a 6th generation family business focused on providing farming and food solutions and developing new plants and technologies to improve the efficiency and sustainability of livestock farmers. Findings from our research and innovation division, Germinal Horizon, have demonstrated improved consistency of herbage supply with more diverse mixtures, with perennial ryegrass (PRG) being out-yielded by PRG+ white clover or a multi-species mixture during the main growing season (June-August). These results are consistent with the ongoing work at Lyons Research Farm (University College Dublin) and Johnstown Castle Research (Teagasc). The sward profile is strongly influenced by fertiliser applied, comparing two different fertilisation rates, legumes accounted for a significantly higher proportion of the overall sward composition (62%) when 150 kg N/ha was applied, compared to 250 kg N/ha (32.5%). Protein concentration and ME value of the sward was dependent on the sward composition. The longevity of the herbs in particular is limited, with a significant change in the dominant species over time. In Year 1, chicory and plantain accounted for 45.6% of the total sward, declining to 23% in Y2.

In recent years, there has been a significant market shift with a growing demand for legumes and herbs as farmers adapt scientific research to reduce environmental impact and maintain productivity and implement robust and rigorously tested scientific research. This is evident through increased sales of legumes and herbs within Ireland.

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### 27. Animal and land performance and soil carbon content of grasslands grazed by dairy x beef steers under set-stocking or cellgrazing over a four-year experiment in Devon

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### Application

The study comparing set stocking (SS) and rotational cell grazing (CG) in a UK-based temperate climate offers insights for optimal grazing practices. It informs livestock productivity, soil health impacts, aiding sustainable land management decisions and policy formulation for improved agricultural practices.

### Introduction

Grasslands are crucial for ruminant feed and various societal benefits such as biodiversity, recreation, and carbon storage, and support millions of cattle and sheep, yielding billions of litres of milk and tons of meat annually in the UK. Grasslands are managed under a variety of stocking methods that exert different impacts on animal, plant, and soil responses. Traditional continuous grazing often leads to overgrazing and uneven pasture use, impacting productivity. Rotational stocking, alternating grazing and rest periods, such as cell grazing, aim for improved productivity and diverse vegetation. Yet, direct comparisons between continuous and rotational grazing in temperate climates are limited. This study aimed to compare set stocking and cell grazing impact on productivity and soil carbon content of a dairy x beef production system.

### Material and methods

This grazing experiment comparing SS and CG took place in Devon (UK) in a 12-hectare field divided into three sections (blocks) totalising six enclosures. SS paddocks were 1.75 ha, while CG paddocks were 1.0 ha, comprising two 0.5-hectare lanes with 21 fence posts delineating up to 42 cells. The entire field was reseeded with perennial ryegrass and white clover in 2013. Autumn-born dairy x beef steer calves, acquired in 2018 (average weight 255 kg) and 2020 (average weight 219 kg), grazed for two consecutive seasons, distributed randomly across treatment groups balanced for weight and breed. SS enclosures were continuously grazed, while CG were rotationally grazed with

animals moving to a new cell every day. The daily allocated grazing area for the CG enclosures varied according to pasture growth rate and animal feed demand in order to maintain pasture quality and optimise grass utilisation. Cattle were winter-housed after the first grazing season. The following April they returned to the same grazing method and were taken through to finish on pasture. Monthly animal weights, weekly pasture covers using a rising plate meter, and annual soil samples (10 cm depth) for carbon content analysis were recorded. Liveweight gain, animal production per hectare and soil carbon content were analysed with a MIXED model with repeated measures in time using the GLIMMIX procedure of SAS 9.2 (2010).

### Results

Overall, the SS steers grew faster (P < 0.0001) than those on the CG system (0.767 vs. 0.600 kg/day) with variation over the years (Fig. 1a). Conversely, land productivity, measured as kg liveweight gained per hectare, was greater in the CG enclosures (687 kg/ha) compared to SS (463 kg/ha), and showed variability across time (Fig. 1b). Notably, the carrying capacity of the CG enclosures increased over time (P < 0.0001), from 1738 to 3035 kg liveweight per ha, whereas it remained relatively constant for the SS, ca. 1300 kg liveweight per ha (Fig. 1c). In 2018, before the first grazing season started, the enclosures allocated to each stocking method had similar (P > 0.05) soil carbon content, averaging 3.35%. There was a general trend to increase carbon content until the third year; however, the SS displayed a decline in soil carbon content, whereas the CG enclosure maintained the carbon levels accumulated in the earlier years (Fig. 1d). This difference in soil carbon content in 2022 could be due to the divergence in botanical composition (*Lulium perenne* L. increased in the CG method over time and decreased in the SS method, data not shown).



Fig. 1. Average daily gain (a), liveweight gained per ha (b), stocking rate (c) and soil carbon content (d) of enclosures grazed by dairy x beef steers under set-stocking or cell grazing methods over a four-year experiment.

### Conclusions

The study concluded that rotational CG demonstrated higher liveweight production per hectare and carrying capacity, while SS exhibited higher individual animal performance. Additionally, CG increased soil carbon levels, highlighting its potential for sustainable grazing practices compared to SS in a temperate climate. Vertical bars are the standard error of the mean.

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### 28. Temporal changes in soil organic carbon in permanent and reseeded pastures grazed by sheep or cattle in southwest England

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### Application

Understanding of how different agricultural interventions influence SOC is crucial for sustainable agriculture and ecosystem preservation.

### Introduction

Ecosystem health combines several indicators, among which SOC plays a pivotal role. Previous research has extensively investigated the impacts of farming interventions and land management practices on SOC content. Existing literature indicates that SOC content is influenced by factors such as land management practices, including ploughing and reseeding. However, gaps persist in comprehensively assessing the effects of different animal grazing species and ploughing frequency on SOC temporal dynamics. This study addressed this gap in knowledge by analysing SOC content in relation to ploughing and animal species grazing over a 10-year period.

### Material and methods

This study was carried out in the North Wyke Farm Platform (NWFP), a large-scale experiment located at Rothamsted Research in southwest England. The NWFP, established in 2010, spans 63 ha, comprising fifteen hydrologically isolated catchments distributed across three farmlets. The experiment monitors a variety of bio-physical variables relevant for assessing sustainability of farming interventions. Between 2011 and 2013, all fields were managed as permanent pastures. From 2013 to 2015, two farmlets underwent ploughing and reseeding with grass only or grass and clover, i.e., improved pasture. In 2019, one of the improved pasture farmlets transitioned to arable management. Every spring, 30 yearling cattle and 75 ewes (and their lambs) are turned to pasture to graze under a continuous stocking method until the end of the grazing season. Some fields are exclusively grazed by cattle until October-November and other fields are exclusively grazed by sheep until December. After cattle are housed, sheep may graze in the cattle grazed fields for short periods. Silage is made from some cattle and sheep grazed fields in May and July-August each year. Soil surveys at 0–10 cm depth were conducted in 2012, 2016, and the yearly from 2018 to 2021, providing SOC content. Farm field event including pasture type (permanent pasture vs improved reseeded- pasture) and the animal species grazing the fields (cattle or sheep) were recorded over time. We analysed SOC content as a function of both management factors and time. We used linear mixed models where the dependent variable was the SOC content, the fixed factors were pasture type, grazing animal species and time in years, and used the fields id as a random factor (all analysis were carried out in R version 4.2.3 and used the lmer package).

### Results

SOC content was affected by the three main factors "species grazing" (P = 0.042), "pasture type" (P < 0.001) and "year" (P < 0.001) and by the interaction effect pasture type × year (P = 0.047). Fields grazed by cattle had greater SOC than those grazed by sheep (5.20% vs 4.67%). As expected, the permanent pasture had greater SOC content than the improved pasture (5.64% vs 4.09%), given the decrease in SOC with ploughing (Fig. 1). In 2012, all the fields were under similar management in term of pasture type and, had similar SOC content (4.59%). The transition to improved pastures demanded ploughing between 2013 and 2015, reducing SOC concentration to 3.89% when assessed in 2016. SOC recovery to pre-ploughing values took a maximum of eight years (Fig. 1). However, the interaction effect was evidenced by the constant increase in SOC in the improved pasture since 2016 and an alternation of increases and decreases for the permanent pasture



Fig. 1. Mean soil organic carbon concentration (SOC, %) across the NWFP between 2012 and 2021 in the fields grazed by cattle or sheep on a permanent (PP) or improved (IP) pasture (Segura et al., 2023).

around 6% since 2016 after a remarkable increase between 2012 and 2016 from 4.58% to 6.02% (Fig. 1). This suggests that the permanent pasture stablised its SOC content around 6%, whilst the improved pasture still has potential for recovering SOC, and that cattle grazing boosted this recovery.

### Conclusions

Under cattle grazing SOC content was higher than under sheep grazing. It demanded a maximum of eight years to recover SOC content to pre-ploughing levels after reseeding. Permanent pastures generally maintained higher SOC than improved ones, but an intriguing pattern emerged; permanent pastures oscillated around 6% after a notable increase in the first four years, suggested and stabilisation of SOC.

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### 29. Association between the sowing site and methanogenic potential of seven forage species

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### Application

Sowing site could modulate enteric methane production by changes in the chemical composition of forages. Therefore, sowing site is a factor to consider when evaluating the methanogenic potential of forages.

### Introduction

As a greenhouse gas, enteric methane ( $CH_4$ ) emissions have a significant environmental impact and represent an energy loss for ruminants. Nevertheless, feeding high quality forages are related to lower  $CH_4$  emissions (Vargas et al., 2022), which are high in crude protein (CP), and low in neutral detergent fiber (NDF). Forage species under the same management, but growing in different locations, exhibit a different chemical composition due to differing environmental conditions of each site, and, therefore, it is of interest to evaluate their  $CH_4$  production. The objective of this experiment was to evaluate the *in vitro*  $CH_4$  production of seven forage species used in ruminant production, growing in two locations contrasting in temperature and rainfall.

### Material and methods

The methanogenic potential of seven forage species was evaluated using a discontinuous fermentation system (batch). The forage species evaluated were Alfalfa (AF; *Medicago sativa*), Bromegrass (BG; *Bromus* spp.), Festulolium (FL; X *Festulolium*), Orchardgrass (OG; *Dactylis glomerata*), Ryegrass (RG; *Lolium perenne*), Tall fescue (TF; *Festuca arundinacea*), and the root bulb and leaves of Turnip (TUR; *Brassica rapa*). Forages species were sown in 2019 at Icalma ( $-38^{\circ}49'11.1''$  S;  $-71^{\circ}17'26.6''$  W; 1160 m above sea level (ASL)) and Pehuenco ( $-38^{\circ}36'44.4''$  S;  $-71^{\circ}7'18.8''$  W; 900 m ASL), both sites located in the Andes foothills, La Araucanía, Chile. A randomized block designs with three blocks was used at each site, and within each block the forage species were randomly distributed. All grass species were harvested in January 2021, while AF and TUR were harvested in March 2021. Samples were dried in oven and ground, and field replicates from each site were pooled by species and incubated separately in bottles (n = 32). The inoculum was a mixture of rumen fluid from two ruminally cannulated Holstein cows and medium of Mould. In a 1:2 volume ratio. The inoculum was transferred to incubation bottles under CO<sub>2</sub> and incubated at 39 °C for 24 h. Gas production (GP) was measured with a pressure transducer and a sample was extracted into previously evacuated exetainers for subsequent analysis of CH<sub>4</sub> and hydrogen (H<sub>2</sub>) concentration by gas chromatography. Bottles were then opened and pH immediately measured. Bottle contents were centrifuged, the supernatants discarded, and the pellets dried to determine the dry matter (DM) disappearance (DMD). In addition, each forage species was analyzed for its content of NDF, CP and ash. Organic matter (OM) was estimated as 100 – ash. The relationship between variables was studied through Pearson correlations I, and visualized using Principal Component Analysis (PCA) Biplot. Significance was declared at P < 0.05, and trend at  $0.05 \ge P < 1$ . Data were ana

### Results

The *in vitro* CH<sub>4</sub> production varied among sowing sites and forage species. In general, the species from Icalma produced in average more CH<sub>4</sub> that the same species from Pehuenco (35.6%). In Icalma, AF and grass species (BG, FL, OG, RG and TF) produced 41.1% and 3.1% CH<sub>4</sub>, respectively. While in Pehuenco, those species produced 67.5 and 12.3% CH<sub>4</sub>, respectively. At both sowing sites, AF produced more CH<sub>4</sub> (52.3%), grass species less CH<sub>4</sub> (-0.3%) and TUR lesser CH<sub>4</sub> (-25.5%) than the overall mean. In case of grass species, RG and TF produced more CH<sub>4</sub> than FL and OG in both sites. The PCA Biplot (Fig. 1) illustrate that 73.2% of the variation was explained by the first two principal components (PC1 and PC2, respectively). The score plot of PC1 discriminated TUR (leaves and root bulb) from other forage species and sowing site. In contrast, score plot of PC2 only discriminated based on sowing site. Independent of components, Icalma had an opposite direction to Pehuenco. The magnitude of vectors are similar among variables with exception of DM, but not directions. The Vectors showed that



Fig. 1. Principal Component Analysis Biplot of chemical composition of forage species and ruminal fermentation variables.

CH<sub>4</sub> production trend to be positively associated with OM (r = 0.45; P = 0.078). In opposite, CH<sub>4</sub> was negatively associated with H<sub>2</sub> (r = -0.83; P < 0.0001) and CP (r = -0.34; P = 0.193). Gas, NDF and pH exhibited lower association with CH<sub>4</sub>.

### Conclusions

The *in vitro*  $CH_4$  production between Icalma and Pehuenco sowing site were dissimilar. There also are difference *in vitro*  $CH_4$  production in forages species, where AF, TF and RG produced more  $CH_4$  than other species and TUR produces less  $CH_4$  than other forages species. The *in vitro*  $CH_4$  are more associated with OM and directly opposite to  $H_2$  and CP.

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### 30. The effect of a biodiverse pasture on the nutritional quality of lamb meat

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### Application

Biodiverse pastures offer a sustainable source of forage for grazing ruminants, but little is known about whether these pastures affect meat nutritional quality. This study reported beneficial effects on nutritional quality of meat from lambs grazing biodiverse vs perennial ryegrass pasture; as such biodiverse pastures should be considered for incorporation into grazing systems.

### Introduction

Within temperate regions the use of monoculture perennial ryegrass (PRG; *Lolium perenne*) for lowland sheep production systems is widespread. However, PRG requires a high input of inorganic nitrogen, and has lower resilience than biodiverse pastures (Hofer et al., 2016), which are considered a sustainable alternative. The impact of biodiverse pastures on animal growth and productivity is inconsistent (Kliem et al., 2018; Grace et al., 2019), and the effect on meat nutritional quality is undefined although previous research suggests that the fatty acid (FA) profile could be improved via higher polyunsaturated FA content (Kliem et al., 2018). Also, the presence of deep-rooting forage herbs in biodiverse swards could improve the trace mineral supply to the grazing animal. The main objective of this study was to investigate the effect of a biodiverse pasture containing 12 different plant species (BP) on lamb growth and meat nutritional quality, when compared with a PRG monoculture.

### Materials and methods

Thirty mule male castrated lambs (mean weight 29.3 kg  $\pm$  0.63 SEM) were grazed for 19 weeks on either a PRG or BP pasture (BP comprising of perennial ryegrass, cocksfoot, festulolium, meadow fescue, red, white and alsike clover, black medick, lucerne, plantain and chicory;

n = 15 lambs per plot). Lambs were weighed weekly, and body condition scored (BCS) from week 6 onwards. Samples of above ground biomass were collected at the beginning, middle and end of the study, and were dried and milled prior to proximate, FA (Juniper et al., 2022) and selenium (Enamorado-Baez et al., 2013) analysis. After reaching the target weight (45 kg) lambs were humanely slaughtered, and tissue samples were taken from *musculus longissimus thoracis*. Tissue was separated into lean and subcutaneous fat prior to homogenisation. Samples were then analysed for FA (Juniper et al., 2022), and lean tissue for trace minerals (iron, selenium, zinc; Enamorado-Baez et al., 2013) and thiobarbituric acid reactive substances (TBARS; Juniper et al., 2022). Pasture composition data was analysed using a two-sample t-test for effects of treatment. Weekly liveweight gain was analysed using an ANOVA MIXED model including fixed effects of treatment, week and random effect of lamb, and meat composition data was analysed using an ANOVA MIXED model with fixed effect of treatment and random effect of lamb.

### Results

There was little difference between nutritional quality of pastures, apart from BP having a higher (P < 0.001) selenium and 18:2 n-6 content, and a tendency for higher ADF (P < 0.1). Weekly liveweight gain was higher (P = 0.017) for PRG than BP (1.34 vs 1.11 kg/week), but there was no overall effect (P = 0.174) of pasture treatment on BCS. Lean and subcutaneous fat tissue from BP lambs contained more (P < 0.05) 18:2 n-6 (Table 1) and zinc (68.6 vs 61.8 ug/kg dry tissue) than lean and subcutaneous fat from PRG lambs. There was no effect (P > 0.05) of pasture on other meat FA (Table 1), trace minerals or TBARS.

### Table 1

Effect of grazing lambs on either a perennial ryegrass monoculture or a biodiverse pasture (12 species mix), on selected fatty acid and trace mineral contents of lean and subcutaneous fat from *musculus longissimus thoracis* (mg/100 g tissue).

	Pasture type <sup>1</sup>			
	PRG	BP	SEM	P <sup>2</sup> Treatment
M. longissimus thoracis				
16:0	2458	2591	184.6	0.608
18:0	2715	2762	219.4	0.88
18:1 cis-9	2935	2987	240.5	0.876
18:1 trans-11	448	489	33.4	0.38
18:2 cis-9, cis-12	187	233	15.1	0.037
18:3 n-3	127	138	11.2	0.492
Total PUFA <sup>3</sup>	561	596	38.2	0.517
VLC <sup>4</sup> n-3	59	57	4.64	0.765
Total lipid	10268	10,639	749.2	0.724
Subcutaneous fat				
16:0	18,993	18,715	659	0.756
18:0	21,135	21,002	886.1	0.912
18:1 cis-9	22,393	20,663	775.7	0.109
18:1 trans-11	4298	4403	311	0.802
18:2 cis-9, cis-12	817	945	36.4	0.014
18:3 n-3	760	813	43.5	0.371
Total PUFA <sup>3</sup>	2909	2742	130.6	0.349
VLC <sup>4</sup> n-3	136	122	12.2	0.392
Total lipid	78,902	76,555	2000.1	0.39

<sup>1</sup> PRG – perennial ryegrass, BP – biodiverse.

<sup>2</sup> Significance of the effect of pasture treatment for n = 15 lambs.

<sup>3</sup> Polyunsaturated fatty acids.

<sup>4</sup> Very long chain n-3 polyunsaturated fatty acids.

### Conclusions

Grazing lambs on a sustainable biodiverse pasture for 19 weeks marginally improved the nutritional quality *m. l. thoracis* meat, due to higher 18:2 n-6 content (without a concomitant increase in TBARS), and zinc concentrations. Further research should focus on including specific plant species in biodiverse pasture to improve nutritional quality of ruminant meat, whilst maintaining pasture resilience and sustainability.

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# 31. Potential use of sheep wool as a soil amendment to mitigate drought effects on red clover (Trifolium pratense) within a circular economy approach for grazing livestock

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#### Application

Pelleted sheep wool, mixed with compost, may help mitigate drought impacts in red-clover swards. The study was a proof of principle experiment as part of a circular economy approach for livestock grazing systems.

### Introduction

The potential for using sheep wool as a fertiliser to support growth has previously been recognised in other plant species (Zheljazkov, 2005). Previous studies demonstrated that using wool as a soil amendment can also increase the moisture content of the potting media (MacKintosh et al., 2023). This study investigated the potential of sheep wool to mitigate the effects of drought using red clover grown in pots in a controlled environment.

#### Methods

Red clover seedlings (n = 30) were transplanted (one seedling/pot) into 15 cm pots, containing different mixtures of raw kemp sheep wool pellets and commercial compost: compost alone (0% wool), 10% wool:90% compost, or 20% wool:80% compost, mixed on DM basis. After a 29-day establishment period, a 14-day experimental phase was conducted where half the plants were maintained well-watered and the remainder were droughted, giving six treatments (five replicates/treatment) in a randomised complete block design. Plants were maintained in controlled environment (20 °C day/12 °C night, and daylength to 10 h) facilities at IBERS. At the end of the 14-day drought period, all plants were harvested and assessed for indicators of drought effects. Relative leaf water content as a measure of turgidity, and herbage yield were assessed as the main indicators of drought mitigation, alongside pot moisture, salinity and leaf area. Data were statistically analysed using Genstat (v.22) [Baird et al, 2022] using ANOVA and Bonferroni *post hoc* where wool content and water exposure were the treatment factors and block number was the blocking factor. Statistical significance was attributed where *P* < 0.05.

#### Results

The relative leaf water content was significantly reduced for droughted plants grown in 100% compost, confirming that plants were drought-stressed (P < 0.05, Fig. 1). The addition of wool to compost increased the relative leaf water content of droughted plants to an extent that was not significantly lower than the well-watered plants that were also grown in 10% or 20% wool (P < 0.05, Fig. 1). Pot moisture content confirmed that the droughting treatment was successfully implemented, with droughted plants in 100% compost having a lower pot moisture content compared to well – watered plants (P < 0.05). Droughted plants grown in the presence of wool had similar pot moisture content to that of the well-watered plants (P > 0.05). However, wool also increased the salinity of the potting media of both droughted and watered plants (P < 0.05). There was no significant effect of wool content or drought treatment on herbage dry matter yield or leaf area.



Fig. 1. Relative leaf water contents (%) at the end of the drought period.

## Conclusion

The data indicate that wool can mitigate the impacts of drought in the time frame studied by allowing the plant to maintain its leaf turgidity, through its effect on maintaining soil moisture content. Due to increased salinity in the potting media where wool was used, further study is required to ensure that wool used as a soil amendment does not have any potential deleterious effects when used over longer time periods. However, given the challenges associated with climate change, it is important to consider methods to combat the effects of drought, and wool used as a soil amendment may provide benefits to forage production, through re-purposing low-grade shorn wool and reducing waste on-farm

## Acknowledgements

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## 32. Tannin variation in tree fodder because of species, plant fraction and month

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## Application

Tannin profiles of tree fodder vary between species, plant fraction and month. Therefore, it is recommended to perform tannin analysis before tree fodder harvest to ensure that the quality and quantity of tannins are appropriate to achieve the desired nutritional and environmental benefits.

## Introduction

Tannins are one of the most abundant polyphenolic compounds in plants, with a multitude of chemically unique entities in nature (Besharati et al., 2022). Condensed tannins (CT) consist of flavan-3-ol subunits linked together to form oligomers and polymers (Besharati et al., 2022). They can reduce enteric methane emissions and improve nitrogen use efficiency and nutritional quality of animal-derived foods (Mueller-Harvey et al., 2019). On the other hand, they may reduce feed intake and digestibility or animal performance when fed in high amounts (dietary concentrations <50 g CT/kg DM are recommended to achieve benefits) (Mueller-Harvey, 2006). However, it is difficult to establish a relationship between CT and their biological activities because of their chemical diversity and the lack of complete understanding of underlying mechanisms (Besharati et al., 2022). For example, procyanidins can be more effective against methanogens, while prodelphinidins have more phenolic groups and can for more H-bonds and higher degree of polymerisation and have improved antiparasitic effects (Mueller-Harvey et al., 2019). Given the increased interest in the use of tree fodder as ruminant feed in silvopastoral systems and the varied effect of tannins on animals, knowing the tannin profile of different tree fodders can promote the beneficial and prevent the damaging- impacts on livestock production. This study aimed to assess the tannin profile (quantitative and qualitative) in three tree fodder species (goat willow, oak, and maple) and their plant fractions (leaves and twigs) throughout the grazing season (June-September).

## Material and methods

Leaf and twig samples were collected from three species (goat willow, *Salix caprea*; oak, *Quercus robur*; field maple, *Acer campestre*) from Elm Farm, an 85 ha organic livestock farm in West Berkshire, UK, with soil types varying from heavy clay loam to sandy loam. Five trees per species (n = 15) were sampled monthly from June – September 2017. The concentration of CT was determined using a Butanol–HCl assay. Qualitative analysis on the molar percentages of flavan-3-ol subunits, mean degree of polymerization (mDP), procyanidins (PC) and prodelphinidins (PD) proportions, and *cis*- versus *trans*- flavan-3-ols subunits was conducted using *in situ* thiolysis assay (Natalello et al. 2020). Invitro true dry matter digestibility (DMD) was determined using the ANKOM Daisy II system. All analyses were conducted in triplicate. Data were analysed using linear mixed models in Minitab Statistical Software 20.2, using species, plant fractions, month, and their interactions as fixed factors, and tree ID (nested within species) as random factor. Tukey's Honestly Significant Difference test was conducted for pairwise comparisons when the effect of a fixed factor was statistically significant (P < 0.05).

## Results

Dry matter content of fodder was highest for maple (484 g/kg fresh), lowest for goat willow (445 g/kg fresh) and intermediate for oak (464 g/kg fresh) (P < 0.001, SE = 7.8). Dry matter digestibility was highest for goat willow (559 g/kg DM), followed by oak (497 g/kg DM) and maple (525 g/kg DM) (P < 0.001, SE = 7.8). Total CT and *trans*- flavan-3-ols contents were highest for goat willow (69.5 g/kg DM); 71.7% of CT), intermediate for oak (35.7 g/kg DM; 64.1% of CT) and lowest for maple (24.1 g/kg DM; 13.2% of CT), while the opposite was observed for *cis* flavan-3-ols when expressed as % of CT (28.3; P < 0.001; SE = 2.9). Procyanidins were highest in maple (73.4% of CT), lowest in oak (45.2% of CT) and intermediate in goat willow (54.7% of CT), while the opposite was observed for prodelphinidins when expressed as % of CT (26.6; P < 0.001, SE = 4.8). When compared with twigs, leaves had higher DMD (639 vs 415 g/kg DM, SE = 7.0, P < 0.001), and total CT concentrations (47.6 vs 38.6 g/kg DM, SE = 3.0, P < 0.001); and lower mDP (3.94 vs 4.47, SE = 0.3, P = 0.002). There was a variation (3.62–5.52, SE = 0.4, P < 0.001), but month did not affect other CT parameters.

## Conclusion

The present study demonstrates that there is a significant effect of species, plant fractions and month on DMD and tannin profile of tree fodder. The differing effects of tannin types on animal metabolism and the high variation between species, tannin contents and profiles should therefore be accounted for when introducing tree fodder to animal nutrition, to achieve the desirable effects by minimizing the risk of anti-nutritional impacts.

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#### 33. Feeding the immune system: Perspectives from the feed additive industry

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The body of science of animal nutrition has entered a new era in which the collective understanding and synergies of other biological systems, has become critical to future advancement. Nutritionists of today and the future must work within an understanding of other biological systems, including the immune system. This requirement presents opportunity to change nutritional applications and offers the feed additive sector, product development opportunities.

When considering the immune status of a production animal, there are two avenues of consideration for practical nutrition. The first of these is the plane of nutrition. The understanding of the energetic requirement for an immunological response, and associated nutrient repartitioning, has been modelled well in recent years (Kvidera et al. 2017a; 2017b). For example, work by Kvidera *et al.* (2017) has demonstrated that the Holstein cow requires >1 kg of glucose for a 12 h active immune response induced by lipopolysaccharide (LPS) infusion (Kvidera et al. 2017a).

Feed additive approaches can be further split into categories. For example, there are direct impacts of postbiotic ingredients. In 2021 the International Scientific Association of Probiotics and Prebiotics (ISAPP) proposed the following definition for the term postbiotic: 'a preparation of inanimate microorganisms and/or their components that confers a heath benefit on the host' (Salminen et al. 2021). Previously the yeast product (non-living yeast ingredients) sector has discussed, at a commercial level, the potential benefits to immune support, however it is only recently that postbiotic modes of action are being characterised both *in vitro* and *in vivo*.

Postbiotic functionalities could be considered as a direct impact however there are some feed additives that could be considered to have an indirect impact. Considering the increased demand for energy at times of stress and during an activated immune response, enzymes, allowing for improved nutrient availability, have a role to play in meeting increased energy requirement within a limited dry matter intake (DMI) of fresh cows. In the same way, silage additives, which improve fermentation profile of ensiled forages, also offer opportunity for maximising nutrient availability per kg of forage DMI (Muck et al. 2018).

A third category of feed additives is better described as a mitigator of potential inflammatory challenge. Both mycotoxins and endotoxins present a challenge to the production animal. The endotoxin challenge is particularly relevant to ruminant nutrition due to the physiology of ruminant animals (McDaniel et al. 2023).

As the scientific frontiers of nutrition and immunology begin to close the gap, the commercial nutrition industry must become technicians of their application. This paper will present a practical understanding of how this can be applied, and how product development is being influenced.

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#### 34. Performances responses improved with methionine balanced diets in transition ewes at commercial farm level

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#### Application

Methionine balancing diet during transition and early lactation periods increased milk yield and composition in dairy ewes improving commercial farm profitability.

#### Introduction

Recent research reported dietary recommendations of digestible methionine in dairy ewes similar to those of dairy cows (2.5% metabolizable protein, Francia et al., 2021). When rumen protected methionine was supplied in lactating dairy ewes' diets, milk protein content increased by 5%. The objective of this study was to confirm, at commercial farm level, the effect of digestible methionine supply on milk yield and composition in dairy ewes.

#### Materials and methods

525 Assaf ewes in a commercial farm, Villafranca de Duero, Valladolid, Spain were feed for 3 weeks before lambing to 3 months after. A TMR diet was fed ad libitum formulated to meet nutrient requirements (INRA, 2007). Treatments were two diets (18.1% CP, NEL = 5.96 K-cal/kg DM): basal diet "CTR" and MS plus methionine supplementation (Metasmart Dry; isopropyl ester of 2-hydroxy-4-methylthio buta-noic acid, Adisseo) provided 71 and 100% of Digestible Methionine requirement, respectively. Metasmart Dry was administered in the milking parlour concentrate with 9 and 12 g/animal/day pre- and post-partum periods. The trial was conducted as a 2-groups randomized block design. Ewes were randomly allocated to experimental treatments. Individual milk yields were recorded daily and milk fat and milk protein monthly.

### Statistical analysis

Data were analysed using the MIXED procedure of JMP for repeated data with treatments as fixed factors. Table 1.

#### Table 1

Lactation performance means, and standard errors rates (±SE).

Prepartum	CTR		MS		P-value		
Lactation	CTR	MS	CTR	MS	Prepartum	Lactation	TRT*Period
Milk, Kg Milk protein, % Milk fat, % Milk protein, g/d Milk fat, g/d	$\begin{array}{c} 2.58 \pm 0.05^{b} \\ 4.62 \pm 0.02^{b} \\ 6.02 \pm 0.05^{a} \\ 121 \pm 1.66^{c} \\ 141 \pm 2.29 \end{array}$	$\begin{array}{c} 3.03 \pm 0.04^{a} \\ 4.74 \pm 0.02^{a} \\ 5.64 \pm 0.05^{b} \\ 133 \pm 1.52^{ab} \\ 144 \pm 2.06 \end{array}$	$\begin{array}{c} 2.77 \pm 0.07^{\rm b} \\ 4.55 \pm 0.03^{\rm b} \\ 6.01 \pm 0.08^{\rm a} \\ 127 \pm 2.39^{\rm bc} \\ 149 \pm 2.58 \end{array}$	$\begin{array}{c} 3.19 \pm 0.05^{a} \\ 4.65 \pm 0.02^{b} \\ 5.62 \pm 0.06^{b} \\ 137 \pm 1.94^{a} \\ 149 \pm 2.58 \end{array}$	0.0015 0.0006 0.8040 0.0099 0.0115	<0.01 <0.01 <0.01 <0.01 0.6474	0.8378 0.4834 0.8951 0.4169 0.4144

## Results

Milk yield is higher in ewes fed MS prepartum (2.97 vs 2.80 kg/d, prepartum: P < 0.05), milk protein content did not differ. Ewes fed MS decreased milk fat content (6.01 vs 5.63%, Lactation: P < 0.01). Milk protein yield increased in ewes fed MS before lambing (132.4 vs 127.5 g/d, Lactation period P < 0.01). Ewes fed MS before lambing had higher milk fat yield (149.5 vs 142.9 g/d, prepartum: P < 0.01).

#### Conclusions

Balancing diet for methionine during transition period before and after lambing improved performances of lactating dairy ewes.

#### Acknowledgments

Francisco González, Raúl González, farmers, and Raúl González advisor.

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## 35. Improving the sustainability of beef production at the Harper Adams University Future Farm: A case study of leveraging nutrition research into practice

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## Application

Findings from current nutrition research can be practically applied on-farm to reduce feed costs and improve beef enterprise sustainability. However, the individualistic nature of each farm case scenario needs to be taken into consideration during this process and the feed industry has a key role in facilitating such improvements.

#### Introduction

Beef production within the United Kingdom has faced considerable challenge over the last number of years, with greater volatility of input prices and an increased emphasis on the greenhouse gas emissions associated with production. Feed not only accounts for up to 75% of the variable costs of beef production, but is also highlighted as a potential vehicle for greenhouse gas mitigation within beef production systems (Nielsen et al., 2013; Eory et al., 2015). There is subsequently a need to examine how the practical implementation of current research can concurrently reduce feed costs whilst improving sustainability within case-specific beef production scenarios.

## Material and methods

This work employed a positivist case-study approach to help generate a multi-faceted, in-depth understanding of how the latest nutrition research can be applied to improve the sustainability of a beef production enterprise (Crowe et al., 2011). The case of study in this instance, consists of the beef production enterprise within the Future Farm (FF) at Harper Adams University. The campus itself, resides within rural Shropshire and in 2022, the Future Farm was established with a view to servicing research, teaching and knowledge exchange, whilst demonstrating best practice and an aim to achieve net-zero by 2030. A component of this change, included the establishment of an intensive dairy beef production enterprise, which finishes British Blue cross dairy steers and heifers from the Universities all-year-round calving Holstein-Friesian dairy herd. An intensive beef system was initially selected due to constraints surrounding grazing and land availability at the time. Between 2022 and 2024, stakeholders have tried to utilise the findings of the latest nutrition research within the FF beef enterprise to reduce feed costs and improve sustainability. Identified topic areas of interest for application included alternative forage-based finishing diets (maize + grass silage versus concentrate-based), forage quality (moderate versus high-quality grass silage) and low protein diets (formulated according to crude protein versus metabolisable protein). The cost implications of these dietary formulations were modelled according to AFRC (1993) in Microsoft Excel and are expressed in relation to a 550 kg steer growing at a rate of 1.45 kg per day.

## Results

The substitution of concentrate with forage had the greatest impact on diet cost and margin over feed, with a predicted increase in margin over feed from £0.11/kg of average daily gain (ADG) to £1.35/kg of ADG when a forage-based (maize and grass silage; 673 g/kg of dry matter forage) total mixed ration was fed as opposed to a finishing concentrate. Formulating according metabolisable protein also increased predicted margin over feed from £1.35 to £1.56/kg of ADG with a decrease in dietary crude protein from 127 g/kg of dry matter to 103 g/kg of dry matter. Due in-part to restrictions on grass silage inclusion (maximum of 160 g/kg of dry matter), the substitution of a moderate quality grass silage (10.7 MJ of metabolisable energy/kg of dry matter) for an excellent quality grass silage (11.9 MJ of metabolisable energy/kg of dry matter) demonstrated the lowest potential to reduce diet costs, with a predicted increase in margin over feed from £1.31 to £1.35/kg of ADG. Consequently, all of these practices have been implemented during diet formulation at the FF beef unit, with heifers and steers finishing at 13.3 and 15.0 months of age, respectively. Indeed, the predicted farmgate greenhouse gas emissions intensity of beef produced from the enterprise is 12.00 kg of CO<sub>2</sub>-equivalent/kg of deadweight, substantially lower that the mean of 20.63 (Range = 13.8 to 26.4) kg of CO<sub>2</sub>-equivalent/kg of deadweight reported by McNicol et al. (2024) for Welsh lowland beef and sheep produce ers. Future target areas for exploration include the incorporation of feed additives, and expansion to include a high performance outdoor dairy beef production system.

## Conclusion

The findings of current nutrition research can be practically utilised on-farm to improve enterprise sustainability whilst reducing costs of production. However, the individual characteristics and constraints of each farm need to be taken into consideration during this process, and the industry plays a key role in facilitating such change whilst improving enterprise resilience.

## Acknowledgments

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#### 36. Co-infection impacts on resistance and tolerance to nematodes differ between different mice genotypes

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Insani Hubi Zulfa. zinsani@sruc.ac.uk KeywordsMice; Genetic resistance; Infection tolerance; Co-infection; Nematodes; *Heligmosomoides* polygyrus

#### Application

The impact of co-infection on the resistance and tolerance to an intestinal nematode was affected by host' genetics, with susceptible hosts benefiting the most.

#### Introduction

There are two mechanisms by which hosts can defend against pathogen infection: resistance, the ability to clear pathogen load and tolerance, the ability to withstand pathogen impact (Raberg et al., 2007). To date, the relationship between resistance and tolerance has mostly been studied in the context of a single infection (Athanasiadou et al., 2015), but dynamics may be different when hosts are exposed to more than one pathogen. The aim of our study was to characterise the variation of host resistance and tolerance to an intestinal nematode of mice (*Heligmosomoides polygyrus*) during co-infection with another intestinal pathogen, Theiler's murine encephalomyelitis virus (TMEV). Host responses against *H. polygyrus* are known to be at least partly, genetically controlled(Oleszak et al., 2004; Maizels et al., 2012). Our hypothesis was tested in genotypes that differed in the susceptibility to *H.polygyrus*; we expected that susceptible mice would suffer more from the co-infection compared to resistant mice.

#### Material and methods

Three strains of mice were used: SJL mice (resistant to *H. polygyrus*), BALB/c mice (intermediate susceptibility to *H. polygyrus*), and C57BL/6 (susceptible to *H. polygyrus*). Mice from each strain were divided into four infection treatment: co-infection (**Co-inf**), *H. polygyrus*-only (**Par**), TMEV-only (**Vir**), and non-infected (**Sham**). Co-inf and Par mice were administered with 0.2 ml of 250 *H. polygyrus* 3rd stage infective larvae (L3) orally at day 0 (D0) whereas 0.2 ml of water was administered for Vir and Sham mice at D0. Co-inf and Vir mice received 0.2 ml of 10^6 PFU TMEV at D8 whereas Par and Sham mice received 0.2 ml of DMEM (Dulbecco s Modified Eagles Medium). Mice were euthanised at **14 dpi** and **42 dpi**; these two time points represent expected nematode establishment and nematode clearance periods respectively, in the resistant genotype. Average daily gain (ADG) and feed intake (FI) were analysed using linear mixed model with ADG or FI as the dependent variable, and mouse strain, infection treatment, week post infection (WPI), and all statistically significant interactions as fixed effects and cage as a random effect. To assess the effect of mice genetics and treatment on the resistance against *H. polygyrus*, as measured by EIC and worm burden, a linear mixed model was used. To assess the effect of mice genetics and treatment on the tolerance against *H. polygyrus*, a linear mixed model was used with carcass weight as dependant variable and parasite burden (total worms and total EIC) as independent variables. Mouse strains, infection treatment, and all statistically significant interactions as fixed effects, and individual mouse as random effect were used in both resistance and tolerance analysis. Statistical analysis was performed in R Studio

#### Results

In the susceptible to *H. polygyrus* C57BL/6 mice, ADG (0.08 g/day) and FI (0.07 g/day) was higher in Co-inf compared to Par, Vir, and Sham at 2 weeks post infection (P < 0.001). However, in the BALB/c mice, co-infection treatment reduced 20% of ADG compared to mice receiving *H. polygyrus*-only at 2 weeks post infection (P = 0.034); 10% of ADG loss was observed in the resistant to *H. polygyrus* SJL mice (P < 0.001). When compared to *H. polygyrus*-only C57BL/6 mice, co-infected C57BL/6 mice had 15% lower EIC (P < 0.001), and 20% worm counts (P < 0.05), whereas Co-inf BALB/c mice showed 5% elevated EIC (P < 0.001) and 10% worm counts (P < 0.05) compared to *H. polygyrus*-only mice. Co-inf did not have any impact on resistance traits in SJL mice. In the second time point tested, 25% reduction EIC (P < 0.001) and 30% decrease in worm burden (P < 0.001) were showed in co-inf C57BL/6 mice compared to *H. polygyrus* only infected counterparts. On the contrary, Co-inf BALB/c mice increased EIC and worm burden up to 25% compared to *H. polygyrus* only mice (P < 0.001). Compared to *H. polygyrus* only SJL mice, Co-inf mice were 5% more tolerant (P < 0.05). Co-inf C57BL/6 mice tended to be more tolerant (P = 0.058) whereas Co-inf BALB/c mice tended to be less tolerant than their *H.polygyrus* only counterparts (P = 0.051).

#### Conclusion

Our data showed that the impact of co-infection with two intestinal pathogens resulted in significant variation on host resistance and tolerance to *H.polygyrus* in three inbred mouse strains. Contrary to expectation, mice susceptible to *H.polygyrus* benefited most from co-infection, as their resistance was improved compared to *H.polygyrus*-only counterparts. On the other hand, mice resistant to *H.polygyrus* showed a benefit in their tolerance compared to *H.polygyrus* only mice. The underlying mechanisms of these co-infection phenotypes are currently investigated.

## Acknowledgement

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#### 37. Gastro - Intestinal parasites in outdoor pigs in Ireland: What factors affect parasitic load?

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#### Application

We must address the high prevalence of some species of gastro – intestinal parasites observed in outdoor pigs to ensure the successful expansion of the outdoor production sector in Ireland.

#### Introduction

Societal concerns about animal welfare in conventional production systems, and European Union policies such as the Green Deal and "Farm to Fork", promoted interest in outdoor/organic pig production systems. However, gastrointestinal parasites pose a threat to domestic pigs in outdoor systems. Left uncontrolled, these can significantly undermine performance, result in substantial economic losses, and potentially lead to pig mortality (Roepstorff et al., 2011). Studies on the prevalence and occurrence of such parasites in Ireland are scarce. Hence, the objective of this study was to identify the specific parasites present in outdoor pigs, to compare parasitic loads across seasons, and evaluate associations between these loads and various pig and management related characteristics.

#### Materials and methods

A total of 61 pig faecal samples were collected from 20 outdoor pig farms located across the island of Ireland on two separate visits during Winter (n = 33) and Summer (n = 28) 2023. Between 2 and 4 spontaneously voided faecal samples were collected per paddock, and mixed thoroughly to achieve paddock level composite samples. These were stored from 0 to 4 °C, then analysed using an improved – McMaster floatation method (described by Taylor et al., 2016). Parasitic egg types were identified by their morphology, the number of eggs counted, and Faecal Egg Count (FEC – number of eggs/gram). Body condition score (BCS) was individually recorded on 432 pigs using a five point scoring system and averaged at paddock level. Data relating to the animal (sex and age) and farm (anthelmintic usage and paddock rotation) were also collected. Data were analysed using non–parametric tests. The effects of season, paddock rotation and anthelmintic treatment on FEC were analysed using Wilcoxson sign rank tests, and the effects of age and sex on FEC were analysed using Kruskal – Wallis tests. Relationships between FEC and BCS were analysed with Pearson correlation tests. R studio (R version 4.2.1) statistical analysis software was used.

#### Results

Four types of gastrointestinal parasitic eggs were identified: *Eimeria*, Strongyles, *Ascaris suum* and *Trichuris suis* (Table 1). Due to the challenge of identifying several species, *Eimeria* and Strongylids were grouped under the broad categories of *Eimeria spp.* and Strongyle. Both *Eimeria spp.* (winter: 82%, summer: 94%) and strongyles (winter: 94%, summer: 82%) had a high prevalence across farms in both seasons. *Ascaris suum* had a prevalence of 35% in winter and 29% in summer. *Trichuris suis* was recorded in one farm (6%) in winter and two farms (12%) in summer. The effects of season, sex, antihelminthic use, and paddock rotation on FEC are shown in Tables 1 and 2. Juvenile pigs (<8 months) had a higher (P < 0.001) *Eimeria spp.* FEC than adults, whereas adult pigs (>8 months) had a higher Strongyle FEC (P < 0.01) (data not shown). The Strongyle load and body condition of pigs was negatively correlated (r = -0.2401, P = 0.001).

#### Table 1

Effect of season and host gender on parasite load (median FEC eggs/g) of pigs (n = 61) across 20 outdoor farms, Ireland.

Species	Season		P-value		Sex		P-value
	Winter	Summer		Male	Female	Mix	
Eimeria spp.	475	3350	<0.001	600	1150	3250	0.001
Strongyle	475	150	0.0237	800	150	300	0.02
Ascaris suum	0	0	0.9321	50	0	0	< 0.001
Trichuris suis	0	0	0.1275	0	0	0	0.5882

## Table 2 Effect of anthelminitics and paddock rotation on parasite load (median FEC eggs/g) of pigs (n=61) across 20 outdoor farms, Ireland.

Species	Anthelmintic u	ıse	P-value	Paddock rotati	on	P-value
	Yes	No		Yes	No	
Eimeria spp.	2775	1025	0.003	1050	4300	0.0006
Strongyle	0	500	<0.001	250	350	0.5275
Ascaris suum	0	0	0.365	0	0	0.2752
Trichuris suis	0	0	0.6853	0	0	0.0002

## Conclusions

Prevalence of *Eimeria spp.* and Strongyle was high, and the seasonal effect on the prevalence was minimal. However, the FEC for both seasons implied that there was a seasonal effect for *Eimeria spp.* and Strongyles. Paddock rotation and anthelmintic usage appeared effective in reducing parasite load (FEC). Male pigs had higher FEC levels of helminth eggs, including Strongyle and *Ascaris suum* and this could be due to the male pigs being rotated less frequently. BCS negatively correlated with the Strongyle FEC, suggesting that the welfare of the animal is compromised when there are high helminth counts. Considering the potential zoonotic threat associated with *Ascaris spp.* and *Trichuris spp.*, further studies are needed to efficiently identify and treat outdoor pigs.

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## 38. Sheep scab: Its diagnosis, presence across, and treatment in, Northern Ireland. Initial results of a pilot knowledge exchange and disease control programme

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## Application

Two-fold lessons can be learnt from this pilot project; the successes that can be achieved through the collaborative nature of the project from farmers to funders, and the documentation of previously unrecognised levels of sheep scab present in Northern Ireland highlighting the need for a sustainable control programme to be developed.

## Introduction

Sheep scab (hereafter, scab), caused by the ectoparasitic mite, *Psoroptes ovis*, is highly infectious and can cause significant detrimental impacts on welfare sheep and emotional stress to farmers caring for them (Crawford et al., 2022). Scab is present in Northern Ireland (NI) and is currently a notifiable disease. However, limited data exists on the prevalence or distribution of the disease (Crawford et al., 2022). A farmer-led group, concerned about the spread of scab, working in NI since 2019, obtained, in partnership, funding from BBSRC through its endemic livestock disease programme to undertake on-farm investigations of suspected cases of scab alongside knowledge exchange (KE) activities (Crawford et al., 2023) as a pilot study to inform future disease control efforts.

## Materials and Methods

Following KE meetings with veterinary surgeons (vets) and farmers, a target was set to engage 100 farmers who considered they may have scab in their flock, or suffered a recent, high-risk, incursion. The programme funded the farmer's private vet to visit the farm, undertake a risk assessment, examine sheep, and obtain diagnostic samples. The vet also advised the farmer on scab prevention and treatment.

Blood samples were taken to detect recent scab exposure via a Pso o2 antigen-based ELISA (Burgess et al., 2012). If visible lesions were identified, the vet was encouraged to take skin scrapes in addition to the blood samples. Even if light microscopy detected live mites, vets were requested to take blood samples to provide a baseline titre to which subsequent serology could be compared if a suspected failure of treatment efficacy arose.

Farmers whose flocks were positive were offered support for the purchase of medicine for flock treatment, up to a financial limit, and in certain high-risk scenarios, some flocks that tested negative were also offered preventative treatment.

## Results

Farmer engagement exceeded project aims, with over 150 farmers enrolling with the scheme and others contacting the helpline or team members privately. These private contacts were mainly driven by farmers seeking advice but unwilling to formally commit to the scheme, because they were reluctant to disclose their flock status to state authorities.

Visits were undertaken on 105 farms. Uptake was from farmers around NI, which resulted in vets from 39 different practices/branches assisting in the scheme's delivery. Scab was confirmed by light microscopy and/or blood samples, in 60 flocks, with their locations shown in Fig. 1. In addition, seven flocks were assigned for further monitoring or testing because initial test results proved equivocal. Blood testing proved vital for detecting scab. Of the 22 flocks which had clinical signs but a negative skin scrape, only five were negative when blood tested.



Fig. 1. Geographical location of flocks participating in the project in which scab was detected (red dots) and those where scab was not confirmed (blue dots).

Plunge dipping in diazinon emulsion was the recommended treatment. Among the sixteen flocks treated with the injectable macrocyclic lactone (ML) products, five subsequently had to be dipped because clinical signs persisted, raising a concern about the failure of treatment efficacy. It was impossible to obtain sufficient live mite samples from these flocks to prove whether the efficacy failure was due to ML resistance. However, suboptimal application (injection) technique and failure to maintain adequate biosecurity were identified in flocks that had used injectable techniques to manage scab.

Scab was identified in some flocks which had access to common grazing, and preventive plunge-dipping was offered to these flock owners, regardless of the results of their flock investigation on the proviso that such treatments were all undertaken in co-ordination with other flock-keepers and before any sheep were returned to the common. Compliance with the scheme's advice on clearing the common and treating all sheep in a coordinated manner was considered to be good.

Post-programme interviews with participating farmers and other stakeholders revealed their relief that someone was tackling scab, that the programme managers were keen to work with the farmers and finally, that the taboo about scab was being broken.

#### Conclusions

The success of the scab group in identifying funding and developing partnerships to deliver the on-farm investigations highlights how researchers, and those funding research can positively impact animal health and welfare by listening to the call from farmers for help with their concerns. The levels of scab identified by the pilot project demands, based on animal and farmer welfare, a concerted effort to identify a sustainable approach to scab control in NI.

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## 39. Predicting, validating and applying mixed parasite infection models for sheep under current and future climate scenarios

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## Application

Assessment and adaptive management of mixed parasite infections on grazing pasture to modify treatment interventions and limit chemical inputs.

#### Introduction

Livestock are often infected with multiple parasites and pathogens, yet control efforts in the host are generally focused on one agent at a time. Furthermore, the importance of the mixed population of infective parasite stages residing on pasture, and therefore risk of future coinfections in the host, are often neglected. This is further complicated by the fact that parasite population dynamics on grazing pastures are not static, but fluctuate spatiotemporally, driven by external factors such as environmental conditions and farm management (Eysker et al., 1998; O'Connor et al., 2006). This complicates day-to-day livestock management and anthelmintic treatment decisions, as strategies must consider current and future infection risk for multiple parasite species.

Climate change is shifting the traditional seasonal patterns of parasite infections and is projected to continue doing so, altering the occurrence of co-infections (van Dijk et al., 2008; Rose et al., 2016) and presenting farmers with additional uncertainty when timing anthelmintic interventions. Improving parasite management strategies to attenuate parasite transmission relies on adequate knowledge of the complex spatiotemporal patterns of multiple parasite species. Our aim was to apply a suite of mathematical models capable of predicting gastrointestinal nematode (GIN) population dynamics for multiple species at the regional, farm and individual field scale, under current and future climate scenarios. These predictions can be used to guide targeted parasite management strategies.

#### Materials and methods

To assess overlapping parasite seasonality and likelihood of co-infections at the regional and farm level in the UK, an egg hatch prediction model for *Nematodirus battus* (Gethings et al., 2015), was used alongside the Q0 spatial predictor and GLOWORM-FL model frameworks for *Haemonchus contortus* and *Teladorsagia circumcincta* (Rose et al., 2015; 2016). Q0 values  $\geq 1$  signify that environmental conditions are conducive to population growth in the absence of host immunity with resultant increased infection pressure. To assess recent predicted seasonality of these parasites in the UK, temperature and precipitation data between 2012 and 2022 were extracted from the E-OBS gridded datasets (Cornes et al., 2018) at 0.1° resolution. Meanwhile, to predict the likelihood of future alterations in species transmission windows, climate data from the UK Climate Projections 2018 (UKCP18) project were assessed.

To predict GIN risk on a field-by-field basis, the model reported by McFarland et al., (2022) was adapted to predict *H. contortus* and *T. cirumcincta* L3 on pasture. Model inputs were collected on a commercial sheep farm in Northern Ireland (NI), during the 2023 grazing season. Pooled faecal samples were collected from batches of ewes and lambs on eight occasions during the 2023 grazing season. A combination of traditional and molecular diagnostics were applied to collected parasite lifestages to examine species composition and relative abundance. These data were used to validate the models and permit scenario based modelling for individual species. To further validate model predictions under current climatic conditions, longitudinal pasture larvae counts were carried out on six selected fields to monitor observed L3 pasture contamination relative to model predictions. Final model inputs consisted of daily total precipitation (mm), daily mean temperature (°C) and predicted total number of GIN eggs deposited on each field per hectare, per day. Model outputs were adjusted to predict daily L3/kg of DM herbage for each of the respective fields.

#### Results

Assessment of model predictions at the regional and farm level suggested parasite species seasonality varied based on geographic location and climatic conditions. Fig. 1 illustrates the differences in predicted *H. contortus* seasonality and the model predicted number of infective L3 larvae on pasture for two farm locations in the UK during the 2020 grazing season. Model predictions using the Q0 and GLOWORM-FL models illustrated that environmental conditions were conducive for the maintenance of *H. contortus* populations in NI (Fig. 1A) and the South-East of England (Fig.1B). However, increased population growth and number of infective L3 on pasture was predicted for the NI farm between June-August 2020 compared to the farm in South-East England. This was likely due to increased precipitation during the summer months in NI, a requirement for *H. contortus* development, motility and survival. Changing shifts in transmission windows were also predicted for the modelled parasite species under climate change scenarios.

Model predictions of GIN risk on a field-by-field basis suggested that parasite species composition varied on fields throughout the grazing season relative to timing and length of the grazing period. Pasture larvae counts often showed agreement with model predictions, however on occasion predicted and observed levels of field L3 contamination did not align. GIN faecal egg counts varied throughout the grazing season relative to sample collection date and application of anthelmintic treatments. Parasite species, including *T. circumcincta, Trichostrongy-lus colubriformis, Oesophagostomum venulosum* and *N. battus* were isolated from faecal and pasture samples and confirmed the occurrence of mixed parasite infections both within the host and on grazing pastures.



**Fig. 1.** Q0 and GLOWORM-FL model predicted population growth and number of infective L3 on pasture for *Haemonchus contortus* for a farm in Northern Ireland (A), and South-East England (B). The top panel in (A) and (B) shows daily predicted Q0 values for each location between January and December 2020. The middle panel in (A) and (B) illustrates the daily average temperature (°C) and daily total precipitation (mm) for each farm location. The bottom panel in (A) and (B) illustrates the GLOWORM-FL model predicted number of L3 on pasture for each farm location when model inputs consisted of 100 parasite eggs per day for the duration of the simulation.

#### Conclusions

Simulation models are important tools to predict general GIN population dynamics, and alongside improved, affordable, species-level diagnostics could provide a valuable resource for predicting pasture infectivity with multiple parasite species. The current study demonstrated how these models can be applied to predict GIN population dynamics at multiple scales including regional, farm and the individual field level. Predictions using current and future climate projections highlighted the variability in seasonality between species at different geographic locations and the likelihood of host co-infection risk. These predictions can be used to develop and assess targeted parasite management strategies that are flexible enough to respond to rapidly changing parasite phenology and farm management.

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#### 40. Identifying and addressing barriers to sustainable control of roundworms in sheep

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#### Application

Farmers lack confidence in advice they receive around sustainable control of gastro-intestinal nematodes in sheep and are unwilling to change practice in case those changes have negative impacts on their businesses.

## Introduction

Gastro-intestinal nematodes (GIN) are common, highly pathogenic parasites of sheep, especially first season grazing lambs. These parasites are traditionally controlled using routine anthelmintic treatments during the grazing season. In recent years, resistance has developed in populations of GIN to all three major anthelmintic classes and multi-drug resistant parasites have been reported on many farms in the UK. Two new anthelmintic classes were introduced in the UK in 2009 (Zolvix) and 2012 (Startect), resistance has already been reported to Zolvix (Bartley et al 2019).

To mitigate the impact of resistance on control of GINs, advice to farmers has changed and is now focussed on identifying risk, using anthelmintics when indicated e.g. by high faecal egg counts or failure to meet weight gain targets and using alternative strategies such as pasture management, mob grazing etc to reduce exposure (https://www.scops.org.uk). However, despite a plethora of industry messages, many farmers do not engage with these messages. The aim of this study was to identify the barriers behind this lack of engagement and, by developing a mathematical model, to demonstrate the impact of sustainable parasite control measures, on a typical farm.

#### Materials and methods

An online questionnaire was used to provide a snapshot of management and worming practices on a cross section of sheep farms across the UK.

Focus groups were held across the UK, either in person or online between December and June 2023. Nine meetings were held, six with farmers, including some 'seldom seen' farmers, i.e. people who had no history of engaging with their Veterinary Practice's knowledge exchange programmes. In addition, one group was held with farm animal veterinarians and two with suitably qualified persons/registered animal medicines advisors (SQP/RAMA). Discussions were recorded and transcribed for thematic analysis.

A mathematical model of a 'typical' sheep farm was developed, informed by data collected through the online questionnaire. The model parameters included growth rates of Texel cross lambs, anorexia, development of immunity, development of larvae on pasture, pre and adult worm burdens, faecal egg counts and meteorological data including rainfall and temperature. The model was evaluated using data from previous studies (Kenyon et al 2013).

#### Results

51 usable responses were obtained from the survey. Upland, lowland and hill farms were represented. The median number of breeding ewes was 300; lambing was most common between March and April and the most common breed was Texel. Almost all farmers used anthelminitics in lambs and triggers included faecal egg counts, not thriving, scour, set interval treatments. Between one and six treatments were used per season. Most farmers set-stocked ewes and lambs until weaning and rotated lambs every 2–3 weeks thereafter.

From the farmer focus groups, there was a great feeling of uncertainty particularly around who and what advice to trust. This uncertainty prompted farmers to follow their established practice. Barriers to change were grouped into four themes:

- (i) practical difficulties around labour availability, time and cost to change practice, convenience to fit worm control into current farm practice.
- (ii) not seeing the need to change current practice was perceived to work, hence 'no need to change',
- (iii) lack of information and support, particularly considering the changing nature of sheep farming, climate etc.

- (iv) a lack of faith in new methods the advice has changed, but where is the evidence that the new advice works? Anecdotal reports of sheep dying when wormers had not been used because of negative faecal egg counts for example.
- (v) There were also positive outcomes, notably an appetite for information and knowledge, an awareness of anthelmintic resistance and its consequences, growing uptake of faecal egg counts which were proving to be beneficial to their businesses.

SQP/RAMAs and vets highlighted the importance of engaging with farmers regularly to encourage adoption of new control strategies. The mathematical model was developed for unparasitized and parasitised lambs over a grazing season and outputs compared to data from experimentally infected lambs. Four treatment scenarios were then evaluated: (i) 4-weekly treatments from weaning to sale; (ii) treatment at weaning and 6 weeks later; (iii) treating 90% of the lambs at 4-weekly intervals; (iv) treatment based on monthly faecal egg counts. The outputs demonstrated how different sustainable worming strategies could be used to maintain growth rates of lambs.

#### Conclusions

There was a sense of shared endeavour amongst farmers, vets and SQP/RAMAs around sustainable control of GIN in sheep. Amongst farmers there was a deep sense of anxiety around leaving lambs untreated and changing their normal worming practices. Farmers were unwilling to take risks that might compromise the health and productivity of their animals, which could impact their business. To address these barriers, more evidence-based studies, both model outputs and on-farm are needed, together with bespoke educational programmes, to convince farmers to focus their efforts onto more sustainable control of GINs.

#### Acknowledgements

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#### 41. Effect of oregano oil on milk yield, methane emissions and feed efficiency of dairy cows

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## Abstract (BSAS Undergraduate Thesis of the Year Winner 2023)

Agricultural systems contribute significant amounts of greenhouse gas emissions (GHG) emissions, being responsible for roughly 30% of emissions, which consist of carbon dioxide, nitrous oxide, and methane (CH4), with the latter of which is released due to enteric fermentation by ruminant livestock and responsible for up to 14.5% of the global GHG emissions. Naturally occurring essential oils (EE) from oregano oil (OO) have the potential to reduce methane production, which is mediated through the rumen microbiome in ruminants, making EE an ideal mitigation strategy that would be useful to production systems including organic. The aim of this study is to assess the effect of OO in the pre and/or postpartum partial mixed ration (PMR) of dairy cows to assess the effect on postpartum milk yield, feed efficiency, and methane emissions of lactating dairy cows. Methane emissions were lower from cows offered OO (*P* 0.005), reducing daily ruminal methanogenesis from lactating cows offered OO by approximately 10% per cow and lowering CH4 emissions per kg of energy-corrected milk (*P* 0.001), milk protein and solids yield (*P* 0.002) compared to cows not offered OO. Moreover, cows offered OO had greater milk (*P* 0.005), energy-corrected milk (*P* 0.015), milk fat (*P* 0.015), and milk solids yield (*P* 0.006), and greater milk lactose concentrations (*P* 0.005) compared with cows not offered OO. Dry matter intake (DMI), live weight and body condition score did not differ between cows offered a PMR with and without OO. In conclusion, offering lactating cows OO in the PMR lowered daily CH4 emissions per cow by 10%, and increased milk and milk fat, and solids yields, without increasing DMI, or affecting the live weight and body condition score of dairy cows. This lowered CH4 emissions per kg of ECM, protein, and solids yield, which improves the sustainability of global food security.

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#### 42. The role of livestock in the economy, and how this is evolving in response to changing context and priorities

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The presentation will consider the role of livestock in the economy, and how this is evolving in response to changing context and priorities. A summary of historic trends and a medium-term outlook for prices, production and trade of the UK's main livestock commodities will be

provided. Then acute changes, as well as slow burning shifts, that are influencing how and when ecosystem benefits and costs associated with livestock are internalised into the market economy are discussed. Finally, the unique challenges of recognising non-market value added within a globally integrated economy are explored.

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43. How can collaborative partnerships between leading bioscience and biotechnology researchers and the UK livestock sector, including farmers and practitioners, drive the development of novel prevention and intervention solutions to endemic diseases?

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With poor livestock health directly correlated to poor productivity, reducing the endemic disease burden of UK livestock is central to reducing carbon emissions from livestock agriculture.

In this session, some of the teams leading the collaborative projects funded through the Endemic Livestock Diseases Initiative share their initial research findings. A panel discussion with project leaders and industry stakeholders working on the Initiative share their experiences of co-creation of knowledge with end users, and explore how effective collaborative research between industry and academia can deliver on-farm impacts to support a healthy, productive and sustainable food system.

The £11.5 million Endemic Livestock Diseases Initiative has been developed in consultation with UK agricultural businesses and policymakers, and is co-funded by BBSRC, Defra, Daera and Scottish Government. The 36 projects funded cover the breadth of digital agriculture, genetics and breeding, farm management, vaccine platforms and technologies, novel therapeutics and co-infection. To date, the Initiative has successfully facilitated the formation of 45 new industry partnerships, forging strong collaborative networks. Moreover, it has engaged with over 170 end-users including farmers, vets and policymakers to ensure that the research outcomes directly address their practical needs.

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## 44. Johnes Disease control in dairy cattle; a critical approach to the use of diagnostic results

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Johne's disease (JD) is a chronic disease of cattle caused by the bacteria Mycobacterium avium subsp. paratuberculosis (MAP), responsible for major financial losses to the British dairy industry. MAP spreads vertically (mother to calf) and horizontal through shedding of the pathogen in the dung.

Early identification and removal of infected cows reduces within herd transmission and aids JD control on the farm. Currently milk antibody ELISA testing carried out every three months is used to provide the requisite diagnostic information. The status of the cow's mother also plays into the chance of infection, vertically or horizontally, and ultimately may affect differences in test performance depending on dam status.

The aim of the project was to evaluate the relationship between the probability of MAP infection based on the milk antibody test results to identify cows posing a risk of MAP vertical spreading and horizontal shedding, ultimately with the aim to inform the farmer on their management choices and through this accelerate Johne's disease control.

Our project team consisted of three commercial laboratories, one veterinary practice, six dairy farms and one academic institute. We calculated the probability of infection on previous test results (MAP antibody ELISA) and by incorporating the dam status. In order to further substantiate the confidence in these probabilities of infection, in the current work we evaluated the performance against the detection of MAP in the blood of the cows, representing the chance of vertical spread, and MAP in the faeces, representing the chance of horizontal spread. Considering the dam status separately allows us to evaluate differences in test performance depending on dam status.

We had a complete set of test results (IS900, MAP02 and Actiphage) from 209 cows. The calculation of the probability of JD infection was based on a median of 7 (Inter quartile range: 5-10) MAP milk ELISA results. 57 cows had at least one positive sample (blood and/or dung). IS900 PCR had 25 test positives, ISMAP02 PCR yielded 27 and the Actiphage 18 test positive cows. 56 cows had only one of the three tests positive, nine cows had two positive tests and two cows had all three tests positive. The backwards stepwise logistic regression was controlled for herd and number of tests and revealed that the IS900 PCR result is more likely to be positive for JD infection based on the probability of JD infection (OR = 8.49, P = 0.033). The ISMAP02 faecal PCR related negatively with a positive dam status (OR = 0.33, P = 0.039). A positive Actiphage blood result relates with the probability of JD infection, but only in cows from positive dams (OR = 4.57, P = 0.046), not from negative dams. This finding could mean that cows from negative dams contain the MAP infection more locally in the gut, whereas cows from positive dams are more likely to allow MAP entering the bloodstream.

This project gained new insights that may improve management of JD, but further work is needed. The end-users, the dairy farmers, are engaged, but very aware that this was a biased sample: farms already doing MAP milk testing. Once the probability calculations are incor-

porated in an algorithm, the predictive ability needs to be evaluated in a larger field trial. This would also be a good opportunity to evaluate the impact of dam status on differences in test performance. This will be crucial in controlling JD in a more advanced and effective way.

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## 45. Helping farmers navigate changing parasite seasonality in ruminants under climate warming

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#### Application

Improved tools for predicting periods of high parasite risk on pasture can enable treatments to be timed more effectively and with less offtarget selection for drug resistance.

## Introduction

Climate change is altering parasite transmission patterns (Cable et al., 2017) and presenting farmers with increasingly variable risk periods (Van Dijk et al., 2008). For some parasites, notably the spring scour worm of sheep, Nematodirus battus, disease forecasts have used historical relationships between soil temperature and larval hatching that have been overtaken by the earlier onset of spring in many years and parts of the UK. Hatching behaviour also seems to be changing, perhaps reflecting parasite adaptation to warmer weather and to management interventions (Melville et al., 2020). New mechanistic models can provide more accurate and robust nowcasts of infection hazard, but research should also consider how these are presented, delivered and applied by farmers and industry partners.

## Material and methods

Mechanistic models of N. battus hatching date based on experimental hatching trials were combined with local mean daily air temperature (Gethings et al., 2015; Hopkinson et al., 2021) to produce risk maps and calendars to alert users to periods of high hazard. User feedback was applied to refine the format of digital risk maps and to evaluate the usefulness of the tools.

#### Results

Predictions showed high regional and inter-annual variability in hatching date (Fig. 1), supporting the need for risk nowcasts and their effective dissemination. These were converted into maps, made available online via SCOPS to provide current information on predicted hatch as well as historical hatching profiles. Online questionnaires of farmers and advisors suggested that using the nowcast enabled more confident and flexible treatments, reducing unnecessary chemical use and disease incidence. Extension of this approach to other parasite groups, however, is more difficult due to the overriding importance of farm-specific management in driving infection levels. This will require more sophisticated co-production and delivery.



Fig. 1. Predicted periods of high Nematodirus battus hatching risk at 11 locations in the UK, showing variation between locations (different rows) and in the same location between years (same row, left and right panels). Darker colours indicate higher risk of hatching.

#### Conclusions

Parasite transmission models are useful tools for predicting high risk periods and helping farmers and their advisors to target treatments accordingly, especially as epidemiology becomes more variable under climate change. The design and communication of such nowcasts,

however, is crucial not only to make them accessible but also to encourage appropriate interpretation. Successful application of digital tools for risk prediction is needed to support farmer adaptation to climate change but requires the early involvement of end users.

## Acknowledgements

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## 46. Factors influencing endemic disease in the Northern Ireland sheep flock

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## Application

This project has demonstrated the potential impact that collaboration between farmers and research organisations can have where a need is identified and driven by the farming community. The project has generated two legacies. Firstly an example for future participatory, codeveloped research, to address questions arising in the farming community that aspire to deliver better livestock health, welfare, and productivity. Secondly, momentum for future control of sheep scab (hereafter scab) in NI through a substantially enhanced understanding of it, alongside a paradigm shift in openness amongst the farming community to discuss scab.

## Introduction

Scab is a notifiable disease and is present in NI. It has a substantial detrimental impact on sheep welfare, the psychological wellbeing of farmers caring for them, as well as an economic cost to the industry (Crawford et al., 2022). Despite this, limited research has been carried out into the distribution of scab throughout NI, or understanding the barriers to better control. Scab has been identified as a taboo subject, with farmers reluctant to report their suspicions of an outbreak in their flock, or seek veterinary advice on diagnosis or treatment; resulting in under-reporting of cases. However, a group of farmers in NI, aware of the impact of scab in their local area, came together with vets and other stakeholders in 2019 to form a group focused on scab control. Realising the limitations of what they could achieve alone, a partnership was established with research and disease control organisations to seek funding to develop a more detailed understanding of the current distribution of scab in NI. This partnership also sought to increase awareness and promote best practice advice, while investigating farming practices, farmer attitudes and knowledge, that may be barriers to control. Data would also be collected to undertake pilot investigations into the economic and environmental impact of scab in NI.

## Material and methods

Three main streams of work were employed to engage the NI sheep sector and gather data:

- 1. A series of six knowledge exchange (KE) events were held across NI on best practice control of scab to stakeholders and vets.
- 2. Following self-reporting of suspicion of disease, individual farm visits (105) were carried out by the farmers own private vet to undertake disease investigation and advise on appropriate treatment.
- 3. A survey was prepared and distributed to 600 NI sheep farmers randomly selected from the national database with a flock size of at least 100 ewes. Data from the 126 completed questionnaires was statistically analysed using an extended theory of planned behaviour (eTPB) and econometric techniques.

#### Results

The regional KE events were attended by 159 farmers and 40 vets. Gaps in knowledge about scab diagnosis and control were highlighted during KE events, along with calls for greater state support to the industry to assist with scab control. Scab was diagnosed in 60 flocks across NI, with a further seven receiving advice to undertake further monitoring and testing. Diagnosis by farmers was primarily based on clinical signs, without veterinary advice. The importance of accurate diagnostic testing was highlighted following the confirmation of scab on 13 (54%) farms based on serology, following a negative skin scrape, in sheep with visible lesions. Following testing of sheep without clinical signs, but at high risk of infestation, based on local disease risks, 10 (29% of flocks tested which did not have clinical signs) returned a positive result, and a further 6 (18%), a non-negative result necessitating further monitoring. Participants raised concerns about injectable macrocyclic lactone (ML) products failing to control scab, while also reporting widespread use of these products for prevention and cure. Use of an ML for 'preventative' purposes was reported by 67 farmers, of which 48% returned a non-negative result following the

farm visit and skin scrape and/or blood testing. Five of the 16 flocks supplied with MLs under the project, subsequently required dipping because of ongoing or recurrence of infestation.

Ouestionnaire responses showed that scab remains an issue of concern in NI and intervention aimed at controlling the disease will benefit from promoting positive attitudes towards blood testing for subclinical diagnosis.

Data obtained from the survey and expert knowledge (Stubbings 2007) recognise that scab is associated with reduced lamb growth rates, increased lamb mortality and loss of ewe body condition. By modelling the consequences of these effects in farm-level assessments, the present study indicates that eradicating sheep scab would, in many cases, reduce the farm carbon footprint by more than 10%, depending on prevalence levels.

The project's impact, in particular on breaking the taboo surrounding scab, and encouraging more open discussion of the disease, was recognised in a national industry award, with stakeholders stating: Participating vet: 'The project provided us with the opportunity to build vital farmer-vet relationships, which have seen us work together in a novel way to treat and eradicate scab, which wasn't being previously achieved. NI sheep industry representative: 'This novel project has achieved the seemingly impossible by removing the stigma surrounding sheep scab; something never done before in Northern Ireland.'

## Conclusions

This collaboration provides a model for addressing a disease, previously taboo. It enabled vets to visit over 100 farms to talk about scab; ensuring its accurate diagnosis and treatment. By starting with the end-user, this approach drove engagement with farmers and vets, underpinned by research and ultimately delivered a positive animals health and welfare impact on-farm.

## Acknowledgements

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## 47. Addressing barriers to sustainable control of gastro-intestinal nematodes in sheep: Exploring novel control strategies using individual-based mathematical modelling

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## Application

We aim to improve control of gastro-intestinal nematodes (GIN) in sheep by understanding barriers to sustainable anti-parasiticide use. Through engagement with farmers, we identify potential interventions and present a mathematical model that tests the likely effect of proposed control strategies on parasite transmission and lamb liveweight gain.

## Introduction

GIN are common and highly pathogenic in sheep, especially first season grazing lambs. They are traditionally controlled using routine anthelmintic treatments. In recent years, populations of GIN, resistant to all three major anthelmintic classes and multi-drug resistant parasites have been reported on many farms in the UK.

To reduce the rate of development of resistance, advice to farmers is now focussed on using anthelmintics only when indicated e.g. by high faecal egg counts (FECs) or failure to meet weight gain targets and using alternative strategies such as pasture management to reduce exposure. Many farmers do not engage with these messages. We aim to identify the barriers behind this lack of engagement and, by developing a mathematical model, to demonstrate the impact of sustainable parasite control measures on a typical farm.

## Materials and methods

An online questionnaire was used to provide a snapshot of management and worming practices on a cross section of sheep farms across the UK. Focus groups on sustainable parasite control were held across the UK, either in person or online between December and June 2023. Nine meetings were held with farmers, including some 'seldom seen' farmers, i.e. people with no history of engaging with their Veterinary Practice's knowledge exchange programmes. In addition, one focus group was held with farm animal veterinarians and two with registered animal medicines advisors. Discussions were recorded and transcribed for thematic analysis.

A mathematical model of a 'typical' sheep farm was developed, informed by data collected through the online questionnaire and evaluated using data from previous studies (Kenyon et al. 2013; Fox et al 2018). We adapt and extend upon existing modelling frameworks that link environmentally driven pasture contamination with parasite burdens (Filipe et al. 2023) to develop an individual-based model that explicitly tracks parasite burdens within individual hosts, enabling consideration of the impacts of management decisions at the level of the farm whilst retaining detail on parasite burden and interventions at the level of individual sheep. Four treatment scenarios were evaluated at three levels of resistance implemented by altering treatment efficacy: (i) Neo-suppressive treatment (NST): 4-weekly treatments from weaning to sale; (ii) Strategic prophylactic treatment (SPT): treatment at weaning and 6 weeks later; (iii) Treatment minus 10 (T-10): 4-weekly treatments that exclude a random 10% of all animals selected from the heaviest half of the flock; (iv) Targeted treatment (TT): treatment of all animals based on monthly composite FECs from ten lambs.

#### Results

The farmer focus groups identified 4 main categories of barriers to change; not seeing any need due to the perceived success of current strategies, a lack of information and support on how to change, a lack of faith in proposed new methods, and practical and logistical issues. There were also positive findings, notably an appetite for information and knowledge, an awareness of anthelmintic resistance and its consequences, growing uptake of FECs which were proving to be beneficial to their businesses. Our mathematical model can be used to help address the first three of the issues described above by exploring the potential efficacy of a range of GIN control strategies.

Our model predicts that reducing the number of treatments can provide similar body weights at a flock level to more intensive treatment regimes (Fig. 1). The NST approach, which administers the most treatments, predicts the greatest body weights across the flock in the



**Fig. 1.** The top plot shows the predicted body weight of untreated lambs. The lower panel shows the estimated difference in body weight of the same lambs under the 4 treatment regimes (columns), at the 3 specified levels of resistance (rows). Grey lines show body weights (top plot) or body weight differences (bottom panel) for lambs; red lines show a cubic regression smoothing spline (top plot) or the median body weight difference (bottom panel) across all lambs; blue lines show times of treatment.

absence of resistance;however, the efficacy of this treatment regime decreases notably under increasing resistance and the latter treatments result in minimal body weight gains. Assuming that the alternative treatment scenarios would at least slow the development of resistance, we would expect better performance over time e.g. comparing NST under 40% resistance to T-10 under 20% resistance.

## Conclusions

Amongst farmers there was a deep sense of anxiety around leaving lambs untreated and changing their normal worming practices for fear it might compromise the health and productivity of their animals, which could impact their business. To address these barriers, we present a mathematical model developed to explore the potential impacts of proposed treatment regimes, showing that any short-term losses due to adopting some of these novel approaches may be offset by slowing the development of resistance. The model was built with flexibility in mind following consultation with stakeholders and can be readily adapted and employed to explore a wide range of novel control scenarios such as weight-based targeting of treatment or rotational/mob grazing. Such model outputs could be used to identify promising control strategies for trial on farm and, together with bespoke educational programmes, to convince farmers to focus their efforts onto more sustainable control of GINs.

## Acknowledgements

We are grateful to the farmers, SQPs and veterinarians who helped in this study.

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# 48. Unravelling *Enterococcus cecorum* infection on UK broiler farms: Correlating clinical signs with genomics, persistence and animal behaviour

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## Application

To combat endemic *Enterococcus cecorum* disease in UK broilers, it is essential to understand the pathogenesis of the organism and to develop tools for early disease detection and prevention. This will ultimately result in improved animal welfare and reduced economic losses. This pump-priming project provided a scientific basis for future research, by starting to address significant knowledge gaps in *E. cecorum* epidemiology. Research outputs can be used further to inform farm management practices related to disease management and prevention. This research will also aid the poultry industry with its antibiotics stewardship programme.

## Introduction

*E. cecorum* infection is an emerging endemic disease in UK and global broiler poultry with significant welfare concerns and results in considerable economic losses to the industry (Wood et al., 2002; De Herdt et al., 2009; Stalker et al., 2010; Dunnam et al., 2023). There are substantial research gaps with regards to *E. cecorum* pathogenesis, source of infection, transmission routes and strain differences in terms of their ability to cause disease. In addition there is a need to detect *E. cecorum* infection earlier in the production cycle to aid disease prevention. The aim of this project was to fill knowledge gaps by conducting research into pathogen genomics, transmission and persistence as well as early detection of disease through behavioural monitoring.

## Materials and methods

Four commercial UK broiler farms (A-D) with a range of historical performances and history of *E. cecorum* infections, were chosen for the study. Farms operated an all- in all-out production system with chicks being placed into disinfected sheds at 1 day of age followed by a 37 to 42 day growth cycle. Water and feed intake and environmental parameters were automatically monitored and final performance data was collected. A sample of birds that had been found dead or were culled for any health reasons, were presented for examination by poultry veterinarians. A total of 725 samples were collected on days -1, 7, 14 and 21 (Table 1). DNA was extracted from all samples and *E. cecorum* was detected using qPCR. Samples were also cultured and

*E. cecorum* was identified using Matrix-assisted laser desorption/ionization (MALDI-TOF). Two *E. cecorum* strains isolated from joint fluid were tested for tenacity at 23 °C and 32 °C on concrete surfaces in a 21 day long *in-vitro* experiment. A total of 180 *E. cecorum* sequences from public repositories (n = 107), APHA archives (n = 74) from 2003 to 2022, and five *E. cecorum* isolated in this farm study were submitted to whole genome sequencing (WGS) and a maximum likelihood phylogenetic tree constructed. The APHA SeqFinder pipeline was used to

## Table 1 qPCR results to detect *E. cecorum* in 725 samples from 4 study farms.

Sample t	уре								
	Chick paper	Chick box swabs	Floor swabs	Wall swabs	Drinker line	Drinker cup	Litter	Caeca	
Days san	npled								
Farm	-1	-1	-1	-1	-1,7,14,21	-1,7,14,21	7,14,21	7,14,21	Total
Α	12/12	0/12	14/14	8/12	8/16	4/8	28/46	12/18	86/138 <b>(62%)</b>
B (4)	4/4*	0/24	3/8	14/24	19/32	8/15	28/86	14/20	90/213 ( <b>42%</b> )
C (4)	4/4*	0/24	2/8	6/24	0/31	2/16	0/92	1/ 35	15/234 ( <b>6.4%</b> )
D (2)	12/12	4/12	2/14	3/12	0/16	2/10	1/46	0/18	24/140 ( <b>17%</b> )

Day -1 is the day before chick placement. Values represent numbers of positive samples against the total number of samples tested.\*Pooled sample from four houses. \*\*Number of houses on farm.

detect AMR genes. Finally, commercially available intelligent camera systems were installed on trial farms to record flock activity patterns, cluster movements, distribution and spread.

## Results

*No E. cecorum* outbreak was observed during the trial period.*E. cecorum* was detected in 215/725 (29.7%) samples by qPCR (Table 1). It was detected on all farms and in all sample types,

including all chick papers suggesting a possible route for entry to farms. qPCR also showed that 99.0% and 88.3% *E. cecorum* positive samples, respectively, from farms D and C were taken at day -1. *E. cecorum* was detected throughout the trial on farms A and B. Three *E. cecorum* were isolated from farm B caecal samples and two from infected birds, which showed sporadic *E. cecorum* infection; but

*E. cecorum* was not isolated from any environmental source, including from farm B. Survival experiments on concrete showed that both *E. cecorum* isolates survived longer at 23 °C than at 32 °C. One isolate survived for longer at both temperatures, and was still viable on day 21. Analysis of WGS data indicated several antimicrobial resistance (AMR) genes conferring resistance to aminoglycosides, macrolides and tetracycline were variably present in isolates. Phylogenetic analysis clustered the majority of UK isolates into three groups which are mostly separated from other non-UK isolates collected worldwide. The groups were widely distributed across all the farms included. Data from the cameras indicated that on farms A, C and D bird activity and distribution patterns were consistent with a healthy broiler population. Analysis of data from a house in farm B revealed an unexpected drop in activity and a decrease in distribution after day 15, signalling a potential health concern. This coincided with a noticeable drop in feed consumption from day 12 and diagnosis of dysbacteriosis by the primary veterinarian.

## Conclusion

This study has highlighted a range of possible environmental reservoirs for *E. cecorum*, by detecting DNA in range of environmental sources. The results indicate that *E. cecorum* may be present as a harmless commensal at low levels within the farm environment, indicating further work is required to understand conditions that promote changes of this commensal to a pathogen that causes large disease outbreaks on UK farms. Bird activity data from cameras could not be linked to *E. cecorum* infection, primarily due lack of *E. cecorum* associated disease on trial farms. Nevertheless the technology shows promises as a reduction in bird activity and change in the distribution was detected on farm B, where gastrointestinal disease was diagnosed.

This project has brought together academia, government bodies, veterinarians, poultry farmers and other stakeholders related to the broiler industry. This collaboration has allowed us to harness expertise and knowledge in all areas relevant to the project and to design trials with scientific outputs that can inform farm management practices related to *E. cecorum* disease prevention and management.

## Acknowledgements

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## 49. Effects of co-infections on Marek's disease in UK poultry farms and development of novel rapid diagnostic strategies

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## Application

Marek's disease (MD) remains a problem in the poultry farms worldwide; co-infections with virulent, avirulent and vaccine strains of *Mardivirus* are common and may complicate the clinical picture. The present work was initiated to understand the prevalence of these co-infections of different Mardivirus species in commercial poultry farms in UK, and to develop rapid diagnostic tests for their detection.

## Introduction

Marek's disease (MD) is a highly infectious viral neoplastic disease of chickens and is endemic to many poultry farms across the globe including UK. The aetiological agent of this disease is Marek's disease virus (MDV), which belongs to *Gallid alphaherpesvirus 2* (also referred to as MDV serotype 1 or MDV- 1). Within the same genus, there are two avirulent strains *Gallid alphaherpesvirus 3* (also referred to as MDV serotype 2 or MDV-2) and *Meleagrid alphaherpesvirus 1* (also referred to as herpesvirus of turkey or HVT) which have been used as vaccines against MDV.

Preliminary studies carried out in the MDV Reference Laboratory (MDVRL) at the Pirbright Institute by real-time PCR have demonstrated co-circulation of high levels of avirulent MDV-2 in commercial poultry farms. Therefore, a BBSRC funded collaborative project with industrial partners (Moy Park Ltd. and Slate Hall Veterinary Practice) was initiated to investigate the prevalence of MDV-2 in commercial poultry farms in UK, the role of MDV-2 in pathology, protection against MD and their effects on flock productivity; develop rapid and sensitive isothermal diagnostic assays; and evaluate the potential of MDV-2 field strains to be used as a recombinant vaccine vector for protection against MDV-1 and other pathogenic viruses affecting poultry. Here, we present our initial findings on the prevalence of co-infection and the development of rapid isothermal based diagnostic tests including Loop Mediated Isothermal Amplification (LAMP), Polymerase Spiral Reaction (PSR) and CRISPR/Cas12a detection.

## Materials and methods

Suspected clinical samples, which included feather tips, spleen, and tumourous tissue (either fresh- frozen or imprinted on FTA cards) and poultry house dust from different commercial poultry farms and backyard poultry were received at MDVRL. The samples were processed for screening of MDV-2 and co-circulation with vaccine strains HVT and CVI988 as well as virulent MDV-1 using ISO/IEC 17025- accredited real-time PCR tests.

For the development of rapid isothermal based diagnostic tests on MDV-2, LAMP reaction was performed at 65 °C for 30 min in a real-time PCR machine or monitored by visual detection of colour change in the positive samples, or through a Lateral Flow Device (LFD). The specificity and sensitivity of the LAMP assays were also examined. PSR was optimised at 63 °C for 60 min, and positive reaction was confirmed by visual detection of colour change. CRISPR Cas12a detection was performed at 37 °C for 10 min in a real-time PCR machine.

## Results

1. MDV-2 is prevalent and co-circulates with other

**Mardivirus strains** 

Samples from 17 different flocks with different ages were tested by MDVRL real-time PCR (Fig. 1). The result shows that: • MDV-2 can co-infect birds that are vaccinated with HVT and CVI988.



HVT MDV-2 CVI988 VMDV

Fig. 1. Prevalence of MDV-2 and co- circulation with other Mardiviruses.



Fig. 2. Isothermal based diagnostic tests on MDV-2 have been developed. A &B, LAMP assay. C, PSR. D, CRISPR-Cas12a detection.

- MDV-2 (like MDV-1 field strains) usually only detected in older flocks.
- MDV-2 can co-infect with MDV-1 field strains.
- 2. Development of isothermal based diagnostic tests on MDV-2
- MDV-2 LAMP assay was specific for detecting MDV-2 viral DNA and did not amplify virulent, or vaccine strains of *Mardivirus* (Fig. 2A). The amplified product was also resolved on microfluidics lateral flow device format and the results also correlated with real-time PCR (Fig. 2B).
- PSR visual detection of colour change for MDV- 2 has been developed and is specific (Fig. 2C).
- CRISPR Cas12 detection has been developed and is specific for MDV-2 (Fig. 2D).

#### Conclusion

Testing of samples submitted from UK poultry farms, using either real-time PCR, or rapid isothermal tests, confirms that MDV-2 is widespread in UK chicken flocks, circulating freely and naturally at high levels. It is prevalent in many older layer, broiler, and breeder chickens, and can be detected in chicken organs, feathers, and poultry house dust. MDV-2 cocirculates with other *Mardiviruses*. It is detected in either presence or absence of virulent MDV field strains, CVI988 and HVT vaccines, and in healthy flocks as well as flocks with clinical signs of MD. It is currently unclear whether these naturally circulating MDV-2 strains provide protection against MD. As this project progresses, we will continue developing and optimising the isothermal tests for MDV-2 for use as simple and rapid on-farm tests to monitor MDV-2 in the field, investigate its influence on flock productivity, immune responses, and disease, characterise MDV-2 field isolates and exploit its potential use as a vector for novel recombinant vaccines.

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## 50. A holistic approach to sustainability starts with youngstock

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Cows play a crucial role in ecosystems due to their ability to turn human inedible protein into human edible protein. Due to the ability of ruminants to digest fibre they can convert grassland or even byproducts into food for human consumption. Unfortunately, ruminants produce methane as a byproduct of fermentation and that has caused focus on reducing emissions. However, we can improve both efficiency and profitability whilst reducing carbon footprint, and that starts with youngstock in both dairy and beef systems.

Effective youngstock rearing can lead to an efficient and profitable cow that stays in the milking herd for longer, reducing emissions per kg of FPCM. This starts with achieving early and adequate intake of high-quality colostrum, followed by effective health protocols and elevated levels of milk feeding.

Early life nutrition can program the metabolism of the cow and determine lifetime productivity. Calves fed on an elevated plane of nutrition produce approximately 225 kg more milk in their first lactation and conceive earlier, therefore had a lower age at first calving than those on a restricted plane of nutrition. This could lead to a 6% reduction in carbon footprint through reduced age at first calving (26 to 23 months) and increased first lactation milk yields (7.5%). Preweaning nutrition also impacted longevity of the cows with 20% vs. 7% remaining at 5th lactation for elevated vs. restricted plane of nutrition, respectively. This can further reduce carbon footprint by reducing replacement rates.

In dairy beef systems colostrum, quality of milk replacer and monitoring are key to growth and health of these animals which determines their success. Metabolic programming also occurs in beef animals when reared on elevated planes of nutrition where these animals grow better and have been shown to have lower treatment rates. Questions still remain to be answered around the effect on slaughter weights and carcass quality.

Once the calf is set up for life through effective rearing, we must look to sustainably feed that efficient animal so that they perform to the best of their programmed potential. Reduction in carbon emissions can be further achieved through precision balancing of diets (reducing waste and formulating for reduced methane emissions) and reviewing sources of raw materials that contribute to increased carbon footprint of feed. A holistic approach to sustainability and carbon footprint is required to satisfy national inventory but also reduce footprint per kg of product. This is intrinsically linked with efficiency and productivity and starts with effective youngstock rearing and management.

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## 51. Environmental enrichment and housing influence on stress during calf disbudding

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## Application

Improving the housing conditions of calves and introducing activities to reduce their stress levels during the process of disbudding.

## Introduction

Disbudding, an important practice in the dairy industry, often exposes calves to significant pain and stress both during and after the procedure, leading to a variety of behavioural and physiological responses like vocalisations and increased heart rate (Cozzi et al., 2015). Two strategies to potentially mitigate this distress are distraction which is engaging calves in alternative behaviours or interactions to divert their attention from stressors and social buffering. Social buffering is the presence or interaction with peers can reduce the stress responses of an individual, suggesting that the company of fellow calves may alleviate the adverse effects of stressful procedures like disbudding (Bučková et al., 2022). Enrichment is a method for reducing discomfort, unpleasant behaviour, and stress in captive animals. Mentally stimulating environments can powerfully combat stress in calves (Moncek et al., 2004).

## Material and methods

This study explored the relationship between environmental enrichment, housing structures and stress indicators in calves. It focused on analysing behaviour and Heart Rate Variability (HRV) during disbudding procedures and health checks. Adopting a  $2 \times 2$  factorial design, 36 Holstein calves (6–12 days old, 35–50 kg at birth) were categorized into four treatment groups. Each treatment group included either four (group housing) or two (pair housing) calves. GE (group housing with enrichment, 4 calves), GN (group housing, no enrichment, 4 calves), PE (pair housing with enrichment, 2 calves), and PN (pair housing, no enrichment, 2 calves). Calves were transferred to the study area 48–72 h after birth. It took approximately two weeks to accumulate a group of eight calves for the group housing treatments. Once these numbers were reached, the calves were then randomly allocated to either enriched or non-enriched conditions within their respective housing groups. Enrichment objects were brush, yoyo, chain and rope toy. Housing dimensions were  $190 \times 240$  cm<sup>2</sup> for pair housing and  $380 \times 240$  cm<sup>2</sup> for group housing. The disbudding procedure used in this study strictly complied to the standard farm management protocols and was conducted by established practices that are routinely implemented on the farm,

without any modifications made specifically for this research. At seven week-age, disbudding was conducted using heated iron with anaesthetic. HRV monitoring was conducted on one sample from each treatment group. Out of the total, 12 calves had their HRV monitored with heart rate monitor chest strap during health checks and disbudding. They were systematically observed during three phases: 1 day before the procedure, during the procedure and 1 day afterwards. Behavioural observations from 2-hour CCTV footages were made using a detailed ethogram. A comprehensive examination of calf heart rates and behaviours during the disbudding process revealed variations.

#### Results

We observed differences across different phases and housing conditions emphasising the significant influence of environmental factors on animal welfare. During the disbudding phases, there were significant behavioural changes in calves. These changes, which were statistically significant (p < 0.001) included positive aspects like calves lying together and engaging in social interactions, as well as negative aspects such as calves lying alone or standing in response to the procedure. Furthermore, the heart rate of the calves also exhibited a change. There was a significant increase in heart rate from before the procedure to during the procedure (p < 0.05) but this situation changed from during the procedure to after its completion (p < 0.001). Analysing the enrichment conditions revealed that calves in nonenriched environments stood more frequently than those in enriched conditions. Additionally, when it came to housing conditions, calves that were pair-housed tended to lie alone for longer durations in comparison to those housed in groups.

## Conclusion

To summarize our findings, this study underscores the importance of enrichment for reducing stress associated with disbudding procedures, which is further supported by our observations. The specific changes in behaviour such as significant shifts in how the animals stand and lie down position provide further evidence of the diverse effects of the treatments used.

#### Acknowledgments

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# 52. The faecal microbiota during the pre-weaning period and its relationship with diarrhoea, health, and performance in dairy heifer calves

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#### Application

Depicting dysbiosis of the hindgut microbiota provides a better understanding of calves with diarrhoea and should facilitate the development of prevention strategies and improvement of animal health and welfare.

#### Introduction

Diarrhoea is a complex disease that results in significant economic burdens to the dairy industry. The relationship between the developing hindgut microbiome and the onset of diarrhoea requires deeper understanding. Many factors influence the development and colonisation of the hindgut microbiome during the pre-weaning period. At this time, the microbiome is unstable and sensitive to disturbances, which can result in dysbiosis, in that a loss in commensal microbes occurs in conjunction with the proliferation of pathogenic species (Chase and Kaushik, 2019). Dysbiosis has been previously linked to diarrhoea and it has been suggested that diarrhoea is the cause of this imbalance (Ma et al., 2020). To better understand the relationships between bacteria within the faecal microbiome pre-, during, and post-disease, a correlation analysis of bacterial genera found to be significant due to health status was performed to better characterize the dysbiosis of the hindgut microbiota and determine if it is the cause or result of diarrhoea in neonatal calves.

#### Materials and methods

Fifty-one spring born Holstein (n = 29, birth weight (BW) 34.7 (SE 0.69) kg) and Jersey (n = 22, BW 25.9 (SE 0.81) kg) heifer calves were observed from birth (day (d) 0) until weaning (d83 (SE 1.04)). Calves were fed 8.5% BW in colostrum, from either, the calf's dam (n = 28) or a mixed source of colostrum ( $\leq 2 \cos s$ ,  $\leq 1 d$ ; n = 23) within 2 hours of birth. Blood was collected via jugular venepuncture at d7 and analysed for serum immunoglobulin G (sIgG) using single radial immunodiffusion. Calf clinical assessments were performed at d7, d21 or disease manifestation, and d83 using a Modified Wisconsin-Madison scoring method. Faecal samples were collected during clinical assessment. Diarrhoea incident was determined using faecal scores and health status (HS) was defined as calves having diarrhoea (n = 27) or healthy (n = 24). Microbial DNA was extracted from 150 faecal samples using a Qiagen Dneasy<sup>®</sup> PowerSoil<sup>®</sup> Pro Kit and extracted DNA was sent to Macrogen for 16S amplicon sequencing. Amplicon sequence data were processed in *R* using *DADA2* and taxonomy

assigned using the SILVA database. Data were further analysed using the *R* packages *Phyloseq* and *MaAsLin2*, and the Benjamini-Hochberg procedure (q) was used to correct for false discovery rate. Amplicon Sequence Variants (ASVs) found associated with HS were checked for normality and homogeneity of variance by histograms, qqplots, and formal statistical tests as part of the UNIVARIATE procedure in SAS 9.4. A Spearman rank-order correlation for non-parametric data was preformed to determine correlations between ASVs and calf performance data including faecal scores. Correlations with a P-value less than 0.05 were considered significant.

## Results

The mean day post-birth for diarrhoea was d22 (SE 0.70); 53% of calves had a diarrhoea incident. There was no difference (P > 0.05) in slgG between healthy and diarrheic calves (Healthy: 43.4 (SE 1.82) g/L; Diarrheic: 37.6 (SE 2.52) g/L). Based on PERMANOVA analysis, calf breed (P = 0.08), colostrum source (P = 0.31), and passive immune status (P = 0.21) had no effect on composition of the faecal microbiota. At disease manifestation, diarrheic calves had reduced bacterial diversity compared to healthy calves, and 24 ASVs were significantly associated (q < 0.05) with HS. *Bifidobacterium* (6.4%) was the dominant genera in healthy calves, while *Alloprevotella* (13.7%) was the dominant genera in diarrheic calves at disease manifestation. *Alloprevotella* had strong negative correlations ( $P \le 0.0001$ ) with *Faecalicoccus, Akkermansia,* and *Intestinibacter*, and a moderate negative correlation ( $P \le 0.01$ ) with *Bifidobacterium, Prevotella\_7, Flavonifractor,* and *Dialister. Bifidobacterium* had a strong positive correlation ( $P \le 0.0001$ ) with *Prevotella\_7* and moderate positive correlations ( $P \le 0.01$ ) with *Collinsella, Faecalicoccus, Flavonifractor, Dialister,* and *Intestinibacter*. A weak negative correlation was observed between slgG and *Alloprevotella* and moderate positive correlations with *Prevotella\_7* and *Faecalicoccus.* Faecal Score displayed moderate negative correlations ( $P \le 0.01$ ) with *Bifidobacterium, Prevotella\_7, Faecalicoccus, Dialister,* and *Intestinibacter.* A strong positive correlation score and *Alloprevotella* and moderate positive correlations with *Prevotella\_7* and *Faecalicoccus.* Faecal Score displayed moderate negative correlations ( $P \le 0.01$ ) with *Bifidobacterium, Prevotella\_7, Faecalicoccus, Dialister,* and *Intestinibacter.* A strong positive correlation between faecal score and *Alloprevotella* was observed. No significant correlations were found between ASVs and average daily gain.

## Conclusion

The reduction in microbial diversity observed in diarrheic calves enabled the proliferation of other bacterial genera. *Alloprevotella*, has not previously been associated with diarrhoea in calves, and is considered a part of the normal intestinal flora. The proliferation of *Alloprevotella* appears to be negatively associated with other genera that are known to be beneficial to gut health (e.g. *Bifidobacterium, Faecalicoccus*). The correlations between ASVs significant at disease manifestation show the relationship between microbes in the hindgut and the influence they have on each other as their presence changes within the hindgut.

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## 53. The association of neonatal calf diarrhoea with and without dehydration on milk feeding and activity variables in young preweaned artificially reared calves

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## Application

An understanding of changes in automatically detected behaviours in neonatal calf diarrhoea (NCD) and concurrent dehydration allows the development of automatic disease detection tools. Early detection of neonatal calf diarrhoea and concurrent dehydration will improve calf outcomes.

## Introduction

NCD is a frequent disease in young calves, which can lead to dehydration. Both conditions are a welfare and production concern. The objective of this study was to identify changes in activity and feeding variables that are associated to NCD, with or without dehydration.

## Materials and methods

One hundred and forty-one calves were moved into group-housing at approximately seven days of age, at which point they entered the trial. The calves were then followed until 25 days of age or three days after the development of NCD. Calves were health scored daily as follows: Wisconsin health score (McGuirk, 2008) for respiratory disease, faecal consistency (Faeces, Table 1), hind leg tail and perineum cleanliness (CLEAN, Table 1) and skin tent elasticity. Calves with a faeces score of two or above were classified as having NCD. Calves that had a return of the skin tent in less than three seconds were classified as NCD-Hydrated (NCD-H). Those with a delayed return of the skin tent were classified as NCD-Dehydrated (NCD-D). To remove any effect of bovine respiratory disease, all calves that had a Wisconsin health score that was classified as diseased (>4) had that day and three days either side removed from the dataset. Rectal temperature (recorded daily as part of the Wisconsin Score) was converted to a four-level categorial variable (Temperature, Table 1).

## Table 1 The criteria for the tail, perineum and hindleg cleanliness, faeces and temperature scores.

	Scoring criteria		
Score	CLEAN	Faeces	Temperature
0	Clean calf or with a small amount of dried faeces on tail/perineum/hind legs	Formed faeces	37.9-38.3°C
1	A large amount of dried faeces or some pasty faeces on tail/perineum/hind legs	Pasty faeces	38.4-38.8°C
2	Wet Faeces on tail/perineum/hind legs	Loose faeces that did not sift through bedding	38.9-39.4°C
3	A very wet tail/perineum or a large amount of faeces on tail/perineum/hind legs	Liquid faeces that sifted through the bedding	≥39.5°C

Milk feeding behaviours were measured using automatic milk feeders (*Biocontrol*). Calves had access to 7 L of acidified milk replacer daily. Activity variables were measured using a triaxial accelerometer (*IceQube*) attached to the left hind leg. The behaviours measured for each calf were; total time at milk feeder, total milk visits, mean milk visit length, mean milk drinking speed, volume of milk drunk each day, mean milk per visit, daily lying time, daily standing time, daily lying bouts, daily standing bouts, total daily motion index, mean lying bout length, mean standing bout length and mean motion index per standing bout.

General linear mixed modelling was carried out using the lme4 library in R studio (Bates *et al.*, 2015). Disease status, season, sex, sire breed type, temperature score, age and the interactions between disease status and age, disease status and season, and disease status and temperature score were tested as fixed effects. Backwards model selection by AIC was carried out using the step() procedure in R. The calf number nested within pen was included in all models as a random effect.

## Results

There were 1125 healthy, 232 NCD-H and 8 NCD-D days for the lying behaviours and 961 healthy, 249 NCD-H and 8 NCD-D days for the milk feeding behaviours available for analysis. Of the fourteen behaviours analysed, seven had residuals that were not normally distributed, despite attempting transformations. The outputs of the final mixed models whose residuals were normally distributed are summarised for the activity and milk feeding behaviours in Tables 2 and 3 respectively.

#### Table 2

The effect of neonatal calf diarrhoea with or without dehydration on activity behaviours of young pre-weaned artificially reared calves<sup>1</sup>. Factors with *p*<0.05 are shown in bold.

Behaviour	Fixed effect	Level	Effect size	Confidence interval	P value
Daily lying bouts (n)	Disease status	Healthy <b>NCD-H</b> NCD-D	Reference - <b>1.100</b> -1.180	Reference <b>1.2451.060</b> 1.396 <b>-</b> 1.000	Reference <b>&lt;0.001</b> 0.052
	Sire breed type	Beef Dairy	Reference – <b>1.097</b>	Reference - <b>0.852 -</b> - <b>0.975</b>	Reference 0.009
	Season	Autumn Winter Spring	Reference <b>1.208</b> <b>1.118</b>	Reference — <b>0.780 —</b> — <b>1.144</b> — <b>1.187 —</b> — <b>1.054</b>	Reference <b>&lt;0.001</b> <b>0.005</b>
Daily standing bouts (n)	Disease status	Healthy NCD-H NCD-D	Reference - <b>1.100</b> - <b>1.183</b>	Reference — <b>1.141–1.060</b> — <b>1.399–1.000</b>	Reference <0.001 0.048
	Sire breed type	Beef Dairy	Reference 	Reference - <b>0.851 -</b> - <b>0.973</b>	Reference <b>&lt;0.001</b>
	Season	Autumn Winter Spring	Reference - <b>1.210</b> - <b>1.118</b>	Reference — <b>0.779 –</b> — <b>1.147</b> — <b>1.186 –</b> — <b>1.054</b>	Reference <b>&lt;0.001</b> <b>0.004</b>
Mean lying bout length (minutes)	Disease status	Healthy NCD-H NCD-D	Reference 1.094 1.219	Reference 1.051–1.139 1.015–1.464	Reference <b>&lt;0.001</b> <b>0.034</b>
	Sire breed type	Beef Dairy	Reference 1.086	Reference 1.010–1.167	Reference <b>0.027</b>
	Season	Autumn Winter Spring	Reference 1.227 1.095	Reference 1.150–1.308 1.026–1.169	Reference <b>&lt;0.001</b> <b>0.020</b>
Total daily activity	Disease status	Healthy NCD-H NCD-D	Reference - <b>132.342</b> - <b>346.630</b>	Reference - <b>201.323-</b> - <b>77.956</b> - <b>852.79767.007</b>	Reference <b>&lt;0.001</b> <b>&lt;0.001</b>
	Sex	Female <b>Male</b>	Reference <b>17.364</b>	Reference - <b>58.99 -</b> - <b>0.487</b>	Reference <b>0.021</b>
	Season	Autumn <b>Winter</b> Spring	Reference – <b>65.545</b> 21.132	Reference - <b>152.15715.029</b> -86.220 <b>-</b> 0.016	Reference <b>&lt;0.001</b> 0.074
	Disease status x Season				<0.001
Mean activity per standing bout	Disease status	Healthy NCD-H NCD-D	Reference 1.233 10.093	Reference - <b>2.519 -</b> - <b>0.407</b> - <b>28.490-</b> - <b>1.086</b>	Reference <b>&lt;0.001</b> <b>0.004</b>
	Sire breed type	Beef <b>Dairy</b>	Reference 1.018	Reference 0.020-3.439	Reference 0.022

<sup>1</sup> The results shown for the models where a transformation was used have been back transformed.

#### Table 3

The effect of neonatal calf diarrhoea with or without dehydration on milk feeding behaviours of young pre-weaned artificially reared calves<sup>1</sup>. Factors with *p*<0.05 are shown in bold.

Behaviour	Fixed effect	Level	Effect size	Confidence interval	P value
Total milk visits (n)	Disease status	Healthy <b>NCD-H</b> NCD-D	Reference <b>0.079</b> 0.055	Reference <b>0.000–0.293</b> –0.880 <b>–</b> 1.999	Reference <b>0.037</b> 0.697
	Age		0.002	0.002-0.003	<0.001
	Season	Autumn <b>Winter</b> Spring	Reference <b>0.004</b> 0.048	Reference - <b>0.118 -</b> - <b>0.008</b> 0.044-0.006	Reference <b>0.001</b> 0.379
	Disease status x Season Disease status x Age				0.005 <0.001
Mean milk visit length (minutes)	Disease status	Healthy NCD-H NCD-D	Reference 1.207 1.674	Reference 1.143–1.025 1.350–2.079	Reference <b>&lt;0.001</b> <b>&lt;0.001</b>
	Age		- <b>0.977</b>	- <b>0.969 -</b> - <b>0.977</b>	<0.001
	Season	Autumn Winter Spring	Reference -0.963 -0.914	Reference -0.932-1.152 -1.253-1.046	Reference 0.516 0.201
	Disease status x Season				0.044

<sup>1</sup> The results shown for the models where a transformation was used have been back transformed.

#### Conclusion

Calves with NCD-H have fewer standing and lying bouts, longer lying bouts, are less active, visit the milk machine more often and have longer visits to milk when compared to their healthy counterparts. Similar results were seen for calves with NCD-D, however dehydration did not have a statistically significant impact on total milk visits and daily lying bouts, most likely due to the small number of NCD-D days. The interaction between disease status and season may reflect a differing response to environmental conditions depending on disease status. The interaction between disease status and age in the number of visits to milk may reflect a differing response to disease as calves grow. The association of NCD-H and NCD-D with changes in behaviour suggests that there is potential for these behaviours to be used in automatic disease detection tools, however factors such as age and season must also be considered.

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## 54. Interaction between colostrum and milk replacer supply on growth and gastrointestinal development of dairy surplus calves

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#### Application

Morbidity, mortality, and irresponsible use of antimicrobials are common concerns in the rearing of dairy surplus calves. Consequently, there is a pressing necessity to identify mechanisms to motivate dairy farmers to improve calf care. The findings of this study will contribute to the recognition of developmental and nutritional factors influencing surplus calf well-being. This, in turn, will enable producers to implement effective and practical feeding strategies that prioritize animal welfare, enhance overall health, and promote future profitability.

#### Introduction

In calves, early postnatal nutrient supply influences growth, future health and performance, and gastrointestinal tract (**GIT**) development (Khan et al., 2007; Ollivett et al., 2012). Colostrum management has been identified to be crucial for calf health and survival. Nevertheless, colostrum has been reported to influence GIT development and digestive and absorptive capacities in neonates (Blättler et al., 2001). However, and to our knowledge, the interaction between colostrum and plane of nutrition and their cumulative effects has not been documented before. Therefore, this study examined the interactions between these two critical early-life interventions and their impact on growth performance and GIT development over time in surplus bull calves as they develop in time.

#### Materials and methods

One hundred and twenty Holstein-Friesian bull calves  $(2.16 \pm 0.99 \text{ d} \text{ of age}; 43.1 \pm 3.56 \text{ kg BW})$  were enrolled. At the dairy farm of origin, calves were fed either 2 or 6 L of colostrum replacer (**CR**), within 12h after birth, aiming to provide a total of 100 or 300 g of IgG, to obtain either failure (**F**) or successful (**S**) transfer of passive immunity, respectively. Upon arrival at the facility, serum IgG levels were measured, therefore; F and S calves were identified accordingly and blocked, and subsequently were randomly assigned to one of two milk replacer (**MR**) feeding schemes: Moderate (**M**; 0.25 Mcal of ME/kg of arrival BW<sup>0.75</sup>, with 15.0% solids/L of MR) or Low (**L**; 0.135 Mcal of ME/kg of arrival BW<sup>0.75</sup>, with 12.5% solids/L of MR). In total, there were 4 treatments resulting from the 2 × 2 factorial design: **FM**, **FL**, **SM**, **SL** (*n* = 30/ treatment). Calves were individually housed and received their MR allowance twice daily in teat buckets until d42, and subsequently gradually weaned until d56. Calves had free access to chopped straw and water during the entire study, and a calf pelleted starter was introduced on d22. To evaluate GIT development and composition, 10 calves/treatment were euthanized at 3 different timepoints (21, 42, and 84 d of age). Other measurements included BW at arrival and weekly thereafter, and daily intakes were individually recorded. Data was analysed by PROC MIXED in SAS accounting for the fixed effects CR, MR, and time, and their interaction, as well as the random effect of block. Time was entered into the model as a repeated statement and initial BW was used as covariable when appropriate.

#### Results

Initial BW was not different across treatments (Figure 1) while serum IgG was greater in S calves (S = 1962.9 vs. F = 941.0 mg IgG/dL). Throughout the experimental period, BW was significantly influenced by both CR (P = 0.012) and MR (P < 0.001) treatments, with a tendency for an interaction between CR, MR, and time (P = 0.09). The BW at the three distinct slaughter ages was influenced by the allowance of MR (P < 0.001), wherein calves receiving moderate levels (SM and FM) consistently exhibited higher weights compared to those receiving low allowances, regardless of colostrum treatment (Fig. 1). Similarly, increased fresh tissue weights were observed in the duodenum, jejunum, ileum, cecum, and colon of SM and FM calves, with the influence of MR being evident (P < 0.05) across the three distinct dissection timepoints. By design, calves receiving moderate levels of MR had greater milk intakes (P < 0.05), while starter intake was influenced by CR (P = 0.05) and throughout the entire experiment, there was a tendency for an interaction between CR and MR (P = 0.09).



**Fig. 1.** Body weight at three different slaughter timepoints of surplus calves. Calves received one of the four treatments: SM=300g IgG + moderate milk allowance; FM=100g IgG + moderate milk allowance; SL=300g IgG + low milk allowance; FL=100g IgG + low milk allowance; FL=100g

#### Conclusions

No morphological difference were observed from CR supply. However, the provision of MR strongly influenced the development of the GIT. Notably, there was an increase in the tissue weight of the intestine in calves receiving more milk. This augmented weight might imply a potentially larger surface area for nutrient absorption, in line with the greater nutrient supply to these calves. Further study of gut tissue morphology may describe more in depth the value of these interventions on the development of gastrointestinal competence of dairy surplus calves.

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## 55. Summer scour syndrome in weaned dairy calves: Case series

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## Application

The data from this study can be used to inform future studies aimed at finding definitive causes of the summer scour syndrome (SSS) in post-weaned calves.

## Introduction

Summer scour syndrome (SSS) is a recently identified pathological condition affecting first-grazing dairy calves in Ireland (Sheehan et al., 2023), the United Kingdom (Swinson et al., 2023), and Australia (Hunnam et al., 2021). The syndrome is characterised by diarrhoea that is unresponsive to treatment, weakness, weight loss and death. Oral and oesophageal ulcerations are observed in some cases. The syndrome is reported to occur in weaned calves within one month post turnout to grass or after moving to new pastures. The primary cause of the syndrome remains unclear, and diagnosis is often made after the common causes of calf diarrhoea at grass (e.g., coccidiosis, parasitic gastroenteritis, bovine viral diarrhoea virus (BVDV), malignant catarrhal fever (MCF), *Salmonella*, rumen acidosis, and copper or molybdenum toxicities) are excluded. The aims of this study were to characterize SSS in weaned dairy calves and determine the conditions under which SSS occurs on Irish commercial farms

## Material and methods

In June 2023, five farms (three dairy farms, one dairy-beef farm, and one mixed dairy and dairy-beef farm) were visited, after referral by private veterinary practitioners with suspected SSS cases. Clinical examinations and thoracic ultrasonography (TUS) were performed on a total of 46 calves (8 to 10 calves per farm) displaying symptoms. Biological samples including blood, feces, and rumen fluid were collected. Blood samples were analysed for ammonia concentrations immediately after collection using a point-of-care analyser (Pocketchem BA, Arkray, Japan) and for haematology profiles, biochemistry, and mineral content, and rumen samples were analysed for ammonia and lactic acid concentrations, using automated analysers. Faecal samples were processed for parasitology analysis using the McMaster technique. A questionnaire by means of interview was conducted with each farmer to record on-farm management practices. Grass and concentrate samples were collected from each farm and analysed for chemical composition, and grass trace mineral analysis. Descriptive statistics (PROC FREQ and PROC MEANS) in SAS 9.4 were used to summarize the distribution of data collected per farm.

## Results

Two farms (dairy-beef and mixed dairy and dairy-beef) were positive for coccidiosis and/or had chronic pneumonia and were designated as non-SSS case farms (NCF), and the remaining farms were designated as SSS case farms (CF). All farms applied gradual (step-down) weaning procedures and calves were fully weaned at 70–84 days of age. Calves on all farms received water *ad libitum*, concentrate supplementation, and forage provision during the pre-weaning period. Calf characteristics and clinical data of calves on CF and NCF are shown in Table 1. The mean rumen fluid pH was similar between CF (6.67–7.09) and NCF (6.43–6.88). Mean ammonia concentrations in rumen fluid ranged from 17.6–29.6 mg/L and 17.2–45.0 mg/L on CF and NCF, respectively. Corresponding blood ammonia concentrations were 5 to 10 times higher on calves from CF compared to NCF (129–223 µmol/L vs. 22–25 µmol/L). Mean blood molybdenum concentrations on NCF were double the concentrations on CF (12.4–13.3 µg/L vs. 6.5–7.5 µg/L) but values were within the normal range. Mean blood copper concentrations were similar between CF and NCF. Grass and concentrate from all farms had crude protein below 20 percent per kg dry matter (DM) and neutral detergent fibre above 40 percent per kg DM. On CF, inorganic nitrogen fertiliser was applied 1 to 3 weeks pre-grazing, whereas on the two NCF, inorganic nitrogen fertiliser was applied 2 to 3 weeks pre-grazing on one farm and no fertiliser was applied on the other.

#### Table 1

Calf characteristics and clinical data collected on CF and NCF.

	CF			NCF	
	CF 1 ( <i>n</i> = 10)	CF 2 ( <i>n</i> = 10)	CF 3 ( <i>n</i> = 10)	NCF 1 ( <i>n</i> = 8)	NCF 2 ( <i>n</i> = 8)
Calf sex (number)	Female (10)	Female (10)	Female (10)	Female (3); Male (5)	Male (8)
Age (SD) in days at turnout	67 (6)	100 (4)	100 (4)	51 (13)	85 (11)
Age (SD) in days at visit	127 (6)	135 (4)	129 (4)	113 (13)	128 (11)
Body weight (SD) in kg	116 (12.4)	123 (11.7)	129 (10.2)	112 (10.8)	103 (21.4)
	(Number calves, an	nd % of total on each farı	n)		
Nasal discharge	5 (50)	2 (20)	2 (20)	3 (38)	6 (75)
Mouth ulcers	4 (40)	1 (10)	0	1 (13)	0
Hyper-salivation	6 (60)	8 (80)	7 (70)	4 (50)	0
Diarrhoea	7 (78) <sup>a</sup>	7 (70)	8 (80)	2 (25)	7 (88)
Pneumonia <sup>b</sup>	0	2 (20)	1 (10)	4 (50)	5 (63)
Poor coat	6 (60)	9 (90)	8 (80)	6 (75)	4 (50)

<sup>a</sup> Faecal sample was not collected from one calf due to rectal emptying (7 out of 9 calves).

#### Conclusion

These preliminary findings suggest that copper or molybdenum toxicity are not primary causes of SSS. High blood ammonia concentrations and the timing and level of inorganic nitrogen fertiliser application to paddocks pre-grazing warrant further investigation.

#### Acknowledgements

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56. Inter-rater agreement of different thoracic ultrasonography techniques performed by novice operators on preweaned dairy calves

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#### Application

In preweaned dairy calves, thoracic ultrasound scanning method, image interpretation and scoring technique are important determinants of the level of agreement, which may be improved in novice operators by using a consistent method and a simplified scoring technique.

#### Introduction

Thoracic ultrasonography (TUS) is a validated method for the identification of lung pathology associated with bovine respiratory disease (Ollivett and Buczinski, 2016). Multiple techniques for TUS are described, each seeking to optimise precision, accuracy and examination duration. However, TUS is an imperfect method for the diagnosis of pneumonia, and differences in examination technique and image interpretation will affect the diagnosis prescribed to individual calves. In this study, we sought to evaluate the agreement between novice raters following basic training when performing different scanning and scoring methods.

#### Materials and methods

A convenience sample of 8 gualified veterinarians within the Farm Animal Health and Production Group at the Royal Veterinary College were enrolled in the study. 24 h prior to the start of the study, TUS was performed on 40 calves by an experienced rater, with the probe passed in a dorsal-to-ventral direction within each intercostal space bilaterally and the results recorded. Thereafter, participants were provided with a one-hour classroom teaching session regarding TUS image interpretation, scoring and scanning techniques. Three published TUS scanning techniques were demonstrated; LUS (Masset et al., 2022), FLUS (Pravettoni et al., 2021) and gTUS (lourguin et al., 2022), based upon their description within the literature. Briefly, to perform LUS the probe was passed systematically throughout the 5th to the 1st and the 5th to the 2nd intercostal spaces on the right and left hand side, respectively. For FLUS, the entire middle and ventral third of the 4th and 5th intercostal spaces were scanned bilaterally, whereas qTUS involved a single movement of the probe from the 9th intercostal space cranioventrally. Upon reaching the heart the probe was advanced dorsally and cranially around it. For each method, participants were asked to score up to 10 different preweaned dairy calves aged between 6-8 weeks within a 45-minute timeframe, with calves in each group selected to ensure a range of healthy and unhealthy individuals. Responses collected included a 6-point TUS score (TUS6) as described by Ollivett and Buczinski (2016), as well as a binary response regarding if the lesion was <1 cm<sup>2</sup> or >1 cm<sup>2</sup> (CE1). Agreement and reliability was assessed for all raters using equally weighted percentage agreement (PA) and Krippendorff's Alpha (KA). Pairwise agreement between raters and the experienced rater was assessed using Cohens Kappa. Interpretation of agreement was based upon Landis and Koch (1977), whereby values of <0.00, 0.00-0.20, 0.21-0.40, 0.41-0.60, 0.61-0.80 and 0.81-1.0 were interpreted as poor, slight, fair, moderate, substantial and almost perfect agreement, respectively. All analysis was performed using R (R Core Team, 2022).

#### Results

Overall PA and KA for TUS6 was 0.31 (95%CI 0.21–0.42, P < 0.05) and 0.17 (95%CI 0.05–0.21, P < 0.05). PA for TUS6 between raters varied by ultrasonographic technique performed. Values of 0.40 (95%CI 0.13–0.68), 0.35 (95%CI 0.13–0.57, P < 0.05) and 0.13 (95%CI 0.02–0.25, P < 0.05) for LUS, FLUS and qTUS were found, respectively. Agreement improved when raters provided a binary response regarding lesion size. For CE1, overall PA and KA were 0.72 (95%CI 0.59–0.86, P < 0.05) and 0.36 (95%CI 0.21–0.52, P < 0.05). A contingency table of Cohen's Kappa agreement for TUS6 between pairs of raters is shown in Table 1., where observer 1 represents the experienced rater, performing a more thorough TUS technique.

## Table 1 Contingency table of Cohen's Kappa agreement for TUS score between pairs of raters.

	1	2	3	4	5	6	7	8
1		0.52	0.38	(-0.02)	0.39	0.47	0.37	(0.10)
2			0.74	(0.01)	0.69	0.67	0.63	(0.20)
3				(-0.03)	0.56	(0.23)	0.59	(-0.04)
4					(0.08)	(-0.07)	(-0.03)	(-0.04)
5						0.49	0.51	(0.13)
6							0.61	(0.14)
7								(0.07)
8								

In all cases equal weighted kappa was performed. P<0.05 in all cases except those displayed in brackets. Values are shaded based upon the extent of agreement, with darker shades corresponding with higher values (greater agreement).

## Conclusions

Inter-rater agreement between participants for TUS6 was fair overall, with marked variation between raters and between technique performed. Agreement was improved when observers provided a binary score based upon lesion size, in comparison to TUS6. We hypothesise that scoring consistency may be improved by the provision of practical training and use of simplified lesion scoring methods. In circumstances where multiple operators are performing TUS, measurement of inter-rater agreement is an essential consideration, in order to ensure the validity of any described outcomes.

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## 57. Effect of live yeast (Saccharomyces cerevisiae) in calf starter feed on the health, and feed efficiency of rearing dairy calves

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## Application

The growth rate and feed efficiency of dairy calves were greater when live yeast was added to the calf starter, which can lower the carbon footprint of, but not antimicrobial use in calf rearing.

## Introduction

Morbidity in young calves is largely attributed to enteric disorders and respiratory disease (Gulliksen et al., 2009) and antimicrobials are an integral part of livestock production. However, human mortality due to antimicrobial-resistant (AMR) bacteria is projected to be 10 million by 2050, eclipsing those due to cancer by 1.8 million annually (WHO, 2019) and the development of natural alternatives to antimicrobials are essential for calves. This study aimed to compare the performance of dairy-bred bull calves offered calf starter feed without and with the addition of live yeast (LY).

## Material and methods

A total of 62 dairy calves were allocated, at 3 ( $\pm$ 1) d of age, to one of two treatment groups according to birth date and live weight (LW) (*n* = 31 calves/treatment) and offered starter without (LW: 50.4  $\pm$  1.09 kg) and with the addition of live yeast (LY) at 0.25 g/kg FM (Vista cell, AB Vista, Ltd.) (LW: 49.3  $\pm$  1.09 kg) for 84 d. Calves were offered pasteurised colostrum up to 3 d of age, housed in pairs, and offered MR at 4 to 6 L/d (Milk replacer at 150g/l: DM 96; CP 23; Oil 20%) between 4 and 70 d of age at fully gradual weaning, along with ad-libitum access to water and calf starter (20% CP and 12.4 MJ ME/kg DM). Daily LW, feed, and water intakes were measured and used to calculate a weekly mean, which along with daily health treatments were normally distributed. These were analysed using the general linear mixed models procedure (GLM ANOVA), applying -LY and +LY use as a fixed effect and animal as a random effect in the model. Differences were assessed by Tukey's test with a confidence interval of 95% and differences were reported at *P* < 0.05.

## Results

Calf growth rate and feed efficiency were greater when LY was added to calf starter, but did not affect calf mortality, electrolyte, and antimicrobial use (See Table 1).

#### Table 1

Mean (±SE) electrolyte and antimicrobial use, dry matter intake (DMI), live-weight gain, feed efficiency, and related carbon footprint of dairy calves offered starter with and without live yeast (LY).

	-LY (± SE)	+LY (±SE)	P value
Mortality, %	0.03	0	0.989
Electrolyte use, d in total	26	24	0.960
Antimicrobial use, d in total	29	20	0.225
Milk replacer intake, kg	58.8 (1.02)	58.2 (1.02)	0.706
Starter intake, kg DM	62.1 (2.19)	61.2 (2.19)	0.785
Cereal straw, kg DM	5.9 (0.364)	5.6 (0.36)	0.515
Total water intake, L/d	41.5 (0.66)	41.3 (0.66)	0.781
Pre-weaning gain (0 to 70 d), kg/d	0.74 (0.016)	0.79 (0.016)	0.033
Post-weaning gain (70 to 84 d), kg/d	0.96 (0.047)	1.09 (0.047)	0.046
Overall daily gain (0 to 84 d), kg/d	0.78 (0.017)	0.84 (0.017)	0.008
Preweaning feed efficiency, DMI/ kg gain	1.67 (0.036)	1.51 (0.036)	0.002
Post-weaning feed efficiency, DMI/ kg gain	2.90 (0.173)	2.47 (0.179)	0.088
Mean feed efficiency, kg DMI/ kg gain	2.15 (0.055)	1.93 (0.055)	0.006
Reduction in feed-related CO <sub>2</sub> -eq, %	0.00 (0.001)	10.13 (0.001)	0.006

## Conclusion

The calf growth rate and FE were greater when LY was added to the calf starter, which can lower the carbon footprint related to feed use in rearing calves. The inclusion of LY in calf starter did not affect calf mortality, electrolyte, and antimicrobial use, which was however minimal overall.

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## 58. Effect of live yeast (Saccharomyces cerevisiae) on the health, and feed efficiency of rearing dairy calves

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#### Application

The growth rate and feed efficiency of dairy calves were greater when live yeast was added to the calf starter, which can lower the carbon footprint of, but not antimicrobial use in calf rearing.

#### Introduction

Morbidity in young calves is largely attributed to enteric disorders and respiratory disease (Gulliksen et al., 2009) and antimicrobials are an integral part of livestock production. However, human mortality due to antimicrobial-resistant (AMR) bacteria is projected to be 10 million by 2050, eclipsing those due to cancer by 1.8 million annually (WHO, 2019) and the development of natural alternatives to antimicrobials that enhance calf health and performance are essential. This study aimed to compare the performance of dairy calves offered a calf starter without and with the addition of live yeast (LY).

### Material and methods

A total of 62 dairy calves were allocated, at 3 ( $\pm$ 1) d of age, to one of two treatment groups according to birth date and live weight (LW) (*n* = 31 calves/treatment) and offered starter without (LW: 50.4  $\pm$  1.09 kg) and with the addition of live yeast (LY) at 0.25 g/kg FM (Vista cell, Abvista, Ltd.) (LW: 49.3  $\pm$  1.09 kg) for 84 d. Calves were offered pasteurised colostrum up to 3 d of age, housed in pairs, and offered MR at 4 to 6 L/d (Milk replacer at 150 g/l: DM 96; CP 23; Oil 20%) between 4 and 70 d of age at fully gradual weaning, along with ad-libitum access to water and calf starter (20% CP and 12.4 MJ ME/kg DM). Daily LW, feed, and water intakes were measured and used to calculate a weekly mean, which along with daily health treatments were normally distributed. These were analysed using the general linear mixed models

procedure (GLM ANOVA), applying -LY and +LY use as a fixed effect and animal as a random effect in the model. Differences were assessed by Tukey's test with a confidence interval of 95% and differences were reported at P < 0.05.

## Results

Calf growth rate and feed efficiency were greater when LY was added to calf starter but did not affect the amount of electrolyte or antimicrobial use (See Table 1).

Table 1

Mean ( $\pm$ SE) feed intake (DMI), live-weight gain, feed efficiency, and electrolyte and antimicrobial use of dairy calves offered starter with (+) and without (-) additional live yeast (LY).

	-LY (±SE)	+LY (± SE)	P value
Electrolyte use, d in total	26	24	0.960
Antimicrobial use, d in total	29	20	0.225
Milk replacer intake, kg	58.8 (1.02)	58.2 (1.02)	0.706
Starter intake, kg DM	62.1 (2.19)	61.2 (2.19)	0.785
Cereal straw, kg DM	5.9 (0.364)	5.6 (0.36)	0.515
Total water intake, L/d	41.5 (0.66)	41.3 (0.66)	0.781
Pre-weaning gain (0 to 70d), kg/d	0.74 (0.016)	0.79 (0.016)	0.033
Post-weaning gain (70 to 84d), kg/d	0.96 (0.047)	1.09 (0.047)	0.046
Mean daily gain (0 to 84d), kg/d	0.78 (0.017)	0.84 (0.017)	0.008
Preweaning feed efficiency, DMI/kg gain	1.67 (0.036)	1.51 (0.036)	0.002
Post-weaning feed efficiency, DMI/kg gain	2.90 (0.173)	2.47 (0.179)	0.088
Mean feed efficiency, kg DMI/kg gain	2.15 (0.055)	1.93 (0.055)	0.006

#### Conclusion

The calf growth rate and feed efficiency (DMI/kg gain) were greater when LY was added to the calf starter, which can lower the carbon footprint of calf rearing. However, the inclusion of LY in calf starters did not affect electrolyte and antimicrobial use, which was required in small amounts.

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## 59. Factors affecting Welsh Lamb meat eating quality

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There is a need to understand the factors which may influence the eating and nutritional quality of Protected Geographical Indication (PGI) Welsh Lamb meat. PGI Welsh Lamb is an important part of the Welsh economy, representing a total value of £171.5 million of Welsh Lamb exports in 2022 (Hybu Cig Cymru, 2022). The Welsh Lamb Meat Quality project was part of the Red Meat Development Programme. Hybu Cig Cymru – Meat Promotion Wales. This work researched on-farm and processing factors influencing meat eating and nutritional quality of PGI Welsh Lamb.

Representative PGI Welsh Lamb samples were sourced from across Wales (n = 624 lambs). Four trials investigated on-farm factors including breed type, lamb gender, muscle cut, lamb finishing diet, daily liveweight gain, seasonality and processing factors including meat ageing period, carcase hanging and packaging. All samples were also assessed for intramuscular fat content and individual fatty acid composition. Nearly 2,000 consumers rated the sensory quality of 13,400 samples in trials. Panels were held at 12 locations across the UK with consumers testing seven samples, rating them on aroma liking, tenderness, juiciness, flavour liking, and overall liking.

Over the whole project, eating quality results indicated that muscle cut and season had the greatest effect on eating quality scores (P < 0.001), with lambs slaughtered in August and November having better eating quality than those slaughtered the following February. Lamb finishing diet and lamb gender had no effect on eating quality. Individual farm influenced on all eating quality attributes (P < 0.001, except Aroma Liking P = 0.011) and nutritional measures (P < 0.001) and this could not be explained by the factors recorded. Slaughter age impacted eating quality, with younger animals having higher consumer sensory scores (P < 0.001). Where consumers were from (i.e. region) also effected sensory scores (P < 0.001). Intramuscular fat (IMF) content was associated with increased consumer scores (P < 0.001). The average omega-6:omega-3 ratio was below 2.0 for all diets, which is considered healthy. The healthiest (lowest) omega-6:omega-3 ratio was derived from the grass finished lambs and the ratio was 0.94 (P > 0.001).

PGI Welsh Lamb meat is healthy, nutritious, and the eating quality was good. Some effects were observed with on-farm and processing factors influencing eating and nutritional quality. Those lambs that were finished on grass and/or forage had higher levels of beneficial omega-3 fatty acids.

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# 60. Prime lamb production from grazed grass – Effects of ewe genotype, whether joined to lamb at 1 year and ewe age on the pattern of exit from flock up to 9 years of age

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## Application

Retaining ewes for more than 6 years results in reduced lamb output, higher ewe mortality and an increased incidence of culling.

#### Introduction

Data from the National Farm Survey show that mean replacement rate on lowland farms in Ireland is 22% whilst ewe productivity has remained relatively static (~1.3 lambs weaned per ewe joined) for the last ~40 years. Replacement ewes represent a major cost in prime-lamb production and Keady (2014) reported that the mean cost, nationally, 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. Since sheep-meat production costs are attributable primarily to the ewe, production costs per lamb can be reduced by increasing the number of lambs produced per ewe lifetime by joining replacements to lamb at 1 year and avoiding non-essential culling. Ewe genotype is a key determinant of ewe productivity (Hanrahan 2001, Keady and Hanrahan, 2018). 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 currently represents the sire of 9.5% of ewes in lowland flocks (Keady et al., 2019). Hanrahan (2001) reported that the number of lambs reared per ewe joined was 0.21 higher for ewes having Belclare sires than crossbred ewes sired by the Suffolk breed. The aim of the current study was to evaluate lifetime patterns in the causes of ewe exit from a flock managed without voluntary culling until 9 years of age, and representing ewes of different genotypes that were first joined as ewe lambs or at 1.5 years of age.

## Material and methods

A total of 424 ewes [157 Belclare (B), 114 B×Suffolk (B×S), 153 >75%S; 2 cohorts] were joined annually, with Charollais rams, starting at 19 months of age. A random 50% of each genetic group had been joined at  $\sim$ 7 months and 85% had produced lambs at 1 year. Ewes only left the flock when culled for physical reasons (e.g., udder and mouth issues, or poor body condition) or when they died. All ewes were shorn at housing in early December (group pens of  $\sim$ 40) and offered grass silage *ad libitum* until lambing. During the 7 weeks prior to lambing ewes were offered concentrate supplement; 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. All lambs were managed as one flock between weaning and slaughter and offered grazed grass as the sole diet. All lambs were slaughtered prior to the end of the grazing season. The data were analysed using the MIXED, LOGISTIC and LIFETEST procedures of SAS, as appropriate.

#### Results

For the Belclare,  $B \times S$  and >75% sewes, the mean number of lambs reared/ewe-joined was 1.62, 1.69 and 1.34 (SE = 0.052) and mean age at exit from the flock was 4.6, 4.9 and 4.8 years (SE = 0.17), respectively. The reasons for culling are shown in Table 1, as a function of ewe age.

Table 1		
Age and reaso	n at culling (% of	ewes joined).

	Ewe age						All	
	2	3	4	5	6	7	8+	
No. ewes joined	424	372	294	219	142	101	57	1609
Died	4.0	6.2	2.0	3.6	4.2	8.9	14.0	18.2
Mastitis/udder	6.4	11.0	11.2	6.8	9.9	11.9	17.5	35.8
Condition	0.5	0.8	6.1	8.2	6.3	5.9	19.3	15.8
Teeth	0.2	0.3	4.4	13.7	4.9	17.8	24.6	19.8
Feet	0.0	0.3	0.3	0.0	2.1	5.9	5.3	3.3
Prolapse	0.7	1.3	1.0	0.0	0.0	0.0	0.0	2.6
Other	0.9	0.5	0.3	2.7	1.4	0.0	7.0	4.5

The percentage of ewes that died was similar up to age 6 but increased thereafter (P < 0.05). The incidences of culling for mastitis/udder issues and poor body condition increased substantially after 7 years of age. As ewes aged the percentage culled for teeth (mouth) and lameness increased. Only 2.6% were culled for uterine prolapse (all prior to age 6). There was evidence for an effect of ewe genotype on the reason for culling: teeth accounted for a greater proportion of the culls of Belclare ewes compared with >75%S ewes (P < 0.10) while poor condition accounted for a significantly greater share of the disposals of >75%S ewes compared with Belclare (P < 0.05). There was no evidence for any difference between the disposal patterns when B × S was contrasted with the average of Belclare and >75%S ewes (i.e., heterosis). Age at first joining had no effect (P > 0.05) on output per ewe, age at culling or culling reason.

## Conclusion

Mastitis/udder was the most prevalent reason for flock exit, followed by teeth, death and poor body condition. As age increased, more ewes were culled for teeth, condition and feet issues. Effects of genotype on reasons for culling were relatively minor and there was no evidence for any effect of age at first joining.

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## 61. Effect of supplementing calcium peroxide on performance and methane emissions in dry ewes

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## Application

Calcium peroxide can be added to the diet of ewes to reduce methane production and intensity without affecting feed intake.

## Introduction

Methane production by livestock has been of major interest due to its significant contribution to anthropogenic GHG emissions and energy loss to the animal. Over the last decade, a variety of dietary manipulation approaches have been proposed to reduce enteric methane emissions in ruminants with limited success mainly due to the requirement of continuous feeding and therefore costs, negative effects on animal production on pasture based systems. Therefore, if feed additives are to become part of a successful abatement strategy for national agricultural systems, it is essential that they are shown to be effective on pasture-based systems. The curent study examined the effect of supplementing calcium peroxide, a novel methane inhibitor, in the diet to reduce methane emissions from enteric fermentation using dry ewes.

## Materials and methods

Sixty Lowland crossed ewes were assigned into a continuous design study for 112 days to assess the effects of feeding calcium peroxide on animal performance and methane emissions. Three groups of ewes (20 ewes/group) were formed and balanced by age, body wight (BW) and body condition score (BCS). The additive was added to the concentrate pellets (dry matter (DM) basis) as follows: control (0.0%), medium (5.0%) and high (7.5%). Limestone was used to balance mineral concentrations in the 3 concentrate pellets. Grass silage was fed libitum, whilst the amount of concentrate was periodically adjusted (20% DM basis). DM intake (DMI) was recorded daily, whilst BW and BCS were recorded weekly. Enteric methane emissions were measured using a GreenFeed unit. Response variables were analysed using REML, with treatment as a fix effect and ewe as random effect (GenStat 21st ed., VSNI Ltd).

#### Table 1

Performance and methane emissions of dry ewes supplemented with calcium peroxide in the concentrate diet.

	Control	Medium	High	SED	P-Val
Initial body weight (BW; kg)	82.8	81.3	80.9	1.04	0.837
Initial body condition score (BCS)	3.33	3.28	3.07	0.193	0.361
Dry matter intake (DMI; kg/d)	1.99	2.04	1.97	0.066	0.562
Silage DMI (kg/d)	1.59	1.63	1.56	0.065	0.564
Meal DMI (kg/d)	0.40	0.41	0.41	0.004	0.252
Average daily gain (kg/d)	0.148 <sup>a</sup>	0.176 <sup>b</sup>	0.183 <sup>b</sup>	0.0119	0.011
Body condition score	3.75	3.78	3.84	0.161	0.879
Methane before experiment (g/d)	48.8	48.1	50.2	1.06	0.775
Methane (g/d)	48.2 <sup>b</sup>	47.0 <sup>ab</sup>	42.9 <sup>a</sup>	1.05	0.040
Methane yield (g/kg DMI)	24.4	23.4	22.2	1.08	0.126
Methane intensity (g/kg BWG)	331.1 <sup>c</sup>	272.9 <sup>b</sup>	239.9 <sup>a</sup>	19.05	<0.001

BWG=Body weight gain.

## Results

Ewes supplemented with medium or high doses of calcium peroxide showed 16% or 19% greater (P = 0.011) average daily gains (ADG) than those in the control diet. The DMI (P = 0.562) and BCS (P = 0.879) did not differ between groups. Methane production was 11% lower (P = 0.040) in ewes consuming the high additive diet when compared to control ewes. Methane intensity was 17.6% and 27.5% lower (P < 0.001) in ewes consuming diets with medium and high additive content than in ewes receiving the control diet. Methane yield did not differ (P = 0.126) between treatments (See Table 1).

## Conclusions

The inclusion of the calcium peroxide at  $\sim$ 1.7% (high dose) of the total diet did not affect feed intake, whilst increased ADG and reduced methane production and intensity in dry ewes.

## Acknowledgments

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## 62. Estimation of standard reference weight of ewes from the Icelandic sheep breed

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## Application

Estimation of standard reference weight of ewes from the Icelandic sheep breed to provide a better basis for determining nutrient requirements and improved sheep production system

## Introduction

Standard Reference Weight (SRW) is a concept that connects frame size, live weight and body condition; and for any particular breed and sex of cattle or sheep SRW is the approximate liveweight achieved by that animal when skeletal development is complete and the empty body contains 250 g fat/kg (CSIRO, 1990); corresponding to body condition score 3 for sheep on the scale described by Russel et al. (1969). The SRW is a useful concept for several purposes: 1) to relate live weight and body condition for mature animals; 2) to define the maturity of growing animals; 3) with the animals estimated degree of maturity, its energy and protein requirements for growth can be estimated with much higher accuracy due to more accurate estimate of the fat, protein and energy content of the gain. To estimate SRW for ewes of the Icelandic breed of sheep, we used data on ewe live-weight (LW) and body condition scores (BCS) from 22 production years, 2001–2022, from the Hestur sheep experimental farm in Borgarfjörður, Southwest- Iceland.

## Material and methods

Live weight (LW) and body condition scores (BCS) were registered at six stages of the annual cycle, defined as following: Post-weaning (18 October); Pre-mating (1 December); Post-mating (4 January); 2-months pregnant (10 February); Mid-pregnancy (15 March); Late-pregnancy (20 April). Each of the set dates varied 1–2 days in either direction. The total dataset contained around 14.000 records of LW and BCS at each stage of the annual cycle. Based on literature studies and a preliminary analysis of the data, it was decided to use for further analysis only data for ewes that had complete records of LW and BCS up to 5 years of age. According to these criteria, data for 1266 ewes were available. Ewe LW at different stages of pregnancy was corrected by formulas describing the weights of the products of conception (Robinson et al., 1976), considering the number of foetuses carried by each ewe and the stage of pregnancy based on recordings of date of mating or lamb birth. The pregnancy-free live weights (PFLW) were then regressed on BCS for data from different ewe age and stages of the annual cycle as reported in Table 1.

Table 1				
Coefficients for the regressions a+b · BCS	s within each yea	ar and stage of the	production cycle	e.

Production stage	year no	age mo	a	b	R <sup>2</sup>	LW at BCS 3.00
Post-weaning	2	17	28.1 <sup>a</sup>	8.93 <sup>B</sup>	0.41	54.91
Pre-mating	2	19	31.7 <sup>ab</sup>	8.33 <sup>B</sup>	0.35	56.65
Post-mating	2	20	30.4 <sup>ab</sup>	8.62 <sup>B</sup>	0.36	56.26
2-months pregnant	2	21	32.9 <sup>b</sup>	8.27 <sup>B</sup>	0.35	57.72
Mid-pregnancy	2	22	29.6 <sup>ab</sup>	9.18 <sup>B</sup>	0.37	57.09
Late-pregnancy	2	23	34.3 <sup>b</sup>	8.47 <sup>B</sup>	0.30	59.68
Post-weaning	3	29	42.6 <sup>c</sup>	6.46 <sup>A</sup>	0.23	61.98
Pre-mating	3	31	42.9 <sup>cd</sup>	7.09 <sup>AB</sup>	0.22	64.21
Post-mating	3	32	43.2 <sup>cd</sup>	7.12 <sup>AB</sup>	0.21	64.59
#### Table 1 (continued)

Production stage	year no	age mo	a	b	R <sup>2</sup>	LW at BCS 3.00
2-months pregnant	3	33	/1 7 <sup>c</sup>	7 70 <sup>AB</sup>	0.25	65.08
2-months program	2	24	-11.7 27.0 <sup>bc</sup>	0.01 <sup>B</sup>	0.25	62.75
wid-pregnancy	5	54	57.0	0.91	0.28	05.75
Late-pregnancy	3	35	37.35	9.415	0.29	65.47
Post-weaning	4	41	45.7 <sup>cd</sup>	6.61 <sup>AB</sup>	0.22	65.48
Pre-mating	4	43	47.8 <sup>d</sup>	6.93 <sup>AB</sup>	0.19	68.63
Post-mating	4	44	47.9 <sup>d</sup>	6.95 <sup>AB</sup>	0.20	68.79
2-months pregnant	4	45	46.6 <sup>cd</sup>	7.64 <sup>AB</sup>	0.24	69.51
Mid-pregnancy	4	46	41.1 <sup>c</sup>	8.97 <sup>B</sup>	0.27	68.05
Late-pregnancy	4	47	44.6 <sup>cd</sup>	8.43 <sup>B</sup>	0.24	69.91
Post-weaning	5	53	47.7 <sup>d</sup>	6.57 <sup>A</sup>	0.21	67.43
Pre-mating	5	55	48.5 <sup>d</sup>	7.31 <sup>AB</sup>	0.19	70.46
Post-mating	5	56	50.4 <sup>d</sup>	6.92 <sup>AB</sup>	0.18	71.12
2-months pregnant	5	57	47.4 <sup>d</sup>	8.07 <sup>B</sup>	0.24	71.63
Mid-pregnancy	5	58	44.1 <sup>cd</sup>	8.88 <sup>B</sup>	0.27	70.70
Late-pregnancy	5	59	43.3 <sup>cd</sup>	9.42 <sup>B</sup>	0.29	71.54

<sup>a, b, c</sup>: Values with different superscript within a column are statistically different, *p*<0.05.

#### Results

Some more complex relationships were tested but none of them gave a better prediction than the simple linear relationship PFLW = a + b + BCS. The resulting regression coefficients a and b within each year and production stage as reported in Table 1 were compared statistically, according to 95% confidence limits. The constant a had generally lower value and the slope b higher value for ewes in year 2 than other age categories in different stages of the production cycle. By using the prediction equations derived by the regressions, LW at BCS 3.00 was calculated for different age categories and production stages, as reported in Table 1.

#### Conclusions

According to the data presented in Table 1 the SRW estimate for ewes of the Icelandic sheep breed, based on LW of fully developed indoor fed ewes at BCS 3.00 is 70–72 kg. The ewes are fully developed at 5 to 6 years of age.

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#### 63. The effect of feeding a calcium peroxide feed additive to mitigate enteric methane emissions in lambs

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#### Application

Calcium peroxide can be added to the diet of ewes to reduce methane production

#### Introduction

Methane is a natural product resulting from enteric fermentation in the rumen forestomach of ruminants. Specifically, 70–80% of methane is produced by rumen methanogens through conversion of carbon dioxide and hydrogen. The latter is a consequence of microbial digestion of plant material resulting in formation of Volatile Fatty Acids (VFAs), which are a source of energy for the animal. Increasing concerns over greenhouse gases produced by livestock and their subsequent heating potential has led to an increase in research into mitigating enteric methane from ruminants. Feed additives, such as 3-NOP, have been shown to reduce methane emissions, but apart from 3-NOP, other potential additives have not been studied to a great extent. Various peroxide-based additives have been screened *in vitro* and shown potential for application as methane mitigating feed additives. This study investigated the methane reducing potential of a calcium-peroxide (CP) based formulation in an early-life lamb study. This study follows on from a previous ewe trial that determined the optimal inclusion rate.

#### Materials and method

Initially, *In-vitro* studies using a rumen-simulating batch culture system were used to incubate varying inclusion levels of the additive with artificial saliva, rumen fluid and grass silage. Following this, seventy pregnant Lowland crossed ewes (35 ewes/group) expecting twin lambs

were selected and fed concentrate pellets (dry matter (DM) basis) as follows: control (0.0%) and CP (7.5%) two weeks prior to lambing. Limestone was used to balance mineral concentrations in the 3 concentrate pellets. At weaning, each of the twin lambs was assigned either the control or treatment pellet, resulting in four treatment groups: Control  $\times$  Control (CC), Control  $\times$  Treatment (CT), Treatment  $\times$  Treatment (TT) and Treatment Control (TC). Grass silage was fed ad libitum to both ewes and lambs, whilst the amount of concentrate was periodically adjusted (25% DM basis). DM intake (DMI) was recorded daily, with body weight recorded weekly. Enteric methane emissions were measured using a GreenFeed unit.

#### Results

*In-vitro* batch culture experiments found the calcium peroxide-based formulation significantly reduced methane emissions compared with the 0% control when added at 0.375%, 0.75%, 1.5%, 2.25% an 3% (p = 0.014). At animal level, a one-way ANOVA (SPSS, 26.0) revealed significant differences among the treatment groups (F = 6.18, p < 0.01). Post-hoc tests using Tukey's HSD indicated that lambs in group TT showed the greatest methane reduction compared to the CC group, although this was not significant (p = 0.94). Lambs born to ewes fed the CP weighed consistently heavier than those born to ewes fed the control diet until weaning (11 weeks) (See Fig. 1).



Fig. 1. Mean lamb weight (kg) on a weekly basis.

#### Conclusions

The supplementation of calcium peroxide has shown great potential in reducing methane emissions and increasing productivity in lambs.

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#### 64. A change in the system: implications of sheep production methods on lamb productivity

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# Application

Hogget ewes may serve as a potential model for breeding sheep to increase productivity in farming systems through minimising the amount of time spent unproductive.

#### Introduction

Sheep are seasonal breeders, bred during the ovulatory period to lamb in spring. Breeding strategies include using sheep one-to-two years of age, known as hogget ewes or ewes, over two years of age to optimise reproductive efficiency. An essential part of the lambing season is the passive transfer of colostrum from ewe to lamb (Övet, 2023). Colostrum contains non-nutrient biologically active substances, such as the immunoglobin G (IgG), linked to the development of immunity, which can only be obtained through passive transfer to the lambs (Viola et al., 2022). Both the quality and quantity of colostrum produced impact the survival and weight gain of the lamb (Agenbag et al., 2021). Breeding hogget ewes within their first year can increase productivity and profitability for some production systems (Gaskins et al., 2005) by reducing the non-productive period (Kenyon and Corner-Thomas, 2022). The aim of the study was to investigate

the productivity of lambs produced by hogget ewes versus ewes. This could influence farming parameters such as flock performance and economic sustainability.

# Materials and methods

The study used New Zealand Romney's (*n* = 76), consisting of 41 ewes, all originating from the same flock, aged between 3 and 5 years old and 35 New Zealand Romney hogget ewes, aged 1 year. The study collected data in February 2023, with animals firstly being weighed using a Rappa weigh crate with TruTest M600 load bars and a TruTest XR5000 weigh head a month prior to the expected lambing date. After each birth, individual bonding pens were used to separate the ewe from her offspring to prevent lamb theft from occurring. Lambs were tagged with an electronic identification (EID) ear tag, and lambs were weighed. Approximately 60 ml colostrum samples were collected three-tosix hours after birth before milk became present. The immunoglobulin content of colostrum was calculated by determining the specific gravity of the fresh colostrum sample using a Brix Refractometer. Lambs at four and eight weeks of age were weighed. Data was analysed using GenStat 22nd edition (VSNi) to examine the significance between age, IgG content of colostrum and daily average weight gain of lamb using a two-way T-test and a one-way ANOVA.

# Results

The prolificacy of ewes was greater, producing single lambs (n = 12/84), twins (n = 32/84), triplets (n = 36/84) and quadruplets (n = 4/84). In contrast, hogget ewes produced only single lambs (n = 29/41) and twins (n = 12/41). The IgG content of ewe colostrum (32.3 g/L) was higher (P < 0.01) than that of hogget ewe colostrum (23.7 g/L). Differences were shown in the trend of lamb weights collected in week four (Fig. 1); hogget lambs had a greater average weight (10.6 Kg) than ewe lambs (7.9 Kg). Lambs born to hoggets with high IgG levels in colostrum maintained a constant weight gain up to eight weeks of age. Lambs born to hogget ewes with low IgG levels gained the least weight within the first four weeks; however, had an accelerated weight gain in the following four weeks. This was similar to the ewe lambs that also demonstrated an accelerated weight gain after week four. However, lambs born to ewes with low IgG levels weighed more at week eight than those born from high-level IgG.



Fig. 1. Average lamb weight gain (kg) from birth to eight weeks of age with standard error of difference bars.

## Conclusion

Utilising New Zealand Romney hogget ewes showed a potential within production systems to produce a profitable system. However, does not provide the same prolificacy and thus is less efficient than ewes in this study flock. This study showed that colostrum IgG levels are not a primary influence on the weight gain of lambs; thus, other factors, such as age-related illnesses, may be present.

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## 65. Sequential minimal optimization regression to describe the dairy sheep lactation in different shape curves

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**Keywords:** Artificial intelligence; atypical curve; ewe; machine learning; milk; wood model

## Application

To understand the characteristics of ovine lactation is a valuable tool in research and at the herd level. It is useful for estimating total production in lactations with incomplete records, making management decisions, implementing and assessing genetic improvement programs, health monitoring, calculating feeding requirements, and economic-administrative aspects.

# Introduction

In the analysis of lactation characteristics in sheep, different production patterns have been identified: typical and atypical lactations (Macciotta et al., 2005). Although techniques such as mathematical modelling and the implementation of machine learning algorithms allow the determination of lactation characteristics, the variability of lactation patterns can affect the quality of fit of these techniques. For this reason, several studies have suggested that shape of lactations should be analyzed separately. The aim of the study was to evaluate the goodness of fit of the Sequential Minimal Optimization Regression algorithm (SMOreg) and a traditional empirical model in estimating the parameters of typical and atypical lactation curves.

#### Material and methods

#### Data base

A total of 1186 monthly records were used, corresponding to 156 sheep lactations from a commercial farm located in Queretaro, Mexico. Total milk yield (TMY), peak yield (PY) and time to peak yield (TPY) were calculated using the centre-day (Sargent, 1968). Descriptive analysis and outlier screening were performed, resulting in a final database of 104 lactations.

#### Mathematical modelling

Lactation curves were fitted to the incomplete gamma empirical model (Wood, 1967), with model parameters estimated using the 'nlsLM' function from the 'minpack.lm' package in the Rstudio. Estimated parameters were then used to obtain predictions for the lactation traits TMY, PY and TPY. The shape of the lactation curve was identified based on the parameters; when parameters b and c correspond to positive values (b < 0 and c < 0), a typical curve is found. The other combinations of b and c represent an atypical curve.

#### Formulation of the machine learning model

We chose to implement the Sequential Minimal Optimisation Regression (SMOreg) algorithm. SMOreg is an algorithm for efficiently solving optimization problems that arise when training a support vector machine, and is proposed to deal with regression problems. Goodness of fit and statistical analysis

Model fit was assessed using correlation coefficient ©, mean absolute error (MAE), root mean square error (RMSE), relative absolute error (RAE) and relative root mean square error (RRSE). Two-way ANOVA is used to evaluate the effect on curve shape and prediction technique. The Tukey test used to compare means at the 95% level of significance.

#### Results

It was found that 51% of the modelled curves were considered atypical. Equations (1) and (2) below show the estimated mean parameters of the Wood model for typical and atypical curves, respectively.

 $Y = (0.3001)t^{(0.5451)} exp^{-(0.0127)t}$ 

(1)

(2)

 $Y = (7.5698)t^{(-0.3962)} exp^{-(0.0001)t}$ 

According to the goodness of fit criteria, the SMOreg algorithm shows a better performance in the estimation of sheep lactation characteristics, with more significant differences in the fit for atypical curves (Table 1). The estimated values for the milk characteristics are shown in Table 2. An effect of the model used to predict TMY was observed, with the Wood model tending to overestimate this trait. For TPY, the results show no significant differences in the estimation for typical curves. However, for atypical curves, the Wood model tends to underestimate this characteristic. In this study, values with p < 0.1 were considered as trends. Therefore, a trend towards overestimation of atypical curves by the Wood model can be observed in the PY estimates.

#### Table 1

Goodness of fit of the Wood model and SMOreg algorithm for the characteristics of lactation TMY, PY, and TPY in typical and atypical shape curves of dairy sheep.

	Total Milk Yield (TMY)			Time to Peak Yield (TPY)				Peak Yield (PY)				
	Typical Atypical			Typical I		Atypical		Typical		Atypical		
	Wood	SMOreg	Wood	SMOreg	Wood	SMOreg	Wood	SMOreg	Wood	SMOreg	Wood	SMOreg
r	0.96	0.96	0.62	0.95	0.36	0.55	0.24	0.68	0.65	0.61	-0.30	0.88
MAE	10.52	8.88	29.64	10.46	19.84	15.63	19.98	8.68	0.24	0.17	6.74	0.12
RMSE (1)	13.21	11.66	62.40	13.97	31.00	20.11	27.37	14.21	0.33	0.25	30.02	0.15
RAE (%)	34.11	28.29	79.86	27.72	94.11	72.60	127.83	53.33	88.24	62.28	2716.19	48.50
RSSE (%)	35.62	31.06	143.67	31.66	129.29	81.81	142.18	71.43	101.99	78.33	9438.84	47.53

\*SMOreg: sequential minimal optimization regression, r: coefficient of correlation, MAE: mean absolute error, RMSE: root mean squared error, RAE: relative absolute error, and RRSE: relative root mean squared error.

#### Table 2

Statistical differences of lactation characteristics for typical and atypical shape curves between the actual lactation and estimated ones by the techniques of prediction (Wood model and SMOreg algorithm).

	Typical		Atypical	Atypical			p value			
	Actual	SMOreg	Wood	Actual	SMOreg	Wood	Technique	Shape	Tx*Shape	
Total milk yield (l) Time to peak yield (day) Peak yield (l)	108.2 <sup>b</sup> 39 <sup>a</sup> 0.991	108.5 <sup>b</sup> 40 <sup>a</sup> 0.999	115.1ª 45ª 0.811	95.4 <sup>b</sup> 22 <sup>b</sup> 0.93	95.8 <sup>b</sup> 21 <sup>b</sup> 0.931	123.1ª 2° 7.644	0.007 0.005 0.075	0.259 <0.001 0.105	0.169 <0.001 0.063	0.362 0.149 0.096

a,b, c Within a row, means followed by a common superscript do not differ significantly in tukey test (p<0.05).

#### **Conclusion and implications**

SMOreg is a powerful algorithm that improves predictions for atypical curves; however, it is necessary to establish techniques to identify this type of pattern within the sheep flock. Therefore, this machine learning algorithm is proposed to be used for better prediction of milk production in dairy sheep systems.

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#### 66. In vitro evaluation of cereal grains and horsebean as ingredients in compound feeds for ruminants

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#### Application

Ongoing efforts to improve feed use efficiency and reduce the environmental burden of ruminant production would benefit from characterisation on of locally available feed ingredients.

#### Introduction

Table 1

Cereal grains and their products constitute a substantial part of livestock feeds in compound feeds. Furthermore, current efforts to substitute soya in compound feeds with locally grown proteins have incentivised the production of pulses/legumes. To this end, the production of horsebean (*Vicia faba*, L.) has increased by about 4 folds in volume from 2012 to 2020 (FAO, 2022) in Northern Europe. Here, we characterized four major cereal grains (i.e., barley, maize, oats and wheat) and horsebean (Table 1) for fermentation kinetics and organic matter degradation (OMD) using the ANKOM RF wireless gas production system (ANKOM Technology, Macedon, NY, USA).

Chemical composition & fermentation parameters.									
Chem <sup>†</sup>	Barley	Maize	Oats	Wheat	Beans	S.E./P <sup>‡</sup>			
СР	101	91.0	128	158	297	-			
NDF	209	93.3	360	142	129	-			
Starch	542	643	419	571	308	-			
Ash	23.0	15.0	21.0	18.0	33.0	-			
Fermentation para	ameters estimates								
A	310 <sup>b</sup>	333 <sup>a</sup>	221 <sup>b</sup>	304 <sup>b</sup>	288 <sup>c</sup>	6.7/**			
В	9.2 <sup>b</sup>	12.0 <sup>a</sup>	7.1 <sup>c</sup>	9.4 <sup>b</sup>	11.6 <sup>a</sup>	0.55/**			
С	2.16	2.15	2.09	2.20	2.07	0.09/ns			
pН	6.17 <sup>c</sup>	6.01 <sup>d</sup>	6.43 <sup>b</sup>	6.10 <sup>c</sup>	6.53 <sup>a</sup>	0.03/**			
ME	9.73 <sup>ab</sup>	9.60 <sup>ab</sup>	8.06 <sup>c</sup>	9.88 <sup>a</sup>	10.1 <sup>a</sup>	0.10/**			
OMD,%	73.8 <sup>a</sup>	74.6 <sup>a</sup>	59.6 <sup>b</sup>	75.2 <sup>a</sup>	76.9 <sup>a</sup>	0.84/**			

 $\dagger$ Comp = composition (g/kg DM); CP=crude protein; Beans=Horsebeans; S.E./P<sup>‡</sup>=standard error /P-value; \*\*=P<0.01; ns=not significant; Means in a row with different superscripts are different at P  $\leq$  0.05.

#### Materials and methods

Gas production was carried out with 1.0 g dry matter of samples (<1.0 mm screen) using 250 mL bottles enriched with 34 mL of rumen fluid (obtained from three rumen canulated standard cows) and 66 mL of buffer solution. The experiment was run twice for 48 h, and in triplicates. After 48 h, pH was recorded, and OMD was estimated from residues. The GP data was fitted to the model of Groot et al. (1996) for calculating asymptotic gas volume (A, mL/ g DM), time in hours to produce 50% of the maximum gas volume (B, h), and a constant related to the shape of the curve ©. All parameters were analysed using Proc GLM in SAS (SAS 9.4 for windows) with significance declared at *P* < 0.05.

## Results

The ingredients differed in fermentation parameters (Table 1 & Fig. 1). Maize achieved the highest gas volume with the lowest endpoint pH and fractional rate of gas production ( $R_{gp}$ ) relative to others. Conversely, oats achieved the lowest gas volume and OMD accompanied by higher pH. For  $R_{gp}$ , the order was oats > barley, wheat > maize and horsebean (Fig. 1b). Oats had the lowest estimated metabolise energy (ME, MJ/kg DM) value probably due to the higher neutral detergent fibre (NDF) from oat hulls.



1

# Fig. 1. Cumulative (a) and fractional rate of (b) gas production

# Conclusion

The faster  $R_{gp}$  of oats may synchronise with the soluble CP from grass silages in the rumen, but oats might restrict ME supply for milk production relative to barley and wheat. The fermentation characteristics of horsebean suggested that it can be a good source of energy in addition to its CP supply.

#### Acknowledgment

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#### 67. Assessing the carry-over effect of enhanced amino acid nutrition of dairy cows in very early lactation

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#### Application

Providing a higher proportion of protein to dairy cows in the fresh period does not benefit milk protein production or nitrogen use efficiency over the first 49 days of lactation.

# Introduction

During the first two to three weeks of lactation (fresh period), dairy cows experience concurrent negative energy and negative protein balance, where dietary intakes do not meet maintenance and production requirements. Consequently, amino acids are mobilised from body tissue reserves and utilised as a substrate for milk protein synthesis. Providing supplemental amino acids during the fresh period, via abomasal infusions of casein, can elicit a sustained, positive effect on milk yield, with this effect potentially persisting into later lactation (Larsen et al., 2014). Therefore, the primary aim of this experiment was to translate these previously reported effects into practical feeding strategies that could be implemented on commercial dairy farms, using UK-representative diets. It was hypthesised that targeted protein supplementation in the first 14 days of lactation would be more beneficial for milk protein yield and nitrogen use efficiency (NUE) compared with supplementing the same amount of protein evenly over the first 49 days of lactation.

# Material and methods

Thirty-six multiparous Holstein-Friesian cows (blocked into two cohorts of eighteen) were paired according to calving date, and within pair, allocated at random to one of two dietary treatments at calving. Dietary treatments were provided for the first 49 days of lactation as total mixed rations (TMRs) and were formulated to provide the same total quantity of CP based on predictions of dry matter intake. Treatment A (control) was formulated to contain 172 g CP/kg DM. Treatment B consisted of two separate TMRs, formulated to contain 222 g CP/kg DM, provided for days 1 to 14 of lactation, and 156 g CP/kg DM, for days 15 to 49 of lactation.

Individual dry matter intake, milk yield and composition were recorded, and blood metabolic profiling was performed (albumin, globulins, total protein, urea, NEFA, BOHB and glucose). Body composition was assessed by measurement of liveweight and body condition score (BCS) and ultrasound scanning was used to estimate the mobilisation of muscle and fat depth from prepartum through to days 14 and 49 of lactation. Data were analysed using linear mixed models, with treatment as a fixed effect, and individual cow and days in milk as random effects.

## Results

Treatment had no effect on average NUE between Treatment A (0.40) and Treatment B (0.39; P = 0.45) or milk protein yield (1.26 versus 1.20; kg/day; P = 0.19) over the first 49 days of lactation. Treatment had no effect on dry matter intake (kg/d; P = 0.43), milk fat yield (kg/day; P = 0.28) and percentage (P = 0.85), milk protein percentage (P = 0.09) or blood composition (P > 0.05 for all parameters assessed). Liveweight and BCS were unaffected by treatment (P = 0.20 and P = 0.35, respectively). There was no effect of treatment on the change in eye muscle depth from the prepartum measurement to day 49 (P = 0.09).

## Conclusions

Offering a greater quantity of CP to early lactation cows during the first 14 days of lactation did not elicit beneficial effects on milk production or composition, or NUE, compared with distributing the same total quantity of CP evenly over the first 49 days of lactation.

## Acknowledgements

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# 68. Effect of supplementation of amino acids on the post-pubertal growth, first lactation performance, and survival of Holstein Friesian dairy heifers

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# Application

The addition of supplemental amino acids (AA), methionine (Met), and lysine (Lys) increases the post-pubertal growth, first lactation milk yield, and survival Holstein Friesian dairy heifers offered an isoenergetic and iso-effective rumen degradable protein (ERDP) diet.

## Introduction

The loss and calving age of dairy heifers are far greater in the UK compared with Europe and the USA (Boulton et al., 2015). Supplementation of AA acids to dairy heifers has received little research attention. This study aimed to assess the effect of AA supplementation on post-pubertal growth and first-lactation milk yield, fertility, feed efficiency, and survival rates of dairy heifers.

## Materials and methods

A total of 76 pubertal heifers, born at the University of Nottingham Centre of Dairy Science (CDSI), were selected at random and allocated at 12 to 12.5 mo. Of age according to birth date and live weight (LW) to be supplemented (+AA) and not supplemented with AA (-AA) Met and Lys for three months. Heifers were offered a perennial grass and maize silage (50:50 DM) based diet, with 2.5 kg/hd/d of supplement that

resulted in an isoenergetic and iso-effective rumen degradable protein diet of enhanced and typical AA concentration. The LW, age at first calving, fertility, milk yield (MY), feed efficiency (FE), and survival were measured from 12 mo. Of age to the end of first lactation.

## Statistical analysis

Data was collated in Excel. The somatic cell count (SSC) was  $Log^{base10}$  transformed, and data was assessed for normality of distribution using Minitab 17.0. The normally distributed data was assessed using ANOVA GLM, while the pregnancy rate was assessed by Chi-square analysis, with -AA and +AA as a fixed effect in the model. Data was reported as means (±SE) with P values and significant differences between means were reported at a P < 0.05.

# Results

Mean LW gain between 12 and 15 mo. And first lactation milk, ECM, FPCM, fat, protein, and solids yield and survival rates were greater for heifers offered +AA, compared with -AA (Table 1). The mean LW gain between 16 and 24 mo., mature LW and age at first calving, pregnancy rate (PR), calving interval (CI), and feed efficiency (FE) did not between heifers offered +AA and -AA.

#### Table 1

Mean growth, lactation performance, and survival rates ( $\pm$ se), of dairy heifers offered post-pubertal diets with (+AA) and without (-AA) supplemented amino acids between 12 and 15 mo. of age.

	+AA	- <b>AA</b>	P Value
Obs., No.	36	36	-
Weight gain 12 to 15 mo., kg/d	1.20 (0.284)	1.00 (0.272)	0.012
Proportion of maturity at parturition, % <sup>1</sup>	86 (1.4)	84 (1.4)	0.206
Age at first calving, mo.	23.7 (0.25)	23.4 (0.26)	0.418
Milk yield, kg/d	35.7 (0.95)	32.8 (0.95)	0.038
ECM yield, kg/d <sup>2</sup>	40.2 (0.37)	38.7 (0.39)	0.003
FPCM yield, kg/d <sup>3</sup>	36.7 (0.79)	34.0 (0.79)	0.016
Milk fat, g/kg	41.3 (0.11)	41.6 (0.11)	0.030
Milk protein, g/kg	35.8 (0.06)	36.0 (0.06)	0.764
Log <sup>base10</sup> SCC, 000 cells/ml	1.6 (0.58)	1.5 (0.48)	0.539
Milk fat, kg/d	1.46 (0.034)	1.35 (0.034)	0.030
Milk protein, kg/d	1.27 (0.031)	1.18 (0.031)	0.042
Milk solids, kg/d	2.72 (0.058)	2.52 (0.058)	0.015
Pregnancy rate, %	87 (6.3)	83 (6.9)	0.345
Calving interval, d	365 (8.5)	354 (8.5)	0.391
Feed efficiency, kg DMI/ kg FPCM yield	0.69 (0.019)	0.70 (0.019)	0.553
Survival, 12 mo. To end of first lactation, %	81	73	0.049

<sup>1 –</sup> Proportion of maturity at parturition was calculated from the live weight / mature weight of  $\geq$  third lactation cows in the closed herd into which the heifers were born. <sup>2 –</sup> ECM – Energy corrected yield, kg=0.327×milk yield kg+12.95×milk fat kg+7.21 x milk protein kg (Sjaunja et al. 1990) <sup>3</sup> – FPCM = Milk kg×((0.1226×Fat %)+(0.0776× Protein %)+0.2534) (Gerber et al. 2010).

## Conclusions

The LW gain from 16 to 24 mo., mature LW and age at first calving, and first lactation PR, CI, and FE did not differ between heifers offered +AA and –AA. However, the addition of AA (Met and Lys) increased LW gain between 12 and 15 mo. And increased first lactation milk, ECM, FPCM, fat, protein, and solids yields, and survival rates. AA supplementation of dairy heifers warrants further study.

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# 69. Effect of essential oils when supplied in feed or via a slow-release rumen bolus on the health and performance of high yielding dairy cows in early lactation

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## Application

The inclusion of essential oils in the feed increases milk yield and milk protein content, whilst the provision via a slow-release rumen bolus decreases DM intake and milk fat content, and increases food conversion efficiency in early lactation, high yielding dairy cows. Essential oils do not decrease the risk of ketosis when blood ketone concentrations are already low.

#### Introduction

Ketosis is a major health problem in the dairy industry, with the prevalence of subclinical ketosis being as high as 28.5% in early lactation in UK herds (Macrae et al., 2019). The development of this disease can cause health, production, and welfare problems in cows, as well as having a substantial negative economic impact (Cainzos et al., 2022). Essential oils are plant-derived bioactive compounds containing volatile compounds that can beneficially manipulate rumen fermentation, reducing the risk of ketosis and increasing performance (Da Silva et al., 2019). Providing essential oils in a slow-release rumen bolus may reduce the potential loss of volatiles and is an easier means to administer to extensively grazing ruminants. There have however been no studies undertaken to determine the effect of essential oils when included in a slow-release rumen bolus or in the diet in 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 performance parameters and blood ketones in dairy cows in early lactation.

#### Materials and methods

Forty-Five multiparous Holstein Friesian dairy cows that were (mean  $\pm$  SE) 11  $\pm$  0.6 d post calving, yielding 38  $\pm$  0.9 kg/d and weighing 697 ( $\pm$ 10.7) kg were randomly allocated to one of three dietary treatments: C: basal total mixed ration (TMR; containing on a DM basis: 0.33 maize silage, 0.21 grass silage, and 0.46 concentrates), TO: basal TMR + essential oil (Agolin, Switzerland fed at 0.9 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, with individual intake measured daily using roughage intake control feeders, milk yield recorded daily, and milk samples collected weekly for analysis by National Milk Laboratories, Wolverhampton, UK. Live weight (LW) and body condition score (BCS) were recorded fortnightly, and blood plasma samples taken via the jugular vein at 1100 h during weeks 0, 2, 4, 6, 8 and 10 for subsequent analysis of  $\beta$ -hydroxybutyrate (BHB), and non-esterified fatty acids (NEFA). The data was analysed by Reml using time, treatment, previous 305-day lactation yield, lactation number (2 and 3+), and days post calving as fixed effects, and cow as a random effect using Genstat (v. 22). The results are provided as the least square mean (LSM) and standard error of the difference (SED). P values  $\leq$  0.05 were considered as significant, and P  $\leq$  0.10 as a tendency.

# Results

The DM intake was 1.3 kg/d lower in cows receiving BO compared to C or TO (P < 0.001; Table 1). Milk yield was 1.1 kg/d higher (P < 0.001) in cows receiving TO than C, with those receiving BO being intermediate. Mean milk fat concentration was 35.2 g/kg and was lower (P < 0.001) in cows fed BO than C, with TO being intermediate. Milk protein content was highest in cows fed TO compared to either C or BO (P < 0.001). There was no effect of treatment on BCS (P > 0.05), however, LW was lower (P < 0.001) in cows on BO compared to TO or C. The feed correction efficiency (FCE) was highest in cows fed BO compared to either C or TO (P < 0.001). There was no effect of treatment on plasma BHB or NEFA concentrations (P > 0.05), with mean values of 0.522 and 0.222 mmol/l respectively.

#### Table 1

Performance of dairy cows fed either a control diet (C), essential oils in the TMR (TO) or essential oils in a slow-release rumen bolus (BO).

	T	reatme	nt		Signific	cance
	С	BO	то	SED	Treatment	Time
DMI (kg/d)	24.6 <sup>b</sup>	23.3ª	24.5 <sup>b</sup>	0.315	<0.001	<0.001
Milk yield (kg/d)	48.9 <sup>a</sup>	49.7 <sup>ab</sup>	50.0 <sup>b</sup>	0.498	<0.001	<0.001
Milk Fat (g/kg)	35.9 <sup>b</sup>	34.3ª	35.4 <sup>ab</sup>	0.960	<0.001	<0.001
Milk Protein (g/kg)	30.7ª	30.3 <sup>a</sup>	31.1 <sup>b</sup>	0.225	<0.001	<0.001
BCS (1-5 scale)	3.09	3.09	3.07	0.049	0.576	0.489
LW (kg)	666 <sup>b</sup>	655ª	673 <sup>b</sup>	4.02	<0.001	0.009
FCE (kg milk/kg DMI)	1.99 <sup>a</sup>	2.17 <sup>b</sup>	2.04 <sup>a</sup>	0.030	<0.001	0.630
Plasma BHB (mmol/l)	0.527	0.562	0.477	0.062	0.241	0.382
Plasma NEFA	0 225	0.210	0.221	0.046	0 770	0.021
(mmol/l)	0.230	0.210	0.221	0.046	0.779	0.921

a-b Means within a row with different superscripts differ (P<0.05). There was no treat x time interaction.

#### Conclusions

Feeding essentials oils in the feed increased milk yield and milk protein content but had no effect on live weight. In contrast, providing essential oils in a slow-release rumen bolus decreased DM intake and milk fat content, and resulted in a lower LW and higher FCE. The provision of essentials oils in the feed or in a bolus did not affect plasma ketone concentrations, although concentrations were low throughout the study across all treatments.

## Acknowledgements

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#### 70. Total tract digestibility and energy utilisation of phenotypically low and high methane intensity dairy cows

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## Application

Long-term methane mitigation strategy by selecting cows with low methane intensity (g CH<sub>4</sub> per kg of energy-corrected milk; ECM).

#### Introduction

Enteric CH<sub>4</sub> from ruminant livestock contributes to 32% of global anthropogenic CH<sub>4</sub> emissions (FAO, 2023) and represents a loss of gross energy intake of 6% on average (Niu et al., 2018). Therefore, reducing CH<sub>4</sub> could theoretically improve animal performance, but recent knowledge suggests otherwise that phenotypically low enteric CH<sub>4</sub> is related to less efficient fibre digestion. A promising strategy to mitigate CH<sub>4</sub> is the selective breeding for low enteric CH<sub>4</sub> intensity cows, i.e., cows low in enteric CH<sub>4</sub> production per kg of milk or energycorrected milk (ECM). In theory, efficient cows require less feed than average and can be expected to produce less CH<sub>4</sub> per unit product at similar level of production (Waghorn and Hegarty, 2011). However, it is unclear whether divergence in CH<sub>4</sub> intensity phenotype is indicative of efficiency in digestion and energy utilization, particularly because individual animals within a species and breed differ in CH<sub>4</sub> production for reasons that cannot be explained by feed intake, the main determinant of CH<sub>4</sub> production. Therefore, the current study aimed to evaluate the total tract digestibility and energy utilization between phenotypically low- and high- CH<sub>4</sub>-intensity dairy cows.

## Materials and methods

Sixteen lactating Norwegian Red dairy cows in their first to fourth lactation (9 primiparous and 7 multiparous) were used in a continuous experiment consisting of 11 days of adaptation and 6 days of data and sample collection (day 12 to 17), including total faces and urine collection for 3 days (day 15 to 17). Cows averaged (mean  $\pm$  SD) 251  $\pm$  30 days in milk, 638  $\pm$  86 kg body weight, and 18  $\pm$  4 kg of daily milk production at the beginning of the experiment. The experiment was conducted in two experimental runs with 8 animals per block. Cows were offered grass silage and concentrate at a proportion of 83:17 on a DM basis during the whole experiment. Feed intake and feed residues were recorded daily and sampled for wet chemical analysis. Gas exchange was estimated using GreenFeed (C-Lock, Inc., South Dakota, USA) over 6 days during data and sample collection with 5-6 hours interval to achieve all hours covering day and night measurement. Cows were milked daily, and samples were analysed for milk composition. According to the CH<sub>4</sub> production expressed per unit of ECM in the experiment, cows were grouped into low- [LM; n = 8; CH<sub>4</sub> intensity of 21  $\pm$  1 g CH<sub>4</sub>/kg ECM] and high- [HM; n = 8; 28  $\pm$  1 g/kg] CH<sub>4</sub> emitters. Linear mixed-effect model from the *nlme* package in R was used to test the differences between the two phenotypes on total tract nutrient digestibility and energy utilization. The model included phenotypes (LM and HM) as fixed effects and individual animal and parity (primiparous and multiparous) as random effects. Inclusion of parity as random factor in the model did not change effect was stated if P < 0.05, or tendency if 0.05 < P < 0.10.

# Results

Total-tract digestibility of DM, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were not statistically different between groups (data not shown, P > 0.1), while the digestibility of NDF and ADF were numerically higher in LM (74% and 71%, respectively) as compared with HM (72% and 69%). The gross energy intake, and energy loss in the faeces, urine, CH<sub>4</sub>, heat, and metabolizable energy intake were not different between the two phenotypes (P > 0.1). However, LM cows were observed to retain more energy for milk production as compared to HM cows (66 and 49 MJ/d, P < 0.05), while there was no difference in energy retention for body mass (P > 0.1).

#### Conclusions

Differences in phenotypes could not be explained by differences in apparent nutrient digestibility but in energy utilization. Cows have similar metabolizable energy available, but cows with lower  $CH_4$  intensity utilize the energy to a greater extent for producing milk. Despite the metabolism of the host animal, phenotypes could potentially be explained by distinct microbiomes or microbial activity, whose analyses are underway.

#### Acknowledgement

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#### 71. Effects of dietary protein levels on ammonia emissions from slurry of dairy cows

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#### Application

Low protein diets can reduce ammonia emissions from slurry of dairy cows.

#### Introduction

Cattle production is a considerable source of nitrogen (N) polution. Dietary manipulation of protein levels is an effective measure to reduce N excretion and slurry ammonia emissions in livestock production. The objective of the current study was to examine the impact of crude protein (CP) level in the diet on ammonia emissions from slurry of dairy cows.

#### Materials and methods

Slurry ammonia emission data used were obtained from a 28-day slurry incubation experiment conducted at the Agri-Food and Biosciences Institute. Slurry samples were collected during a digestibility trial involving 12 lactating dairy cows which were offered TMR diets comprising grass silage and concentrates in a 0.5/0.5 ratio (DM basis). Three diets differing in CP content (14.0, 15.5 or 17.0% CP, DM basis) were offered (4 cows/diet, for details please see Craig et al., 2024). Faeces and urine (no acid added) from a single sample day were collected separately from each cow and samples stored at 4 °C for 6 days. A15 kg slurry sample was then created for each cow by mixing faeces and urine (in a 30 l bucket) in the same proportion (by weight) that they were excreted in on the designed sampling day. Buckets were then stored in a controlled temperature chamber at 14 °C, with a lid covering approximately ¼ of the surface of the bucket. Ammonia emission measurements were carried out at 0, 3, 6, 24, 48, 72 and 96 h following mixing, and subsequently at weeks 1, 2, 3 and 4. Prior to each measurement time the lid was placed on each bucket for 1 h before the measurement of air ammonia concentration in the head space using a photoacoustic gas monitor. The data were analysed using GenStat (21st ed., VSNI Ltd).

#### Results

Ammonia production increased rapidly following mixing of manure and urine, peaking at 24 h with the low CP diet and at 72 h with the medium and high CP diets, before decreasing gradually until the end of the measurement period (Fig. 1). Each increase in dietary CP level increased ammonia emissions during the experiment period (Fig. 1), except for the measurements in week 3 between the 2 high CP diets. Reducing dietary CP levels from 17.0% to 15.5% and from 17% to 14.0% reduced ammonia emissions by an average of 41% (range from 36–44%) and 64% (range from 61–68%), respectively. Similarly, reducing diet CP from 15.5% to 14.0% reduced ammonia emissions by 38% (range from 33–40%).



Fig. 1. Ammonia emission patterns from slurry of dairy cows offered diets containing different CP levels.

# Conclusions

Reducing dietary CP levels is an effective mitigation strategy to reduce ammonia emissions from dairy production systems. The impacts of lower protein diets on dairy cow performance and welfare/health are currently being examined within this project.

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## 72. Effect of offering concentrates with differing human edible fractions on dairy cow performance

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# Application

The human edible fraction of the diet of mid lactation dairy cow can be successfully reduced without negatively impacting on energy corrected milk yield.

## Introduction

A growing world population and unavailability of arable land for agricultural expansion may threaten global food security in the coming decades. It has been suggested that making greater use of human inedible feeds in dairy cow rations would release more land for human food production and also reduce food wastage. A previous study by Münnich et al. (2018) examined the impact of substituting individual grains with by-product feeds on milk production. The current study was designed to examine the effects of replacing cereal grains and soyabean meal with by-product feeds on milk production and feed efficiency of dairy cows offered a grass silage based diet.

## Materials and methods

A two period changeover design study using 20 mid lactation cows (mean of 157 days calved) examined the effects of offering concentrate containing a high proportion of human edible ingredients (HE) or human inedible ingredients (HI). Both concentrates were formulated to be isoenergetic and isonitrogenous, while differing in their ingredient composition. HE contained significant quantities of cereals and soyabean meal in HI were replaced with by-product feeds such as rapeseed meal and distillers dark grains. A common grass silage (mean DM, 375 g/kg; CP, 126 g/kgDM, metabolizable energy, 11.5 MJ/kgDM) was offered throughout the study as a partial mixed ration comprising 63 % grass silage and 37 % concentrate on a dry matter (DM) basis. Cows also received 0.25 kg/day of a commercial concentrate at each milking. Feed intakes, milk yields, milk composition and liveweights were recorded during the fourth week of each period. The human edible fraction of feeds was calculated using the methodology described by Wilkinson (2011). Feed conversion efficiency (FCE) was calculated for each treatment as kg energy corrected milk yield (ECM) per kg DM intake (DMI). Nitrogen (N) use efficiency (NUE) was estimated via milk N output as a proportion of total N intake. Edible feed conversion ratio (eFCR) was defined as human edible food output as a proportion of human edible fractions described by Ertl et al. (2015) under current standard extraction allowances.

## Results

The human edible fraction of HE and HI were estimated to be 0.54 and 0.18 respectively. Cows offered HI had significantly higher total DMI than animals offered HE (P < 0.05). There were no significant differences between the treatments in terms of ECM yield or liveweight. However, animals offered HE produced milk with a higher milk fat and protein concentration than cows offered HI (P < 0.001). While FCE and NUE were similar for both treatments, eFCR (energy) and NFP (protein) was higher among cows offered HI (P < 0.001) (See Table 1).

#### Table 1

Performance of cows offered concentrates high in either human edible or human inedible based ingredients.

	Human edible concentrate	Human inedible concentrate	SED	P-value
Total DMI (kg/day)	19.9	20.6	0.21	P<0.05
ECM yield (kg/day)	32.8	32.8	0.38	0.926
Milk fat (g/kg)	48.2	46.1	0.51	P<0.001
Milk protein (g/kg)	36.3	34.8	0.24	P<0.001
Liveweight (kg)	640	643	3.6	0.461
FCE	1.65	1.61	0.026	0.149
NUE	0.35	0.34	0.006	0.119
eFCR (energy)	1.26	3.36	0.011	P<0.001
NFP (protein) (g/cow/day)	352	701	13.3	P<0.001

# Conclusions

Mid lactation cows offered concentrates high in human inedible ingredients had similar ECM yield as cows consuming concentrates high in human edible ingredients. Human edible feed conversion efficiency improved with increased inclusion of human inedible ingredients in the concentrate.

# Acknowledgments

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

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## 73. Concentrate feed based on ammoniated barley can replace soymeal in dairy cow diets

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## Application

The use of ammoniated cereal grains can replace soymeal in lactating dairy cow diets allowing minimal recourse to imported ingredients.

## Introduction

Cereal grains and their byproducts are ideal sources of energy for milk production. But at higher inclusion levels, the rapidly fermentable carbohydrates of these products pose risks for increased ruminal disorders (e.g., acidosis). Alkalisation (or ammoniation) of grains is expected to increase protein, modulate rumen pH with improved buffering capacity, and improve fibre digestibility due to conducive rumen pH for microbes. Such a product currently being used and tested under different conditions is alkalised barley containing Home n' Dry pellets (Alkagrain<sup>®</sup>, Alkasystems; Dugdale Nutrition, LAN, UK). We tested the interactive effects of replacing alkalised barley for soymeal in concentrates for dairy cows fed two contrasting silages differing on organic matter digestibility (**OMD**) on feed intake and milk yield.

## Materials and methods

Forty lactating Norwegian Red dairy cows of mixed parity were allocated to four groups based on parity and initial milk yield. The groups were randomly assigned to one of the four dietary groups using a  $2 \times 2$  factorial design [(2 concentrates: alkalised barley based = **ABB**; and soymeal based = **SMB**); and 2 silage qualities: early cut = **high-OMD** vs. late cut = **low-OMD**)]. For concentrates, ABB replaced SMB quantitatively (i.e., w/w), whereas the low-OMD cows received more of the concentrates ( $\simeq 1.2 \text{ kg DM/d}$ ) in compensation for the silage quality. The trial lasted for 63 days: 21 d of adaptation and 42 d of data collection (intake, milk yield and milk composition). Energy corrected milk (**ECM**) was calculated using milk yield and its composition (Sjaunja et al., 1991). The recorded data were compared using repeated measures ANOVA of Proc Mixed in SAS (SAS 9.4 for windows) with autoregressive (AR1) covariance structure. Statistical significance was declared at *P* < 0.05.

## Results

No concentrate by grass silage interaction effects were observed except for starch intake (Table 1). Intake of silage and total dry matter did not differ between the concentrate groups. However, silage intake was marginally lower (P = 0.08), and concentrate intake was significantly higher (P < 0.001) for the low-OMD. Milk yield and ECM were not affected by concentrate type, but cows fed low-OMD silage produced lower (P = 0.045) ECM than cows fed high-OMD.

## Table 1

Feed chemical composition, feed intake and performance.

	Concenti	rate	Grass silages		Effects o	f (P - values)		
Feed composition	ABB	SMB	low-OMD	high-OMD	s.e.	Concentrate	Grass silage	Conc. X silage
Dry matter, g/kg fresh	873	875	273	225	-	-	-	-
Crude protein, g/kg DM	171	172	119	151	-	-	-	-
Starch, g/kg DM	495	391	-	-	-	-	-	-
NDF, g/kg DM	168	203	519	467	-	-	-	-
Intake; kg DM/day								
Grass Silage	12.3	12.3	11.9	12.7	0.31	ns	0.08	ns
Concentrate	7.5	7.8	8.3	7.1	0.13	ns	< 0.001	ns
Total dry matter	19.8	20.1	20.1	19.7	0.35	ns	ns	ns

(continued on next page)

Table 1 (continued)

	Concentra	ate	Grass silage	S	Effects of	(P – values)		
Starch	3.72	3.07	3.66	3.13	0.08	<0.001	<0.001	0.031
Crude protein	3.03	3.07	2.91	3.20	0.07	<0.001	ns	ns
Milk yield and ECM; kg/day								
Milk yield	26.6	26.7	25.9	27.5	1.03	ns	ns	ns
ECM	29.9	29.7	28.7	30.9	1.07	ns	0.045	ns

s.e.=standard error of the interaction term; NDF=neutral detergent fibre; ns=not significant; Conc. X silage=interaction between concentrate & silage.

#### Conclusion

Ammoniated barley can replace soymeal in concentrates without effects on feed intake, milk yield and ECM. The lower ECM on low-OMD silage, even with more of the concentrates, highlight the importance silage quality.

#### Acknowledgment

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## 74. Effect of offering rapeseed-based diets differing in crude protein content on dairy cow performance

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#### Application

Soya can be sucessfully replaced with rapeseed meal (RSM) in dairy cow diets.

#### Introduction

Reducing CP levels in dairy cow diets can improve nitrogen (N) use efficiency (defined as N output in milk/N input; NUE), and reduce N loss to the environment. A total diet CP level of between 15 and 16% has been suggested as optimal to maintain milk production while improving NUE (Barros et al., 2017; Lavery et al., 2022). Alongside environmental pressures to reduce diet CP levels, there is increasing retailer and societal pressure to remove soybean meal (SBM) from dairy cow diets. Therefore, the current study examined the impact on cow performance and NUE of replacing SBM with rapeseed meal (RSM) in diets differing in CP levels.

# Materials and methods

A continuous design study using 88 Holstein dairy cows (28 primiparous) examined three RSM based diets containing either 14% (R140), 15.5% (R155) or 17% (R170) CP ((dry matter (DM) basis). A fourth 'conventional diet' containing soya had a total diet CP (DM basis) of 15.5% (C155). Diets R140, R155, R170 and C155 supplied 93, 98 103 And 106% of metabolise protein (MP-N) requirements, respectively. Cows were enrolled post-calving and remained on treatment until day 252 of lactation. A common silage (mean DM, 307 g/kg; CP, 119 g/kg DM); metabolise energy, 11.2 MJ/kg DM) was offered across all treatments throughout the study. Concentrates offered were isoenergetic, while differing in their ingredient composition. Silage and concentrates were offered in the form of a total mixed ration (50:50 DM basis). Blood samples were taken at weeks 4, 8, 12, 16, 20, 28 and 36 post-calving. The weekly means of individual cow dry matter intakes (DMI) and performance data were analysed using REML, with cow as the experimental unit and week as the repeated measure (GenStat 21st ed., VSNI Ltd).

#### Results

Within the RSM diets, DMI, milk protein content, fat plus protein yield, milk urea and plasma urea content increased with increased CP content (P < 0.001; Table 1). Compared to the other two RSM diets, milk yield (P < 0.001) was increased with R170, while milk fat (P = 0.002) was increased with R140. Compared to the RSM diets, DMI was higher with C155 (P < 0.001), while milk yield and fat plus protein yield (P < 0.001) with C155 did not differ from R170. Milk fat content with C155 was not different from R140, while milk protein with C155 was not different from R140 and R155. Milk urea and plasma urea content of C155 was intermediate between R155 and R170 (P < 0.001). Plasma  $\beta$ -hydroxybutyrate concentrations were greater in C155 compared to the RSM treatments. Bodyweight and condition score were unaffected by treatment. Compared to C155, NUE was greater with the RSM treatments, with R140 having the greatest NUE (0.36).

#### Conclusions

There was a positive production response to increasing levels of total diet CP with the RSM diets, reflecting an undersupply of MP-N with R140 and R155. Soya-bean meal can be removed from diets with no loss in performance.

# Table 1 Performance of cows offered either rapeseed-based diets differing in crude protein content or a conventional diet.

						P-Value		
	R140	R155	R170	C155	SED	Trt (T)	Week (W)	$(T \times W)$
Total DMI (kg/d)	21.2 <sup>a</sup>	22.0 <sup>b</sup>	22.9 <sup>c</sup>	23.5 <sup>d</sup>	0.28	<0.001	<0.001	0.318
Milk yield (kg/d)	31.5 <sup>a</sup>	32.7 <sup>a</sup>	34.8 <sup>b</sup>	34.4 <sup>b</sup>	0.81	< 0.001	< 0.001	0.573
Fat (g/kg)	49.8 <sup>b</sup>	48.4 <sup>a</sup>	48.6 <sup>a</sup>	49.8 <sup>b</sup>	0.46	0.002	< 0.001	0.999
Protein (g/kg)	34.7 <sup>a</sup>	35.8 <sup>b</sup>	36.6 <sup>c</sup>	35.1 <sup>ab</sup>	0.36	< 0.001	< 0.001	0.131
Fat + protein yield (kg/d)	2.64 <sup>a</sup>	2.75 <sup>b</sup>	2.94 <sup>c</sup>	2.91 <sup>c</sup>	0.054	< 0.001	< 0.001	0.987
Milk urea (mg/kg)	74 <sup>a</sup>	86 <sup>b</sup>	114 <sup>d</sup>	97 <sup>c</sup>	1.9	< 0.001	0.218	< 0.001
Plasma βHB (mmol/L)	0.52 <sup>a</sup>	0.49 <sup>a</sup>	0.49 <sup>a</sup>	0.58 <sup>b</sup>	0.025	< 0.001	< 0.001	0.279
Plasma NEFA (mmol/L)	0.21	0.22	0.24	0.21	0.016	0.160	< 0.001	0.316
Plasma urea (mmol/L)	2.22ª	2.68 <sup>b</sup>	3.78 <sup>d</sup>	3.00 <sup>c</sup>	0.091	< 0.001	< 0.001	0.206
Milk N/N intake (NUE)	0.36 <sup>c</sup>	0.34 <sup>b</sup>	0.34 <sup>b</sup>	0.33ª	0.004	<0.001	<0.001	0.684

DMI; dry matter intake, NEFA; non-esterified fatty acids, βHB; β-hydroxybutyrate, NUE; nitrogen use efficiency.

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# 75. Adoption of science on farms and behaviour change

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The adoption of science on farms and behaviour change in agriculture can face various barriers and common challenges however, key is to understand these to put into place potential strategies to influence positive change.

Some barriers to the adoption of science on farms include limited access to the scientific information and lack of training and education. With this, it is important to increase communication and accessibility to scientific information in a tailored way to suit the needs of farmers such as workshops or practical hand-on learning. There may also be some financial or infrastructure resource constraints stopping farmers adopt science, which financial incentives, subsidies, or grants in providing access to the new scientific technology could make more affordable to the farmer.

Despite highlighting the most up-to-date scientific evidence and financially assisting the adoption of science on farm, there can still be resistance to change away from traditional practices. Showcasing real life practical stories by engaging early adopters can help to demonstrate the success of implementing new science. Key for agricultural workers and vets is the practical demonstration of new technologies on farm. However, there is also the social science of behaviour change, where it is comfortable to continue to do what is always done. Lack of motivation, fear of failure, time constraints, resistance to external influence and ineffective communication all have an influence on behaviour change. On farms, some of the methods used are consistently repeating the same message so that farmers see the change actually as a normal practice, continuing to highlight the economic and time saving benefits of change and bringing farmers together to talk about the success of new technologies and how the work put into change has paid off with long term efficiency gains. This message must be consistent across all those that have an influence on farm.

In all cases, a collaborative approach involving farmers, researchers, policymakers, and local communities for the successful adoption of science on farms and behaviour change in agriculture.

This session touches on many of these points including sustainability, profitability and behaviour change. The talks investigate using data driven tools to enhance farm sustainability, examine farm profitability and impacts of seasonality, and look at behaviour change on farm through surveys and examining how vets and farmers can work better together.

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## 76. Can adopting seasonal grazing to protect grassland degradation also conserve the microbial communities?

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# Application

Seasonal grazing (SG) is applied to protect the grassland degradation. We aimed to study the microbial community under seasonal vak grazing to study how the changes in grazing regime would impact the diversity and composition of the microbial community.

# Introduction

Grasslands cover over 40% of the terrestrial ecosystem and are among the most vulnerable ecosystems due to climate change and anthropogenic activities (Liu & Lu, 2021). Livestock grazing is the largest land use of the grassland (Yao et al., 2023). Grazing changes the soil's physical and chemical properties by trampling, removing plant biomass, and the addition of feces and urine. To protect the grassland ecosystem, several strategies are adopted such as stripped grazing, separating paddocks for different seasons, and adjusting the stocking rate to provide rest to the grassland for recovery (Khalil et al., 2019). Soil microbial communities are an important component of the ecosystem that runs the biogeochemical cycle and keeps the nutrient balance. The changes in the environment (such as rainfall and temperature, animal activity) and physiochemical properties of soil (such as pH, soil carbon, nitrogen, etc.,) can highly influence the diversity, composition, and functioning of soil microbial community (Yao et al., 2023).

# Aim and methodology

Seasonal yak grazing has been applied in the alpine meadows along with two other grazing regimes including all-year grazing (A-YG) and no grazing (NG), at Magu Research Station of Lanzhou University in Magu County, Gansu Province, China (33.96°N, 101.83°E; 3500 m a.s.l.) since 2016. We sample the soil (composed of five cores) during the peak growing season from each season's grazing pastures (three replicates). The bacterial and fungal communities' analyses were carried out by standard protocols of Majorbio Bio-Pharm Technology Co. Ltd., (Shanghai, China) by using 338F and 806R primers for bacteria and ITS1 and ITS2 primers for fungi. The online platform of Majorbio Bio-Pharm Technology was used to determine the microbial alpha diversity (Shannon index) and beta diversity via Principal Co-ordinates Analysis (PcoA) using the Bray-Curtis dissimilarity matrix.

# Results

The bacterial and fungal alpha diversity (Shannon) was observed to be higher at A-YG and NG than SG. The SG decreased the alpha diversity of microbes in alpine meadows but the change was not significant (Fig. 1a & b). The grazing changed the beta diversity of both bacteria and fungi (p < 0.01) (Fig. 1c & d). The bacterial and fungal bacterial communities of summer grazing were clustered separately in PcoA. The composition of bacterial and fungal phyla had a different response to the grazing. The bacterial phyla did not show significant variation



Adopting Seasonal Grazing For Grassland Ecology Protection: A Microbial Perspective

Fig. 1. Effect of seasonal, all-year, and no grazing on the bacterial and fungal alpha diversity (a-b), beta diversity (c-d), and community composition (e-f) in alpine meadows.

in response to the different grazing practices (Fig. 1e). The differential biomarker taxa in the bacterial community were 131 with the highest in A-YG (39) followed by autumn (26), summer (24), winter (17), NG (14), and spring (11). On the other hand, the fungal community composition was highly affected by the changes in grazing. The Ascomycota were the highest in soil grazed during summer and lowest during the winter season. Basidiomycota, on the other hand, was highest in winter and lowest in summer and all-year grazing pasture (Fig. 1f). In the fungal community, 89 differential biomarkers texa were observed, with the highest in NG (31) followed by autumn (25), A-YG (13), summer (12), spring (5), and winter (3). Livestock grazing changes the soil chemistry and plant community resulting in changes in the microbial richness and diversity in soil (Ma et al., 2019). Warm and cold grazing both change the composition of bacteria and fungi differently as different taxa respond differently to changes in environment resulting in different diversity responses during different seasons of grazing (Zhang & Fu, 2021). Livestock grazing has a significant effect on the beta diversity of bacteria and fungi than the alpha diversity (Jing et al., 2023). Ma et al. (2022) reported that seasonal grazing has no significant effect on the alpha diversity of bacteria and fungi and the overall ecosystem.

#### Conclusion

Based on the findings, we conclude that A-YG increased the diversity which can change the overall functionality of the grassland ecosystem. SG also alters the microbial community significantly and might lead to changes in the ecosystem and loss of biodiversity. Other studies support that seasonal grazing might be appropriate for protecting grassland biodiversity. Further in-depth studies with a large sampling area and diverse grasslands understand how seasonal grazing affects the grassland ecology.

#### Acknowledgments

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## 77. Food Futures: A data and science driven tool to enhance farm sustainability

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#### Application

This unique tool, co-created with scientists, policy and industry partners, can enhance farm sustainability by facilitating the dissemination and uptake of research findings, as well as supporting positive behaviour change and evidence-based policy development. Commercial application is a key aspect of this work, by including it into farm quality assurance schemes that can be used by the industry and consumers to drive higher integrity and trust food systems.

#### Introduction

Quantifying and enhancing the sustainability of agri-food production is key to protecting existing markets and securing new ones. Economic, social and environmental sustainability have to be quantified, meaning a large set of varied data are required. As part of the Agri-Food Quest 'Food Futures' project, a holistic, data-driven tool has been developed to measure, verify and report whole farm sustainability. This paper presents findings from the application of the tool to Northern Ireland (NI) farms.

#### Materials and methods

Scientifically robust indicators and metrics of sustainability were developed to measure economic, social, (i.e. farm family wellbeing) and environmental sustainability at farm level. Importantly, the latest research findings were incorporated in the Food Futures tool, to inform the selection and definition of more than 100 indicators and their sustainability scoring system. Specifically, responses to each indicator (eg slurry application methods, % of soils at optimum pH) are scored on a scale of 0–10, with 10 representing either best practice, the optimum status or the most efficient farms.

Data were then collected from 30 ambassador dairy, beef and sheep farms in NI since 2019 using a comprehensive questionnaire to inform the Food Futures tool. In 2022, an interactive dashboard was created to facilitate the use of the tool by (a) optimising data capture, (b) providing quantified feedback on the level of performance using graphics and traffic light systems and (c) providing targeted advice and practical options to improve on-farm sustainability. In 2022, Food Futures worked with the Livestock and Meat Commission (LMC) to successfully test a bolt-on tool (shorter than the full Food Futures tool) on more than 160 randomly selected Farm Quality Assured (FQA) beef and sheep farms in NI. The purpose of the bolt-on exercise was to run a second pilot for the Food Futures programme to 1) obtain more data from a wider range of farming conditions across NI, 2) seek feedback from participants (both assessors and farmers) and 3) further test the digital dashboard.

# Results

Initial results indicated that there was clear potential to enhance further the sustainability at farm level. For example, among all 162 farms surveyed as part of the FQA scheme (mostly beef and sheep enterprises), less than 50% of the farms surveyed had assessed their soil health by taking regular soil samples across the farm. Using splash plates was still the most common method to apply slurry (>80%). Only 55% of the study farms had a succession plan in place and more than 35% felt that they were unable to take regular time off. The level of uptake from these FQA farms, done on a voluntary basis, was lower than among the ambassador farms; nevertheless, the quality of the answers provided was excellent, with very few issues or missing observations. Since the initial group of 30 ambassador farms completed the full survey from the start of the programme, their active participation in both the development and use of the data-driven tool has already resulted in positive behavioural change to further improve their sustainability credentials. For example, 33% of these farms planted more trees and hedgerows, 22% adopted lower emission slurry spreading techniques and more than 20% of them increased the frequency and extent of their soil sampling regime to inform fertiliser application on-farm.

## Conclusions

The platform developed in this project enables users to visualise key strengths and weaknesses, set up realistic targets, identify and implement actions and explore relationships among key metrics. Building on the successful proof of concept developed as part of this project, there is now a need to further automate data entry requirements by establishing further links with existing dataflows and schemes. Continued transparency, scientific robustness and partnership with industry and government will facilitate a smarter use of on-farm data to deliver improved sustainability credentials.

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# 78. Implications of beef price seasonality on the profitability of Irish beef production systems

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## Application

Seasonality of Irish beef production influences beef prices which affects the profitability of beef production systems.

## Introduction

Irish beef is produced under production systems (dairy-beef and suckler) that incorporate large amounts of grass, both grazed in-situ and ensiled in the animals' diet (typically representing 80–90% of total feed budgets). This has resulted in spring-calving systems dominating and production systems with a competitive cost advantage through a lower reliance on purchased concentrates. However, the resulting seasonality of beef supply, with a peak in finished animal sales in autumn, has led to seasonal price trends. Thus, beef price tends to decline in mid to late autumn while highest prices, coinciding with lower numbers of finished animal sales, occur in early summer. The aim of this present study was to evaluate the effect of seasonality of beef price on the profitability of a range of production systems differing in age/-date of slaughter, by analysing data relating to the period 2013 to 2022.

## Material and methods

Average weekly beef prices for steers slaughtered at Irish beef processing plants over the ten-year period 2013–2022 were obtained from Bord Bia (The Irish Food Board; Bord Bia, 2023). These data were used to determine the seasonality of beef price, defined as the ratio of monthly price to annual average price, across carcass conformation (U, R, O, P) and fat classes (2, 3 and 4). To quantify the effect of seasonality on the profitability of beef cattle production, four beef finishing systems were modelled. These systems represented March-born late maturing crossbred steers, which were either light ('L', 480 kg) or heavy ('H', 580) at purchase in early September in their second season (i.e. 18 months of age) and which were either finished at pasture (P) or following an indoor finishing period (I). The pasture finishing systems involved finishing animals at 20 months of age in late October having been supplemented with 4.5 kg concentrate for 60 days (HP) or at 28 months of age in June with no supplementation during finishing (LP). The indoor finishing systems involved finishing animals on a silage + 4.5 kg concentrate diet at 22 months of age in January (HI) or at 25 months of age in April (LI). Prevailing prices for inputs and fixed/ overhead costs were applied. Beef carcass price of €5.06 was assumed with seasonality effects for each of the years 2013 to 2022 quantified separately providing for a total of four (systems) x ten (seasonal pricing effects) scenarios.

## Results

Analysis of historical carcass price data from 2013 to 2022, showed pronounced intra- and inter-year price fluctuations, and underlined the historically high prices which prevailed in 2022. The data indicated a general trend for beef prices to increase from January until May/June



Fig. 1. Beef carcass price ratio for Irish R3 steers for the period 2013-2022 (error bars signify max and min price ratios).

# Table 1 Ranking of profitability (€/head) for finishing systems purchasing light (L) and heavy (H) steers finishing off pasture (P) or indoors (I).

Year	LP	Ц	HP	HI
2013	1	2	4	3
2014	1	2	4	3
2015	1	3	4	2
2016	1	2	4	3
2017	1	3	4	2
2018	1	2	4	3
2019	1	3	4	2
2020	1	3	4	2
2021	1	4	2	3
2022	1	3	2	4
Average margin (€/head)	343	8	-24	-6

before decreasing towards the year-end (Fig. 1). Large differences in margins ( $\epsilon$ /head) were seen between systems years, with the highest margins for LP (Table 1). This mainly reflects lower feeding and animal purchase costs in that system. However, the longer period on farm for this system (ten months) compared to the other systems has implication s for the number of animals that can be accommodated and hence profitability per hectare. The yearly impact of price seasonality led to differences in the ranking of the remaining three systems.

## Conclusion

Beef production systems that maximise the utilisation on of grazed grass and minimise the use of purchased concentrates are more profitable than those involving more prolonged indoor feeding periods. The data also highlights the importance of beef price seasonality on the profitability of Irish beef systems.

## Acknowledgements

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#### 79. The effect of production system and price changes on profitability of dairy farms

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#### Application

The AFBI Dairy Systems Model can be used for the technical and economic analysis of different dairy systems.

#### Introduction

Dairy farming is the largest agriculture sector in Northern Ireland in terms of output and contribution to overall GDP. The majority of milk produced in Northern Ireland is sold as internationally traded dairy products and milk price is, therefore, strongly connected to global dairy

commodity prices. This export dependency can leave Northern Ireland dairy farms exposed to the high levels of volatility experienced by global dairy commodity prices. Furthermore, the recent price volatility in major dairy farm inputs such as fertiliser and concentrate has created profound challenges for farmers in their farm planning decisions. However, farm simulation models can be useful to investigate the effects of variations in production, price and policy parameters on farm performance and profitability. This study aimed to determine the effect of production system and price changes on the profitability of dairy farms.

# Materials and methods

The model is a whole farm, single year, static, deterministic simulation model that facilitates the technical and economic evaluation of dairy production systems. It operates with a monthly time step and was developed in Microsoft Excel. The model is empirical and uses data from production research experiments to specify coefficients and production functions (e.g. grazed grass dry matter digestibility and energy content, milk yield and the monthly proportions of grazed grass and grass silage in the diet). Where alternative production functions are available, the model structure allows these to be used. The user must define the farm land area owned and the production system choices. Dietary components consist of grazed grass, grass silage and concentrate (which is purchased when required). The model consists of four sub models namely; the farm system, animal nutrition, feed supply and financial. Three different dairy systems were examined in this study – Low, Medium and High. In the Low system, cows calved in the Spring and produced 6,000 L per lactation. In the Medium system cows calved in the Autumn and Spring and produced 8,000 L per cow. In the High system cows calved all year round and produced 10,000 L per cow. Farm size was assumed to be 70ha and stocking rate was set at 170 kg organic N/ha. The effects of changing milk, concentrate, contractor, fertiliser and electricity prices and interest rates on the profitability of the three systems was analysed.

#### Results

As shown in Table 1 the Medium system was found to have the highest net profit per farm and cow, the Low system had the smallest net profit per farm and cow. However, net profit per litre was highest for the Low system.

#### Table 1

Profitability of dairy systems using the AFBI Dairy Systems Model

	Low	Medium	High
Sales (£/farm) <sup>1</sup>	243,602	301,793	358,616
Variable costs (£/farm) <sup>2</sup>	105,708	151,325	215,526
Gross margin (£/farm)	137,895	150,468	143,090
Fixed costs (£/farm)	102,881	110,956	117,397
Net profit (£/farm)	35,013	39,512	25,693
Net profit (£/cow)	347	403	270
Net profit (ppl)	6	5	3

<sup>1</sup> Milk price 35ppl,

<sup>2</sup> Concentrate price £350/t, Fertiliser price £390/t.

As shown in Table 2, all price changes had the biggest effect on the High system and the smallest effect on the Low system, reflecting the level of inputs and milk sales from these systems. Overall in this analysis milk price changes had the largest effect on profitability. A 10% change in milk price caused net profit to change by 62%, 69% and 129% for the Low, Medium and High systems, respectively. Changes in concentrate price had the second largest effect on profitability. A 10% change in concentrate prices caused net profit to change by 15%, 20% and 43% for the Low, Medium and High systems, respectively. Changing contractor prices by 10% caused net profit to change by 9%, 11% and 26% for the Low, Medium and High systems, respectively. Changes in fertiliser prices had a relatively low effect on profitability. A 10% change in fertiliser price caused net profit to change by 5%, 5% and 8% for the Low, Medium and High systems, respectively. Changing interest rates by 10% caused net profit to change by 7%, 8% and 13% for the Low, Medium and High systems, respectively. Change in electricity price caused net profit to change by 7%, 8% and 13% for the Low, Medium and High systems, respectively. A 10% change in electricity price caused net profit to change by 2.9%, 2.7% and 4.5% for the Low, Medium and High systems, respectively.

#### Table 2

Effects of changing milk, concentrate, contractor, fertiliser and electricity price and interest rates on the net profit of dairy systems using the AFBI Dairy Systems Model.

Impact on farm net profit (£)	Low	Medium	High
Milk price (+/-10%)	21,651	27,338	33,377
Concentrate price (+/-10%)	5,283	7,848	11,116
Contractor price (+/-10%)	3,096	4,365	6,729
Fertiliser price (+/-10%)	1,736	2,039	2,174
Interest rate $(+/-10\%)$	2,625	3,087	3,433
Electricity price (+/-10%)	1,000	1,077	1,145

#### Conclusions

The AFBI Dairy Systems Model has been developed which can be used to analyse how changes in physical and economic factors affect farm performance. Going forward, this will provide an important research tool informing and supporting decision-making at the farm level in Northern Ireland. The application of the model in this study found that the Medium system (autumn/spring calving, 8,000 l/cow) was the most profitable. Profitability of the High system was the most sensitive to price changes and the Low system the least.

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# 80. A little less conversation, a little more action please: The need for implementation planning and assistance to bridge the research-practice gap in the sheep sector

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## Application

Agri-veterinary practice has to advance, with substantial on-going research activity seeking to understand changes needed to meet existing, and emerging challenges and opportunities. However, not explicitly considering how research outcomes are practically integrated into daily farming risks research outputs failing to deliver their potential, perpetuating the research – implementation gap.

## Introduction

Considerable resources are invested in research activities annually, however, rarely is research tied specifically to an implementation plan. In the case of influencing change in the care of farmed livestock, both farmers and their veterinary surgeons need to understand the need for change, what the new practice should be and how to implement it (Michie et al., 2011). However, many examples exist within the sheep industry where previous research has highlighted specific issues and proposed solutions, yet these issues still cause major concerns. As part of a wider project on medicine stewardship in sheep in Northern Ireland (NI) we sought to identify if specific examples of the research – implementation gap existed, in the context of the lived experience of NI sheep farmers and vets. We ultimately aim to avoid past failures in translating research into change through reflecting on where this has previously happened.

## Materials and methods

Utilizing mixed research methods, a literature review, scoping questionnaire and semi-structured interviews (n = 27 farmers; n = 15 vets) were followed by discussion groups (n = 13 farmers; n = 2 vets). These sought participants' views on, and experiences of, medicine use in the NI sheep flock. Participants were sheep farmers and farm animal veterinary practitioners working in NI.

The interviews and discussion groups were electronically recorded and transcribed, or contemporaneous notes were made when permission for recording was not obtained. Transcripts and notes were re-read, coded inductively and exemplar quotations identified.

## Results

The review of extant literature and the findings of 122 questionnaires revealed four main foci which were then considered in light of farmer and vet beliefs and behaviours for further targeted action: (i) control of infectious lameness; (ii) administration of oral antibiotics to neonatal lambs; (iii) prevention of abortion due to Chlamydia abortus; (iv) and sheep scab control.

Lameness was identified by farmers and vets as the main driver for antibiotic use in sheep flocks, reflecting findings elsewhere. Vaccination to prevent footrot was reported by 21% of survey participants. Farmers and vets expressed that better lameness management was something they wished to achieve. However, awareness of the 5-point-plan (Clements and Stoye, 2014) among farmers was low and little evidence was seen or reported of a co-ordinated, multi-factorial response to manage lameness, despite evidence in the literature going back some 50 years on the key action points needed to minimise infectious lameness (Abbott, 2000).

Farmers participating in this study recognised that routine oral antibiotic administration was something they would like to avoid, but some found it inevitable that at some point during lambing they would administer antibiotics. Others freely admitted not knowing why they undertook the practice at all. Some farmers and vets expressed apprehension about lambing seasons in the future without such products, despite the long-standing evidence of the efficacy of colostrum (Hodgson et al., 1992). Farmer frustration at being unable to source oral antibiotics was reported, alongside vets selling alternative antibiotics for oral administration. This frustrated vets who were promoting better hygiene and colostrum management for watery mouth prevention.

Use of a vaccine, which has been available in various iterations for 60 years, (Entrican et al., 2012), to prevent enzootic abortion of ewes (EAE) was reported by 48% of questionnaire participants. Vets reported how, through concerted effort, they could persuade farmers to stop using injectable antibiotics for prevention of EAE, however, these efforts were impeded by vaccine availability issues, farmers' unprepared-ness, and other vets who were willing to supply prophylactic parenteral antibiotics for abortion prevention.

When addressing sheep scab, farmers highlighted the following: a lack of understanding of disease control, which has been a problem for decades (Spence, 1951); a strained relationship with the state authorities which hindered reporting and seeking help; and an inability to obtain the necessary certificate to purchase and administer organophosphate dip. Hopelessness in the face of neighbours who refused to control scab in their flocks was also described.

# Conclusions

Key evidence-based recommendations to address these foci have been available for decades; yet these four areas still present concern for flock-keepers today. Reasons for failure to adopt research outputs could be considered under existing models of behavioural change including failure of knowledge exchange; a feeling of, or actual inability to deliver change; as well as external restrictions on farmer behaviour. Therefore, consideration needs to be given to how change can be brought about in addition to what needs to change. This discussion must be centrally focused on the end user: the farmer.

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## 81. Transition cow management and health in Irish dairy herds: Results from an on-line survey addressed to farmers

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#### Application

The present qualitative and quantitative survey study was conducted to describe farmers' perception of the transition period and reported disease prevalence and management strategies, to ultimately inform and guide quantitative research aimed at optimising transition cow health and management in Irish dairy farms.

#### Introduction

The transition period involves the change from dry and pregnant to lactating and not pregnant in dairy cows. This transition involves numerous physiological, immunological and metabolic challenges for the cow. Suboptimal management during this period has been associated with increased disease incidence, poorer production, poorer reproduction, and higher herd removal rates. Considering the unique nature of the Irish dairy production system (predominantly pasture-based and spring calving), the transition period challenges are potentially different to that of other systems.

## Materials and methods

An online survey consisting of 19 multiple choice and open-ended questions was distributed via text message to clients of Teagasc (The Agriculture and Food Development Authority in Ireland) dairy advisory services (n = 3,899) in autumn 2022. The survey questions addressed three main areas: 1) Farmers' perception of the transition period, 2) Disease prevalence, and 3) Management practices.

#### Results

#### **Study population**

A total of 525 responses suitable for analysis were obtained in this study. Respondents managed spring-calving (84%), split-calving (13%), all year round calving (2%) and autumn calving herds (1%), and defined their feeding strategy as grazing high input (providing yearly >1 ton of bought-in feed/cow; 52%) or low input (providing yearly <1 ton of bought-in feed/cow; 48%). On average, respondents' herd size was 135 cows (interquartile range = 78 to 162 cows).

## Perception of the transition period

Fresh calver cow diseases (e.g. milk fever, held cleanings, metritis, displaced abomasum, ketosis) were ranked first on importance (based on incidence and/or impact on the herd) by 49% of the respondents; the remainder of farmers ranked first mastitis (27%), lameness (17%), or infectious disease (e.g. Johne's, respiratory disease; 7%). The majority of respondents considered freshly calved cows health "critical" (86%) and correct management during the dry period essential for future cows' health and performance (90%).

## Disease

Freshly calved cows (within the first 3 weeks after calving) were reported to have the highest incidence of disease by most of the respondents (58%). Highest disease incidence was most commonly observed in late calving cows (48%) and multiparous cows (52%) with a significant cohort of respondents indicating that they observe the highest incidence of disease in cows all throughout the calving season (41%) and in cows of any parity (43%). Most respondents reported "occasional cases but no major effect on herd performance" for milk fever (73%), metritis (72%), retained placenta (69%), displaced abomasum and/or digestive problems (62%), and ketosis (61%). While fatty liver (70%), grass tetany (60%), dystocia (49%), acidosis (48%) and subclinical ketosis (40%) were reported as "not a problem" for respondents' herds. Remarkably, milk fever and subclinical hypocalcaemia were reported as being a "significant problem" (regularly treating severe cases with some cows lost/culled; 13% and 8%, respectively) or a "routine problem" (regularly treating cows to control the issues; 2% and 1%, respectively) by some respondents. Nearly half (48%) of respondents reported treating 1 to 3% of their herd for milk fever, and 17% reported treating 4 to 6% of their herd for the condition. Similarly, most of the respondents (56%) reported treating between 1 and 3% of their herd for retained placenta, but a considerable cohort (11%) reported treating between 4 and 6% of their herd. Less than 1% of the cows were reported to be treated for metritis (52%), displaced abomasum and/or digestive problems (72%), ketosis (73%), and grass tetany (83%).

# **Management practices**

*Dry cow management.* Most respondents reported to body condition score cows throughout the dry period (74%), managing cows in more than one group (56%), supplementing magnesium (52%), and providing feed sources other than silage to close-up cows (48%). Less commonly implemented management practices included calcium supplementation to close-up dry cows (35%), vitamin D supplementation to close-up dry cows (23%), low potassium diet to dry cows (20%), provision of a general dry cow mineral in the diet (14%), and acidifying the diet of close-up dry cows (6%).

*Fresh cow management.* Most respondents reported to keep freshly caved cows indoors for a period after calving (68%) and high-risk cows' calcium supplementation at calving (57%). Less commonly implemented management practices include once-a-day milking for a few days after calving (36%), magnesium supplementation in diet (26%), calcium supplementation in diet (18%), vitamin D supplementation in diet (10%), all cows routine calcium supplementation at calving (8%), fresh cows are provided with general minerals (3%), and provision of feed sources other than forages to fresh cows (2%).

## Conclusions

In conclusion, Irish dairy farmers acknowledge the importance of fresh cow diseases and that correct management during the transition period is important for future performance of the dairy cow, therefore supporting the need of transition cow health and management research within the Irish dairy production system. This survey has identified milk fever and subclinical hypocalcaemia, as well as body condition score management during the dry period and calcium supplementation at calving as health conditions and management practices that merit research efforts to respectively decrease their prevalence and optimise the benefits of their implementation in Irish dairy farms.

## Acknowledgements

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# 82. Working with vets and farmers in Wales to promote pro-active animal health planning to control sheep lameness

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Lameness is a significant issue on UK farms with surveys suggesting that over 3% of ewes are lame at any one time; with footrot and contagious digital dermatitis as predominant causes of lameness. Footrot alone has been shown to cost individual farmers between an average of £3.60 per ewe on the farm with >10% lameness costing up to £6.35 (Winter and Green, 2017) or even as much as £14 (Lovatt, 2014) per ewe in the flock per year.

Effective pro-active health planning hinges on developing relationships between vets and farmers. A key aim of the Hybu Cig Cymru/Meat Promotion Wales Red Meat Development Programme, Flock and Herd Health Planning Project (Stoc+) was to facilitate this process. Animal health priorities were co-identified by farmers with their Stoc+ vets at an initial health planning consultation that built on existing health plans. Across the whole Stoc+ project of 360 Welsh farmers, half of the farmers with sheep, identified with their vet, that sheep lameness was one of their top three priorities. To research this issue further, all Stoc+ farmers with sheep were invited to take part in an additional pilot project to address lameness in their flocks. Forty-three farms across Wales participated in this veterinary-led lameness project between August 2022 and March 2023. It included online entry and exit surveys, interactive in-person and online farmer meetings, phone and text support for farmers to encourage the collection of flock lameness data. All farmers attended an initial on farm workshop at which they identified and discussed their current use of the 'Five Point Plan'. They were given training on lameness diagnosis and treatment and at each workshop were a number of engaged Stoc+ Vets. Bespoke veterinary lameness action plans were developed for each farmer based on the tried and tested 'Five Point Plan' (Clements and Stoye, 2014) and incorporating recent research into lameness prevention and treatment.

Farmers assessed the level of lameness in their flocks at the start and end of the project. Using a T-test for two independent means, it was shown that lameness prevalence across flocks fell significantly over the course of the project: from 4.9% to 2.0% for ewes (t-value = 3.49; p < 0.001), from 4.9% to 2.2% for lambs (t-value = 2.21; p < 0.05) and from 15.6% to 6.1% for rams (t-value = 3.65; p < 0.001) (Fig. 1). Farmer satisfaction as to their ability to identify and treat flock lameness increased over the course of the project (Fig. 2), with 97% of participants claiming the project had increased their understanding of how to control lameness (Fig. 3). There was a visible shift in farmer perception of



Fig. 1. Box and whisker plot to show the improvement in farmer-reported lameness prevalence in each category of sheep from the initial survey (Sept 22) to the feedback survey (March 23).



Fig. 2. Farmer responses in feedback survey as to how satisfied they were with their ability to control lameness before joining and since partaking in the project.



their antibiotic usage (Fig. 4) with an increase in those stating that they were using little or an appropriate amount of antibiotics in their flock and a decrease in those stating they used more than they were comfortable with from 38% to 8% of farmers. This project has demonstrated how focused veterinary engagement, with an emphasis on Plan-Prevent-Protect (Lovatt et al., 2019) and the Five Point Plan can lead to significantly reduced levels of sheep lameness, increased levels of farmer confidence and reduced levels of



Fig. 4. Direct comparison between farmer responses to the question asking how comfortable they were with the quantity of antibiotics that they use in their flock.

antibiotic use. Targeted and appropriate use of antibiotics to treat sheep lameness formed part of the project dissemination. This project fed into the wider Sheep Antibiotic Guardian Group's 'Healthy Feet Happy Sheep' (Sheep Antibiotic Guardian Group, 2023) campaign. Dissemination of the outcomes included the provision of resources such as bilingual videos and online materials for vets and for farmers at Hybu Cig Cymru roadshows, in further education colleges and within veterinary practice Flock Health Club meetings.

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#### 83. Achieving European protein demands using ethanol co-products

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Driven by consumer demands for healthier and more environmentally friendly options, the European food market is shifting towards more sustainable, homegrown sources of plant-based protein. The European Commission predicts that by 2050 the demand for plant-based protein will increase by 50% and recently stated, "We will improve our food security by reducing our dependencies on key imported agricultural products and inputs, in particular by increasing the EU production of plant-based proteins". Given that Europe is only 27% self-sufficient in high quality protein, this presents a significant challenge for the animal feed industry.

Establishing a new protein crop for Europe would take a decade to establish elite, geographically adapted varieties, adopt new agronomic practices, and establish appropriate supply chains. There are significant advantages to embracing traditional crops and practices. The dry grind ethanol industry is an international business converting traditional crops into renewable fuel and co-products. Distillers Dried Grains and Solubles is a valuable feed co-product from the dry grind ethanol process but was never designed for any specific purpose and has limited application across a wide range of livestock species. However, co-products *per se* are a unique category of feed materials that do not compete with food and when used in feed contribute to a circular economy.

A novel mechanical separation technology (The Maximised Stillage Co-products ( $MSC^{IM}$ ) has been installed in several corn ethanol plants. The product has been successfully tested in over 50 trials, and commercially across the full range of livestock, in aquaculture and diets for companion animals. Corn fermented protein (CFP) ranges in protein concentration from 52% crude protein (CP) *as is* to over 60% CP when higher protein crops such as wheat are used as feedstock. The term corn fermented protein references that the product is derived from an initial fermentation lasting up to 70 hours. A high proportion of the yeast generated during fermentation is recovered in the MSC process (approx. 24% spent yeast in dry matter of 52% CFP).

The MSC CFP product has been used to replace a variety of protein materials in feed formulation, particularly imported soy-based products, and without exception, been proven to be an ideal replacement protein with additional benefits from spent yeast and fermentation characteristics. Current production of CFP, which is regulatory compliant, is over one million tons per annum. These new protein co-products

align well with the goals of the circular economy and can contribute a large volume of high quality, home-grown, non-GM plant protein. Utilizing these co-products meets the growing consumer demand for plant-based protein while also reducing environmental impact. Corn Fermented Protein is the first commercially viable, new high-concentration, vegetable protein for the feed industry in the past 20 years.

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## 84. Insect sentience & welfare

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Humans farm and slaughter over a trillion insects every year, compared to "only" 80 billion conventional livestock. These insects are routinely heated, frozen, starved, or minced to death. Yet, despite the vast number of individual animals involved, and despite industry practices that raise massive welfare concerns, debates about animal welfare almost universally ignore farmed insects. As a result, there is no insect welfare legislation or comprehensive best practice guidelines. How has this situation arisen? One justification is that insects are not sentient – they cannot feel pain or pleasure, and respond to noxious stimuli purely by reflex. In this presentation, I will challenge this view and outline the evidence for sentience in insects.

As a case-study, I will discuss our research on motivational trade-offs in bumblebees (*Bombus terrestris*), which offers evidence for pain. We investigated whether bees' attraction to sucrose reduced their avoidance of noxious heat. Bees could choose between either unheated or noxiously-heated (55 °C) feeders with different sucrose concentrations. Bees avoided noxious feeders when the unheated feeders contained high sucrose concentrations, but used the noxious feeders more as the sucrose concentration at unheated feeders decreased. As contextual information modulated bees' responses to noxious stimuli, these responses could not be simple reflexes. The bees' decisions also relied on learned colour cues, so the trade-off involved processing in the brain, rather than just peripheral processing. These findings are at least consistent with a capacity for pain in insects – a conclusion that raises serious concerns about farmed insect welfare.

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# 85. Dose-response effects of diets partially replaced by food processing industrial by-products on in vitro ruminal fermentation and methane production

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## Application

By-products derived from the food processing industry have the potential to replace the conventional ingredients in ruminant feed, as well as improve production performance, energy and nitrogen utilization efficiency, reducing methane emissions and thereby making farms more effective and environmentally friendly.

## Introduction

There are 1.3 billion tons of food lost or wasted every year around the world, approximately one-third weight of global food production (Calicioglu et al., 2019). In the UK, the quantity of food waste without recycling reaches 15 million tonnes annually (234 kg/person/year or 50% of food), nearly half of which is disposed of in landfilling (Wrap, 2015). It is globally acknowledged that addressing the environmental impact of their disposal is imperative. Simultaneously, there is a pressing challenge in the resource competition between food for human consumption and feed for animal. The resolution to transform the waste into feedstuff for livestock is becoming an increasingly prevailing trend. Prior to feeding ruminants with by-products, more accurate information is needed on their nutritive value and potential to reduce emissions. Therefore, the aim of this study was to explore *in vitro*, the effects of various by-products from the food processing industry on rumen fermentation.

## Material and methods

Materials of different by-products, including red apple pomace(AP), green apple pomace(AP2), hempseed cake(HC), coffee hulls(CH), coffee grounds(CG), spent mushroom compost (SMC) and distillers Dried Grains with Solubles (DDGs), were collected from various food processing industry in N. Ireland, freeze-dried and ground at 1-mm screen 2. Tested by-products were incubated *in vitro* in doses of 100, 200, 300 g/kg. These amounts replaced equal amounts of the mixed ration (500 mg), which was composed of grass silage and concentrate (70:30). AP, AP2, CH, CG and SMC were designed to replace the silage portion, while HC and DDG were designed to replace the concentrate portion. Incubations were done anaerobically at 39 °C for 24h in jars containing buffered rumen fluid from 3 dairy cattle from the abattoir. Gas production, pH, methane(CH<sub>4</sub>) concentration in headspace gas samples, ammonia(NH<sub>3</sub>-N) and volatile fatty acids (VFA) in the fermentation medium, were measured at 24h. Each of the 21 treatments (7 by-products  $\times$  3 doses) was repeated in two independent *in vitro* runs. For statistical analysis, all data were analyzed by the SPSS (Version 20.0, IBM). A linear mixed model (LMM) was used to evaluate the effects of by-products on rumen fermentation, with by-products, dose and interaction as fixed effects, run as random effect. General linear model (GLM) was used to compare the by-products effect within the dose considering run as random factor. Linear and non-linear effects of dose within test by-products were evaluated by orthogonal polynomial contrasts.

# Results

After 24 h fermentation, based on LMM, gas production, pH and NH<sub>3</sub>-N were significantly affected by different by-products and doses (P < 0.001), while tendency were found in CH<sub>4</sub> production affected by doses. There were no significant effects on VFA caused by dose(P > 0.05) but by-products (P < 0.001), and the CG and SMC have the lowest concentration among all sorts of VFA. For GLM, CH<sub>4</sub> production decreased linearly with the increases in doses of all by-products, (P < 0.05), while for CH<sub>4</sub> production per unit of degradable dry matter, the AP2, HC, CH, CG and SMC didn't show linear trends (P > 0.05). The CH<sub>4</sub> production decreased by 29%, 33.1%, 33.9%, 31.0%, 30.4%, 27.6% and 28.9% at the dose in 100-300 g/kg, when diets were replaced with AP, AP2, HC, CH, CG, SMC and DDGS, respectively. Gas production and *in vitro* dry matter degradability respond similarly to CH<sub>4</sub> production (P < 0.001), except the AP and AP2 (P > 0.05). Doses had significant impact on NH<sub>3</sub>-N concentration in nearly all by-products, often leading to a positive linear dose response (P < 0.001), while AP2 had a quadratic response (P < 0.001).

# Conclusions

Based on our *in vitro* study, the by-products tested were proved to be a potential option for replacing conventional feed ingredients but not exceeding the dose at 200 g/kg, especially apple pomace and hempseed cake, making it promising to apply these by-products on ruminant feed. Further *in vivo* studies are needed to be done to confirm these results on  $CH_4$  production, but also explore the effect on the quality of animal products (milk. Etc.).

# Acknowledgements

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## 86. In vitro fermentation characteristics of four agro-industrial protein by-products compared to soyabean meal

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# Application

The alternative to soyabean meal agro-industrial by-products can be used in animal diets due to the reduced embodied carbon footprint but a further reduction in methane production was not confirmed in the present study.

## Introduction

Soyabean meal (SBM) is one of the most common protein feeds in animal rations due to its high nutritional value and commercial availability (Sasu-Boakye et al., 2014). However, there is concern over the environmental impact of growing soya (land use and degradation, water consumption, and transportation costs (Song et al., 2021). Agro-industrial co-products made from locally grown crops likely carry a lower embodied carbon footprint than SBM and, if high in unsaturated fatty acids, may directly reduce enteric methane emissions. Given the recent concerns around methane emissions from livestock production, most being from rumen fermentation, and the strong effect of animal diet on such emissions, the potential of new feeds for reducing methane emissions is also essential to assess their overall sustainability. The present study aims to assess *in vitro* the effect of four agro-industrial by-products (maize residue (MR), wheat distillers' grains (WDG), brewers' spent grains (BG), and corn steep liquor (CSL)) on gas and methane production, compared to SBM.

# Material and methods

Four food industry by-products either dried and ground (MR; WDG; BG; and SBM as control treatment) or liquid (CSL), were incubated with dried and ground grass silage and wheat in Wheaton flasks, giving a total of 1.0 g of total mixed ration (TMR), with a protein content of 40 g/kg DM. Negative control flasks with no TMR substrate were also included. In each flask, 90 mL buffer and 10 mL rumen fluid were

mixed, followed by sealing and incubation at 39 °C. Total gas volume was measured using headspace gas pressure at 2, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h. The experiment was run three times. Gas pressure readings were used to calculate gas volume and a sample (10 mL) of gas was collected for methane via the port valve for analysis using gas chromatography (Bruker 450-GC). At 72 h, the flask content was filtered, and the residue was oven-dried at 100 °C for 4 h. In vitro Dry Matter Digestibility (IVDMD; g/kg) was computed based on residual DM. Curves were fitted to the cumulative production data. The filtrate underwent VFA analysis using GC. Data were analysed (Rstudio) using a general linear mixed model analysis of variance that included treatment as a fixed effect and the run as a random effect. Tukey's Honestly Significant Difference test was used for pairwise comparisons where treatment effects were significant (P < 0.05).

# Results

Cumulative gas and methane productions expressed as ml/DM or /digested DM after 72h, and the methane production as % total gas production did not differ (P > 0.05). IVDMD was lower (P < 0.001) in the BG compared to all other treatments, and in the MR compared to CSL. The fractional rate of degradation (/h), estimated at 50% of asymptote, was lower (P < 0.001) in the BG, WDG, and MR compared to SBM, and CSL. The extent of degradation (%) estimated at 0.04/h and 0.025/h passage rates, was lower (P < 0.01) in the BG than in the other treatments. Rumen fluid VFA profile parameters were not significantly affected by the treatment (P > 0.05).

## Conclusions

Despite the lower embodied carbon footprint of the tested agro-industrial by-products, compared with soyabean meal, the overall cumulative gas and methane production between them, was not significantly different. Brewers' spent grains had lower *in vitro* dry matter digestibility, but this may not raise concerns because previous animal studies using brewers' grains in beef and lactating dairy cow diets, did not show any adverse effect on animal productivity and feed digestibility (Moate et al., 2011; Duthie et al., 2015).

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# 87. Chemical composition and in vitro gas production evaluation of brown and red sub-Antarctic macroalgae as feed for ruminants

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# Application

The inclusion of seaweeds in ruminant diets could potentially play a viable role in ruminant diets as a source of protein and energy.

## Introduction

Macroalgae can be used for several purposes including, for instance, human consumption, soil fertilizers and animal feed, due to their diverse range of metabolites (de Freitas et al. 2020). The objective of this study was to evaluate different macroalgae found in the Chilean sub-Antarctic Region, in terms of their chemical composition and in vitro ruminal fermentation.

# Material and methods

Four algae (Lessonia flavicans, Gigartina skottbergii, Ulva lactuca and Macrocystis pyrifera) and one alfalfa sample were used. Chemical composition, in vitro gas production (Theodorou et al. 1994) and methane production were determined. In a first incubation, 0.800 g DM of each ingredient was used and incubated in triplicate at  $39^{\circ}$ C. The gas volume (ml gas/g DM) was recorded at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h using a pressure transducer (model 8804 HD). After the incubation period (96h), dry matter disappearance (DMD96h mg/100mg), relative gas production (RGP, ml gas 96 h)/(mg/100 mg DMD 96 h) were determined. The concentration of short chain fatty acids (SCFA) according to Getachew et al. (2002) and the microbial biomass production (MBP) according to Blümmel et al. (1997). To determine CH<sub>4</sub>, 0.200 g DM sample of each ingredient was incubated in 100 mL glass syringes in triplicate in three incubation runs (Theodorou et al., 1994). Gas volume (mL gas/200 mg DM) and CH<sub>4</sub> were measured after 4, 8, 12 and 24 h of fermentation. A completely randomized design and Tukey's test were used when significant differences between treatments were observed (P < 0.05).

## Results

Table 1 shows the chemical composition of the macroalgae. OM content, (g/kg) was higher for alfalfa (P < 0.0001), followed by G.skottsbergi. and lower for M.pyriphera, as for CP content (P < 0.0001), it was higher for U.lactuca, followed by alfalfa hay and lower for G.skottsbergi. *In vitro* gas production (ml gas/ gDM) at 96 h was lower (P < 0.001) for G.skottsbergi, followed by M.pyriphera and L.flavicons, however G.skottsbergi and M.pyriphera show the highest DMD 96h (mg/100mg), being lower for U.lactuca. The four algae produced lower amounts of methane (ml CH<sub>4</sub>/g DM) compared to alfalfa hay (P < 0.0001), however, L.flavicons and M.pyriphera were the one that presented the lowest amounts in the hours evaluated.

Table 1

Chemical composition (g/kg DM), methane production (ml CH<sub>4</sub>/g DM), and in vitro rumen gas kinetics (ml gas/g DM) and fermentation profile of different macro alga as apotential use in ruminat diets.

Item	G.skottsbergi	M.pyriphera	L.flavicons	U.lactuca	Alfalfa hay	SEM	P-value
OM,g/kg	744.63b	561.97e	693.77c	641.92d	899.13a	0.638	0.0001
CP,g/kg	86.00e	141.55c	111.86d	185.91a	154.50b	1.054	0.0001
EE, g/kg	17.68a	3.00d	1.65e	14.34b	8.57c	0.188	0.0001
ml gas/g DM							
6h	4.44c	7.78bc	4.42c	10.92b	16.31a	0.976	0.0001
12h	6.82d	12.45c	10.87cd	20.74b	40.41a	1.206	0.0001
24h	11.55d	14.68d	25.13c	33.42b	73.66a	1.621	0.0001
48h	20.97d	32.99cd	41.18c	58.56b	102.28a	3.142	0.0001
96h	25.88d	59.14c	48.83c	82.51b	118.28a	4.567	0.0001
ml CH <sub>4</sub> /g DM							
3h	0.27b	0.25b	0.03b	0.34b	5.26a	0.407	0.0001
6h	0.21b	0.21b	0.03b	0.30b	7.39a	1.486	0.0184
9h	3.61b	0.59c	0.09c	1.65b	7.81a	1.79	0.0522
12h	0.61b	0.62b	0.15b	1.13b	6.77a	0.513	0.0001
24h	8.53b	0.88b	0.18b	1.42b	37.18a	3.09	0.0001
DMD,mg/100mg	68.49a	67.62a	41.60b	14.72c	44.64b	0.765	0.0001
ME, Mj/kgDM	7.89e	11.27c	10.28d	15.07b	16.02a	0.101	0.0001
MCP, mg/g	679.80a	669.81a	404.98b	132.48c	413.95b	7.226	0.0001
SCFA	0.05d	0.06d	0.10c	0.14b	0.32a	0.007	0.0001
N-NH3,mg/dI	26.91a	21.05b	21.47b	30.67a	31.07a	2.293	0.0258

#### Conclusion

The inclusion of U. lactuca suggests a viable alternative in diets for sheep, while G. skottsbergisi has a higher in vitro Dry matter digestibility, it has a lower nutritive value and fermentation at the rumen *in vitro* level. *In vivo* studies are suggested to evaluate its possible use in ruminant diets.

#### Acknowledgements

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# 88. Effect of replacing urea direct supplementation with urea-protected additives on ruminal fermentation and rumen ammonia concentration in beef cattle

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#### Application

The use of slow urea liberators can reduce the risk of alkalosis in the first hours, improving the utilization of N-NH<sub>3</sub> at rumen level in beef cattle.

# Introduction

Rumen bacteria can convert non protein nitrogen (NPN) sources into microbial crude protein if the amount of energy is available. Feed grade urea (FGU) is the most used NPN source in beef cattle diets (Cherdthong and Wanapat, 2010; Kertz, 2010). An *in vivo* study was conducted to determine the effects of feeding feed grade urea (FGU) or slow-release urea (SRU) as a replacement for true protein supplements (soybean meal, SBM), and control diet (CTR, no N inclusion sources) in beef cattle diets to see if N-NH<sub>3</sub> concentration is reduced at the ruminal level during the first few hours, decreasing the risk of alkalosis.

# Material and methods

Five beef cattle of 577 ± 48. 75 kg live weight, provided with ruminal cannula, which were fed a conventional diet (concentrate/corn silage, 60: 40 as DM) and the following treatments were used, treatment 1, Control diet (Ctrl-without any N-additives), treatment 2, Soybean meal (SBM) as reference protein source (Ctrl-SBM); treatment 3, Urea-based diet, treatment 4, SRU Optigen-based diet (replacing Urea); treatment 5, SRU-Biuret based diet (replacing Urea); treatment 6, Urea with the inclusion of HyUrit at 25% of DMI (Urea+ HyUrit). Randomly distributed to one of six experimental treatments, in a 5x6 incomplete Latin square design, treatments. The diet was isoenergetic and isoproteic with 11.4 MJ ME/kg DM and 14 % CP. Each experimental period lasted 30d, of which 23 days were used for diet adaptation and five days for sample collection (intake and digestibility), the last two days were used for rumen dynamics testing (NH<sub>3</sub> and pH) at 0 (just before feeding), 0.5, 1, 2, 4, 6, 8, 12, and 24 h after feeding. Each heifer received each treatment once during each of the six periods. Animals were weighed at the beginning and end of each experimental period. The additives were offered daily in a single dose at 08:00 h in the morning. Animal performance variables such as dry matter intake (DMI), digestibility, average daily weight gain (ADG), gain/feed ratio, chemical composition of the diets, ruminal fermentation profile and nitrogen utilization were considered.

## Results

Mean live weight (568.7 ± 45 kg), mean daily weight gain (1.8 kg/d), DM intake (14.32 kg/d), OM intake (12.6 kg/d), NDF intake (8.15 kg/d) and ADF intake (3.53 kg/d) were similar among treatments (P > 0.05), however, significant differences (P < 0.001) were observed for dry matter digestibility coefficient, with SBM, Optigen and Biuret being superior to HyUrit, and the digestibility coefficient (P > 0.05) for OM, NDF and ADF were similar among treatments. In the *in vivo* N-NH<sub>3</sub> dynamics (mg/dl), HyUrit 25% shows the lowest N-NH<sub>3</sub> concentrations (P < 0.001), with respect to the rest of treatments, the highest N-NH<sub>3</sub> concentrations for hours 0.5, 1 and 2 after feeding were for feed grade Urea, Optigen and Biuret followed by control and SBM diets (See Table 1).

#### Table 1

Values of *in vitro* ruminal ammonia (N-NH<sub>3</sub>,mg/dl) as a function of time and replacement of soy bean meal (SBM), feed grade Urea (FGU) and SUR sources in beef cattle on iso-protein and iso-energetic diets.

N-NH <sub>3</sub>	Control	SBM	FGU	Optigen	Biuret	HyUrit 25%	SEM	P value
0h	9.83a	7.37b	3.68c	4.21c	6.20b	1.52d	0.328	0.0001
0.5h	10.71c	14.25c	30.28b	30.26b	45.88a	1.98d	1.053	0.0001
1h	12.42c	14.73c	29.02b	30.75b	38.88a	7.98d	0.957	0.0001
2h	10.47d	9.94d	24.90a	13.03c	16.39b	7.40e	0.086	0.0001
4h	1.08d	3.85c	16.72a	5.08c	8.14b	3.96c	0.353	0.0001
6h	0.90d	5.95b	10.43a	2.32c	6.21b	5.22b	0.279	0.0001
8h	0.86e	5.41b	3.91c	1.96d	6.53a	5.74ab	0.185	0.0001
12h	1.00f	4.03cd	4.31c	3.55d	5.29b	6.24a	0.167	0.0001
24h	0.93d	5.15ab	3.54c	3.74c	4.67bc	6.08a	0.274	0.0001

SBM, soya bean meal; FGU, feed grade urea.

## Conclusion

The addition of HyUrit25% diminish the release of  $N-NH_3$  in the rumen at concentrations below 8 mg/dl, which diminish the risk of ammonia intoxication compared to the addition of feed grade urea, Optigen and Biuret.

## Acknowledgements

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# 89. Available former foods, co-products and by-products with potential for incorporation into monogastric livestock diets within the UK

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#### Application

Increasing reliance on sustainable feed resources achieved through the valorisation of former food products (FFPs), as well as co- and byproducts resulting from UK's different manufacturing processes, can mitigate the environmental burdens and economic costs associated with the production of livestock feed.

## Introduction

Feed production is the largest contributor to the environmental and economic impacts associated with most livestock production systems. Rising global demand for animal products is predicted to double livestock production by 2050, intensifying competition between humans and animals for crops, land, and water use. Global livestock production systems rely heavily on unsustainable plant-based protein to fulfill dietary protein requirements, with pigs and poultry as key, competitors for human-edible crops. Using FFPs, co- or by-products has the potential to benefit local producers and the wider economy by reducing the pressures on land required for feed-crop cultivation/landfill, environmental and water footprint of livestock feed production, and by avoiding costs associated with conventional crop production, while simultaneously valorising and recognising on the value of readily available products which otherwise would be lost or incur disposal costs. An estimated 650,000t of FFPs are processed annually in the UK for animal feed, along with 800,000t of surplus food that could be suitable for use in animal feed (WRAP, 2016). To optimise these sustainable sources, we must identify what is available and in what quantities and evaluate its suitability as a feed ingredient.

#### Methods

A multistep approach was adopted to build an inventory of potential alternative feed ingredients from FFPs, co-, and by-products (Fig. 1). The initial phases of this research project encompassed a systematic approach to identify and prioritise the key characteristics or criteria when considering a potential feed ingredient for the diets of non-ruminant livestock. These preliminary steps (Steps 1 and 2) involved a meticulous process of identifying and prioritizing the critical characteristics and criteria through consultations with stakeholders in the animal feed industry and researchers specialising in this field. Step 3 entailed extensive desk-based qualitative research aimed at sourcing and collating information on available products. This phase was characterised by direct engagement with various sectors of the industry, including animal feed companies, feed mills, supermarkets, large-scale and local food producers/processors, nutritionists, waste management companies, etc. This was complemented by a thorough review of both published scientific and grey literature (news articles, industry



Fig. 1. Overview of methodology used to construct and populate the alternative ingredient inventory.

websites, and published government reports). This multi-pronged approach not only facilitated the identification of areas of focus and sources of information but also broadened the scope of the search for potential feed ingredients. To foster broader communication and acquire additional insights, questionnaires were prepared and distributed within industry working groups. With the amalgamation of information from these various sources, an extensive inventory of potential feed ingredients was meticulously constructed and populated with a wide array of data, including details on product generation, availability, stability, safety, regulatory implications, acceptability, environmental impact (assessed using Life Cycle inventory sourced from suppliers or assigned based on the GFLI database), functionality, incorporation, and cost related attributes.

# Results

Over 60 companies across the UK livestock feed industry and the food and drinks production industry were directly contacted to identify FFPs, co- or by-products. Responses and data from four major feed suppliers and one algae-based company, the survey generated an inventory of 17 FFPs and 21 co-products. Among these, 17 FFPs and 21 co-products showed potential for pig diets, and seven FFPs and one co-product were found suitable for poultry diets. Most of the ingredients identified came from the brewery/distillery (17) and baking (10) industries. The inventory registers 14 products with crude protein (CP) over 20%, the highest being Brewers yeast (CP 35–40%, lysine 2.7%). Nineteen ingredients were liquid or moist, which limits their widespread use across pig systems in their current form. Information on availability, location, further processing requirements, and cost of the products was limited or unavailable, especially for Wales and Northern Ireland. Ingredients in the inventory have a lower environmental impact (ranging 5.2–406.2 kg CO eq/tonne product) than imported South American soyabean meal (3050 kg CO eq/tonne product, Tallentire et al. 2017).

# Conclusion

In conclusion, collation of information presented in this report has identified significant gaps in the information available in the public domain pertaining to FFPs, co-/by-products within the UK feed industry. To progress the utilisation on of these products as sustainable dietary ingredients for non-ruminant animals, it is imperative to establish a co-ordinated UK-wide strategy aimed at systematically identifying and categorising FFPs, co- and by-products. Such an approach will facilitate the advancement of processing and development techniques, ultimately promoting the utilisation on of these underutilised resources.

# Acknowledgements

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# 90. Lucky 7 Angus, eco-beef, Davinci Cattle

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In 1895 James Jensen started a five-generation ranch, from this came Lucky 7 Angus. That first winter he lived in a dugout on the side of a hill and shoveled snow off the grass to feed his 3 horses and 7 cows in what is called the Nation's Icebox, Boulder, Wyoming. Jim Jensen took over as the 4th generation and started raising Angus seedstock that were better adapted to survive in tough conditions. Lucky 7 Angus then became the leader in feed efficiency, high elevation PAP, and now in raising cattle that are Environmentally Friendly.

For any business to be sustainable it must be profitable. By implementing management and genetic selection you can make cattle that eat less per pound of beef produced, live longer and healthier, and thus make the rancher more profitable.

The second necessary condition for sustainability is to have the consumers demanding your practices. In today's world it is important to be Environmentally Friendly, which is what those same cows accomplish. These cattle also produce more pounds of beef with less Carbon output.

Lastly, it is important to show Agricultures roll in feeding the world as well as let them see the changes we are willing to make to make the world a better place.

By creating a new breed, Davinci Cattle, we will feed a growing population, and help save the world one herd at a time.

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# 91. The effect of feeding Ascophyllum nodosum to finishing beef cattle on performance, gas emissions and rumen fermentation

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# Application

The dietary provision of brown macroalga has the potential to reduce enteric methane (CH4) expelled from ruminants. The mechanism for reducing methane (CH4) may be due to phenolic compounds, specifically phlorotannins found exclusively in brown seaweeds.

# Introduction

Several macroalga (seaweed) species have been assessed in-vitro for their effects on rumen fermentation and subsequent CH4 mitigation. The red seaweed Asparagopsis taxiformis has been shown to reduce CH4 by up to 80% in-vivo1, however as an invasive species its cultivation in the UK is undesirable. As a result, native temperate species with differing modes of action are being investigated.

The brown species Ascophyllum nodosum (ASCO) yielded reductions of up to 10% when included at 8% dry matter (DM) in-vitro, whilst literature indicates that reductions could be greater with 15% being observed2. Other studies indicate that ASCO provision could improve rumen fermentation through supporting protein metabolism, reducing bloat and having anthelmintic activity3. However, there are limited studies investigating the effects of ASCO supplementation in-vivo, particularly in beef cattle. The aim of this study was to assess the impact of 4% DM ASCO provision in the diet on CH4 production, daily liveweight gain (DLWG), feed intake and carcass traits in finishing beef cattle. As ASCO has a high iodine content, 4% inclusion was chosen to ensure no negative effects on animal health.

# Materials and methods

Thirty-six finishing beef cattle of four breeds and two sexes were evaluated over a 100-day period. Cattle were allocated into two experimental groups 'control' (CON) and 4% DM (ASCO) balanced by liveweight, breed, sex and age. The cattle were housed in four pens (two pens of each sex) with nine animals per pen. A ration of 80:20 forage:concentrate was provided for the first 7 d of the study, 70:30 between 7–14 d and remained on a 60:40 ration for the rest of the trial period. In the ASCO group, the forage portion was reduced by 4% DM to account for the ASCO provision.

Methane and hydrogen emissions were recorded using GreenFeed units (C-Lock Inc., Rapid City, SD, USA), which were placed in each pen for a two-week training period prior to starting the treatment diet and a minimum of 49 days in each pen during the study. Feed intake was recorded real-time using Biocontrol feed boxes (BioControl A/S, Rakkestad, Norway) and mean daily intake each week calculated. Liveweight and body condition scores (BCS) were recorded bi-weekly, with average daily gain (ADG) calculated through regression. Rumen fluid samples were collected post-slaughter and assessed for volatile fatty acid (VFA) via gas-chromatography analysis and ammonia (NH3) content according to the method of Chaney and Marbach4. The carcass weight and kill out % was recorded at slaughter and marbling and fat colour of the meat was visually assessed by a trained technician using Meat Livestock Australia standard assessment chips four days post slaughter. The VFA and NH3 data sets were assessed for homogeneity and normality before the completion of one-way ANOVA analysis with Bonferroni post-hoc tests using GraphPad Prism version 5.

Methane emissions were evaluated as CH4 production (g/day), CH4 yield (g CH4/kg DMI) and CH4 intensity (gCH4/g LWG) and predicted as a linear mixed model (using REML estimation method) in GenStat with fixed effects of age, breed, sex and treatment. Pen, animal nested in pen and GreenFeed unit were included as random effects.

## Results

No statistically significant differences were observed for DM intake (P > 0.05), ADG kg/day (P > 0.05) or BCS (P > 0.05) across dietary treatments. The dietary inclusion of 4% DM ASCO had no significant effect on CH4 production (P > 0.05), CH4 yield (P > 0.05) or CH4 intensity (P > 0.05). The ASCO treatment numerically improved mean marbling score, but no statistical significance was observed in this or other carcass traits. No statistically significant differences were observed for NH3 (P > 0.05) or VFA (P > 0.05) production across treatments.

## Conclusion

The in-vivo inclusion of 4% DM ASCO into a finishing beef cattle ration had no effect on animal performance or gas emission variables. Whilst the use of brown seaweeds is favourable in temperate climates, the economic viability for minimal CH4 reductions and negligible production effects suggests that research focus should remain on red species for CH4 mitigation in ruminants. However, the potential effects of meat from animals supplemented with ASCO on human health parameters warrant further investigation.

## Acknowledgements

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# 92. Associations between data derived KPI's and gross margin in Welsh and English beef suckler herds

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## Application

Key performance indicators of beef suckler production can be used to predict the gross margin of both lowland and less favoured area suckler herds in Wales and England.

#### Introduction

Key performance indicators (KPI's) within livestock production systems can be used to inform both production and financial efficiency and aid on-farm decision making (Jones et al., 2021). This is done by collecting data on farm, calculating the KPI, and benchmarking to determine year on year performance or compare with industry recommendations. A recent report by Huang et al., (2022) highlighted that nearly half of the farms in their study were underperforming when they conducted a KPI review with farms scoring highly on KPI's tending to score highly on business resilience. While farming systems can be very diverse, gross margin offers the ability to measure the gross income less the variable costs on an enterprise basis. Therefore, the aim of the present study was to analyse data from the Farm Business Survey (FBS) and determine the relationship between calculated KPI's and gross margin per head of suckler cows in two different farming system types, lowland and LFA.

#### Materials and methods

Data was extracted from FBS which is the annual stratified survey of farms ran by Aberystwyth University in Wales and the Department for Environment, Food and Rural Affairs in England. After searching the literature for key performance indicators used to describe suckler cow performance, data was extracted for the year 2021–2022 using MATLAB from survey results and ten key performance indicators were calculated as described below. A total of 130 lowland and 315 LFA suckler farms were used for the data analysis. The FBS defines LFA or Low-land as holdings where grazing livestock account for two thirds of their total standard output (see Table 1).

#### Table 1

Results of the stepwise regression. Significance of coefficients: 0 \*\*\*\* 0.001 \*\*\* 0.01 \*\* 0.05 '.' 0.1 or 'ns' not significant.

	Changes in mean gross margin (£) given one unit change in KPI					
KPI	LFA		Lowland			
Variable Costs per cow, (£)	-0.6160	***	-0.51442	***		
Cow Mortality, %	-12.1437	**	-15.45502	***		
Replacement Rate, %	-2.9926	*		ns		
Vet and Med per cow, (£)		ns	0.05405	***		
Concentrates per cow, (£)		ns	1.03706	***		
Forage Area per cow		ns	67.15824	*		
Total calves per cow		ns	-68.85293			

Data quality was checked using histograms and qq plots. Farms with multiple key performance indicators missing were removed and data was also tested for normal distribution. Statistical analysis was conducted using RStudio and forward and backward stepwise linear regression was conducted. Possible predictors of the gross margin out of the following independent variables on a per cow basis; Vet & Med  $(\pounds)$ , Variable Cost  $(\pounds)$ , Concentrates  $(\pounds)$ , Gross Margin $(\pounds)$ , Forage Area (ha), Calf Mortality (%), Grazing units, Cow to Bull Ratio, Total calves, Cow Mortality (%), Replacement Rate (%).

#### Results

Average gross margin per head for Lowland sucklers was £256.20 and £284.98 for LFA suckler systems. Key performance indicators significantly associated with gross margin per head of suckler cows were cow mortality, variable costs, vet and med, concentrates and forage area with R2 = 0.4183 and 0.6125 for LFA and Lowland farms, respectively.

#### Conclusions

This data analysis has shown that on- farm livestock performance measured through KPI's can be used to predict the gross margin of suckler cow enterprises. The results can be used to illustrate and emphasise the financial gain farmers could achieve by measuring and benchmarking the performance of their suckler herd. Further work is needed to investigate which KPI's are going to be most advantageous in relation to production system type.

#### Acknowledgements

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#### 93. Analysis of environmental factor effects on rumen microbiome multi-omics traits in beef cattle

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#### Application

The study highlights that breed, diet, and additives were essential factors shaping the composition of a major number of ruminal metabolites, microbial genes, and microbe genera, but for some these specific interactions between factors were also significant with sometimes large effect value.

#### Introduction

Productivity of ruminant animals depends on the anaerobic microbial ecosystem to ferment and convert indigestible forages into premium meat products (Leahy et al., 2010). A comprehensive meta-analysis evaluated 98 enteric methane mitigation options (Arndt et al., 2022). Of the five solutions that decreased absolute methane emissions while maintaining animal productivity, only three were able to boost animal productivity while reducing product-based methane emissions. A lack of knowledge of rumen microbiomes and environmental interactions may be partially responsible for the inconsistency of the outcomes obtained from various methane mitigation strategies. The study aims to understand the impact of environmental factors, including host genetics, diet, and additives, on the rumen microbiome from a multi-omics perspective.

#### Material and methods

The study used metabolome and metagenomic data from rumen samples of 30 beef cattle recorded by SRUC (Roehe et al., 2016). The rumen samples corresponded to four different beef cattle breeds, two diets, and four feed additives. The detailed experimental design is as previously reported (Rooke et al., 2014). Metabolome: 115 metabolites with relative concentrations >0.001% were selected. Metagenome: 1461 microbial genes with relative abundance >0.001% were selected, and 1178 microbial taxonomies (genus level) with relative abundance >0.001% were selected. The study used the SPSS Generalized Linear Models module to treat each multi-omics trait (e.g., metabolite/microbial gene/microbial genus) as a dependent variable. The contribution and significance( $\alpha = 0.05$ ) of the main effects and interaction effects of environmental factors for each dependent variable were then obtained. Boxplots show the distribution of main effects and interaction effects contribution (P < 0.05) for each omics trait.

#### Results

For the metabolite, Fig. 1 shows that Diet had the lowest contribution of all main effects. Among interaction effects, Breed\*Diet\*Additive significantly affected the greatest number of metabolites, but the overall contribution was at a low magnitude. The interaction effect with the highest average impact contribution was Breed\*Additive.

Breed significantly contributed to more than 40% of variations of 267 microbial genes. Diet significantly affected the relative abundance of 607 microbial genes, which was 42% of the microbial genes in our dataset. Among the interaction effects, Diet\*Additive affected the highest number of microbial genes and expressed the highest overall contribution.

For microbial taxa, Breed explained 34.5% of the relative abundance of 258 microbial genera, which was the highest among the main factors. Diet was the factor that significantly affected the largest number of microbial genera. Among the interaction effects, Diet\*Additive significantly affected most microbial genera. Breed\*Addive had a higher overall contribution among interaction effects.

#### Conclusions

The Breed effect showed the largest average contribution to multi-omics traits. Diet significantly affected equal or more levels of each multi-omics trait but at a lower effect value than Breed. Generally, the interactions between factors affected lower levels of each multi-omics trait, than the main factors, but sometimes with large effect value, as found for Breed\*Additive effect on the variation of 13 metabolites.

#### Acknowledgements

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Fig. 1. Boxplots of the effect value of environmental factors and their interactions on each of the multi-omics traits. (The number in the box represents the number of levels of each multi-omics trait that were significantly affected by certain main or interaction effects).

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94. An investigation into the effect of mechanical brush provision on the behaviour, cleanliness and performance of finishing beef cattle

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## Application

Provision of a mechanical brush offers behavioural and performance advantages for housed finishing beef cattle.

## Introduction

High standards of animal welfare are fundamental in sustainable livestock production systems. Finishing beef animals indoors is commonplace, although housing facilities for beef cattle are often designed to optimise resource use efficiency with little consideration for the behavioural needs of the animals. As market demand for higher on-farm animal welfare standards continues to grow, more research is required to identify cost-effective solutions which can be implemented on beef farms to improve animal welfare. The objective of this study was to assess the effects of mechanical brush provision on the behaviour, cleanliness and growth performance of finishing steers.
# Materials and methods

This study was carried out on a commercial beef farm in Northern Ireland. On day 0 of the study, 72 continental steers were weighed and randomly assigned to one of two groups. 1 group (n = 36) were assigned to a pen containing a mechanical brush, while the other group (control; n = 36) were assigned to a pen containing no brush. The pens were identical in design, dimension and bedding management. Over the 60-day study period, both groups were managed identically and offered the same total mixed ration *ad libitum*. Animal behaviour was recorded by one trained observer for 12 hours each week using instantaneous scan sampling at 10-minute intervals. Cattle were cleanliness scored every 2 weeks using a 5-point evaluation system, with 13 individuals randomly selected at each scoring opportunity. Cattle were reweighed at the end of the 60-day finishing period, prior to being loaded for transport to slaughter. Individual daily liveweight gains (DLWG) were calculated and carcase weights and conformation grades were provided by the processor. Behaviour data was subjected to the Chi-squared test using IBM SPSS Statistics (version 27) to detect between group differences. Weight gains and carcase characteristics were analysed using analysis of variance. Cleanliness scores were also compared between groups.

## Results

Brush provision significantly increased lying and eating times (P < 0.05) and reduced the amount of time spent standing idle (P < 0.001) and walking (P < 0.05). The frequency of self-grooming behaviour was not significantly different across treatment groups (P > 0.05). Cattle provided with a brush engaged in fewer displacement and mounting behaviours (P < 0.05) and expressed stereotypic behaviours less frequently (P < 0.001) than those in the control group. There was no difference in cleanliness scores between groups. Cattle in the brush treatment group had significantly (P = < 0.05) higher DLWG's (+0.21 kg/day on average) than those in the control group. Cattle provided with a brush also tended to have better conformation scores although this difference was not significant (Table 1).

## Conclusion

Results from this study support the implementation of mechanical brushes in housing facilities for finishing beef cattle. The behavioural and performance advantages identified warrant further research into enrichment objects for housed beef cattle.

## Acknowledgement

This study was completed with the support of South West College.

### Table 1

Effects of mechanical brush provision on beef cattle performance parameters.

Parameter	Treatment		SEM	P-value
	Brush	No Brush		
Start weight (kg)	538	540	8.2	0.869
Final weight (kg)	656	645	8.9	0.390
DLWG (kg/d)	1.84	1.64	0.059	0.018
Cold carcass weight (kg)	377.3	374.5	5.71	0.737
Dressing proportion (g/kg)	0.575	0.581	0.0044	0.361
Conformation score*	10.9	10.4	0.23	0.078
Fat Classification	6.6	6.3	0.25	0.346

\* Conformation based on EUROP converted to 15-point scale (1=P-, 15=E+).

\*\* Fat classification based on 1-5 converted to 15-point scale (1=1-, 15=5+).

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## 95. Dietary supplementation with rapeseed oil and cake on animal performance, methane emissions, and digestibility of beef cattle

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## Application

Ireland has an ambitious target of reducing agricultural GHG emissions by 25% by 2030, which includes a recommended 10% reduction in enteric methane. Many potentially effective dietary additives are not feasible due to availability, requirement for regulatory approval, transient efficacy or cost. Practical and cost-effective dietary based methane mitigation regimens are necessary to reduce carbon footprint and improve the circularity of nutrient usage in beef cattle rations.

## Introduction

Oilseed rape (*Brassica napus* L) is a popular break crop in Irish cereal production. Its high fat containing by-products have been suggested to be effective in reducing enteric methanogenesis in ruminants (Bayat et al. 2022). Rapeseed oil is rich in fatty acids including oleic (C18:1), linoleic (C18:2), palmitic acid (C16:0) and linolenic acid (C18:3), which are putatively responsible for the observed  $CH_4$  inhibition

(Lewinska et al. 2015). Additionally, high fat diets have been shown to positively affect intramuscular fat content of meat (Hess et al. 2008). With high rapeseed oil by-products such as cold pressed rapeseed cake, there is an opportunity to also improve nutrient circularity by reducing reliance on imported ration ingredients for finishing diets. The objective of this study was to holistically examine the effect of fatty acid supplementation from different physical forms of rapeseed (cold pressed rapeseed cake and rapeseed oil) on dry matter intake (DMI), average daily gain (ADG), enteric gaseous emissions, haematological profile and poly unsaturated fatty acid (PUFA) content of *longissimus dorsi* muscle of beef cattle on a winter finishing diet.

## Materials and methods

For the experiment, 54 crossbred Charolais steers and heifers; with an initial body weight (BW) of 384 kg (SD 39.5) at Teagasc Grange, Animal & Grassland Research and Innovation Centre were offered concentrate feed at 41% of total DMI (exclusive to silage) containing one of the following: 1. Unsupplemented control (CON), 2. Rapeseed cake (RSC) or 3. Rapeseed oil (RSO). Dietary inclusion of rapeseed oil and cake (2.5 and 14.5%, respectively) was balanced on oil, total fatty acid, PUFA and mono unsaturated fatty acid (MUFA) content. Cattle were offered their respective diets with grass silage on an *ad libitum* basis during which feed intake and gaseous emissions analysis (GreenFeed emissions monitoring system – GEM) were recorded over 88 consecutive days, including a 17 day pre- baseline period. Cattle were weighed weekly to calculate ADG. Ultrasonic muscle and fat deposition measurements were recorded at day 1 and 87. Blood serum samples were collected from the jugular vein on day 10, 53 and 88 of the experiment. A muscle biopsy was collected from 15 animals per treatment from the *longissimus dorsi* muscle on day 89 to assess the fatty acid composition. Statistical analysis was performed on SAS (version 9.4) where repeated measures was used to assess changes in gaseous emissions, DMI, and blood metabolites over time. Linear regression was used to calculate ADG. For muscle and fat deposition measurements and fatty acid composition of the *longissimus dorsi* muscle, data was analysed using the mixed-model procedure (PROC MIXED). The probability value for statistical significance is *P* < 0.05.

## Results

Cattle achieved a mean DMI of 7.44 kg/day (SD = 1.3) and there was no statistically significant difference between treatments (P > 0.05). Cattle fed RSO and CON had a higher ADG than RSC (1.24, 1.17 and 1.05 kg/day respectively). A total CH<sub>4</sub> reduction of 15.52 and 15.91 g/day was observed in RSC and RSO supplemented cattle, respectively, compared to CON. This equated to a lower CH<sub>4</sub> intensity of 2.01 and 2.79 g/kg DMI for RSC and RSO compared to CON, and 0.03 and 0.04 g/kg BW, respectively. The production of CO<sub>2</sub> in the rumen was not affected however, H<sub>2</sub> was reduced by RSO supplementation. There was an increase in serum concentrations of HDL and LDL cholesterol for RSC and RSO (P < 0.05). Diets had no effect on muscle and fat accretion, and did not alter the fatty acid composition of the muscle (P > 0.05).

	CON	RSC	RSO	S.E.M	P value
DMI; kg	7.5	7.61	7.74	0.012	0.22
ADG; kg	1.17 <sup>a</sup>	1.05 <sup>b</sup>	1.24 <sup>a</sup>	0.027	*0.01
Start weight; kg	383.9	384.3	383.6	5.61	0.95
Middle weight; kg	421.0	418.9	424.3	5.84	0.41
End weight; kg	465.3	459.5	464.9	5.66	0.54
Gaseous emissions					
CH <sub>4</sub> ; g/d	199.11 <sup>a</sup>	183.59 <sup>b</sup>	183.20 <sup>b</sup>	0.661	*0.021
H <sub>2</sub> ; g/d	0.50 <sup>a</sup>	0.49 <sup>a</sup>	0.43 <sup>b</sup>	0.004	*0.031
$CO_2$ ; g/d	6978	6718	6843	15	0.25
CH <sub>4</sub> ; g/kg DMI	26.61 <sup>a</sup>	24.60 <sup>ab</sup>	23.82 <sup>b</sup>	0.108	*0.002
CH <sub>4</sub> ; g/kg BW	0.47 <sup>a</sup>	0.44 <sup>b</sup>	0.43 <sup>b</sup>	0.001	*0.005

DMI = dry matter intake, ADG = Average daily gain, BW = body weight.

# Conclusion

Supplementing beef cattle diets with either rapeseed oil or rapeseed cake resulted in a similar reduction in ruminal  $CH_4$  emissions of approximately 8% when compared with an unsupplemented control diet. Animals supplemented with RSO achieved this without any notable effect on performance, however RSC reduced ADG. Rapeseed derived ingredients altered HDL and LDL cholesterol concentrations in the blood. Rapeseed oil has potential as an anti-methanogenic feed supplement with no impact on animal performance, showing consistent efficacy over the duration of the experiment and expeditious implementation due to no regulatory approval requirements.

## Acknowledgements

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## 96. Preliminary investigation of the mineral and fatty acid composition of grass-fed Dexter beef

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## Application

Beef from Dexter breed cattle is well renowned for its notably high marbling content, yet this breed has received very little scientific investigation in term of meat quality. This preliminary study offers insight into the mineral and fatty acid (FA) composition of commercially produced, grass-fed Dexter beef in the context of nutrient provision to humans.

## Introduction

Beef contributes an array of important and highly bio-available nutrients to the human diet, amongst which certain minerals and Fas have particular importance such as iron (Fe), zinc (Zn), selenium (Se) and omega-3 Fas. Dexter cattle are a rare dual-purpose breed of miniature cattle originating from Ireland with mature weights of up to 350 kg for cows and 450 kg for bulls (McMahon, 2016), and are predominantly reared in extensive, pasture-based systems on small-scale holdings. There has been very little scientific research carried out on the Dexter breed, particularly with regard to meat quality despite the notable high marbling content of beef produced from Dexter breed cattle. Thus, the aim of this preliminary study was to determine and characterise the mineral and FA profile of extensive grass-fed Dexter beef.

## Materials and methods

Seven commercially-reared Dexter breed cattle were slaughtered in September-October 2018, with an average slaughter age of 30 months and carcass weight of approximately 130 kg. Cattle received a grass only diet with a high magnesium salt lick at grazing, and a grass hay-lage diet during winter housing. Cattle were slaughtered locally to the farm at Long Compton abattoir located in Warwickshire, UK. Carcasses were aged for three weeks prior to cutting, at which point samples of *longissimus dorsi* muscle were collected. Subcutaneous fat, intermuscular fat and connective tissue were removed and the remaining muscle tissue freeze-dried and ground for laboratory analysis. Minerals were extracted using a nitric-perchloric acid digestion with concentrations of Fe, potassium (K), magnesium (Mg) and phosphorus (P) determined by ICP-OES and copper (Cu), manganese (Mn), Se and Zn by ICP-MS. FA methylation and quantification was carried out as per Lee et al (2012).

#### Results

Grass-fed Dexter beef contained 'nutritionally significant' concentrations (i.e. >15% of the nutrient reference value (NRV) per 100 g) of K, P, Fe and Zn (Fig. 1). Concentrations of Mg, Cu, Mn and Se were not 'nutritionally significant', although Se was very close to the threshold of 15% of NRV. Mean ( $\pm$ SD) total FA content of the Dexter beef analysed was 5.26% ( $\pm$ 1.25); comprising 42.0% ( $\pm$ 2.77) saturated Fas, 49.3% ( $\pm$ 2.88) monounsaturated Fas, 7.15% ( $\pm$ 0.948) polyunsaturated Fas. Mean concentration of  $\alpha$ -linolenic acid (ALA) was 54.2 mg per 100 g ( $\pm$ 7.19) and below the minimum of 300 mg of ALA per 100 g required for labelling as a 'source of' omega-3 Fas. Mean summative concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was 19.1 mg per 100 g ( $\pm$ 1.66), again below the minimum of 40 mg of long-chain omega-3 Fas' then the 'source of' omega-3 Fas. However, if docosapentaenoic acid (DPA) were included in the sum of 'long-chain omega-3 Fas' then the 'source of' threshold is exceeded, with the mean summative concentration of EPA, DHA and DPA being 44.8 mg per 100 g ( $\pm$ 3.45) (Fig. 2).



Fig. 1. Mean mineral concentrations (solid bars  $\pm$  SD) in commercially produced, grass-fed Dexter beef compared to 15% of the nutrient reference value (NRV) for each mineral ( $\times$ ).



Fig. 2. Mean summative concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) in commercially produced, grass-fed Dexter beef compared to concentrations required per 100g of product to be labelled as a 'source of' or 'high in' omega-3 fatty acids.

## Conclusions

Grass-fed Dexter beef provides nutritionally significant amounts of K, P, Fe and Zn and can be labelled as a source of these minerals. However, it does not meet current nutritional claims rules in the UK/EU for labelling as a 'source of' omega-3 Fas based on EPA and DHA alone. Labelling grass-fed Dexter beef as a 'source of' omega-3 Fas would require a rule change so that DPA is included in the definition of 'longchain omega-3' Fas alongside EPA and DHA. Further work is required to determine the extent to which small-scale, locally-grown and extensively-reared meat, such as grass-fed Dexter beef, can contribute to healthier, more sustainable and more equitable human diets.

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# 97. Effect of farrowing pen hygiene (standard vs. optimal) and creep feeding regime (dry pelleted starter diet vs. liquid mixture of milk replacer and starter diet) on pig feed intake and growth

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## Application

Creep feed dry matter intake of piglets was increased when a liquid mixture of starter diet and milk replacer was offered but only when optimal hygiene conditions existed in the farrowing accommodation. This dietary strategy increased pre-weaning growth and weaning weight and improved pen hygiene increased creep feed dry matter intake.

Introduction: Piglet weaning weight (ww) can be increased by increasing pre-weaning creep feed intake. The objective was to evaluate the effect of providing dry pelleted starter diet (DPS) or a liquid mixture of milk replacer and starter diet (LMR+S) to suckling pigs housed in farrowing accommodation of standard or optimal hygiene on pre- and post-weaning feed intake and growth.

## Material and methods

The experiment was a  $2 \times 2$  factorial arrangement with factors being pen hygiene (standard or optimal) and creep feeding regime (DPS or LMR+S). The standard hygiene environment was obtained by thorough washing with water and <18 hours drying. The optimal hygiene environment was obtained by a thorough washing with water and <18 hours drying. The optimal hygiene environment was obtained by detergent application, followed by a thorough water wash, chlorocresol-based disinfectant application and 6 days drying. Both cleaning protocols were implemented prior to entry of the sows to the farrowing rooms. Prior to entry of sows, 16 pens from each hygiene environment were swabbed for determination of *Enterobacteriaceae* counts and total bacterial counts. On day (d) 107 of gestation, 87 sows were weighed and blocked on the basis of parity, previous number of piglets weaned and bodyweight before being randomly assigned to one of the four treatments. By 48 hours post-farrowing, litter size was standardised within block. From d11 to weaning at d28 DPS (3 mm diameter) was offered. Between d4-9 piglets on LMR+S (~15% dry matter) treatment were offered liquid milk replacer only; thereafter a liquid mixture of milk replacer and an increasing proportion of starter diet was offered up to weaning. Suckling pigs were weighed on d4 and d28 of age and total dry matter feed intake (DMI) was recorded for the entire suckling period. At weaning, single-sex pen groups (12 male or female pigs of even weight per pen; n = 12 pens/treatment) were formed, blocked on sex and ww and followed until target slaughter weight (130 ± 1.9 kg). Data were analysed using PROC MIXED (v9.4, SAS Institute Inc.) with

creep feeding regime (DPS or LMR+S), pen hygiene (standard or optimal) and their associated interactions as fixed effects. Block was a random effect and piglet birth weight (for pre-weaning parameters) and ww (for post-weaning parameters) were included as co-variates, when significant in the model. The litter/sow was the experimental unit prior to weaning and pen group was the experimental unit post-weaning. Pre-weaning mortality per litter and days required to reach target slaughter weight from weaning to slaughter were analysed using PROC GLIMMIX with multinomial distribution. The Tukey-Kramer adjustment was applied for multiple comparison of means and differences were considered significant when  $P \le 0.05$ .

## Results

At entry of sows to farrowing accommodation, optimal hygiene pens had lower *Enterobacteriaceae* counts (0.4 vs  $3.5 \pm 0.10 \log_{10} \text{CFU/cm}^2$ ) and total bacterial counts (2.1 vs  $4.9 \pm 0.14 \log_{10} \text{CFU/cm}^2$ ) compared to standard hygiene pens (P < 0.05). There was a creep feeding × hygiene interaction for ww. Weaning weight was higher for LMR+S compared to DPS in both standard and optimal hygiene environments (Table 1; P < 0.05). At weaning LMR+S piglets were heavier than DPS piglets (8.9 vs  $8.3 \pm 0.12$  Kg; P < 0.05). There was a creep feeding × hygiene interaction for DMI from d4-28 of lactation; DMI was increased by LMR+S in the optimal but not the standard hygiene environment (Table 1; P < 0.05). The DMI of piglets was higher in the optimal compared to the standard hygiene environment (475 vs  $326 \pm 34.8$  g; P < 0.05). The ADG was higher for LMR+S compared to DPS (289 vs  $272 \pm 6.4$  g/day; P < 0.05) but was not affected by pen hygiene (P > 0.05). The proportion of piglet deaths per litter between d4-28 was reduced by  $1.01 \pm 0.50\%$  with LMR+S (P < 0.05). Post-weaning, pigs originating from optimal hygiene farrowing pens had numerically higher ADG and reached target slaughter weight 3.8 days earlier (P < 0.05) than pigs from standard hygiene pens.

#### Table 1

Effect of creep feeding regime (DPS or LMR+S) and farrowing pen hygiene (standard or optimal) on pre-weaning piglet feed intake and growth [Least square means ± pooled standard errors of the mean (SEM)].

Creep feeding	DPS <sup>1</sup>		LMR+S <sup>1</sup>			P-value		
Hygiene	OPTIMAL	STANDARD	OPTIMAL	STANDARD	SEM	Hygiene	Creep feed	Hygiene * Creep feed
No. Sows/litters Bodyweight (kg)	22	23	21	21				
Day 4	2.1	2.2	2.3	2.2	0.09	0.78	0.32	0.62
Day 28 (weaning)	8.3 <sup>ab</sup>	8.2 <sup>a</sup>	8.9 <sup>c</sup>	8.7 <sup>bc</sup>	0.18	0.35	<0.01	0.03
Total dry matter intake (g/pig)	374 <sup>a</sup>	343 <sup>a</sup>	576 <sup>b</sup>	310 <sup>a</sup>	49.2	0.01	0.09	0.02
Average daily gain (g/day)	274	269	295	283	7.9	0.26	0.01	0.62

 $^{a-c}$  Values within a row that do not share a common superscript differ significantly at *P*<0.05.

<sup>1</sup> Dry pelleted starter diet from d11-28 (DPS) and a liquid mixture of milk replacer and starter diet from d4-28 (LMR+S).

## Conclusion

Total creep feed dry matter intake of suckling piglets was increased by feeding a liquid mixture of milk replacer and starter diet but only under optimal hygiene conditions. The liquid mixture of milk replacer and starter diet increased pre-weaning growth and weaning weight and reduced pre-weaning mortality. Optimal pen hygiene increased total creep feed dry matter intake of suckling piglets and reduced the days to slaughter.

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# 98. Risk of weaner pigs developing ear necrosis – Preliminary investigation of associations with pre-weaning ear biting behaviour and other early life characteristics

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#### Application

The etiology and risk factors for ear necrosis are largely unknown, which hampers efforts to prevent and treat the disease. This study attempts to elucidate some of the risk factors, which can help improve pig health and welfare.

## Introduction

Ear necrosis is a major welfare concern in recent years but we have poor understanding of the risk factors and causes. These are likely multifactorial but one theory proposes ear biting as a risk factor for the disease whereby trauma to the ears caused by biting behaviour enables infection by opportunistic pathogens (e.g. Staph aureus). Another hypothesis is that the infection arises from an internal systemic imbalance resulting in immunosuppression. Such an imbalance could be inherent to the pig (e.g. birthweight) or caused by dietary inadequacies/ weaning stress, etc. Pigs start directing behaviour to their littermates' ears early in life and progress to more traumatic biting as they age. This pilot study investigated the role of ear biting behaviour performed by piglets in the week prior to weaning and other early life characteristics in the development of ear necrosis (EN) post-weaning.

## Materials and methods

A total of 138 piglets born to 11 sows were housed in conventional farrowing crates with plastic slatted floors, one solid plastic heating mat, and one plastic toy attached to the wall. At weaning (28 d of age), they were mixed into 13 same sex pens of 9–10 pigs with single space feeders, plastic slatted floors, and two rubber floor toys. At three weeks of age, pigs were individually marked on their backs with hair dye to facilitate individual behaviour observations. All occurrences of ear biting behaviour were recorded by direct observation with continuous sampling (3-minute sessions; n = 24 minutes total/litter) over three days in the week prior to weaning. The identity of the bitten pig was recorded, and the average number of bites per observation session was calculated for each pig. Pigs were individually inspected for EN weekly from weeks 2 to 6 post-weaning (until transfer to finisher accommodation), and scored 0 to 5 according to severity. Scores were collapsed into 1] presence or absence of EN; 2] the maximum score the pig achieved during this time (MaxScore). Data were analysed using SASv9.4 (PROC Glimmix and Spearman correlations) to investigate associations between pre-weaning ear biting behaviour, birthweight, and litter characteristics (total piglets born, litter size at d1, mortality rate from d1 to day of behaviour observation) and EN.

## Results

While 58.7% of pigs developed EN post-weaning, the majority received mild scores (Table 1) and no pigs received a score of 5. The average number of ear bites per 3 min observation session was 0.36 bites/litter. This equates to an average of 1.5 ear bites per minute occurring in a single litter when piglets are active in the week prior to weaning. There was no apparent association between ear biting and EN (P > 0.05). While total born (P > 0.05) and litter size at d1 (P > 0.05) were not associated with EN (data not shown), mortality rate had a moderate positive association with both EN of any severity ( $r_{(136)} = 0.21$ , P = 0.01), and EN MaxScore ( $r_{(136)} = 0.21$ , P = 0.02). Additionally, pigs that developed EN of any severity had higher birthweights than those that did not develop EN (1.57 ± 0.312 vs. 1.44 ± 0.293 kg, P = 0.03).

#### Table 1

Descriptive data (mean ± SE) of ear biting behaviour pre-weaning and early life characteristics and ear necrosis (EN) development post weaning.

	Maximum EN score					
Variable	0	1	2	3	4	
Pigs (no., %) Ear bites/3min obs (no.) Birthweight, kg Litter mortality. %	57 (41.30) $0.39 \pm 0.39$ $1.44 \pm 0.29$ $11.50 \pm 10.11$	$43 (31.16) \\ 0.32 \pm 0.42 \\ 1.54 \pm 0.29 \\ 15.66 \pm 10.04$	15 (10.87) 0.37 ± 0.30 1.58 ± 0.35 15.97 ± 10.10	$18 (13.04) \\ 0.34 \pm 0.29 \\ 1.62 \pm 0.29 \\ 16.31 \pm 9.77$	5 (3.62) $0.50 \pm 0.16$ $1.54 \pm 0.39$ $18.39 \pm 8.46$	

## Conclusion

The lack of an association between ear biting behaviour and EN could relate to the small number of pigs in the study and to the limited number that developed severe EN post-weaning. It may also be that the numerous stressors imposed on the pig at weaning outweighed any effect of behaviour recorded pre-weaning. Five pigs with an EN score of 4 received on average the numerical highest number of ear bites pre-weaning. This suggests the need for further research with more animals before rejecting the hypothesised link between ear biting behaviour and EN. The link between EN and higher birthweights supports on-farm findings where pigs with higher weaning weights had an increased risk of EN (Busch et al., 2008). Likewise, Diana et al. (2019) showed that the fastest growing pigs were more likely to develop EN. This suggests the possibility of an imbalance in the biological requirements of fast growing animals (e.g. nutritional, space etc.). Our pre-liminary findings indicate that further research on early life characteristics would help us better understand the role of internal, systemic factors in EN development.

## Acknowledgements

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## 99. Sex effects on porcine microbiota impact on alternative treatments for post weaning diarrhoea

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## Application

Differential microbiota composition between males and females may indicate that sex-specific therapeutical intervention approaches could improve their efficacy.

# Introduction

We previously described that the broad-spectrum antimicrobial-alternative peracetic acid (PAA) derived by precursor hydrolysis was able to decrease diarrhoetic symptoms in piglets (Galgano et al., 2023), in similar fashion as zinc oxide (ZnO). Here, we further explored possible interactions between these interventions and piglet sex.

## Materials and methods

Four treatments (control, 3100 ppm in-feed ZnO, 50 ppm and 150 ppm of in-water PAA), were administered to 6 pens/treatment (14-day trial) with 2 pigs per pen, 6 rooms (4 pens/room) and balanced for sex. In-water PAA treatments were prepared daily from mixing different ratios of PAA precursors. We report here data on stomach and caecal microbial composition via 16S rRNA sequencing (515Fb-816Rb). Linear mixed model was carried out in R via using the package lme4 for performance and MaAsLin2 for taxonomical data, including fixed (treatment and sex) and random effects (rooms/pens/pigs).

## Results

The taxonomical analysis of the composing genera through the gut locations analysed revealed both compositional differences between sexes and in response to treatments in males and females. In the stomach, the relative abundance of both *Fibrobacter* and *Sharpea* variated significantly when analysing the microbiota of males and females (P < 0.05, Q < 0.05). Moreover, 97 genera were significantly differentially abundant as a consequence of treatment administration in either males or females. Of these, *Actinobacillus* was found to be significantly less abundant (P < 0.05, Q < 0.05) in the males given 150 ppm of PAA ( $0.1\% \pm 0.1\%$ ) compared to all other treatment-sex combinations ( $0.8\% \pm 0.6\%$ ). In the caecum, the relative abundance of 27 genera was significantly different when comparing the microbiota of males and females in general (P < 0.05, Q < 0.05), amongst which *Lactobacillus* was significantly enriched in females ( $23.57\% \pm 8.53\%$ ) compared to males ( $14.21\% \pm 8.63\%$ ). In parallel, 135 genera were significantly differentially abundant (P < 0.05, Q < 0.05) in the interaction of treatment and sex. Amongst these genera, *Ruminococcus* was less abundant in males given ZnO ( $0.09\% \pm 0.07\%$ ) compared to all other treatment-sex combinations, and in the caecal content of females administered ZnO ( $0.42\% \pm 0.42\%$ ) compared to control females ( $1.65\% \pm 1.04\%$ ) (Fig. 1).

## Conclusions

Treatments targeting the modulation of gut microorganisms could achieve different effects in males and females, likely due to differences in microbiota composition between the two sexes. Amongst others, we found that 150 ppm of PAA resulted in a reduction of *Actinobacillus* in the



B Caecum Ruminococcus spp.

🛢 0ppm 🛢 ZnO 🛢 50ppm 🖨 150ppm





stomach of male pigs. Interestingly this genus had previously been found in association to infection in porcine epidemic diarrhoea (Tan et al., 2020), likely pointing towards a beneficial effect of this PAA level of inclusion in males. In parallel, we found that *Lactobacillus*, whose enrichment in the lower gut is usually associated with healthier pigs was more abundant in females than males in general, whilst probiotic genera, such *Ruminococcus* (Sun et al., 2019) were reduced in both females and males given ZnO. Our findings could thus indicate that interventions based on the modulation of the gut microbiota may benefit from being sex-tailored in order to enhance their therapeutic effect.

#### Acknowledgments

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## 100. Does removing Zinc Oxide affect post-weaning blood chemistry?

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### Application

This study shows that removing Zinc Oxide (ZnO) from post-weaning diets does not negatively affect C-reactive protein (CRP), Haptoglobin (Hp), Tacrolimus (TAC) or Cortisol levels in the blood.

## Introduction

There is an urgent need to seek alternative strategies to cope with the absence of ZnO in piglet diets. The European swine industry has been banned from using ZnO due to its effect on the environment. Modifications of Crude Protein (CP) and Crude Fibre (CF) have been suggested as nutritional measures to reduce the risk of post-weaning diarrhoea (PWD) in the absence of ZnO. This work sought to evaluate the impact of different post-weaning diets varying in levels of CP, CF and the addition of dietary supplements on piglet post-weaning performance.

## Materials and methods

720 pigs, housed in mixed-sex pens (10 pigs/pen, 12 pens/treatment) and weaned at 28 days of age, were offered one of six treatments (T) constructed of a Starter 1 diet (16.25 MJ/kg DE, 1.65 % Lys) for 13 days. Treatments were: **T1**, conventional diets (20% CP, 2.11% CF) with ZnO (2500 mg/kg); **T2**, conventional diets, no additional ZnO; **T3**, conventional diets with antibiotic (Apramycin,100 mg/kg), no additional ZnO; **T4**, 18% CP diets (3.5% CF), no additional ZnO; **T5**, 16% CP diets (3.5% CF), no additional ZnO; **T6**, 18% CP diets (2.49% CF) and supplemental amino acids (commercial product), no additional ZnO. All treatments were formulated at the same Energy/Lysine ratio. On day 0 and 7 blood samples were taken from two average piglets per pen (one male and one female). Samples were collected in 2 ml lithium heparin tubes, centrifuged at 7000 rpm for 15 min, and frozen at – 22 °C pending analysis. Data were analysed in R studio using the nlme package to perform GLM models. Body weight was included as a covariate and where appropriate, batch was included as a random factor in the models. The alpha level of significance was set at *P* < 0.05.

#### Results

Cortisol concentrations were significantly lower in the T1 treatment compared to T3 at day 7 (P < 0.05). Tendencies were observed on C-reactive protein (CRP; P = 0.057) and Haptoglobin (Hp; P = 0.067) on day 0 and 7, respectively. Both of these were a result of low concentrations of CRP and Hp in the T4 treatment compared to T2 (CRP), and T4 to T6 (Hp) (Table 1).

#### Table 1

The effect of dietary treatment on plasma C-reactive protein (CRP), Haptoglobin (Hp), Tacrolimus (TAC) and cortisol concentration in the blood of piglets at Day 0 or 7 of age (mean ± SEM).

	Day 0				Day 7			
	CRP (mg/L)	Hp (g/L)	TAC (mmol/L)	Cortisol (µg/dL)	CRP (mg/L)	Hp (g/L)	TAC (mmol/L)	Cortisol (µg/dL)
T1	8.17	0.261	0.216	5.16	7.54	1.08	0.170	1.37ª
T2	13.7	0.294	0.239	4.54	8.36	0.721	0.186	1.58 <sup>ab</sup>
Т3	12.7	0.375	0.228	3.72	16.1	1.01	0.178	2.13 <sup>b</sup>
T4	6.52	0.110	0.210	3.51	9.87	0.540	0.177	1.54 <sup>ab</sup>
T5	11.6	0.357	0.214	3.52	14.0	0.843	0.185	2.09 <sup>ab</sup>
T6	10.1	0.220	0.250	4.52	15.5	1.24	0.171	1.97 <sup>ab</sup>
SEM	1.78	0.1040	0.0161	0.540	4.950	0.2130	0.0110	0.206
Treatment	Probabilities 0.057	0.521	0.380	0.188	0.626	0.067	0.862	0.029

<sup>a,b</sup> Means within in a column with different superscripts differ significantly (P<0.05).

## Conclusions

The nutritional interventions used in this study had little effect on biomarkers of inflammation and oxidative stress. There was only a significant effect on cortisol, however, it is worth bearing in mind that cortisol is a highly variable measurement (Larzul et al, 2015).

## Acknowledgments

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## 101. Exploring the effects of dietary amino acid levels on the social behaviour and performance of commercial pigs

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### Application

Manipulating Lys and Trp content of pig feed could help to reduce harmful social behaviours, with a possible positive effect on production measures.

### Introduction

Negative social behaviours between pigs, such as ear biting, can cause acute and chronic stress, result in lesions and compromise pig welfare. There is growing interest in the manipulation of amino acid levels in pig diets as a nutritional strategy to affect behaviour (Meer et al., 2017). In the current study, we focused on lysine (Lys) and tryptophan (Trp). Lys is the first limiting amino acid in the majority of pig diets and Trp is the primary precursor of serotonin. To ensure commercial relevance, effects of diet on behaviour should be explored in the context of production. Here, we determined the effects of varying dietary Lys and Trp levels on the social behaviour and performance of pigs.

## Material and methods

2,293 PIC Camborough<sup>®</sup> barrows with a mean starting weight of  $11.87 \pm 1.35$  Kg were used in a randomised complete block design (block = wean date and weight) with a 2 × 3 factorial arrangement of the following feeding treatments: 1) Standardised ileal digestible (SID) Lys levels: 100% Lys = diets with 100% PIC requirement at the average point of the growth phase (LH), vs 80% Lys = diets with 80% PIC requirement at the beginning of the growth phase (LL); and 2) Trp to Lys ratio of 0.210 (TH), 0.185 I or 0.160 (TL). Pigs were randomly allocated across the 6 treatments over 3 starting dates (wean dates). The pig's behaviour, the number of pigs showing signs of ear, tail and flank lesions and the severity of those lesions and the severity and location of lesions caused by fighting were recorded over a period of 10 weeks. Feed intake and weight gain were recorded. Statistical analysis was carried out using R Software. Pen was the experimental unit and non-significant fixed effects and interactions were dropped from models. Treatment 6 was stopped after feeding phase 2 after reaching behavioural and production endpoints. Only behaviour and production data from treatments 1–5 were analysed. For lesions, data from all 6 treatments was analysed. Behaviour observations were analysed using principal component analysis. Both ear lesions (total and severe ear lesions) and fight lesions scores were adapted into binary data sets (presence and absence of lesions, lesion score above and below median score respectively) and then analysed using a generalised linear mixed model. Production characteristics were calculated from raw data and then analysed using linear mixed models.

## Results

We found that the effect of Lys tended towards significant (F = 3.499, df = 1, p = 0.066) but there was no effect of Trp on PC1 (activity) (F = 0.326, df = 2, p = 0.723). We found no effect of Lys or Trp on PC2 (social behaviour) (Lys; F = 0.004, df = 1, p = 0.953, Trp; F = 0.987, df = 2, p = 0.379). We found there to be a significant effect of Lys (F = 4.806, df = 1, p = 0.032) and barn (F = 9.028, df = 1, p = 0.009) on ear biting behaviour. Pigs on lower Lys diets showed higher levels of ear biting behaviour.

We found a significant effect of the Lys score week interaction on the number of pens showing ear lesions ( $X^2 = 18.408$ , df = 2, p < 0.001) and a significant effect of both the Lys Trp interaction ( $X^2 = 6.997$ , df = 2, p = 0.030) and the Lys score week interaction ( $X^2 = 6.574$ , df = 1, p = 0.0104) on the number of pens showing severe ear lesions. Pigs on LH/TM and LH/TL had significantly lower incidences of severe lesions that other treatments. We found an effect of pig size ( $X^2 = 36.487$ , df = 2, p < 0.001) and score week ( $X^2 = 22.464$ , df = 1, p < 0.001) on fight lesions but no significant effect of Lys ( $X^2 = 0.071$ , df = 1, p = 0.790) or Trp ( $X^2 = 2.919$ , df = 2, p = 0.232).

There was a significant effect of Trp (F = 3.507, df = 1, p = 0.036) and feeding phase (F = 2406.377, df = 5, p < 0.001) on pigs average daily feed intake (ADFI), pigs on the TM diet had a significantly higher ADFI than pigs on the TL diet. However, we found no effect of Trp on average daily gain (ADG) (p = 0.227). We found a significant effect of the Lys feeding phase interaction on ADG (F = 157.73, df = 11, p < 0.001). At feeding phases 2, 3 and 4, pigs on the LH diet had a significantly higher ADG than pigs on the LL diet. We found a significant effect of the Lys feeding phase interaction on feed conversion ratio (FCR) (F = 5.450, df = 5, p < 0.001). In feeding phases 1, 2, 3 and 4 pigs on the LH diet had a significantly lower FCR than pigs on the LL diet.

## Conclusions

Results suggest that dietary Lys levels affect the behaviour of pigs, specifically low lysine levels increased ear biting behaviour which is a significant issue. Lys seems to have the greatest effect on the pig weight gain and while there is evidence that the TH pigs did consume more feed, it was not enough to compensate for the effects of the Lys deficit. Tail and flank biting were not common in this population, but the potential remains for Lys levels to affect this behaviour in other populations and so deserves further exploration especially as EU producers are encouraged to maintain intact tails.

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## 102. The interaction between stocking level and loose enrichment material on pig performance

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#### Application

A deeper understanding of how loose enrichment material and stocking levels impact the production parameters of terminal line pigs may provide producers with the necessary insight to make informed decisions on increased welfare implementations.

#### Introduction

To meet the welfare standards defined in EU animal welfare legislation, pig producers need to adapt their current management practices. This is particularly the case in relation to tail docking. At the same time, it is essential that pig enterprises remain profitable. Straw is the gold standard as enrichment for pigs but it is not always available in Ireland. Thus this study compared straw (n = 12 pens) with three alternative manipulable enrichment materials; Haylage (n = 12), Hay (n = 12) and Grass (n = 11), with the hypothesis that responses of the pigs to the various materials would not differ. As stocking density 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, 0.41 m<sup>2</sup>/pig; finisher: 1.24, 1.00, 0.83 m<sup>2</sup>/pig, respectively), and hypothesised that pigs in lower stocked pens would have improved performance, and reduced incidence of tail biting outbreaks.

#### 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  $4 \times 3$  factorial design. Enrichment was provided via a mesh rack hung on the front wall of the pen. Pens of pigs were weighed at three time points; when transitioning from the weaner phase to the finisher phase (Transfer; six weeks post-weaning), three weeks later (Mid-finish), and the day prior to slaughter (Week 21). Pig were fed ad libitum, and feed delivery recorded daily at pen level. Metrics included in analysis were pig weight, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). An outbreak of tail biting was considered when at least one pig had an open wound on its tail and an intervention was put in place (e.g. addition of supplementary enrichment). Data were analysed using SAS v9.4 accounting for repeated measurements, and considering the pen as the experimental unit.

#### Results

The different enrichment materials did not affect pig weights either over the entire experimental period, or at any time point (P = 0.15). However there was an interaction between time point and stocking levels (P = 0.006), and at Week 21 this effect of stocking level was significant (P < 0.05); pigs kept in pens of 12 were lighter at this time ( $127.1 \pm 1.1 \text{ kg}$ ) than those in pens of 10 or 8 ( $131.0 \pm 1.0 \text{ kg}$ ,  $130.4 \pm 1.1 \text{ kg}$ , respectively). The materials also had no significant effect on the ADG, ADFI or FCR. There was an interaction between stage and stocking level (P = 0.04), whereby in the weaner stage ADG of pigs kept in pens of 8 ( $0.613 \pm 0.014 \text{ g/day}$ ) was higher than those kept in pens of 12 ( $0.530 \pm 0.014$  g/day; P < 0.01), and tended to be higher than those in pens of 10 ( $0.556 \pm 0.014$  g/day; P = 0.06). An interaction between stage and stocking level was also observed in the ADFI (P = 0.06), with pigs in pens of 10 ( $2.54 \pm 0.03$  g/day) having a higher ADFI than those in pens of 12 ( $2.39 \pm 0.03$  g/day) in the finisher stage. When considering the FCR, there was no interaction between stocking levels and stage. However in the finisher stage there was a tendency for an effect of stocking density (P = 0.06), with pigs in pens of 12 ( $2.016 \pm 0.026$ ) having a numerically lower FCR than those in pens of 8 ( $2.064 \pm 0.025$ ) or 10 ( $2.105 \pm 0.025$ ). With regard to tail biting outbreaks, 97 were recorded during this study, of which the majority occurred in Haylage pens (38.14%), followed by Hay (31.96%), Straw (22.68%), and then Grass (7.22%). Within stocking levels, the largest portion of outbreaks occurred in pens of 12 (67.01%), followed by pens of 10 (21.65%) and 8 (11.34%).

# Conclusion

The results indicate that the materials used had virtually the same effect on the weight gained by the pigs, intake, daily gain and efficiency of feed conversion. Keeping pigs at lower stocking levels however seemed to have a positive impact on the weight gained, but this only became significant near the end of the finishing period when there was less space in the pen. Furthermore, pens stocked at the highest stocking level performed poorer on average than lower stocked pens when comparing the ADG and ADFI, indicating that lower stocked pens may reach slaughter weight sooner than higher stocked pens. When considering tail biting outbreaks, Grass seemed to perform better than other materials and the occurrence of outbreaks seemed to increase the more densely the pens were stocked.

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# 103. Ammonia emissions from finishing pigs in fully slatted systems

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# Application

The ammonia emission value of 1.55 kg/pig place/year and 16.9% TAN (total ammoniacal nitrogen) will be presented to the United Kingdom (UK) Ammonia Inventory with a view to updating the standard value for finishing pigs. The updated emission value will provide an accurate baseline of ammonia emission.

# Introduction

Ammonia gas is a pollutant which results in a detrimental effect on areas of special scientific interest with the loss of biodiversity, negatively affecting the environment and reducing carbon sequestration ability. Ammonia is also a significant source of nitrous dioxide, a potent greenhouse gas, and there is a global drive to reduce ammonia emissions (Phillipe et al., 2011). Ammonia emission from pig production in the UK is relatively small and the industry has been proactive in reducing emissions. To do this even more effectively, it is important to have an accurate understanding of ammonia emissions from pigs in modern systems. The current emission factor in the UK Inventory (2021) is mainly based on historic data. Since it was derived, dietary crude protein (CP) has been reduced and production efficiency has increased resulting in lower nitrogen (N) excretion. However, the standard value for ammonia emission has not been updated and therefore the baseline value for ammonia emission is unknown, making it difficult to understand the effect of ammonia mitigation strategies or to make informed decisions on business expansion. The objective of this study was to quantify ammonia emissions from finishing pigs on fully slatted systems to update the value in the UK Ammonia Inventory (2021).

# Material and methods

Ammonia emissions were measured from two pig units in Northern Ireland; at AFBI Hillsborough (Site 1) and a pig unit at Antrim (Site 2). Both units were similar in structure, pen size, ventilation and management and are representative of indoors finishing pig units across the UK. The study followed the principles of the Verification of Environmental Technologies for Agricultural Production (VERA) Test Protocol for Livestock Housing and Management 2.0. Ammonia emissions was assessed over three years and measurements were conducted over five different time points and over a range of pig weights (41-121 kg). At each time point, continuous measurements were taken for a minimum of 24 hours. Ammonia concentrations were recorded using tuneable diode laser absorption spectroscopy (Solus Ammonia Analyser). Site 1 had two rooms and Site 2 had four rooms with sampling tubes placed on each fan outlet in each room and at two inlets. Ammonia emissions were measured every 15 minutes. The ventilation rate at each fan outlet was calculated which allowed for calculation of room ventilation rate (m<sup>3</sup>/hr) and conversion of ammonia concentrations from ppm to g/d/LU and kg/pig place/year. Total ammoniacal nitrogen (% TAN) was calculated from ammonia emissions kg/pig place year using the value of 10.8 kg/pig place/year for N excretion for finishing pigs derived from Beattie et al. (2020). Statistical differences between sites were tested for using ANOVA with each measurement taken as a replicate.

# Results

Although there was a numerical difference, there was no significant (P = 0.205) difference between ammonia emissions or %TAN between the two sites (1.80 and 1.46 kg/pig place/year, SED = 0.26 and 19.7 and 15.9%, SED = 2.90 for site 1 and 2 respectively). The mean ammonia emission across the sites was 1.55 kg/pig place/year and the mean %TAN was 16.9%. Ammonia emissions ranged from 0.59 to 3.31 kg/pig place/year and %TAN ranged from 6.5 to 36.2%.

# Conclusion

The non-significant difference in ammonia emissions between the two sites indicates that ammonia emissions are comparable across different finishing units. The range in ammonia emissions and of %TAN is in keeping with those from other studies, but the mean values are lower than those in the current UK Ammonia Inventory (2021). Ammonia emissions in kg/pig place/year are 46% lower and % TAN is 42% lower. These reductions are a reflection of the lower dietary CP now being offered to finishing pigs and the increased efficiency of modern genetics. Having an accurate quantification of ammonia emissions from finishing pigs on slatted systems will enable more effective implementation of mitigation strategies.

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## 104. New technologies and data science applied on commercial sheep properties in Australia

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Data from new technologies in extensive sheep production offer great opportunities to improve animal welfare, sustainability, and profitability. Production of both wool and sheep meat is largely affected by growth rate, body condition, and survival of both young and adult animals. The present work focusses on experiences and results on the development and utilisation of an automatic monitoring system of live weight, pastures, and survival of grazing sheep in commercial properties of Australia. These data were then used to develop real-time prediction models of performance and survival. Data was collected across 7 commercial sites over 3 years in Merino, Poll Dorset, and Border Leicester. Of all technologies evaluated, automatic in-paddock weighing provided the most practical and useful information for the detection of missing and dead animals, changes in live weight, lambing events, lactation status, maternal parentage, and mortality risk. The survival risk models can also consider other factors in the real-time monitoring system such as disease prevalence and dog predation, amongst others such as genetic information. Low nutritional status monitored through live weight loss during the dry season and lactation was the largest welfare and profitability risk even though other factors such as disease and dog predation are also important.

The monitoring system provides automatic monitoring of live weight and growth rate, body condition, automatic mothering up, nutritional management, and animals present in the paddock. The latter is more accurate if water is used as the attractant instead of supplemental mineral blocks and it is also affected by the forage characteristics and size of the paddock, amongst others.

The use of remote monitoring technologies in extensive sheep production systems could play a critical role in the viability of these systems into the future. The automated system developed can be tailored to multiple purposes such as nutritional management, reproduction, genetic improvement, and animal welfare.

## **Conflict of interest**

The authors declare no conflict of interest other than the funding stated below. Acknowledgements

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## 105. Genomic selection for a sustainable future in dairy farming

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As global agriculture pivots towards sustainability, the convergence of genomic selection and innovative phenotyping technologies emerges as a potent force for shaping the next generation of breeding values. This approach allows farmers, with no additional cost or

effort, to actively breed for sustainability, thereby reducing their environmental footprint. Dairy breeding programs have curtailed methane emissions per litre of milk produced over the years by selecting for milk production traits, laying a foundation for a more sustainable future. However, there is pressure to further reduce emissions from the dairy sector. The release of the Sustainability Index in Australia, encompassing feed efficiency, longevity, profitability, and sustainability, is projected to lead to a 5% reduction in emissions by 2050. Yet, to achieve more ambitious global emission targets, the development of breeding values using individual methane records becomes paramount, potentially reducing emissions by 20–30% by 2050. Genomic selection has enabled the Australian dairy industry to use precision phenotyping in smaller genotyped populations to develop recent breeding values. However, genomic selection remains a "numbers game" requiring large reference populations with high-quality phenotypes to deliver reliable breeding values for farmers to make informed decisions for selective breeding. Therefore, deploying a multitude of predictors enhances methane phenotyping, with wearable low-cost sensors, mid-infrared spectroscopy from routine milk recording, and assessments of rumen microbiome and metabolites/fatty acids serving as illustrative examples. Integrating artificial intelligence into genomic selection to provide better decision-making tools is another emerging area. Navigating the challenges of measurement in commercial herds promises not only economic sustainability and license to operate, but a substantive contribution to global sustainability goals.

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## 106. The emerging role of integrated sensor technologies for research and commercial application in rangeland beef production

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In the rangelands of Australia, beef cattle producers steward over half of the nation's landmass. With an increasing focus on sustainable practices, these producers are now harnessing sensor technologies to revolutionise beef production. This shift is significant in both research and commercial spheres, with technologies such as remote weighing systems, satellite pasture estimation, and animal health sensors leading the way.

Remote weighing systems offer real-time monitoring of sheep and cattle growth, reducing the need for physical handling and improving animal welfare. This technology enhances efficiency in commercial operations and is pivotal in research settings for intensive and accurate data collection.

Satellite feedbase estimation is crucial in sustainable land management, providing essential information on pasture quantity and availability, and offering an insight into land condition across vast landscapes. This data is key to preventing overgrazing and preserving rangeland ecosystems.

Health and welfare sensors are transforming livestock management standards. These sensors enable early detection of health issues, maintaining consistent quality in beef production and ensuring commercial viability.

The individual application of each sensor offers unique insights unattainable through traditional management observations. Yet, the true value emerges in their integration, providing a comprehensive benefit for both researchers and the industry.

Integrating these technologies will influence both short and long-term decision-making. Short-term, they guide infrastructure improvements, optimising resource use such as water and fencing. In the long term, the data informs genetic advancements and animal husbandry practices, shaping breeding programmes to develop more resilient and efficient cattle, thus enhancing the sustainability of beef production systems.

The integration of these sensors will enable the capture and analysis of previously inaccessible data. This advancement provides a level of precision in livestock management that informs more proactive management decisions, optimising grazing strategies, improving disease prevention, and enhancing animal welfare in both research and commercial contexts.

Case studies demonstrate the transformative impact of these technologies in the Australian beef industry, highlighting how their practical application not only enhances current practices but also paves the way for future advancements in sensor technology within livestock management. Such developments hold the promise of further refining and evolving industry practices, leading to greater efficiency and sustainability.

In conclusion, the adoption of integrated sensor technology in rangeland beef production marks a significant move towards a more sustainable, resilient, and technologically advanced rangeland livestock industry. It represents the integration of economic and ecological goals, ensuring the sustainability of the industry and the ecosystems it relies upon.

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## 107. Livestock in a circular European food system

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Food production systems have an immense and extensive impact on the crucial Earth systems. One promising future redesign, which is gaining increased attention, are circular food systems. In circular food systems, the use of plant biomass for human consumption is prior-

itized to meet human nutritional requirements (as such, losses are avoided when transforming plant-based calories or proteins into animal-based calories or proteins). Furthermore, waste is minimized – i.e. food waste, by-products, crop residues, human excreta and overconsumption of nutrients. If avoiding waste is not possible it should be utilized (recycled) in the most sustainable way. For example, during food processing, by-products such as potato peals, wheat middling's or soybean meals are produced. If these by-products are used as a fertilizer, the use of artificial fertilizer will potentially decrease, while if by-products are used as feed for farm animals, inedible biomass for humans will be transformed into e.g. valuable food and manure.

We used a biophysical data driven optimization model to explore the effects of adopting such different circularity principles in the EU27 +UK. We showed that redesigning the European food system based on circularity principles could bring environmental benefits for Europe and the world. Agricultural land (arable land and cropland) can be reduced by 71% and greenhouse gas emissions by 29% per capita while producing enough healthy diets within a self-sufficient European food system. Healthy diets are defined by securing enough nutrients on a daily basis but also includes food intake recommendations to avoid overconsumption of foods that are linked to dietary diseases.

The effect on livestock is significant: beef cattle is reduced by 91%, pigs by 78%, broilers by 79% and layers by 33% while dairy and fish show relatively small changes. Feed-food competition is largely reduced and the feed of the animals contain of food losses and waste, grass, by-products and fodder crops produced in rotation with edible-crops. The share of food losses and waste in animal feeds largely differs across animal types: from zero in ruminant feed to large shares in fish and monogastric feeds. Although animal numbers are largely reduced, their role remains important in terms of providing essential nutrients (e.g., vitamin B12). The consumption of animal-based proteins would be around 40% versus 60% plant-based proteins. The overall reduction in animal-protein intake per capita is around 50%, i.e., from 49 g per person per day to 24 g per person per day.

To conclude our work shows that the combination of avoiding overconsumption and healthy eating while recycling residual food system streams has the potential to greatly improve human and planetary health. However, transitioning the EU's food system towards circularity implies sequential changes among all its components – in other words a radical redesign of the food system.

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## 108. Effect of feeding brown seaweed to dairy cows on the ammonia emissions from slurry during storage

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## Application

Feeding brown seaweed to dairy cows reduces ammonia (NH<sub>3</sub>) emissions from slurry during storage.

## Introduction

Agriculture, particularly dairy cow farming is a major contributor to NH<sub>3</sub> emissions. Animal slurry releases substantial amounts of NH<sub>3</sub> into the atmosphere, especially during the storage, handling, and field application of slurry. The release of NH<sub>3</sub> has negative environmental impacts including acidification, eutrophication, and production of particulate matter which affects human health (Mahmud et al., 2021). To mitigate these emissions and reduce the environmental footprint of dairy farming, alternative nutritional strategies are being explored, including brown seaweed species. Seaweed not only offers a sustainable feed source but also provides carbohydrates that promote gut health and contain bioactive compounds beneficial for animal well-being (Makkar et al., 2016, Muizelaar et al., 2023). Recent studies have investigated the potential of seaweed to reduce methane emissions from ruminants (De Bhowmick and Hayes, 2023). However, there are no studies on the potential of seaweed to reduce NH<sub>3</sub> from slurry in dairy cows. One of the mechanisms by which seaweed can mitigate NH<sub>3</sub> emissions is through the presence of phlorotannins, which bind to proteins, inhibit their degradation in the rumen, and reduce NH<sub>3</sub> emissions from animal waste. This study aimed to investigate the impact of the inclusion of brown seaweed in the diet of dairy cows on NH<sub>3</sub> emissions from slurry storage, providing valuable insights into the potential of seaweed as a sustainable solution in dairy farming.

## Material and methods

Fifteen late lactation multiparous Holstein-Friesian cows were evaluated in a 3 (diet)  $\times$  3 (period) Latin square design experiment with 21 days/period. The three groups of cows (five cows/group) were balanced by parity, body weight, and average milk yield. The animals were blocked into five subgroups with three cows per subgroup according to their parity, bodyweight, milk yield and body condition score, and then the three cows within each subgroup were randomly allocated to the three treatment diets. Total Mixed Ration diets consisted of 40% concentrate on a dry matter basis with 60% grass silage as the control diet, 56% grass silage and 4% dried Himanthalia elongata in the whole seaweed diet, and 56% grass silage and 4% Himanthalia elongata extract in the seaweed extract diet. Each cow was equipped with a specialized system for separate collection of urine and faeces (Katongole and Yan, 2022). From each cow within each treatment group, 12 kg of faeces and 8 kg of urine were collected separately based on the 2:3 ratio of urine to faeces typically observed in dairy cows (McIlroy et al., 2019). Samples were collected in triplicate and following the mix of corresponding faeces and urine, the slurry was placed in 30-litre plastic containers. The static chamber method was used to measure NH<sub>3</sub> emission (Baral et al., 2023). NH<sub>3</sub> was measured over six weeks. To assess the cumulative NH<sub>3</sub>-N flux among the treatments, a one-way analysis of variance was conducted. Subsequently, post-hoc Tukey tests were performed to identify any significant differences in the means of the treatments. A significance level of *P* < 0.05 was considered statistically significant for all tests conducted.

## Results

Daily NH<sub>3</sub> flux (mg N/m<sup>2</sup> per day) over the 36-day measuring NH<sub>3</sub> from the slurry is shown in Fig. 1. Fig. 2 shows the cumulative NH<sub>3</sub> flux (mg N/m2) among the groups. In comparison to the control group, the whole seaweed and seaweed extract groups reduced NH<sub>3</sub> emissions by  $43.92 \pm 4.82\%$  and  $29.93 \pm 1.95\%$ , respectively. There was a significant difference in pH levels of slurry among the groups (P < 0.05), while there was no difference in dry matter content (P > 0.05). Additionally, there was a highly significant difference in the ash and organic matter content among the groups (P < 0.001).



Fig. 1. Daily NH<sub>3</sub> flux in the control, whole seaweed and seaweed extract groups ( $\Delta$  indicating the NH<sub>3</sub> flux after stirring on the same day).



Fig. 2. Cumulative NH<sub>3</sub> flux across control, whole seaweed and seaweed extract groups.

## Conclusions

This study demonstrated that including brown seaweeds in the diet of dairy cows could be a viable and novel strategy for mitigating NH<sub>3</sub> emissions during slurry storage. Future research should focus on a realistic storage period of 4–6 months and explore the potential of different seaweed species and the mechanism of action of phlorotannins on NH<sub>3</sub> reduction.

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## 109. A simulation study comparing methane emissions from dairy cow farming in Greece at three reference years

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## Application

Overall methane emissions from dairy cattle in Greece decreased significantly the last 20 years due to considerable increases in productivity.

### Introduction

Ruminant production is considered a major contributor of anthropogenic methane emissions that affect global climate change. However, the constant increase in cow productivity, a common feature of the dairy industry in the developed countries, leads to more efficient use of natural resources and to a mitigation of environmental burdens. The aim of the present study was to assess and compare methane emissions from dairy cow farms in Greece at three different time-points representing the evolution of the local dairy sector.

## Material and methods

Daily direct (enteric) and indirect (manure) methane emissions were estimated using the equations proposed in NASEM (2021). The reference years chosen were 1985, 2003 and 2022, due to similar overall annual milk production (650,000-660,000 tons). Number of dairy cows kept were based on EUROSTAT and FAO data (219,000, 170,000 and 88,000, for the three reference years, respectively); 85 percent of those were considered to be lactating and 15 percent being dry at any time of the year. Heifer inventory (2-6mo, 7-12mo and 12-1<sup>st</sup> calving) was calculated considering replacement rates of 0.20, 0.25 and 0.30 while age at first calving was set at 30, 28 and 26 months. for the three reference years, respectively. For all the aforementioned animal groups, available data from surveys and PhD projects were used to formulate typical rations for each reference year, meeting net energy and metabolizable protein requirements (INRA 2007). Cow bodyweights were set at 500 kg, 600 kg and 700 kg; daily milk yields were set at 10.0 kg, 12.5 kg and 25.0 kg; and heifer average daily gains were set at 600 g, 700 g and 800 g for the three reference years, respectively. Feedstuffs used included: 1985, alfalfa hay, wheat straw, corn grain, wheat bran, cottonseed cake and supplements; 2003, corn silage, alfalfa hay, wheat straw, corn grain, soybean meal, sunflower meal, molasses and supplements; 2022, corn silage, grass silage, alfalfa hay, wheat straw, corn grain, soybean meal, canola meal, molasses, rumen-inert fat and supplements. For 1985, restricted feeding was considered while for 2003 and 2022, rations were formulated for ad libitum intake. For all rations, dry matter and gross energy intake, as well as crude fat, crude protein, neutral detergent fiber, digestible neutral detergent fiber and hemicellulose content (percent dry matter) were calculated, as they are required in NASEM 2021 equations for the estimation of methane emissions (enteric, Mcal/d; manure, digestible volatile solids, kg/d). Year 1985 was used as baseline and estimations for 2003 and 2022 are reported as a percentage of 1985 emissions.

## Results

Direct daily methane emissions (Mcal/d) were 959,606, 976,107 and 577,176, for years 1985, 2003 and 2022, respectively; indirect methane emissions (digestible volatile solids, kg/d) were 1,174,305, 1,125,365 and 650,230, respectively. There was little change in methane emissions between 1985 and 2003. The transformation/modernization of the dairy sector was well under way in 2003 but cow numbers had not been substantially reduced at that point and milk output per cow had just started to increase. However, 2022



Fig. 1. Estimated methane emissions from the dairy cow sector in Greece, at three reference years with similar annual milk production.

methane emissions were approx. 40 percent (enteric) and 45 percent (manure) lower than those of 1985 (Fig. 1), due to the considerable increase in cow productivity (x2.5) which led to a decrease in cow numbers by 60 percent. To put it in another way, under current dairy farming conditions almost 1,000,000 tons of cow milk can be produced in Greece (an increase of 33 percent) with methane emissions at 1985 levels.

## Conclusion

Enhanced cow productivity and efficient use of natural resources allow the production of increased amounts of human food with methane emissions similar to those of 40 years ago. This was achieved without the application of specific methane emission mitigation strategies which are now at the verge of widespread adoption by the dairy industry. A communication campaign must be developed, to present these facts to consumers and legislators alike.

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# 110. Calcium peroxide as feed additive: Effects on gaseous emissions during cattle slurry storage

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## Application

This work is important to show the environmental effects of Calcium Peroxide, as a cattle feed additive, on the resultant slurry storage.

## Introduction

Livestock production systems, especially manure management lead to environmental issues such as the release of greenhouse gases (GHG) and air pollutants such as ammonia (NH<sub>3</sub>). Agriculture, specifically livestock production, accounts for over 80% and 60% of the total global NH<sub>3</sub> emissions, respectively (Behera et al., 2013). Feeds and feed additives can determine the composition and quantity of manure produced, thereby controlling what enters the manure management chain at the housing, storage or land spreading stages. Feed additives such

as seaweeds (Roskam et al., 2022; Alvarez-Hess et al., 2023), linseed oil (Doreau et al., 2018) and Calcium Peroxide ( $CaO_2$ ) (Graham et al., 2022; 2023) have reduced methane when added to cattle diets. Limited studies extend gaseous measurements from feed additives to the manure management stage. The potential of  $CaO_2$  for methane mitigation in beef cattle is new and on the rise. This study therefore examined the downstream effects of adding  $CaO_2$  in varying quantities and form on ammonia and GHG emissions from resultant cattle manure during long-term storage.

## Materials and methods

Urine and faeces were collected from the animals during a feeding trial. Briefly, 16 animals were divided into four treatments of varying quantities/forms of Calcium Peroxide (CaO<sub>2</sub>) (CAP) – Control (CON), CAP Low (1.35% CaO<sub>2</sub>), CAP High (2.25% CaO<sub>2</sub>) and CAP High Pellet (2.25% CaO<sub>2</sub> with a pelleted coarse ration) of the overall diet. All animals were fed a 60:40 forage to concentrate diet.

The faeces and urine of each treatment were mixed and sieved to form a homogenous slurry in a ratio that produced a dry matter (DM) of 6% (Bourdin et al., 2014). Sub-samples were collected before the experiment for slurry characteristics analysis. 1.6 kg of the mixed slurry was then transferred to a 2 L-capacity urine container for a laboratory-scale incubation in a temperature-controlled growth chamber. The temperature and relative humidity of the chamber were set to 12°C and 76% respectively. Each treatment was replicated five times in a randomised block design. To simulate air movements during manure storage in a slatted shed, 10 holes were drilled into the lids of the 2 L containers.

Ammonia and GHG emissions were sampled three times a week at the start of the experiment and then reduced to twice a week. Ammonia was sampled using a dynamic chamber technique with a photoacoustic gas analyser while the GHG emissions were measured with a static chamber technique as described by (Kavanagh et al., 2019; Connolly et al., 2023). pH and temperature readings were also taken every sampling day.

### Results

This experiment is ongoing, early results indicate that CaO<sub>2</sub> impact slurry characteristics, GHGs and NH<sub>3</sub> emissions from the resultant manure. The final results will be presented at the conference.

### Conclusion

This work is ongoing.

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# 111. Two for the price of one? Moving to dual-purpose dairy breeds has little impact on total greenhouse gas emissions from cattle production

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## Application

Dual-purpose breeds are suggested as an alternative to the separate UK dairy and suckler herds, yet potential reductions in total greenhouse gas emissions (GHGe) are relatively small.

#### Introduction

Over the past century, traditional dual-purpose cattle breeds in the UK have been replaced by specialized high-yielding dairy breeds (e.g. Holstein Friesian, HF). Half of UK beef is sourced from dairy cattle, with the remainder being produced by suckler beef cattle. Under current GHGe accounting methods, dairy-beef has a lower GHGe because maternal emissions are allocated to dairy production. Shifting to dual-purpose breeds selected for both milk and meat production may therefore reduce total GHGe from UK cattle if an increase in dairy-beef displaces suckler beef. Norwegian Red (NR) cattle, which are selected less intensively for milk production and with a greater emphasis on health, fertility and carcase yield are a potential alternative breed.

The objectives of this work were to model the effect of replacing HF cattle with a dual-purpose breed (NR) on:

- a) Milk and beef production from the UK dairy herd
- b) Total GHGe from the UK cattle herd (dairy and beef)

## Materials and methods

Literature reviews were undertaken to source data on the relative performance of HF and NR dairy cattle and their purebred and crossbred calves, using appropriate search terms in internet databases (Science Direct, PubMed, Google Scholar and CAB Abstracts). These data (Table 1), in conjunction with UK-specific dairy and beef performance data, were used to populate two deterministic models adapted from Capper and Cady (2020) and Capper et al. (2021). Based on population demographics, metabolism and nutrient requirements of dairy and beef cattle, the models quantified the GHGe impact of changing the UK dairy herd from primarily HF to NR. Emissions were quantified per 1 million metric tonnes (t) of energy-corrected milk (ECM; current UK dairy production is 14.9 million t/annum) plus 53,755 t carcase weight (CW) beef, based on a 50:50 dairy-beef:suckler beef split in the control (HF) population. System boundaries extended from the production of feeds to ECM and CW beef at the farm gate. Cropping data were sourced from national databases and feed GHGe data from the GLFI (2023) database (see Table 2).

#### Table 1

Comparative key performance indicators of Holstein and Norwegian Red cattle.<sup>1</sup>

	Holstein	Norwegian Red
Energy corrected milk yield (kg/d)	27.5	24.7
Lactation length, d	331	323
Mature bodyweight, kg	570	537
Calving interval, d	391	383
Lactations in the herd	3.6	4.2
Cow mortality, %	6.3	3.5
Heifer replacement rate, %	27.8	23.8
Age at first calving, mo	26.6	26.9
Cows producing a live calf/yr, %	86.6	91.5
Calf birthweight, kg	42.2	39.7
Pre-weaning calf mortality, %	7.2	4.0

<sup>1</sup> Data sourced from 18 published papers.

#### Table 2

Effects of changing the UK dairy population from Holstein to Norwegian Red cattle on GHGe from dairy and beef cattle if total dairy and beef production were kept constant.<sup>1</sup>

	Holstein	Norwegian Red	% difference
Dairy cows, '000 head	118.8	132.7	11.7
Dairy heifers, '000 head	75.3	72.4	-3.16
Total dairy cattle, '000 head	194.3	205.6	5.94
Dairy GHGe, kg CO <sub>2</sub> e/kg ECM	1.478	1.512	2.29
Prime beef from dairy herd, t CW	21,057	29,258	39.0
Cull cow beef from dairy herd, t CW	7,124	7,303	2.51
Dairy beef GHGe, kg CO <sub>2</sub> e/kg beef CW	17.17	17.65	2.81
Suckler cows, '000 head	96.4	64.8	-32.8
Prime beef from suckler herd, t CW	21,057	14,157	-32.8
Cull cow beef from suckler herd, t CW	4,516	3,036	-32.8
Suckler beef GHGe, kg CO <sub>2</sub> e/kg beef CW	32.44	32.44	-
Total dairy (milk) GHGe, t CO2e	1,477,819	1,511,690	2.29
Total dairy beef GHGe, t CO <sub>2</sub> e	361,446	516,034	42.8
Total suckler beef GHGe, t CO <sub>2</sub> e	783,477	526,746	-32.77
Total beef GHG, t CO <sub>2</sub> e	1,144,923	1,043,050	-8.90
Total cattle industry GHGe, t CO <sub>2</sub> e	2,622,742	2,554,740	-2.59

<sup>1</sup> Milk production 1,000,000 t ECM ; beef production 53,755 t CW.

## Results

Maintaining total milk production from NR cattle required an 11.7% increase in dairy cow numbers because of the lower ECM yield. This was partially offset by health improvements that reduced the number of heifer replacements required (-3.16%), to give an overall increase in dairy cattle numbers of 5.94%. With concurrent increases in resource use, this increased total dairy GHGe by 2.29%. Changing from a HF to a NR dairy population reduced total beef GHGe by 8.90%. This was primarily driven by a 32.8% reduction in suckler cows as the increased NR herd size shifted more dairy calves into beef. Nevertheless, the overall reduction was moderated by NR cattle having a lower growth rate than HF cattle, which increased the emissions intensity by 2.81%. Changing from a HF to a NR dairy population reduced total GHGe per 1 million t of milk plus 53,755 t beef by 2.59% ( $-68,002 \text{ t CO}_2\text{e}$ ). In context, this reduction would be equal to removing  $\sim$ 37,600 UK cars from the road, based on 0.152 kg CO<sub>2</sub> per km driven (Department for Business Energy & Industrial Strategy, 2019) and 11,909 km driven per year.

# Conclusions

Changing to NR cattle would confer a relatively small change in GHGe from the UK cattle industry, although the absolute quantity of emissions reduced is not inconsiderable when compared to transport emissions. Producer behavioural and cultural changes required to switch from HF to NR might also render this conversion unfeasible. Future efforts should be targeted at improving health and reproduction, reducing age at slaughter and improving grassland management, which would be expected to confer greater reductions in GHGe from suckler cattle production (Taylor et al., 2020).

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## 112. Characterization of greenhouse gas emissions from lactating Holstein dairy cows in East China

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## Application

China has a large population of dairy cows, whereas there is lack of GHG emissions data. The quantification of enteric methane (CH<sub>4</sub>) emissions from lactating dairy cows is of great importance to develop national emission inventories as well as to develop mitigation approaches, and thus contribute to the transformation of livestock production systems in China.

## Introduction

Livestock is a prime anthropogenic source of  $CH_4$  emissions from the agricultural sector, accounting for 18% of global GHG emissions, of which ruminant livestock is responsible for 93% of all livestock GHG emissions globally, and the dairy cows have the largest GHG emissions. However, China lacks a local dairy cow GHG emissions database, and before this experiment, neither did China have the latest animal  $CH_4$ emission detection equipment (GreenFeed system). Furthermore, China did not measure the gas emissions from a large herd of cows. The purpose of this study was to accurately determine the GHG emissions of lactating Holstein dairy cows under normal feeding conditions using the GreenFeed system. This would lay the foundation for determining the  $CH_4$  and  $CO_2$  emissions of lactating Holstein dairy cows in East China, as well as facilitating further studies on the GHG emission characteristics of lactating Holstein dairy cows and locally applicable GHG emission reduction approaches in China.

## Materials and methods

The 153 lactating Holstein dairy cows were selected and were housed in a barn. The parities of these animals ranged from 2 to 5, the days in milk from 104 to 182 d, and milk yield from 25.9 to 53.7 kg/d. he cows had free access to drinking water and saltlicks, and were fed a basal TMR with a forage:concentrate ratio of 40:60 on a dry matter (DM) basis, and the composition of the TMR was the same throughout the experiment (Table 1). The TMRs were provided three times daily in 4:3:3 proportions by an automatic feed wagon to guarantee ad libitum

#### Table 1

Ingredient and chemical composition of the basal diet fed to lactating Holstein dairy cows.

Items	Content, % of DM
Ingredient composition <sup>a</sup>	
Corn silage	21.1
Alfalfa hay	14.75
Oatgrass hay	3.46
Dandelion hay	0.69
Steam-flaked corn	14.77
Soybean meal	14.25
Corn flour	11.8
Beet pulp	5.11
Whole cottonseed	4.39
Rapeseed meal	2.56
Extruded soybean	1.64
Mineral-vitamin premix <sup>b</sup>	5.48
Calculated chemical composition	
OM	97.8
СР	16.78
EE	5.48

#### Table 1 (continued)

Items	Content, % of DM
NDF	32.75
ADF	22.09
Ca	0.82
Р	0.46
NE <sub>L</sub> , MJ/kg	7.46

<sup>a</sup> DM dry matter, OM organic matter, CP crude protein, EE ether extract, NDF neutral detergent fiber, ADF acid detergent fiber, Ca calcium, P phosphorus, NEL net energy values were estimated based on NRC (2001).

<sup>b</sup> The premix contained 140g/kg of Mg, 122g/kg of Ca, 93g/kg of Na, 50g/kg of K, 48g/kg of Fe, 24g/kg of P, 2g/kg of S, 999mg/kg of Zn, 580mg/kg of Mn, 360mg/kg of Cu, 180,070 IU of VA, 30,000 IU of VD and 601 IU of VE.

intake (aiming at 10% refusals), with feeding times of approximately 08:30, 15:30, and 23:30 h. The whole experiment was completed in 120 d with measurements of enteric  $CH_4$  and  $CO_2$  emissions, DM intake, milk production, milk composition, and body weight of cows. There were four experimental periods, each 30 d long, and in experimental periods 1–4, 40, 40, 40, and 33 cows were randomly selected for measurement, respectively. Two GreenFeed units (C-Lock Inc., Rapid City, SD, USA) were permanently available for measuring gas emissions from cows according to the methods of Huhtanen et al. (Huhtanen et al., 2015). More than 20 valid data points were ensured for each cow, and the average value was calculated as the final data using Microsoft 365 Excel package; otherwise, it would be eliminated (see Table 2).

#### Table 2

Feed intake, milk production and composition and carbon dioxide emission of lactating Holstein dairy cows (n<sup>a</sup>=111).

Items <sup>b</sup>	Mean	Minimum	Maximum	SD <sup>c</sup>
Animal description				
Age, months	51.7	36.3	89.8	12.7
Parity number	2.8	2	5	1
Days in milk, d	138	104	182	19
Metabolic weight, kg	136.5	116.4	160.1	9.5
Dry matter intake, kg/d	23.1	17.6	33.7	2.6
Milk production and composition				
Milk yield, kg/d	38.1	25.9	53.7	6.9
Feed efficiency, kg/kg	1.65	0.62	2.32	0.29
Milk fat yield, g/d	1414	545	2222	272
Milk protein yield, g/d	1247	446	1991	236
ECM yield, kg/d	37.2	31	55.1	6.7
Gas emissions				
$CH_4(CO_2eq), g/d$	8304	5392	11190	1151
CH <sub>4</sub> (CO <sub>2</sub> eq)/DM intake, g/kg	259.4	227.1	492.6	48.3
CH <sub>4</sub> (CO <sub>2</sub> eq)/ECM, g/kg	229.5	149.8	455	48.1
Total CO <sub>2</sub> , g/d	19201	14412	24145	2004
Total CO <sub>2</sub> /DM intake, g/kg	831.5	575.2	976.7	84.1
Total CO <sub>2</sub> /ECM, g/kg	531.1	343.3	1095.6	102.8

<sup>a</sup> n, number of observations in the data set.

<sup>b</sup> Feed efficiency=milk yield/dry matter intake (kg/kg). ECM Energy-corrected milk (kg/d)=milk yield (kg/d)×[(38.3×fat (%)×10+24.2×protein (%)×10+16.54×lactose (%)×10+20.7) / 3140]. CH<sub>4</sub>(CO<sub>2</sub>eq) (g/d)=the CH<sub>4</sub> emission in the experiment was expressed as CO<sub>2</sub> equivalent using the equation as CH<sub>4</sub>(CO<sub>2</sub>eq) (g/d)=CH<sub>4</sub> emissions (g/d)×25. DM intake=dry matter intake (kg/d). Total CO<sub>2</sub> (g/d)=CH<sub>4</sub>(CO<sub>2</sub>eq)+CO<sub>2</sub> production.

<sup>c</sup> SD=standard deviation.

#### Results

Mean herd responses throughout the study were as follows: 111 cows completed all experimental processes, while 42 cows were rejected because they were sick or had not visited the GreenFeed system 20 times. On average, lactating days of cows were  $138 \pm 19.04$  d, metabolic weight was  $136.5 \pm 9.5$  kg, parity was  $2.8 \pm 1.0$ , dry atter intake (DM intake) was  $23.1 \pm 2.6$ kg/d, and milk yield was  $38.1 \pm 6.9$  kg/d. The GreenFeed system revealed that CH<sub>4</sub> production (expressed in CO<sub>2</sub> equivalent, CO<sub>2</sub>eq) was found to be 8304 g/d, CH<sub>4(CO2-eq)</sub>/DM intake was 359 g/kg, CH<sub>4(CO2-eq)</sub>/energy-corrected milk (ECM) was 229.5 g/kg, total CO<sub>2</sub> production (CH<sub>4</sub> production plus CO<sub>2</sub> production) was 19,201 g/d, total CO<sub>2</sub> /DM intake was 831 g/kg, and total CO<sub>2</sub>/ECM was 531 g/kg.

#### Conclusions

This study demonstrates that total CO<sub>2</sub> emissions from lactating Holstein dairy cows in East China averaged 19,201 ± 2,004 g/d, 831 ± 84 g/ kg·DM intake, and 531.1 ± 103 g/kg·ECM, respectively.

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## 113. Precision livestock farming (PLF) approaches to sustainable productivity

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Precision livestock farming (PLF) offers an opportunity to sustainably increase animal productivity, improve animal welfare and health, whilst reducing environmental impact. A potential improvement of animal productivity can be achieved using real time monitoring and management systems, alerting the farmer when problems arise. Production related information generated from PLF systems can therefore enhance farm management in a situation of scarce labour, but we should consider if it is appropriate to attempt to entirely replace the farmers own knowledge and experience; the level of displacement of human input should be considered for optimal integration of PLF in real farm systems. Training and support are therefore important in adoption of PLF systems in commercial farming practice.

Implementation of sensor systems and automation can improve herd management and reduce labour requirements (Steeneveld and Hogeveen, 2015). However, many sensor systems also have the potential to provide targeted information about other, more complex traits (Friggens and Thorup, 2015) in addition to the detection of health problems and fertility events by offering alerts in response to changing response to environmental conditions or physiological challenge. Common sensor data, such as milk production and activity time series, can be used to predict a complex trait such as lifetime resilience and achieving more sustainable productive lifespan targets (DeVries, 2020). One step toward optimization of farm management with respect to longevity would be the identification of animals that have a high probability of completing several lactations, or, more specifically, that are "resilient" (Ranzato, 2022). Resilient animals can be considered as animals that avoid early culling by coping well with the farm's management conditions (Friggens et al 2022). These animals reproduce easily, produce consistently, and react well to imposed challenges and (physiological) stress (Ahlman et al., 2011).

Simultaneously, additional benefits of these technologies can be generated from the calculation of precision phenotypes and their use for the characterization to understand overall and relative performance of animals within the farm context and comparing genetic/environment interaction (Royal et al., 2000). Adriaens et al (2020 and 2021) described how biologically meaningful proxies can be constructed for the cow's physiological status from the high-frequency milk yield and activity dynamics provided by commercially available sensor systems. These proxies offer potential decision support for breeding, culling and treatment events to optimise herd sustainable productivity (Cabrera 2018). Further potential exists for this approach to be translated into continuous monitoring of animal welfare as PLF technologies offer timeseries data, such as perturbations in daily milk yield or cow activity from accelerometer outputs, that may provide appropriate opportunities for tracking welfare trajectories. This approach might offer practical solutions that will complement the current state of the art such as 'Welfare Quality' assessments which require time-consuming human observations and are challenging to achieve at more than restricted annual spot-check level.

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#### 114. Grazing systems for robotic milking: the impact of daytime pasture access on dairy cow performance

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## Application

This research shows that daytime pasture access for robotically milked dairy cows can have a negative impact on cow performance.

## Introduction

As the dairy industry strives to improve labour efficiency, cow management and cost of production, the interest and adoption of robotic milking technology has grown. However, challenges exist in relation to the integration of this technology into a pasture-based system. Previous research has highlighted that full-time summer grazing can have a detrimental impact on milking frequency and animal performance (Lyons et al., 2014). Grazed grass continues to be the most cost-effective feedstuff for Northern Irish dairy farms and thus there is a need to

utilise this valuable resource within robotic milking systems. This research investigates the use of daytime access to pasture in comparison to a fully housed system for high yielding dairy cows milked via a robotic milking system.

## Materials and methods

Fifty spring-calving dairy cows (12 parity 1, 38 parity 2+) were on a continuous design experiment during summer 2021. Treatment groups were balanced for lactation, days in milk, milk yield, liveweight, body condition score and the Predicted Transmitting Ability (PTA) for milk, fat, protein and Profitable Lifetime Index (£PLI). Two treatments consisted of (i) full-time housing, (ii) daytime pasture access for 16 hours per day. All cows were fed a total mixed ration (TMR) via feedboxes (15 kg DM grass silage:3 kg DM concentrates) and concentrates to yield through the robot (at a rate of 0.45 kg/L above M+25 for cows and M+20 for heifers). In addition, grazed cows were offered 2-way grazing from 4am to 8pm each day. During the 63 day study, cow performance, feed intake, milking behaviour, cow activity and grazing efficiency were monitored. Statistical analysis were conducted using Genstat (21<sup>st</sup> Edition). Number of paddock visits and percentage of cows grazing which were analysed as a linear mixed model. All other analysis were completed using a linear mixed model with repeated measures. The correlation between time points was accounted for using an AR model of order 1, with the exception of intake and grazing data which used an antedependence model of order 1.

## Results

Milk yield (P < 0.001) and milk protein (P < 0.01) were significantly greater for housed cows yet milk fat did not differ significantly (P < 0.05) (Table 1). Number of milkings (P < 0.001) and number of refusals (non-milking visits) (P < 0.001) were significantly greater for housed cows (Table 1). TMR intake and concentrate intake were also greater (P < 0.001) for housed cows than for grazed cows. The grazed cows showed a greater preference for morning grazing than afternoon grazing demonstrated by a greater number of paddock visits (1.26 vs 1.01, P < 0.001) and cows grazing (90.9% vs 74.8%, P < 0.001). Grazing utilisation rate did not differ (P > 0.05) between morning and afternoon paddocks. Margin over feed costs was greater (P < 0.001) for housed cows. However, 29% of grazed cows had a margin over feed costs what was greater than the mean (£6.49) for the housed group.

#### Table 1

The impact of daytime pasture access on dairy cow performance.

	Grazed	Housed	SED	P value
Milk yield (L/d)	28.76	31.97	5.442	<0.001
Milk fat (%)	3.69	3.88	0.101	NS
Milk protein (%)	3.38	3.51	0.0457	< 0.01
Number of milkings	2.87	3.46	0.0409	< 0.001
Number of refusals	4.56	8.92	0.564	< 0.001
TMR intake (kg DM/d)	10.69	14.34	0.494	< 0.001
Total concentrate intake (kg DM/d)	7.96	10.42	0.663	< 0.001
Margin over feed costs (£/cow/d)	5.20	6.49	0.290	<0.001

## Conclusion

This study showed that daytime pasture access can result in a decline in milk yield, which may have been impacted by the reduced number of milkings and/or lower concentrate intake for grazed cows. Margin over feed costs demonstrated that at the level of performance achieved in this study, housing robotically milked cows was more economical than offering daytime pasture access. However, the variability within the grazed group illustrates that there is potential for some cows to perform well or better at grass. Further investigation will identify the key drivers of this, which could be used to further refine grazing systems for robotic milking or develop a methodology to preselect animals that are suitable for these systems.

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#### 115. Using machine learning for imputation and predicting finisher performance in commercial pig production

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## Application

The application of this research is to modernise and optimise pig production by harnessing data, technology, and advanced machine learning techniques for monitoring and prediction purposes. This enables farmers to efficiently allocate resources such as feed and labour while minimising wastage.

# Introduction

The growing global population and climate challenges require livestock production systems to increase yields whilst also maintaining acceptable welfare standards. Therefore, accurately allocating resources such as feed and labour whilst minimising waste will be a top priority for livestock farmers. To achieve this, the collection and utilisation of high-quality quantitative data for monitoring and prediction purposes are imperative. Traditionally, predictive analytics in pig production have relied on general linear models to predict performance, using multiple predictor variables. However, these models often struggle to handle complex relationships between factors and non-linear data that often arise when multiple predictor variables are used (Comrie 1997). Recently, machine learning (ML) algorithms have emerged as an alternative to address these issues. Machine learning algorithms learn from historical data to make predictions, with their accuracy improving as more data is included. The aim of this study is to use ML algorithms on data collected from commercial farms to (1) address missing data issues, (2) obtain more accurate predictions compared to classic linear regression models and (3) identify the most important early-life predictor variables to predict finisher performance.

## Materials and methods

Data were collected in four batches under commercial conditions at multiple sites of Karro Food Group Ltd. A total of 313 sows were balanced across parities 1 to 6 and were serviced with four different sire lines (A- Pietran synthetic terminal sire, B- Hampshire synthetic terminal sire, C-White synthetic terminal sire, and D-Hampshire synthetic terminal sire). A total of 5225 piglets were individually ear-tagged at birth. Individual birth weights (BWT) were collected from batches 2, 3 and 4, and individual weights were recorded for all batches at finishing entry (week 9; WK9) and finishing exit (week 23; WK23). Abattoir data including, net cold weight (NCW) (kg) and carcass lean meat (LM) (kg), were collected. Data underwent pre-processing, involving the encoding of categorical data, and outlier removal using the zscore algorithm. Batch 1 BWT was not measured, and data was missing completely at random for WK9, WK23, NCW and LM. The level of missing data gathered under commercial conditions in this study mirrors authentic on-farm conditions, thereby presenting a dynamic realworld context for predictive analysis. To address missing data, imputation was performed using ML algorithms from the Python scikit-learn library, including linear regression, Decision tree regression (DT Regression), k-Nearest Neighbour regression (KNNR) and Support Vector (SV) regression, Random Forest Regression (RFR), Extreme Gradient Boosting (XGBoost) regression, Light Gradient Boosting (LGBM) regression, Adaptive Boosting (AdaBoost) regression, Stacking Regression (SR) and Voting Regression (VR). A complete case dataset was randomly shuffled into 5 subsets were used to train and test the algorithms with the most accurate imputation algorithm selected using the relative root mean square prediction error and concordance correlation coefficient (You et al 2023). A final imputed dataset was made for downstream prediction analysis. The same ML algorithms used for imputation were used for finishing performance prediction. All model evaluations and statistical analyses were performed using Jupyter Notebook (Project Jupyter, USA). The accuracy of downstream prediction algorithms was assessed using mean absolute error (MAE), root mean squared error (RMSE), and the mean absolute percentage error (MAPE). The best predictor variables are listed in order of importance from highest to lowest.

## Results

See Table 1.

#### Table 1

Imputation and downstream analysis of BWT (required for finishing predictions) and finishing variables.

Imputation			Downstream analysis				
Variable	Missing data	Top imputer	Top predictive algorithm	RMSE (LR metric)	MAE (LR metric)	R <sup>2</sup> (LR metric)	Variable importances
BWT	22.3%	SR_SVR					
WK9	13.8%	SR_LR	SR_SVR	3.33 (3.54)	2.53 (2.75)	0.33 (0.24)	imp_BWT, PAR, SL, Batch, TLS, MED, Sex
WK23	23.6%	SR_LR	SR_LR	9.45 (10.1)	7.09 (7.70)	0.29 (0.17)	imp_BWT, batch, SL, PAR, TLS, Sex, MED
NCW	48.3%	RF	SVR	6.66 (7.25)	4.85 (5.57)	0.29 (0.17)	Rep, imp_BWT, SL, Sex, PAR, TLS, MED
LM	48.3%	RF	SR_SVR	1.84 (2.04)	1.26 (1.46)	0.22 (0.05)	SL, Sex, Batch, TLS, imp_BWT, PAR, MED

SR\_SVR = Stacking regression with SVR as the meta-model, SR\_LR = Stacking regression with LR as the metamodel, imp\_BWT = imputed BWT, SL = Sire line, TLS = Total litter size, PAR = Parity, MED = Medicated, LR accuracy metrics are presented in brackets for comparison with top performing model.

## Conclusions

This study highlights the application of ML algorithms in imputing large datasets and predicting finisher performance. Our findings indicate that ensemble models such as stacking regression and random forest regression were the most accurate for imputation. The best algorithms for predicting finishing performance were stacking regression algorithms with LR and SVR as meta-models and SVR, outperforming other methods, including linear regression, by capturing underlying data patterns more efficiently. Our findings also reaffirm the critical role of BWT in influencing finishing performance outcomes.

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# 116. An investigation of the impact of virtual fencing technology on sheep welfare and behaviour

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# Application

Virtual fencing use is being advocated for a range of cattle and sheep grazing systems. This technology is promoted as enhancing grazing management. However, important knowledge gaps remain regarding its application. This study investigated the impact of virtual fencing on the welfare and behaviour of lowland and hill breeds of sheep.

## 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 environmental sustainability, particularly conservation grazing. Real-time GPS monitoring of animal location may also be beneficial for animal welfare and management (Umstatter, 2011). The technology typically uses 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 warning to turn around. This is followed by an electric pulse if it continues beyond the VF. Through associative learning after a short training period, the animals learn the correct response to the audio cues (Lee et al., 2009). However, an electric pulse may cause stress for an animal, raising concerns on the impact of VF systems on welfare and behaviour (Animal Welfare Committee, 2022). Therefore, this study aimed to determine the behaviour and welfare effects of VF across different breeds of sheep.

## Material & methods

Ninety-six dry ewes (average 4.5 years old) were split into 3 cohorts in a  $2 \times 2$  factorial design experiment (breed: lowland or hill, fencing: physical or virtual). Each cohort comprised 4 treatments: lowland control (LC), lowland VF (LVF), hill control (HC) and hill VF (HVF) (n<sub>group</sub> = 8, n<sub>cohort</sub> = 32). Groups were balanced on breed, bodyweight and temperament. All ewes were fitted with Nofence collars (Nofence, Norway). The experimental period lasted 38 days, including an initial 10-day training period for the VF groups followed by a 28-day grazing period where groups were moved to fresh pasture weekly. The training of the VF groups was done with a single VF line in their pasture and two VF lines for the remainder of the study. On days 0, 10, 24 and 38 ewes were weighed, assessed for temperament, reactivity in isolation and flight response using the isolation box method (based on Atkinson et al., 2022). Temperament, vocalisations, 180° head turns, number of jumps, single feet lifted, headbutts and ground scratches were recorded (Hero Session 5, GoPro,USA) over a 2-minute period in the weighing crate (1.25\*0.5 m). Flight response was measured as the time taken for the ewe to leave the weighing crate and cover 1.70 m distance. An attention bias test (based after Monk et al., 2020) was performed at the end of the study, using an umbrella as the 'threat' for 10 seconds, and behavioural reactions were recorded for 2 minutes. Vocalisations, number of zones crossed, latency to eat, time spent looking at the 'threat corner' and time spent at entry/exit doors were recorded. Activity data (unitless) reported from the VF collars provided information on overall movement patterns, based on an accelerometer registering along 3 axes. Statistical analyses were conducted using R (version 4.2.2) and the package "rstatix" with the individual variables analysed according to their distribution and homogeneity of variances: ANOVA (post-hoc student t-test), Kruskal-Wallis (post-hoc Dunn test), Welsh ANOVA (post-hoc Games-Howell). The data reported are presented as mean ± standard error.

## Results

Preliminary results (cohort 1) showed that VF did not have an effect on bodyweight. There was a difference in the activity data between breeds and treatments, with higher activity levels recorded in hill ewes than lowland ewes, and higher activity in LC than LVF (on average 33,800.98  $\pm$  2,717.43 (LC), 26,243.74  $\pm$  3,370.09 (LVF), 35,5862.13  $\pm$  4,332.35 (HC), 33,984.01  $\pm$  3,567.42 (HVF) [*F* = 59.319. *P* < 0.001]). Regarding the isolation box test, no differences were observed between breeds or treatments (Table 1). Attention bias test results showed no behavioural differences between breeds or treatments. On average across treatments, vigilance behaviour was 32.04  $\pm$  3.38 s, attention to the 'threat' corner was 4.07  $\pm$  0.74 s, number of eating bouts was 0.57  $\pm$  0.14, latency to eat was 82.89  $\pm$  8.93 s, number of zones crossed was 10.07  $\pm$  1.78, time spent standing at the entry door was 4.18  $\pm$  0.85 s, and time spent standing at the exit door was 5.75  $\pm$  1.07 s.

#### Table 1

Isolation box parameters by date & by treatment (Mean±standard error).

-									
Parameter	LC	LVF	MC	MVF	p-value				
Prior training (Day 0)									
Vocalisations	0.25±0.25	0	0	0	0.39				
Jumps	0	0.71±0.53	0	0.13±0.13	0.25				
Single feet	9.29±3.28	13.14±4.11	10.71±1.95	12.25±3.42	0.62				
Headbutts	3.71±1.81	1.43±0.91	0.71±0.44	5.63±3.46	0.69				
Scratching	0	0	0	0	NA				
180° head turns	0.50±0.33	0.25±0.25	1.00±0.50	0.50±0.50	0.37				
Temperament	2.00±0.38	1.88±0.40	2.75±0.37	2.88±0.58	0.28				
End training (Day 10)									
Vocalisations	5.75±1.81	1.50±1.05	2.00±0.76	2.29±0.44	0.077				
Jumps	0.25±0.16	0.13±0.13	0.14±0.13	0	0.62				
Single feet	8.63±1.67	14.25±3.32	6.86±3.05	9.67±1.58	0.13				
Headbutts	1.13±0.99	1.50±1.05	0.57±0.40	0.17±0.14	0.94				
Scratching	1.00±0.76	0.25±0.16	0.14±0.13	0.67±0.58	0.92				
180° head turns	1.25±1.00	0.50±0.33	0.29±0.17	3.29±1.79	0.37				
Temperament	3.13±0.30	2.50±0.27	3.00±0.29	3.29±0.17	0.25				
2 weeks in (Day 24)									
Vocalisations	1.13±0.85	1.38±0.89	1.00±0.61	0.63±0.38	0.99				
Jumps	0	0	0	0	NA				
Single feet	5.63±1.59 <sup>ab</sup>	14.25±3.94 <sup>b</sup>	5.14±3.21 <sup>a</sup>	7.00±2.88 <sup>ab</sup>	0.047				
Headbutts	0.38±0.38	0.13±0.13	0	0	0.58				
Scratching	0.25±0.25	0.13±0.13	0	0	0.58				
180° head turns	0.63±0.42	1.50±0.53	1.00±0.65	1.50±0.42	0.26				
Temperament	2.25±0.16	2.38±0.18	1.57±0.19	2.25±0.41	1.00				
End (Day 38)									
Vocalisations	1.25±0.82	0.71±0.53	0.50±0.30	0.63±0.32	0.99				
Jumps	0	0	0	0.25±0.25	0.45				
Single feet	7.63±2.23	14.86±4.48	4.50±1.20	6.38±1.53	0.087				
Headbutts	0.25±0.25	0	0	0.13±0.13	0.64				
Scratching	0.88±0.52	0.67±0.43	0	0	0.12				
180° head turns	0.25±0.25	1.29±0.34	1.17±0.41	1.88±0.64	0.10				
Temperament	2.50±0.27	2.71±0.17	2.00±0.00	2.38±0.26	0.13				

#### Conclusion

Preliminary results indicate that VF did not negatively impact ewe behaviour or welfare, yet it seems that breeds behave differently to the technology. These results need to be confirmed with the bigger data set, combined with cortisol analysis. However, these preliminary findings give confidence that the technology works for containing both hill and lowland sheep within a set boundary which should encourage the uptake of the technology.

#### Acknowledgements

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## 117. Machine learning algorithms for the prediction of EUROP classification grade and carcass weight, using 3-dimensional measurements of beef carcasses

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#### Application

Currently in the United Kingdom, many abattoirs rely on subjective techniques (visual assessment) for grading carcasses. Video image analysis systems coupled with machine learning techniques have the potential to be adopted in abattoirs, offering an objective approach to classification.

## Introduction

Mechanical grading can objectively classify beef carcasses, however, it is not widely adopted, often due to the infrastructure and equipment costs. Purpose-built imaging systems, requiring limited infrastructure, can extract 3D measurements of beef carcasses, which can be used to predict carcass characteristics, reducing cost. Objective classification can increase trust in the payment system and provide financial incentives for cattle to be finished at the target classification grade. This has the potential to reduce the number of over-finished cattle, increasing farm profitability and reducing greenhouse gas emissions.

## Materials and methods

A time-of-flight camera, installed in a commercial abattoir in Scotland, captured 7-seconds worth of video per carcass, immediately prior to grading. Thirty-Five point cloud frames were captured per beef carcass and processed in real time, with algorithms developed by Innovent Technology Ltd., using Halcon Image Processing Library (MVTech Software GmbH, Munich, Germany). Forty-four 3D measurements were extracted from 285,109 images of 17,250 carcasses. The 3D measurements were averaged on a per carcass basis giving one data row per carcass, before being split into training and test datasets (70:30). The training dataset was used to build random forests (RFs) and artificial neural networks (ANNs), for the prediction of cold carcass weight (CCW), EUROP conformation class and fat class, using fixed effects (CCW, sex (steer or heifer), breed type (continental or British) and kill date) or fixed effects and the 3D measurements. Grid combinations of model parameters were used to optimise model performance. The best model was then used to predict the dependent variables from the test dataset. The R<sup>2</sup> and RMSE for the predicted CCW values were calculated, and a confusion matrix was built to identify the number of correctly predicted classes for conformation and fat.

## Results

Results for CCW, conformation and fat classes predicted using the best RFs and ANNs are displayed in Table 1. Including the 3D measurements improved accuracies across traits and techniques, compared to including only fixed effects. The best models resulted in moderate-high accuracy for the prediction of CCW ( $R^2 = 0.72$  for RFs, 0.68 for ANNs), conformation class (accuracy = 71% for RF, 77% for ANNs), and fat class (accuracy = 57% for RFs and ANNS).

The increased accuracy for conformation and CCW, resulting from including the 3D measurements, suggests a strong relationship between the 3D measurements and these traits. Including the 3D measurements for the estimation of fat class resulted in a relatively small increase (2%) over the fixed effects model, suggesting that the measurement add minimal predictive power.

#### Table 1

Prediction accuracies for random forests and artificial neural networks for the estimation of cold carcass weight, conformation, and fat class, using fixed effects (FE) and 3D measurements (3DM).

	Random Forests		Artificial Neural N	etworks
	FE	FE + 3DM	FE	FE + 3DM
Cold Carcass Weight				
R <sup>2</sup> (prediction accuracy)	0.24	0.72	0.20	0.68
RMSE (kg)	36.48	22.10	36.86	23.81
Conformation				
Correctly classified classes (%)	64%	71%	65%	71%
Over/under-scored by one class (%)	34%	28%	33%	28%
Fat				
Correctly classified classes (%)	55%	57%	55%	57%
Over/under-scored by one class (%)	40%	28%	28%	30%

## Conclusion

Three-dimensional imaging technology and machine learning techniques (random forests and artificial neural networks) can be used to predict cold carcass weight, conformation class and fat class, in line with EUROP classification standards, with moderate-high accuracy. The machine learning techniques resulted in improved prediction accuracies compared to previous multiple linear regression models, built using the same dataset (Nisbet et al. 2024).

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# 118. Development and validation of a fully automated 2D imaging system generating body condition scores for dairy cows using machine learning

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# Application

Autonomous livestock monitoring applications offer opportunities for consistent and objective assessment of various health and welfare indicators, labour-demanding and time-consuming tasks when dependant on humans.

# Introduction

Monitoring body condition of dairy cows at specific stages of their lactation is a valuable aid to improve feed efficiency, fertility, and milk production and to minimize metabolic disorders. Despite that being emphasized over the last decades, most farms rarely conduct regular body condition scoring. CattleEye Ltd (Belfast, UK) has recently developed a fully automated system for lameness detection using a 2D camera mounted over an exit race (Anagnostopoulos et al., 2023). Our aim was to develop and evaluate the performance of a machine learning algorithm that would generate real-time body condition scores (BCS) using the same equipment.

## Materials and methods

The study was conducted in 7 dairy farms located in west England and Wales which were equipped with a camera. Herd size ranged from 600 to 2,300 lactating Holstein cows. Manual BCS scoring (MAN\_BCS) data for training of the algorithm was collected by 5 human assessors (HAs) who performed a total of 34 whole-milking-herd BCS sessions. A total of 34,150 MAN\_BCS with correct cow identification were recorded using the 1-5-point scale method with 0.25 increments (Ferguson et al., 1994). The algorithm was developed using the training dataset comprising various farms, which allowed us to build robust image datasets for predicting a cow's BCS. During one milking at the same days the HAs visited the farms, top-down videos of the cows exiting the milking parlour were gathered and processed by a pre-trained cow detection and pose estimation pipeline to track and identify the key points on each animal. An ordinal regression model was used when developing the algorithm. A state-of-the-art deep learning architecture, EfficientNetV2, was used to form the algorithm's backbone. Subsequently, data for testing was collected in 4 of the participating farms at least 2 months after the last training session to allow conditions to change. The MAN\_BCS collected by one HA in the milking parlour, who performed two whole-herd BCS sessions on each farm 30 days apart, were used as the ground truth. At the end of the study, MAN\_BCS were merged with the automated BCS (CE\_BCS) using the cow ID, resulting in 9,657 single BCS. A total of 3,817 cows were scored twice 30 days apart and the change in their BCS ( $\Delta$ BCS) was calculated. To assess the intra-observer reliability, the HA visited the same farm 24 h after a whole-herd BCS scoring session to score a subset of the same cows. Automated BCS (CE\_BCS) recordings from the same days were also stored to assess the precision of the automated system at the same farm. The intra- and inter-rater agreement between MAN\_BCS and CE\_BCS for the single BCS and the  $\triangle$ BCS was estimated by calculating the weighted kappa ( $\kappa_w$ ) for the exact score agreement and the percentage agreement (PA) at the 0.00, ±0.25 and ±0.50 -unit of BCS error range. Moreover, a Bland-Altman plot was created to examine for any systematic or proportional bias of system. The MAN\_BCS of 3 HAs were also used to evaluate the inter-rater agreement between humans. Finally, we measured the backfat thickness (BFT) of 111 randomly selected cows using ultrasonography. Passing-Bablock regressions were used to assess the relationship of MAN\_BCS and CE\_BCS with BFT.

## Results

The system had an almost perfect repeatability with a  $\kappa_w = 0.99$  with 96.2% PA at the exact score agreement, while the HA's intra-observer agreement produced a  $\kappa_w = 0.94$  with 68.1% PA at the exact score agreement. The  $\kappa_w$  for the exact agreement between single MAN\_BCS and CE\_BCS was 0.69, indicative of substantial agreement, while PA at the 0.00, ±0.25 and ±0.50 -unit error was 44.4, 84.6 and 94.8 %, respectively. A minimal systematic bias was observed (-0.09-unit) with a proportional bias at the extreme scores. Regarding  $\Delta$ BCS, the  $\kappa_w$  for the exact agreement between MAN\_BCS and CE\_BCS was 0.20, while PA at the 0.00 and ±0.25 error was 45.7 and 88.2%, respectively. The  $\kappa_w$  between HAs was 0.77-0.82 and the PA at the 0.00, ±0.25 and ±0.50 -unit error was 29.7-53.4, 75.3-95.8 and 91.7-99.4%, respectively. A strong linear relationship of both MAN\_BCS and CE\_BCS with the BFT measurements was observed, producing Spearman's rank correlation coefficients of  $\rho = 0.91$  and 0.75 (P < 0.001), respectively.

## Conclusions

Based on our results, automatically generated BCS by the system can predict single BCS and changes in BCS with sufficient accuracy, similar to that observed between trained scorers. Collecting more training data at the extremes could improve the system's accuracy at the low scores, where it was found to be lower.

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## 119. Evaluation of a fully automated video surveillance system for cattle lameness detection using machine learning

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## Application

An automated system that could reliably identify lame cows would be a valuable tool for lameness management in modern dairy herds.

## Introduction

CattleEye Ltd (Belfast, UK), has recently developed and commercialized an automated lameness detection system using a 2D security camera placed over an exit race. The system generates mobility scores via a machine learning algorithm on a scale 0-100, with each 25-points increments representing one grade on the 4-grade (0-3) AHDB mobility scoring method. Our aim was to evaluate the performance of this system using manual mobility scores and foot lesion records as reference.

## Materials and methods

The study was conducted in 8 dairy farms located in west England and Wales. Herd size ranged from 600 to 2.300 lactating Holstein cows. Four experienced veterinarians (VETs) performed a total of 29 whole-herd mobility scoring sessions using the AHDB scoring method. On each session, individual daily scores of the last week recorded by the system (CE) were stored and analysed at the end of the study. The weekly average score was calculated and converted to represent a binary score of lame versus non-lame cows. Agreement between the binary converted manual mobility scores (MAN) and CE scores was assessed using simple percentage agreement (PA), Cohen's kappa ( $\kappa$ ) and Gwet's agreement coefficient (AC). Moreover, the same VET was present during 17 professional foot trimming sessions in 3 of the participating farms. These sessions included both routine and therapeutic trims and the VET was blind to which cows were presented. The VET recorded the presence and graded the severity of any sole haemorrhage (SH), sole ulcer (SU), white line disease (WL), toe ulcer (TU), digital dermatitis (DD), interdigital phlegmon (IP) and interdigital hyperplasia (IH) cases in all four feet. Cows were binary classified as bearing potentially painful lesions that would impair their mobility or not, in two different ways. Cows with at least one case of SU grade  $\geq 2$ , WL grade 3, TU, stage M2 of DD and IP grade 2, were considered as having lesions of HIGH severity, while cows with at least one case of SU, WL grade > 2, TU, stage M1 or M2 of DD, IP and IH grade 2 were considered as having lesions of MODERATE & HIGH severity. Lesion records were then matched with the weekly average CE scores, resulting in a dataset of 991 cows. The same VET also mobility scored a subset of 340 cows in 2 farms 1-3 days prior to foot trimming. Accuracy (ACC), sensitivity (SE) and specificity (SP) were calculated for both CE and the VET in predicting the presence of lesions of HIGH or MODERATE & HIGH severity. Finally, individual daily CE scores covering a period of 90 days prior to the trimming sessions were retrieved. Two linear mixed models were fitted to assess retrospectively the association of the lesions status (HIGH or MODERATE & HIGH severity) with the daily CE scores on a continuous scale (0–100). Lesions status, farm, time, time × lesions status interaction were fitted as fixed effects and the random effect of each cow was considered for the repeated scores.

## Results

A total of 27,082 mobility scores were available, after merging the weekly average CE and MAN scores using the cow ID. The agreement between binary CE and MAN scores produced overall PA,  $\kappa$  and AC ranging from 81.5% to 86.3%, from 0.23 to 0.41, and from 0.76 to 0.83, respectively. Prevalence of HIGH and of MODERATE & HIGH severity lesions was 9.6% and 43.6%, respectively. ACC, SE and SP of CE and MAN in predicting presence of HIGH severity, yielding a combination of 0.83, 0.40 and 0.88, and 0.80, 0.53 and 0.83, respectively. Accordingly, ACC, SE and SP of CE and MAN in predicting presence of MODERATE & HIGH severity, were 0.60, 0.23 and 0.92, and 0.60, 0.29 and 0.87, respectively. Finally, continuous CE scores were historically increased in cows with lesions of HIGH and of MODERATE & HIGH severity by an overall difference in estimated marginal means of 9.4 points (95% CI: 7.0-11.7, *P* < 0.001) and of 3.9 points (95% CI: 2.6–5.3, *P* < 0.001) compared to the non-affected ones, respectively.

## Conclusions

The agreement between MAN and CE scores was in concordance with that previously reported between experienced human scorers assessing mobility of cows live (Linardopoulou et al., 2022; Anagnostopoulos et al., 2023). Further investigation of farm and cow-level factors that potentially influence the predictive ability of CE in identifying cows bearing potentially painful foot lesions is needed. The association of historical CE scores with foot lesions even of moderate severity creates an opportunity for early intervention before the development of severe pathologies.

## Acknowledgements

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## 120. Automated weight estimation of broiler chickens using 2D computer vision

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### Application

To develop an automated camera-based system to measure the live weight of broiler chickens.

## Introduction

The measurement of bird live weight during the production cycle is a key management practice in commercial broiler farming. However, there are concerns over the accuracy and practicalities of current methods of weighing broiler chickens (i.e. manual sampling and platform weighers) (Nyalala et al., 2021). Automated camera-based weighing systems could overcome these challenges by offering a non-invasive method of obtaining live weight data (Amraei et al., 2017). This paper proposes the use of low-cost, overhead cameras combined with computer vision techniques to automatically weigh broiler chickens. The main objectives were to explore the potential of image features from 2D video analytics, together with regression modelling, to calculate the live weight of broilers and to establish the impact of age and posture (i.e. sitting/standing) on the accuracy of weight estimation.

## Material and methods

The study was approved by the School of Biological Sciences (Queen's University Belfast) Research Ethics Committee (reference number MHLS 20-128). Data were collected in one commercial broiler house in Northern Ireland containing 28,000 mixed sex birds of the Ross 308 broiler line. A temporary passageway was created in the house and an overhead camera (GeeKam action camera, Shenzhen Bodalong Technology Co. Ltd, Guangdong, CN) was placed at a height of approximately 2m from the floor (Fig. 1). Birds were selected from the flock at random and a reference weight was obtained by manually weighing each bird in a container connected to a digital scale (Dr Meter Fishing Scale, Shenzhen Thousand shores Technology Co. Ltd, Guangdong, CN) before placing them in the temporary passageway and allowing them to walk under the camera. This procedure was performed when birds were 23 (n = 21 broilers) and 35 (n = 23 broilers) days old and weighed between 0.57 to 2.98 kg. Sample sizes were limited to minimise disturbance in the house. A feature analysis was performed using two types of feature sets relating to bounding box and automatic segmentation information (Fig. 2). The bounding box feature sets included width, height, ellipse axes, ellipse area, posture and age. The segmented feature sets included ellipse axes, convex area, eccentricity,



Fig. 1. Schematic diagram of the camera setup used to record broilers of varying weights.



Fig. 2. Two types of feature sets were generated to predict broiler weight using (a) bounding box and (b) segmentation methods.

perimeter and age. The relationship between the 2D video features and the reference weight (i.e. the manual weight) was evaluated using six multivariate regression models (linear, robust linear, interaction, pure-quadratic, quadratic linear and nonlinear Hougen-Watson models). The results from the best performing feature sets and regression model are presented.

## Results

For both the bounding box and segmentation feature sets, the "minor axis + age" feature set produced the lowest error of weight estimation. The best segmentation feature set had a mean absolute error (MAE) of  $103.9 \pm 85.7$  g (or mean relative error (MRE) of  $7.9 \pm 5.9$ %), whilst the MAE of the bounding box feature set was  $88.8 \pm 82.5$  g (MRE of  $6.4 \pm 4.5$ %), when the feature sets were fed into an interaction linear model. The results indicated that the posture feature did not improve weight estimation whilst age improved the performance of all models.

## Conclusion

The analysis of 2D image features using video analytics and regression modelling is a promising method of automatically measuring broiler live weight, whilst causing minimal disturbance to the birds. The system could estimate the weight of individual broiler chickens with a mean relative error of  $6.4 \pm 4.5\%$ . Further work is needed to refine and test the approaches on different age groups of broiler chickens in a non-experimental farm setting.

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## 121. Utilizing machine learning models to predict dry matter intake in dairy cattle via herd and milk production data

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#### Application

Machine learning models based on herd and milk production data can be used to predict dry matter intake in dairy cattle.

#### Introduction

A key aspect of a sustainable dairy industry is the efficient use of feed resources. Considering the increasing availability of information generated by digitalisation technology, utilising this data with machine learning (ML) algorithms may help stakeholders identify more efficient dairy animals (Hubbart et al., 2023). While dry matter intake (DMI) is a widely adopted metric to evaluate the feed efficiency of cattle, it is challenging to determine the intake of individual cows in large-scale agricultural systems (Seymour et al., 2019). Therefore, there is interest in applying easily obtainable data to ML algorithms to develop models to predict DMI accurately. Potential data that are relevant to DMI include milk yield and composition, and herd data such as body weight, body conditioning score and lactation week (Vazquez & Smith, 2000). This study aims to use ML methods to predict the DMI of Holstein dairy cows by utilizing routine milk production and and herd information available on farms.

#### Materials and methods

Data from five studies conducted at the Agri-Food and Biosciences Institute in Hillsborough was used. All studies commenced at calving and involved a feed-to-yield concentrate allocation approach. All cattle were provided with a diet, comprising of grass silage and concentrates. While additional concentrates were given to every cattle through an out-of-parlour feeding sytem. Data collected included milk yield, milk fat, protein and lactose content, milk fat-to-protein ratio, energy corrected milk (ECM) yield, lactation numbers (1, 2, 3, or 4 or more), weeks-in-milk, live weight, body conditioning score (BCS), and DMI. A total of 4403 weekly cow records were collected. This study applied eight machine learning algorithms to predict DMI using the data collected. Linear methods included linear model (LM), generalised linear model with stepwise selection based on evaluating the AIC values (GLM\_StepAIC), non-linear methods using support vector machine (SVM), and k-Nearest Neighbours algorithm (k-NN). Decision trees and ensemble decision methods, classification, and regression tree (CART), bagged classification and regression trees (Bagged CART), random forest (RF) and stochastic gradient boosting (SGB) were also applied. Model performance was then evaluated using mean absolute error (MAE), root mean square error (RMSE) and coefficient of determination (R<sup>2</sup>). Fisher's Least Significant difference (LSD) test of the accuracy of machine learning (ML) algorithms was performed to evaluate the MAE, RMSE and R<sup>2</sup> values to determine the difference in performance between the models. All analysis were undertaken using R v4.3.1.

## Results

Using various machine learning methods to predict DMI, the random forest algorithm resulted in the most accurate models with the lowest mean RMSE and MAE value, while having high  $R^2$  value (see Table 1).

#### Table 1

Summary statistic (Minimum, Mean, Maximum) and Fisher's Least Significant difference (LSD) test of the accuracy of machine learning (ML) algorithms to predict DMI in cattle using mean absolute error (MAE), root mean square error (RMSE) and coefficient of determination (R<sup>2</sup>).

Model	MAE	MAE			RMSE			R <sup>2</sup>		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	
SVM	1.51	1.66 <sup>a</sup>	1.83	1.93	2.20 <sup>a</sup>	2.45	0.68	0.73 <sup>a</sup>	0.78	
RF	1.44	1.60 <sup>ab</sup>	1.77	1.85	2.13 <sup>a</sup>	2.38	0.70	0.75 <sup>a</sup>	0.79	
SGB	1.47	1.63 <sup>b</sup>	1.73	1.85	2.16 <sup>a</sup>	2.38	0.70	0.74 <sup>a</sup>	0.78	
LM	1.53	1.67 <sup>c</sup>	1.83	1.93	2.21 <sup>b</sup>	2.48	0.68	0.73 <sup>b</sup>	0.76	
GLM_StepAIC	1.53	1.67 <sup>c</sup>	1.83	1.93	2.21 <sup>b</sup>	2.48	0.68	0.73 <sup>b</sup>	0.76	
k-NN	1.61	1.73 <sup>d</sup>	1.97	2.06	2.27 <sup>c</sup>	2.64	0.63	0.71 <sup>c</sup>	0.76	
Bagged CART	1.60	1.79 <sup>e</sup>	1.95	2.04	2.35 <sup>d</sup>	2.62	0.64	0.69 <sup>d</sup>	0.75	
CART	1.91	$2.14^{f}$	2.29	2.42	2.74 <sup>e</sup>	2.96	0.51	0.58 <sup>e</sup>	0.66	

Min; Minimum, Max; Maximum.\*\*different superscripts in the mean MAE, RMSE, RF columns represent significant (P<0.05) difference in LSD test between herd regions.

## Conclusions

The present study demonstrated that routine records available on farms can be effectively utilised with machine learning algorithms to develop a DMI prediction model. While algorithms varied in performance, the SVM algorithm had the lowest MAE that was significantly different from the other models. SVM, RF and SGB models have the lowest RMSE and highest R<sup>2</sup> values that were significantly different between the other models. Therefore, SVM, RF and SGB models has the ability to provide great accuracy when predicting DMI for dairy cows and has the potential to improve nutritional management in the dairy sector.

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#### 122. Farm animal welfare and the environment: friends or foes?

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The relationship between improving animal welfare and addressing environmental problems is complex, and the extent to which they align or conflict depends on specific practices, policies, and contexts. Practices that enhance animal welfare can align with sustainable agricultural methods. For example, pasture-based systems, organic or agroecological farming benefit both animal welfare by facilitating more natural behaviours and the environment by reducing reliance on resource-intensive farming practices and improving biodiversity. High welfare production systems also produce healthier animals with better longevity thereby reducing resource consumption (and reliance on antibiotics). However, such systems are criticised as not being productive enough to feed the world. On the other hand, intensive farming methods are seemingly more efficient in their use of carbon-based resources but they prioritise high production at the expense of animal welfare. Interestingly they may also damage other aspects of the environment such as water quality or biodiversity. For example, the production of animal feed associated with such systems contributes to deforestation, habitat loss, and overuse of land and water resources. This highlights potential conflict between the current singular focus on anthropogenic climate change and other dimensions of environmental protection. It is likely that in balancing such issues, we may need to adopt systems offering greater benefits to animal welfare and non-climate change associated aspects of environmental protection in spite of higher greenhouse gas emissions. The concept of One Welfare acknowledges the interconnectedness of humans, animals and the environment and provides a framework for achieving a harmonious balance between animal welfare and environmental concerns.

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#### 123. Effects of a customized trace mineral supplementation on health status and mineral metabolism in seven dairy cattle farms

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# Application

Zinc (Zn), copper (Cu) and manganese (Mn) are added to the feed of dairy cows as a supplement to the basic ration (forage, cereals, etc.) to cover their physiological needs, improve their health and optimize their productivity. Excessive use, however, wastes non-renewable mineral resources, is potentially toxic in animals and in the soil, which receives dejecta with high concentrations of metals.

## Introduction

Imprecise supplementation of Zn, Cu and Mn can result in nutritional imbalances, economic losses, and environmental impacts. Because mineral content in forages is extremely variable compared to total digestible nutrients or protein, proposing customized mineral supplementation is a great of interest. The objective of this work was to assess the effect of a customized mineral supplementation for Cu, Zn and Mn on health status and mineral metabolism in 7 commercial dairy herds.

# Material and methods

Seven commercial dairy herds in Auvergne-Rhône-Alpes Region, France with in average 89 dairy cows per herd were selected for this study. In each farm, forages and all ingredients of the basal diets were collected each month and analyzed by ICP-MS for mineral content (Zn, Cu, Mn, S, Mo and Fe). Labels of the mineral feeds were also collected with a focus on Cu, Zn and Mn. New mineral feeds were formulated by farm to provide a total intake of Zn and Mn of respectively 80 and 70 ppm/kg DM (+11% and -20% of Zn and Mn supplemented on average). The total intake of Cu recommended was between 9.5 and 11 ppm/kg DM (-50% on average of Cu supplemented) depending on the level of Cu antagonists int the basal diet (S, Mo and Fe). The dairy cows were fed the new mineral feeds for a total duration of 6 months. In each farm, blood samples were collected three times on 10% of the animals (n = 47; T0=beginning, T1 = 3 months and T2 = 6 months after the beginning of the trial). Blood samples were analysed for Cu, Zn (Colorimetry technique) and Mn (ICP-MS) concentrations in plasma, the activity of SODe and the oxidase activity of ceruloplasmin (Colorimetry technique). The effects of the customized mineral supplementation on the later parameters were explored through ANOVA integrating in the statistical model the treatment (T0 vs T1 vs T2) and the farm as fixed effects and the cow nested within the farm as a random effect.

## Results

Before supplementing with the new mineral feeds, there was a global tendency to over supplement the diets in Cu (on average +182% of the requirements). Cu in plasma did not differ among the treatments (P = 0.079) and was maintained within the biological thresholds (13,65 µmol/L; Hussein and Staufenbiel, 2012). Zinc in plasma significantly increased (P = 0.000; T2 > T1 > T0; +21%). No statistical differences were observed regarding Mn in plasma for T0 and T2, however, T1 was significantly lower (2,92, 2,82 and 1,96 µg/L respectively). Ceruloplasmin activity significantly increased in T2 compared to T0 (P = 0.001; 7628 vs 5708 mU/mL). No statistical differences were observed regarding the activity of SODe (P = 0.135).

## Conclusion

These results showed that Cu, Zn and Mn requirements of dairy cattle can be covered with a customized mineralization without inducing any deficiencies or metabolic problem. Last but not least, environmental and economic aspects should be taken into account in such studies.

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## 124. Needle-free intradermal vaccination, an opportunity to improve commercial pig welfare

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## Application

Promoting high quality animal welfare for UK commercial pigs, using a needle free vaccination device.

## Introduction

Livestock welfare has been placed at the forefront of both consumers and farmers which has increased the demands to promote positive welfare. This has driven the need to research strategies to improve animal welfare. Currently the UK has over 10,000 pig holdings, with piglets being vaccinated during production to prevent disease. Effective vaccinations are required for challenging pathogens, porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* that impact the health of pigs, the fattening phase and cause economic losses (López-Lorenzo et al, 2021). Vaccinations are commonly delivered by intramuscular injections which present multiple welfare concerns. Intramuscular needle vaccinations are associated with pain, especially from the reuse of needles, spread of disease and public health issues. An alternative method is intradermal vaccination which can be achieved using needle-free devices that have been reported to reduce the negative effects imposed by needle syringes (Có-Rives et al, 2023). Previous studies have demonstrated vaccines delivered using needle-free devices stimulate both a humoral and cell-mediated immune response and improve piglet welfare (Temple et al, 2020; Dalmau et al, 2021). This study aimed to explore the welfare benefits of a previously unreported intradermal needle-free vaccination on a commercial UK pig unit.

## Materials and methods

Piglets (181) were split into two experimental groups across two trials (i) intramuscular (n = 91); receiving Ingelvac CircoFLEX<sup>®</sup> (ii) Intradermal (n = 90); receiving Mhyosphere<sup>®</sup> with a needle-free device Hipradermic 3.0. Piglet behaviours were observed at 1h or 24h post vaccination (n = 600) using scan sampling, to assess piglet welfare. All observations were recorded using a CCTV system, using cameras placed above the experimental pens. Vocalisations of piglets during the point of vaccinations were recorded by observers. Piglet weights were recorded on the day of vaccination and one-week post-vaccination (piglets approx. 5 weeks old) to assess growth. Data was analysed using GenStat 22<sup>nd</sup> edition (VSNi) to examine behaviours (Two-way ANOVA), weights (T-tests) and vocalisations (Chi-square).

# Results

Piglets vaccinated using either the intramuscularly or intradermally route showed no statistically significant difference between the behaviours observed (Table 1). A higher percentage of piglets in the intradermal vaccination group, were standing or sitting versus those vaccinated intramuscularly at both 1h and 24h post vaccination. Piglets were observed spending more time drinking and feeding 24 hrs compared to 1 hr post-vaccination (P < 0.001). Piglets vaccinated via the intramuscular route (51.1%) vocalised more compared to the intradermal route (43.8%), although this difference was non-significant (p = 0.329). Pre-vaccination weights were similar across the two treatments; intradermal (7.71 ± 1.64 kg) and intramuscular (7.39 ± 1.79 kg), and post-vaccination intradermally vaccinated piglets were heavier (8.49 ± 1.79 kg) compared to intramuscular piglets (7.58 ± 1.79 kg).

#### Table 1

Behaviours of piglets at two time points post-vaccination with either an Intradermal (ID) or Intramuscular (IM) vaccine.

Behaviour (% observed)	ID		I	М	_		
(,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	1hr	24hr	1hr	24hr	Vaccine SED <sup>1</sup>	Time SED <sup>1</sup>	V× T SED <sup>1</sup>
Laying sternally	41.7	31.5	39.6	33.5	4.7	4.7	6.7
Side Lying	8.1	7.5	11.2	8.3	1.4	1.4	1.9
Moving	24.6	24.8	26.5	20.0	2.6	2.6	3.8
Tail and Ear Biting	1.5	2.2	1.8	4.3	0.7	0.7	1.1
Drinking or Feeding	2.8	10.7	2.3	12.5	1.4	1.4***	2.0
Standing or Sitting	21.4	23.3	18.6	21.4	4.6	4.6	6.6

 $^{1}$ SED = Standard Error of Difference

\*\*\* indicates significance at P<0.001

<sup>1</sup>SED = Standard Error of Difference.

\*\*\* indicates significance at P < 0.001.

## Conclusion

The use of a needle-free device to deliver a vaccine through a intradermal route did not reveal adverse effects on piglet welfare. Piglets in the intradermal group demonstrated an increase in weight that may be associated with avoiding invasive and stressful procedure. These findings support the literature that needle-free devices offer an alternative that provides advantages over needle-based delivery methods of vaccines.

#### Acknowledgement

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## 125. How does temporary use of the farrowing crate effect piglet mortality and the performance of lactating sows?

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## Application

Crating sows for 4 days post-farrowing reduced piglet mortality by approximately 4% compared with sows housed loose in the pen, resulting in an extra 0.6 piglets weaned per litter.

#### Introduction

There is growing pressure to remove crates and cages from animal production to improve welfare and this includes the farrowing crate, originally designed to protect piglets from sow overlying. There is concern within the pig industry that removal of the farrowing crate may result in an increase in piglet mortality therefore reducing uptake (King et al., 2019). Temporary crating may provide an agreeable compromise by protecting piglets at their most vulnerable during farrowing and early lactation, whilst providing freedom of movement for the sow during the remainder of the lactation period. The aim of this study was to determine the effect of different temporary crating lengths on piglet mortality and sow lactation performance.

## Materials and methods

Ethical approval for this study was granted by the University of Leeds Animal Welfare and Ethical Review Body. This study used a total of 500 sows and their litters (8229 piglets) over 14 batches at the National Pig Centre, Leeds, UK. Sows were allocated to one of three treatment groups 1) loose housed across farrowing and lactation (n = 167), 2) crated *ca* 24h before farrowing + 2 days of lactation (n = 166), 3) crated *ca* 24h before farrowing + 4 days of lactation (n = 167). Sows were housed in fully slatted farrowing pens (JFL15, JYDEN, Denmark;  $2.4 \times 2.4$  m). Piglets were weighed and ear tagged for identification within 24 hours of birth, at an average day 7 (week 1) and at weaning (approx. 28d). All piglet pre-weaning mortality including cause and date of death was recorded. Data were analysed in SPSS Statistics 29 using the general linear model function with treatment, parity and room (nested within batch) as factors. Covariates (e.g. piglet age, litter size) were included for each model and removed in a step wise basis where not significant. Data are presented as estimated marginal means. Bonferroni *post-hoc* tests were used to identify differences between means.

### Results

Pre-weaning mortality was highest for loose housed sows (Fig. 1). Piglets that died were younger (P < 0.001) and of a lower body weight (P = 0.008) on average for the loose sows (3.99 days, 1.48 kg) than those that were crated for either two (5.14 days, 1.51 kg) or four days (7.00 days, 1.72 kg).



Fig. 1. Piglet mortality (%) for litters of sows that were loose housed, or crated approximately 24h before farrowing and for either two days or four days of lactation. Colours within each bar represent the cause of death.

Sows crated for four days after farrowing weaned larger litters than those that were crated for two days or those housed loose, however the litter was *ca* 3kg lighter at weaning (Table 1). No interactions between parity and treatment were observed but there was a significant effect of parity throughout (P < 0.001).

#### Table 1

Lactation performance of sows. a-b different superscript letters denote differences between means (P<0.05). Significant values (P<0.05) and trends (P<0.10) are shown in bold.

	Loose (n=167)	Crated + 2 days (n=166)	Crated + 4 days (n=167)	SEM	P value Treatment
Total born sum (kg) <sup>A</sup>	24.7	24.7	24.8	0.230	0.855
Total born average (kg) <sup>A</sup>	1.42	1.42	1.43	0.013	0.925
Born alive sum (kg) <sup>A</sup>	23.3	23.3	23.4	0.223	0.885
Born alive average (kg) <sup>A</sup>	1.44	1.43	1.44	0.014	0.891
Week 1 sum (kg) <sup>C</sup>	38.3	37.6	37.1	0.409	0.095
Week 1 average (kg) <sup>c</sup>	2.82 <sup>a</sup>	2.75 <sup>ab</sup>	2.72 <sup>b</sup>	0.029	0.042
Week 1 average daily gain (g/d) <sup>c</sup>	0.194 <sup>a</sup>	0.187 <sup>ab</sup>	0.181 <sup>b</sup>	0.003	0.003
Wean sum (kg) <sup>D</sup>	115.8 <sup>a</sup>	115.2ª	112.9 <sup>b</sup>	0.705	0.009
Wean average (kg) <sup>D</sup>	8.63 <sup>a</sup>	8.62 <sup>ab</sup>	8.49 <sup>b</sup>	0.050	0.073
Wean average daily gain (g/d) <sup>E</sup>	0.288	0.288	0.283	0.002	0.145
Number weaned <sup>F</sup>	13.1 <sup>a</sup>	13.4 <sup>a</sup>	13.7 <sup>b</sup>	0.128	0.001

<sup>A</sup> controlling for number born, <sup>B</sup> controlling for number born alive, <sup>C</sup> controlling for number of pigs and piglet age, <sup>D</sup> controlling for number weaned and week 1 weight, <sup>F</sup> controlling for weaning age.

#### Conclusion

Restricting the movement of sows in the early lactation period reduced pre-weaning mortality compared to those allowed to farrow and lactate freely within the pen. Analysis of the effects of housing on sow stress and behaviour are on-going and required to support management decisions.

#### Acknowledgements

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## 126. Lameness induced behavioural changes in dairy cows: independent of time of day and temperature effect

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#### Application

Behavioural changes can serve as indicators for detecting lameness in dairy cows. However, there is limited clarity regarding what behavioural changes are specifically attributed to lameness.

#### Introduction

Knowing and understanding the behavioural indicators could allow for development of an effective lameness detection systems. Particularly when other factors inducing natural behavioural alterations such as temperature and time of day are involved. Therefore, this study aimed to investigate behavioural changes attributed to lameness in dairy while accounting for temperature and time of day effects.

#### Materials and methods

Data (total records: 14,587) were collected at 12 Dutch dairy farms in 2021 and 2022 from summer to early autumn. Visual behavioural observations (15 minutes per observation) were carried out per farm on healthy and lame cows on pasture for a week. For statistical analysis, duration (fraction of the portion of the 15-minutes) and frequency (counts per 15minutes) of different behaviours were computed from the processed data and used as response variables. The response variables were then tested against the predictors: health status (lame or healthy), time of day (time from start of observation), and temperature (maximum wet bulb). The behaviours included were: grazing, lying, standing, ruminating and walking. An array of quantile regression models to estimate the medians were then developed to examine how response variables relate to the predictors and their interactions. Various combinations of potential predictors which could explain a specific behaviour were tested. Models with the lowest AICs were selected regardless of the number of predictors involved and considered a result for a particular behaviour.

#### Results

Lame cows displayed increased walking and lying durations compared to healthy cows. Inversely, healthy cows increased rumination duration, while lame cows decreased when temperatures were average and hot. Interestingly, colder temperatures led to longer rumination in lame cows compared to the healthy. All cows decreased standing duration in average and hot temperatures, with lame cows consistently
standing less. Grazing durations decreased for both groups in average temperatures in the early morning, with hotter days resulting in more grazing during midday. Even with these adjustment, lame cows consistently grazed lesser durations than healthy cows. Lame cows also showed increased lying frequency and decreased walking as well as standing compared to healthy cows. In contrast, all cows increased grazing and reduced rumination at midday when temperature were average and hot.

#### Conclusion

Despite temperature and time of day effects, differences in behaviour between lame and healthy cows persisted. Suggesting that some behaviours could be linked to lameness despite the influence of temperature and time.

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### POSTER ABSTRACTS

#### 127. Development of a novel biosecurity risk assessment scoring tool for Irish pasture-based dairy farms

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#### Objective

To develop a biosecurity scoring tool to be used on Irish dairy pasture based farms.

#### Materials and methods

As part of a joint Teagasc/UCD project a risk assessment tool has been designed focusing on pasture-based dairy production in Ireland. This online risk assessment tool contains 4 sections; Risk of disease entry, Speed of spread of disease, Diagnosis of infection and Vaccination/ Baseline resilience. A biosecurity questionnaire with 75 questions was drafted based on the relevant literature. The responses to questions were weighted by expert opinion using the best-worst scaling (BWS) system in the software Conjointly. The BWS allocates scores to each answer to produce an overall score. Experts in biosecurity carried out the weighting process in three online sessions. The five expert groups included veterinary practitioners (n = 5), ECBHM (European College of Bovine Health Management) (n = 7) members, Department of Agriculture Food and Marine (DAFM) (n = 14) specialists in biosecurity, Animal Health Ireland's (AHI) Technical Working Group (TWG) (n = 8) for biosecurity and the project team (n = 5). In total 39 experts took part in the weighting process.

Each group was given a tutorial on how the software assigned scores. The experts assigned "best" and "worst" to multiple biosecurity practices. For example, "*Clinical disease outbreaks are always investigated*" was considered "best" practice, while "*Clinical disease outbreaks are never investigated*" was ranked the "worst" management practice. They were asked to do this independently based on their expert opinion without knowing the responses of other experts. No two experts received the same set of practices. Scores were assigned to subcategories; risk of disease entry, speed of disease spread and diagnosis of infection.

From the sessions, auto-generated scores based on the experts' opinions were assigned to each on-farm practice. This allowing for individual farm score based on the farmers' biosecurity practices. The score highlights areas of high – moderate – low risk. These risk levels will be demonstrated using a traffic light system for visual representation (see Fig. 1).

This audit tool will be implemented on spring calving, eProfit Monitor farms to assess biosecurity status. Reports will be sent out to farmers and followed up with an on-farm visit (see Fig. 2).

Clinical disease outbreaks are always investigated	0.3400717
Clinical disease outbreaks are sometimes investigated	0.14883585
Clinical disease outbreaks are rarely investigated	-0.28057971
Clinical disease outbreaks are never investigated	-0.47957127

Fig. 1. Best Worst Scaling scores obtained based on clinical disease outbreaks



	rour neru	Averagence
Diagnosis	75	76
Introduction risk	82	74
Speed of spread	73	78

Fig. 2. Individual farm score derived from BWS weightings, represented using a traffic light system

#### Results

Following completion of the three online sessions with 39 above experts the software assigned best and worst scores to 53 biosecurity practices and an overall farm score. An Individual farm report can be generated based on farmers responses in correlation with weightings derived from the BWS process.

#### Conclusion

This novel approach to assigning biosecurity scores harnesses the merits of both expert opinion and conjoint analysis. Ireland's dairy cow population growth has led to concerns surrounding biosecurity. This project will determine current national biosecurity status and assess the impact of interventions.

#### Acknowledgements

We thank the experts who took part in this project, participating farmers, Teagasc KT, ICBF and the Teagasc Walsh Scholar Fellowship.

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#### 128. High sample throughput SNP genotyping of cattle using the standard BioTools X9 high throughput genomics system

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#### Introduction

The Standard BioTools<sup>M</sup>X9 <sup>M</sup> High Throughput Genomics system is the ideal solution for high sample throughput SNP genotyping for plant and animal studies because it offers outstanding data quality and an efficient workflow available. The system routinely achieves 99% or higher call rates and takes only four hours to obtain results.

The performance of the X9 High Throughput Genomics system is demonstrated using cattle samples obtained with the Performagene™kit from DNA Genotek. The samples have been tested against a parentage panel of 96 SNPs that were developed by a USDA-led cattle consortium.

#### Materials and methods

All genetic applications start with the collection of DNA samples. Blood samples are expensive to collect, as well as complex and costly to ship, store and process. Hair follicles and ear punches are inefficient to

process and can provide low quality and quantity DNA, resulting in failed sample and a requirement to recollect, incurring additional cost, effort and time.

Performagene eliminates these challenges by:

- Easy, reliable, and cost-effective collection, transportation and processing
- Barcoded for full sample traceability
- Efficient sample preparation and extraction of high quality, high quantity DNA
- DNA is stable for 1 year at room temperature

High-Throughput, Automated Workflow for Animal Genotyping and Parentage Testing

- 1. Sample mix is loaded in the sample inlets
- SNP Assays are loaded in the assay inlets
- 2. The 9,216 individual reactions are assembled automatically by the X9 system, dramatically reducing reagent and pipetting requirements
- 3. Allele-specific PCR products are generated and imaged in the X9 High Throughput Genomics system

#### SNP Genotyping Experiment

The X9 High Throughput Genomics system was used to genotype 48 cattle nasal samples in duplicate against a 96 SNP parentage panel. The nasal swab samples were obtained using the Performagene kit from DNA Genotek and STA was used, although not required.

#### Results

- The SNP genotyping results demonstrate the excellent performance of the X9 High Throughput Genomics system using cattle samples.
- Results have shown 99.3% call rates, with the majority of no calls coming from one sample.
- 99.9% call rates and 100% concordance are achieved in duplicates after that sample had been removed from the analysis.

#### Conclusions

- SNP genotyping experiments using Performagene cattle nasal samples have demonstrated 99.9% call rates and 100percent concordance on the X9 High Throughput Genomics system.
- The workflow for Dynamic Array IFCs requires only a few hours of total time and minutes of actual hands on-time.
- The X9 High Throughput Genomics system has been designed to be easily integrated into a high throughput laboratory.

The X9 High Throughput Genomics system provides the ideal solution for high sample.

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#### 129. In vitro evaluation of Urochloa brizantha extracts as a potential feed additive to modulate microbial diversity

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#### Application

The use of hydroalcoholic extract (HE) reduced the relative gene expression of archaea, therefore, it may be interesting to further investigate this product and its effect as a feed additive.

#### Introduction

Antibiotics in animal feed can promote livestock growth but have controversial effects as they can lead to the emergence of antibiotic-resistant strains that can endanger the health of human and livestock. In addition, the use of antibiotics is prohibited in some regions of the world, such has been completely banned in Europe since 2006. In this context, recent studies indicate that secondary metabolites, such as saponins, could be used as a potential natural alternative to additives in the favourable modulation of rumen fermentation (Qu et al., 2023). Forages often present saponins like dioscin/protodioscin, soyasapogenin, diosgenin, soyasapogenol, and yamogenin (Low, 2015). However, their role in ruminal metabolism and microbial diversity is poorly understood in a ruminant model. Thus, the objective of this study was to evaluate the effects of *U. brizantha* extracts on microbial diversity of an *in vitro* experiment over 24 h incubation.

#### Materials and methods

The *in vitro* gas production was performed using the semi-automatic technique measurement, and after 24h of incubation was collected 7 mL of ruminal fluid for microbial quantification by PCR. The experimental design used was completely randomized in a factorial arrangement  $(2 \times 4 + 2)$ , with two main effects (two types of extracts: ethanolic, EE with 99% of ethanol and hydroalcoholic, HE with 70% of ethanol, and four levels: 50; 100; 150 and 200 mL of extract/kg of DM) plus the additional treatments that were, a positive control (MON, 25 ppm of monensin - Rumensin<sup>®</sup>, Elanco Brasil) and negative control (CTL, no inclusion of additives). Thus, there were 10 treatments, with three replicates within each experimental unit, which were evaluated by PROC GLIMMIX from SAS (2004). The means were compared using orthogonal contrasts at the 5% significance level, to verify the effect of the extract (EXT), treatments with EE and EH extracts, and all doses: 50, 100, 150,

and 200 mL/kg DM were grouped. Genomic DNA was extracted with the PureLinkTM Microbiome kit following the manufacturer's recommendations. DNA quality was assessed using a NanoDrop One/One spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and agarose gel electrophoresis. Relative quantification by real-time PCR was performed to estimate the number of methanogenic archaea, protozoa, and bacteria of the species *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* concerning the total number of bacteria in the samples. All real-time PCR reactions were performed in duplicate in the CFX96TM thermal cycler (Bio-Rad) in a final volume of 10  $\mu$ L reaction per well containing 2x qPCRBIO SyGreen Mix (PCRBiosystems, London, UK), 5 ng/ $\mu$ l DNA, and the forward (F) and reverse (R)primers at concentrations of 300 nM for Archaea (F – AATTGGAKTCAACGCCGGR; R – TGGGTCTCGCTCGTTG) and Total Bacteria (F – CGGCAACGAGCGCAACCC; R – CCATTGTAGCACGTGTGTAGCC), 100 nM for protozoa (F – GCTTTCGWTGGTAGTGTATT; R – CTTGCCCTCYAATCGTWCT), and 200 nM for *Fibrobacter succinogenes* (F – GTTCGGAATTACTGGGCGTAAA; R – CGCCTGCCCCTGAACTATC) and *Ruminococcus flavefaciens* (F – CGAACGGAGA-TAATTTGAGTTTACTTAGG; R – CGGTCTCTGTATGTTATGAGGTATTACC). A negative control was evaluated on each plate to detect possible contaminants or the formation of primer dimers. The efficiency of each primer was evaluated based on a linear regression model using serial dilution of a pool of DNA samples. The efficiency of PCR amplification was calculated according to the equation: E = 10(-1/slope), and the relative quantification (RQ) values were determined by the method of Pfaffl (2001). The total 16S rRNA gene amplified by the total bacteria primer was used as an endogenous gene for data normalization (Denman; Mcsweeney, 2006).

#### Results

No differences were observed (P > 0.05) between treatments regarding to relative gene expression of archaea, *Ruminococcus flavefaciens*, and protozoa. Only relative gene expression of *Fibrobacter succinogenes* was different (P = 0.02) between EXT (0.67) and CTL (1.00) with a 33% reduction. There was an effect of the type of extract, archaea relative gene expression was greater in EE (0.93) than HE (0.73). There was an effect of the type of extract, the relative gene expression of archaea and *Ruminococcus flavefaciens* were higher in EE concerning HE. However, occurred opposite effect for protozoa, EE (0.90) was lower than HE (1.37).

#### Conclusions

The *U. brizantha* extracts were not antiprotozoal efficacy. The HE was better than EE in reducing archaea which can influence the reduction of methane. However, this study was conducted as an in vitro method, therefore, an *in vivo* study will be needed to verify the effects of HE on microbial diversity and methane emission in ruminants.

#### Acknowledgements

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#### 130. What links between steroidal saponins of Urochloa brizantha and rumen metabolism

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#### Application

The inclusion of extract in the diet altered in vitro rumen metabolism, but these differences were not reflected in methane production.

#### Introduction

The livestock sector is an important source of animal protein for human consumption, but also a contributor to GHG emissions. So, several strategies have been studied to mitigate methane (CH<sub>4</sub>) through the modulation of ruminal fermentation. Saponins are triterpene or steroidal glycosides present in many plants and due to their properties, saponins are finding application in the chemistry and pharmaceutical industry as supplements for health foods, and potential anti-cancer treatments for humans (Wina; Muetzel; Becker, 2005). However, their role in the metabolism and function are poorly understood in animal models. In Brazil, beef cattle are predominantly pasture-based, and the genus *Urochloa* corresponds to approximately 85% of cultivated pastures, and presents steroidal saponin. Thus, the objective of this study was to evaluate the effects of *U. brizantha* extracts on rumen fermentation and methane production *in vitro* experiment over 24 h incubation.

#### Materials and methods

The *in vitro* incubation was conducted in bottles of 160 mL with 0.5 g of substrate (70:30 corn silage: concentrate), put in TNT bags (100 g/ cm<sup>2</sup>), and inoculated with 25 mL of inoculum (50:50 solid: ruminal liquid) and 50 mL of buffered mineral solution for 24 h. Eight Texel × Santa Ines sheep cannulated were used as donors. The experimental design used was completely randomized in a factorial arrangement ( $2 \times 4 + 2$ ), with two main effects (two types of extracts: ethanolic, EE with 99% of ethanol and hydroalcoholic, HE with 70% of ethanol and four levels: 50; 100; 150 and 200 mL of extract/kg of DM) plus the additional treatments that were, a positive control (MON,

25 ppm of monensin - Rumensin<sup>®</sup>, Elanco Brasil) and negative control (CTL, without inclusion of additives). Thus, there were 10 treatments, with three replicates within each experimental unit, which were evaluated by PROC GLIMMIX from SAS (2004), and the means were compared using orthogonal contrasts at the 5% significance level. To verify the effect of the extract (EXT), treatments with EE and HE extracts and all doses: 50, 100, 150, and 200 mL/kg DM were grouped. The in vitro gas production measurements were made at 0, 4, 8, 12, 16, 20, and 24 h post-inoculation using the semi-automatic technique measurement, 2.0 mL of the gases were collected for methane determination by the chromatographic method. After 24 h of incubation, the flasks were placed in containers of iced water to interrupt the fermentation process, and samples of the rumen liquid were collected for determination of short-chain fatty acids (SCFs) and ammoniacal nitrogen (N-NH<sub>3</sub>).

#### Results

The inclusion of EXT in the diet changed the standard ruminal fermentation concerning CTL treatment. The EXT treatment produced a higher proportion of acetate (C2; 70.64%) and a lower percentage of propionate (C3; 14,10%) concerning CTL (63.05%; 18.04%, respectively). For butyrate production (C4), there is no difference between EXT and CTL (P > 0.05), but EXT (9.79%) had higher production concerning MON (8.24%). This result is contrary to what was expected with the inclusion of saponins that provide an increase in propionate, and lower acetate and butyrate (Kholif, 2023). A lower proportion of propionate will make greater amounts of carbon and hydrogen available in the rumen, which can consequently be used to produce methane (Johnson and Johnson, 1995). There was no difference (P > 0.05) in N-NH<sub>3</sub> content between EXT and CTL treatments, however, EXT (31 mg/dL) was higher than MON (23 mg/dL). No differences were observed (P > 0.05) between treatments for CH<sub>4</sub> production, and the average was 19.3 mL CH<sub>4</sub>/g of degraded dry matter.

#### Conclusions

The inclusion of extract in the diet alters in vitro rumen metabolism producing higher acetate and ammoniacal nitrogen, and lower propionate without altering methane production. New studies need to be carried out with the use of extracts in animals, to verify the effect of *Urochloa brizantha* extracts as a food additive.

#### Acknowledgments

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#### 131. Assessing baselines for environmental credentials in hill and upland sheep systems where productivity gain is the main driver

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Hybu Cig Cymru – Meat Promotion Wales', Red Meat Development Programme Hill Ram Scheme supported farmers in Wales to breed hill sheep to improve productive performance. As part of this project a bespoke maternal hill selection index. was developed focussing on detailed performance recording and using DNA analysis to facilitate the development of genomic selection. These farmers are pro-actively driving efficient production in hill and upland farming systems in Wales. As part of this project, environmental audits were completed to set baselines to evidence the sustainability credentials of these systems and to recommend improvements.

A total of 28 farms participated in the environmental audits. Of these 20 were involved in both the carbon and biodiversity audits, 5 farms a biodiversity audit alone and 3 farms only the carbon audit.

Biodiversity audits were conducted through detailed ecological surveys based on Chartered Institute of Ecology and Environmental Management (CIEM) methods by FWAG Cymru on 25 farms during a dry summer and autumn between the 26<sup>th</sup> of July and the 27<sup>th</sup> of November 2022.

Farms ranged in size from 9.3 to 446 hectares excluding any grazing rights on common land. A total of 10 farms were in the Glastir Advanced agri-environment scheme including 4 in Glastir Organic. Others participated in the Glastir Woodland Creation and Small Grants, and Coed Cadw schemes for hedge restoration, hedge planting, tree planting and the Eryri National Park.

Carbon audits were undertaken on 23 Welsh hill sheep farms, between August and November of 2022 with 17 of the farms also having a suckler beef enterprise. The face- to- face data collection and subsequent audit estimated individual farm carbon emissions using the 'Agrecalc' carbon calculator. The soil sequestration module produced an indication of the soil carbon sequestration using geospatial data, grass-land management, grazing management and cultivation practice. The calculator assesses the farm's sequestration (or sometimes emissions) via soil carbon on a whole farm basis, by enterprise and by product. The biodiversity audit mapping information enabled wood-land areas and hedgerow lengths to be used in 'Agrecalc'. A carbon plan for each farm identified and discussed key areas for reducing carbon emissions The 'Agrecalc' database was used to benchmark the farms against businesses with the same enterprise type to gauge carbon efficiency and identify areas for improvement.

The total farmed area of those surveyed was over 4,000 hectares (excluding common grazing) with 43% identified as 'habitat' land including semi-natural grassland/rush pasture, upland mosaics/acidic grassland/bogs, marshy grassland and flower rich meadows. In addition, there was 440.8 ha or 10.8% of the land in woodland and scrub. The sole grazing of upland areas, totalled 954 hectares for the 25 farms in addition to the vast areas of common grazing. Hedges were universally present on all farms surveyed with a combined total 16.54 kilometres of hedgerows. There were over 27,100 metres of dry-stone walls with many covered with mosses, ferns and lichen. These functional bound-aries provide habitats for a range of wildlife including nesting birds such as, redstarts, wheatears, great and blue tits, little owls and wrens as well as voles, mice, rats, hares and rabbits.

The average carbon footprint excluding sequestration across the 23 farms was recorded to be  $28.69 \text{ kgCO}_2\text{e/kg}$  dwt for the sheep enterprise and for beef, the average carbon footprint across the 17 farms was  $37.61 \text{ kgCO}_2\text{e/kg}$  dwt. These are both below the averages of comparable systems within the 'Agrecalc' database The range on the farms was considerable. The average carbon footprints including sequestration was recorded to be  $5.97 \text{ kgCO}_2\text{e/kg}$  dwt for sheep and  $5.73 \text{ kgCO}_2\text{e/lg}$  dwt for beef.

	Group average	Agrecalc average kgCO <sub>2</sub> e/kg dwt	Group lowest	Group highest
Whole Farm CO2e emissions per kg farm output inc. soil carbon sequestration (kg CO2e / kg output)	4.60	N/A	-12.88	78.88
CO2e emissions per kg output sheep enterprise Excluding sequestration (kgCO <sub>2</sub> e/kg dwt)	28.69	32.47 (good hill ewe flock) 40.29 (extensive hill ewe flock)	20.25	49.51
CO2e emissions per kg output beef enterprise <i>Excluding sequestration</i> (kgCO <sub>2</sub> e/kg dwt)	37.61	42.73 (Spring calving upland suckler cow) 51.80 (Spring calving hill suckler cows)	12.53	51.80

Feed efficiency was a key area highlighted to reduce the carbon footprint through better quality forage and using analysis to calculate any feed rations. However, improving maternal genetics of these flocks is an important part of increasing efficiency across all the key traits. Hill sheep adapted to their environment and utilizing poor quality forage would positively influence the carbon footprint.

#### Conclusion

The farmers' livestock production methods together with agri-environment incentives have shaped and maintained the rich biodiversity of these hill and upland areas. Each farm contributes to the safeguarding and enhancing of the landscape we value whilst producing stock of high quality for the red meat market. The eco-systems services delivered by these habitats include the management of our landscape, wild-life, water courses, soils, historic sites and features, public access routes and areas, and existing carbon stocks. There are opportunities to reduce emissions and increase sequestration on these farms but they compare well when benchmarked against farms of similar types and are forging ahead with genetic improvement for important production traits to improve efficiency whilst continuing to support ecological biodiversity through ruminant agriculture.

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#### 132. The impact of yeasts as feed supplement on the blood haematological and serum indices of West African Dwarf goats

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#### Application

Yeast supplementation in the diets of ruminants can be seen as one of the possible ways of reducing the health challenges facing these animals during the dry season in most tropical countries. Table 1.

Table 1	
The chemical composition of diets, Haematological and Serum indices of WAD goats fed diets supplemented with yeast.	

Yeast inclusion level in the diets								
Item	T1 (0%)	T2 (100%)	T3 (100%)	T4 (50:50%)	T5 (70:30%)	Con	Cassava peel	Grass
Chemical compositi	on (%)							
DM	89.45	89.31	89.29	89.45	90.15	88.49	90.31	91.08
Ash	12.71	13.03	12.66	11.85	11.92	13.33	15.76	11.48
CP	12.16	11.13	11.01	12.43	10.92	11.48	8.38	9.80
NDF	50.53	53.46	53.62	51.45	47.33	50.39	50.18	62.47
ADF	31.85	34.01	34.46	31.97	34.04	28.68	37.14	47.62
ADL	4.00	4.15	3.79	4.02	2.92	4.09	2.94	4.34
Haematological and DMI (g/d)	Serum indices	of WAD goats 276.82ª	265.00 <sup>b</sup>	260.62 <sup>bc</sup>	273.00ª	<b>SEM</b> 1.52	<b>P value</b> 0.000	*Ref
Haematological indic	`es							
$RBC \times 10^{12}/L$	7.54	9.30	9.82	8.46	9.12	0.64	0.153	8-18
WBC $\times 10^{9}/L$	5.86 <sup>c</sup>	7.62 <sup>ab</sup>	7.42 <sup>b</sup>	6.70 <sup>bc</sup>	8.54 <sup>a</sup>	0.25	0.000	5-14
Hgb (g/L)	71.80	82.60	88.00	77.80	81.60	5.78	0.391	70-150
PCV (%)	17.52	21.20	21.98	19.12	20.56	4.49	0.148	22-38
Serum indices								
Alb (g/L)	30.38	31.17	26.65	25.95	26.88	1.31	0.026	24-39
TP (g/dl)	7.58	7.35	6.67	6.94	6.62	0.21	0.010	6.4-7.0
GLO (g/L)	45.70	42.55	40.03	43.58	38.97	1.72	0.069	27-58
GLU (mmol/L)	1.89	2.23	2.05	1.73	2.06	0.22	0.559	2.78-4.16
TC (mmol/L)	1.85	2.11	2.12	1.81	1.88	0.11	0.150	1.63-2.79

Con; Concentrate, DM; Dry matter, CP; Crude protein, NDF; Neutral detergent fibre, ADF; acid detergent fibre, ADL; acid detergent lignin, DMI; Dry matter intake, RBC; Red blood cell, WBC; White blood cell, Hgb; Haemoglobin, PCV; Packed cell volume, Alb; Albumin, TP; Total protein, GLO; Globulin, GLU; Glucose, TC; total cholesterol.

#### Introduction

During the dry season in most tropical countries, ruminants good health conditions are been challenged by poor feeding systems, parasites and diseases, global warming, and heat stress. To reduce these health challenges, yeast supplementation as a probiotic has been identified to be capable of maintaining animals' good health condition through increased immune systems, maintenance, and balance of gut microbiota (Kulkarni et al., 2022). Among these yeasts; *Saccharomyces cerevisiae* –SC and *Aspergillus oryzae* –AO, are most commonly used as they are capable of achieving these benefits with little or no detrimental effect on the animals. However, these benefits can be limited by factors such as probiotic type, amount, stage of growth, diet type, etc. This experiment determined the impact of yeasts (SC & AO) as feed supplements on the blood haematological and serum indices of West African Dwarf (WAD) goats.

#### Material and methods

One-way analysis of variance was used to determine the effect of dietary (*Megathyrsus maximus* hay – 70%: concentrate – 30% mixture) supplementation with two yeast (SC & AO) at varying levels (0%, 100% SC, 100% AO, 50% SC: 50% AO, and 70% SC: 30% AO) given at 5 g/day/animal on the blood haematological and serum indices of West African Dwarf-WAD goats. The concentrate is composed of cassava peel – 29%, maize – 7%, Palm kernel cake – 27%, wheat bran – 27%, Groundnut cake – 8.30%, bone meal – 0.40%, salt – 0.3% and premix – 1%. Twenty-five WAD goats were randomly allocated to the 5 dietary treatments (n = 5) for 112 days which includes the adaptation period (14d), and were fed twice daily at 10.00 am and 4.00 pm at 5% of their body weight. On the last day of the experiment, blood samples (10 ml) were collected from each animal via jugular vein puncture. About 5 ml was discharged into sterile vacutainer tubes containing ethylene diamine tetracetic acid (EDTA) for haematological study, while the remaining 5 ml was discharged into sterile vacutainer tubes without EDTA to allow blood clotting at 4–5 °C, centrifuged at 3000 rpm for 15 min for serum analyses. Sub-samples of the feed and test ingredients were taken for chemical analyses. The blood haematological and serum data were then statistically analysed using a one-way analysis of variance in Minitab16 software and means were separated using Tukey's post hoc test.

#### Results

The yeast significantly (P < 0.05) increased the white blood cells (WBCs) of the animals while it did not influence the other parameters measured. However, animals on the T5 diet recorded the highest WBCs concentration.

#### Conclusion

The yeast improved the immune system of the animals through increased WBCs that are within the normal concentration found in healthy goats. Supplementation of the yeast up to 70 SC: 30% AO is capable of improving the immune system of the animals towards maintenance of good health condition.

#### Acknowledgements

Tertiary Education Trust Fund and Federal University of Agriculture, Abeokuta, Nigeria for the fund and facility used.

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#### 133. Hourly distribution of time of calving in spring-calving suckler cows

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#### Application

The hourly distribution of time of calving in spring-calving suckler cows was highly variable but when averaged across the 16 individual herd-years, revealed a relatively uniform distribution. Similarly, when expressed in terms of three 8-h periods, based on spring-time 'dark-ness' and 'daylight', the mean distribution was broadly equal. This implies that, on average, the requirement for calving supervision is similar 'around the clock'.

#### Introduction

Parturition (calving) is an exceptionally busy and labour-intensive period on-farm, and working with suckler cows, especially at 'night', can pose an increased safety risk to farmers. There is limited literature available on the timing of calving in the suckler herd. The present study was undertaken to quantify the hourly distribution of time of calving during the '24-h (h) day' for beef  $\times$  beef and beef  $\times$  dairy suckler beef cows taking into consideration cows requiring assistance at birth.

#### Material and methods

Time of calving was recorded for 1535 calvings over 16 individual herd-years for suckler cows in spring-calving herds at Teagasc Grange. The dataset comprised of beef  $\times$  beef (n = 910) and beef  $\times$  dairy (n = 625) cow breed types and included primiparous (first-calvers, n = 632; mean post-calving weight, 541 kg) and multiparous (parity of  $\ge 2$ , n = 903; mean post-calving weight, 624 kg) animals. The beef breeds represented in the cows were Charolais (CH), Limousin (LM), Hereford (HE), Aberdeen Angus (AA), Belgian Blue (BB), Saler (SA) and Simmental (SI), and Holstein-Friesian (FR) in the dairy crossbreds. The cows were bred to Aberdeen Angus, Blonde d'Aquitaine, Belgian Blue, Charolais, Hereford, Limousin and Simmental sires. The cows were accommodated indoors each year and generally offered moderate dry matter digestibility grass silage *ad libitum* (straw included occasionally) with a mineral and vitamin supplement daily pre-partum. Time of daily feeding was usually in the morning. The cows were moved to individual straw-bedded calving pens pre-calving (approximately 1 to 7 days). Data were checked for normality and homogeneity of variance by histograms, q-q plots, and formal statistical tests as part of the UNIVARIATE procedure of SAS, using SAS software Version 9.4 (SAS Institute Inc. Cary, 2016). The variables were summarised using PROC FREQ and PROC MEANS.

#### Results

Calving times were expressed hourly over the '24-h' (h) day. Mean hourly percentage of calvings ranged from 2.7 to 5.3, with large variation evident within hour. The hourly distribution of calving over the 16 herd-years is presented in Fig. 1. The mean hourly calving pattern over 24-h ranged from 2.7% at 1700 h to 5.3% at 1400 h, with large variation evident within each hour across the herd. When the 24-h day was divided into 'darkness' (1600–0800 – 16 h) and daylight" years (Fig. 1). (0800–1600 – 8 h) the mean hourly percentage of calvings was 4.1 and 4.4, respectively, corresponding to a distribution of 65 and 35% for those time periods.



Fig. 1. The hourly distribution (mean percentage with SD, indicated by the bars) of calvings throughout the '24-h' day for the 16 herd-years (orange bars, 0800–1600= 'daylight' and blue bars, 1600–0800='darkness')

When the daily calving pattern was divided equally into three 8-h periods; 'night' (2300–0700), 'morning-day' (0700–1500) and 'eveningday' (1500–2300), the mean distribution of calving times was 33, 35 and 32%, respectively, albeit with large variation within each period (21–46, 27–46 and 22–46%, respectively) across the herd-years. Cow breed type, calf sex, calf birth weight and calving difficulty did not appreciably influence the distribution of calving.

#### Conclusion

It is concluded that, on average, time of calving for spring-calving suckler cows is relatively evenly distributed throughout the 24-h day, albeit large variation is evident across individual herd-years. Cow breed type, calf sex and calf birth weight did not influence the distribution of calving. The distribution of unassisted and assisted calvings were equally distributed.

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#### 134. Rhinoceros milk composition and the effect of lactation stage on milk components

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#### Application

A knowledge of the milk composition of rhinoceros cows at different stages of lactation was crucial to formulate a milk replacer for orphan rhinoceros calves.

#### Introduction

A century ago, the five living rhinoceros species were abundantly distributed throughout Asia and Africa. The World Wide Fund for Nature (WWF) estimated that approximately 500,000 rhinos roamed Africa and Asia at the beginning of the 20th century, but that numbers have declined to 70,000 by 1970. Since 2013 only about 29,000 rhinoceros of all kinds survive in the wild (Metrione and Eyres, 2014). More than 80% of these, including those in captivity, are of the Ceratotherium simum species, commonly known as the white rhino. As a result of international interest in the conservation of the species, as well as the involvement of numerous organisations, game reserves and game farmers, rhinoceros numbers have increased since 2007. The increase was most notable in the case of white rhinos where the number of animals has increased from just over 11,000 in 2007 to almost 20,000 in 2013 (Metrione and Eyres). Due to the overexploitation of rhino horn in Eastern countries, poaching of rhinos for their horns remains a constant problem. Many rhino calves are left orphan when their dams are slaughtered by callous criminals. In order to formulate a milk replacer that would meet the nutrient requirements of rhino calves of different ages, a knowledge of the milk composition of rhinoceros cows at different stages of lactation is needed. A trial was done to obtain the required information.

#### Material and methods

Eighteen lactating southern white rhinoceros cows (*Ceratotherium simum simum*) were used in the study which was conducted in the Northern Cape and North West Provinces of South Africa. Mean days in milk (DIM ± SE) of the three groups were  $80.5 \pm 21.4$  (E, early),  $243.3 \pm 57.8$  (M, mid) and  $533 \pm 24.1$  (L, late lactation). Cows were sedated for regular veterinary examinations which included dehorning. Milk samples were collected under sedation from both teats by palpation and mild pressure and transferred to 50 mL milk collection tubes. Because milk was collected in the field on a game farm, they were chilled in a cooler box and transported to a cooling facility on the farm as soon as possible and stored at 4 °C until analyzed within 48 h after collection. Data were subjected to a one-way ANOVA and differences were declared significant at P < 0.05. The milk was analyzed for proximate composition, minerals, and total amino acids.

#### Results

Total solids (g/kg) decreased significantly from 99.8 (E) to 93.7 (M) and 91.3 (L) as DIM increased. Similarly, milk fat (g/kg) decreased (P < 0.05) from 8.4 (E) to 3.2 and 3.0 (M and L) and milk protein (g/kg) decreased from 14.1 (E) to 10.5 (M) and 9.5 (L). The lactose content (mean 67.8 g/kg) was not affected by DIM. The content of all the minerals decreased as lactation increased, but the effect of DIM was significant only for K (767 to 483 g/kg from E to L), Na (357 to 253 g/kg from E to L), Cu (0.20 to 0.12 mg/kg from E to L), Zn (2.24 to 1.9 mg/kg from E to L) and Mn (0.44 to 0.07 mg/kg from E to L). The only amino acids that changed significantly as lactation progressed were Met, Thr and His which decreased, but total amino acids (g/L) were not affected by DIM and means were 10.9 (E), 10.5 (M) and 10.0 (L).

#### Conclusion

It was concluded that the composition of white rhinoceros milk changed as lactation progressed and that the concentration of most of the milk components that were measured decreased with increased DIM, but lactose and total amino acid contents remained constant.

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#### 135. Dietary supplements reshaping the rumen bacterial community of yak (Bos grunniens)

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A complete understanding of the rumen microbial population and their transformation when exposed to diverse dietary regimens is vital to improving the health, welfare, and overall productivity of livestock

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#### Introduction

The Yak (*Bos grunniens*), a vital ruminant on the Qinghai Tibetan Plateau, holds a pivotal role in the daily lives of local herdsmen, providing them with essential resources such as milk, meat, fuel, and economic benefits. Yaks predominantly rely on local alpine pastures to fulfill their nutritional needs but undergo slow growth, weight loss, and potential fatalities due to seasonal forage variations, particularly in the cold season, causing economic losses (Dong et al., 2006). In recent years, herdsmen have turned to oats-hay, and concentrate as supplementary feeds during the cold season to address these challenges. Studies have emphasized the key role of the rumen microbiota in the health and productivity of ruminants and the diet emerges as one of the most influential factors affecting the rumen microbiota (Guo et al., 2020). Therefore, the objective of the current study was to investigate the response of the rumen bacterial community in yaks to dietary supplementation during the cold season.

#### Materials and methods

Twenty-one female domestic yaks, all aged 3 years, were randomly assigned to the three groups: a pure grazing, group, a grazing with oats hay supplement group, and a grazing with concentrate supplement group. Rumen contents were collected to assess the rumen bacterial community after 90 days. Total metagenomic DNA was isolated and 16S rRNA gene sequencing was executed using universal primers for V4 region. After sequencing, primer sequences, barcodes, low-quality sequences, and chimeras were truncated using Qiime software, and clean reads were clustered into Amplicon Sequence Variants using 99% identity. Taxonomy was assigned using the Greengenes database to the representative sequences and diversity analysis was performed. The normality of the data was checked using the Shapiro–Wilk test. Analysis of similarities was performed in R studio by using the Anosim function of the vegan package to examine the grouping variations of each group. Significance was declared at P < 0.05 and P values were modified using a false discovery rate to exclude false-positive results.

#### Results

Higher bacterial diversity was observed in grazing and concentrate supplement groups. The dominant phyla observed in all three groups included Bacteroidetes, Proteobacteria, and Firmicutes. A higher abundance of fiber-degrading genera was observed in the grazing yaks and oats hay supplement group. At the same time, fatty acid-producing genera were dominant in the concentrate supplement group.

#### Conclusions

Dietary supplementation did not exert a significant impact on the relative abundance of the dominant phyla within all groups. Nonetheless, the abundance of certain bacterial genera displayed a closer association with the varying dietary regimens.

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#### 136. The effect of dry cow footbaths on the prevalence of digital dermatitis post calving; a randomised controlled trial

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#### Application

Footbaths are an important part of digital dermatitis (DD) control but dry cows are rarely footbathed as frequently as milking cows. This presents a potential gap in DD control. Animals subjected to footbathing during the dry period were less likely to develop DD post-calving, in our study.

#### Introduction

Footbaths have been widely used on dairy farms as a prevention measure against DD but limited controlled studies supporting their efficacy are available. Experimental footbath studies are challenging to undertake (Cook, 2017). An increase in the prevalence of DD lesions post calving is often reported (Solano et al., 2016), and therefore foot hygiene during the dry period may be critical in DD control. On most farms, dry cows (i.e. non-lactating cows) are subjected to far less frequent footbath regimes compared to their milking counterparts. We aimed to investigate the impact of footbathing during the last month of the dry period on the prevalence of DD post-calving, particularly for "active" lesions.

#### Materials and methods

A single commercial dairy herd was selected for convenience and the close to calving dry period pen was split in two, with individual automatic footbaths (Hoofcount Ltd, Preston UK) installed on each side. The footbath placed in the control group remained empty, while the treatment group footbath was filled with a solution of 1% formalin. Both were automatically cleaned every 50 cow visits. Animals entering the footbaths automatically received part of their daily concentrate allowance and were held for up to 3 minutes. Animals were enrolled weekly, approximately 30 days before their expected calving. Animals were sorted by date of expected calving and alternately assigned to either the control or treatment group. The hind feet were examined in a foot-trimming crush for presence of DD lesions using the M-stage scoring system (Berry et al., 2012) at enrolment and again approximately 7 days post calving. Analysis was performed using R (4.0.1) and differences between treatment and control groups were investigated with chi-squared tests, applying Yates' continuity correction. A contingency table of active DD lesion (M1, M2 and M4.1) frequencies at the second assessment for the two groups was used to calculate the Relative Risk and the Number Needed to Treat (NNT).

#### Results

In total, data from 54 animals were available for the preliminary analysis. The difference in proportion of cows with active DD lesion at the second assessment between groups was statistically significant (Table 1). Animals in the control group were 3.71 (95%CI: 1.18-11.7) times more likely to have an active DD lesion post-calving. The NNT was 3.19 (95%CI: 1.86-11.21).

#### Table 1

Number and proportion of animals within parity and Digital Dermatitis infection groups for the control and treatment groups.

	Control	Treatment	chi-squared
	(N = 28)	(N = 26)	(P-value)
Parity	8 (29%)	7 (27%)	
0	20 (71%)	19 (73%)	
Animals with active lesions at enrolment	7 (25%)	4 (15%)	0.02
Animals with active lesions at post-calving	12 (43%)	3 (12%)	
Animals with M-2 at enrolment	3 (11%)	3 (12%)	0.07
Animals with M-2 at post-calving	5 (18%)	0 (0%)	

N: Number of animals in each group, Parity: primiparous (0)/ multiparous (1), chi-squared: results of two-tailed chi-squared tests applying Yate's correction with P-value< 0.05 signifying statistically significant difference.

#### Conclusions

A 1% formalin solution footbaths during the last month of the dry period appears to reduce the prevalence of active DD lesions during the first week of lactation. Foot skin swabs collected from these animals during both assessments will be used to investigate the impact of footbathing on foot skin microbiome. The expected study duration is two years and the groups will be swapped by the end of the first year to correct for any potential pen effect.

#### Acknowledgements

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# 137. Meta-analysis of heritability estimates and pathway enrichment analysis of candidate genes revealing the genetic landscape of feed efficiency in Holstein dairy cows

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#### Application

A thorough understanding of the genetic factors that influence the feed efficiency of dairy cows is a prerequisite for planning and implementing selective breeding programs. The results of this study will be useful for developing future genomic selection breeding to improve feed efficiency of dairy cattle and sustainability of dairy production.

#### Introduction

Improving the feeding efficiency of dairy cows is a key component of optimizing land resource utilization and meeting the demand for high-quality protein. Advances in genomic methods and omics techniques have made it possible to breed more efficient dairy cows through genomic selection. However, a comprehensive understanding of the genetic background in dairy cows is necessary to proceed with breeding programs. The aim of this study is to enhance existing knowledge of the genetic landscape of feed efficiency in Holstein cows, including reported heritability estimates, genes, genetic markers, and pathways. To achieve this, a meta-analysis of collected heritability estimates was performed to assess the feasibility of incorporating feed efficiency traits into breeding programs. Additionally, a pathway enrichment analysis was conducted to elucidate the gene regulatory mechanisms involved in feed efficiency.

#### Material and methods

A random-effects model based on Comprehensive Meta-Analysis was applied to integrate feed efficiency heritability estimates from 27 studies using the R package 'meta' (Borenstein et al., 2010). For typical feed efficiency traits (e.g., residual feed intake, dry matter intake, energy balance), a meta-analysis with a comparable number of studies was performed to investigate feed efficiency heritability in a large scale.

A total of 164 reported candidate genes with were summarised from 20 studies, a false discovery rate under 0.05 was considered statistically significant. Genes from three sources were annotated: 1) candidate genes mapped around reported genetic markers within 1 Mb interval (500 kb upstream and 500 kb downstream), 2) mapped genes in the associated window, and 3) differentially expressed genes. Genomic coordinates for genes were converted using the assembly ARS-UCD1.2 of the bovine genomes as reference. Gene list was then enriched to discover key gene regulatory network or biological mechanisms by two functional enrichment analysis tools: gProfiler (https://biit.cs.ut.ee/gprofiler/gost) and DAVID (https://david.ncifcrf.gov/summary.jsp). Functional information was retrieved from the following sources: Gene Ontology and Kyoto Encyclopedia of Genes and Genomes, Benjamini adjusted P value was considered statistically significant.

#### Results

The integrated heritability with 95% confidence intervals by meta-analysis for residual feed intake (17 studies), dry matter intake (18 studies) and energy balance (6 studies) were found to range from 0.15-0.24, 0.28-0.40 and 0.11-0.33, respectively. All three FE traits in meta-analysis were observed to possess moderate heritability estimates (heritability estimates > 0.20). Meta-analysis results showed high heterogeneity ( $I^2 > 80\%$ ).

Across two annotation databases, pathway enrichment signals for feed efficiency complex were highly enriched in the purine ribonucleoside triphosphate metabolic process, metabolism of mitochondrion organization, ATP metabolic process and Oxidative phosphorylation (Fig. 1: Top terms and mechanisms for feed efficiency complex enriched by Gene ontology).



Fig. 1. Top terms and mechanisms for feed efficiency complex enriched by Gene ontology.

#### Conclusion

The heritability estimates generated from meta-analysis implies feed efficiency traits are well within acceptable boundaries for inclusion into breeding programs. Pathway enrichment results are highly consistent with earlier findings in the reported genetic markers or associated windows. Overall, feed efficiency complex was found to be related to diverse biological processes and pathways, suggesting feed efficiency complex traits are highly polygenic.

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#### 138. Mycotoxin occurrence and management practices of grass silages across Great Britain: A survey

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#### Application

Animal health, performance and profitability can be negatively affected by mycotoxin contamination of grass silages. A greater knowledge of management and forage factors that pre-dispose grass silages to mycotoxin production can be used to improve animal health and performance.

#### Introduction

Field conditions, sward growth stage and clamp management can all influence the fermentation of silages (Borreani et al., 2018). Poor fermentation and management at feed out can lead to a deterioration in the nutritive quality of the forage, and an increased likelihood of mycotoxin production from fungal contaminants such as *Aspergillus, Fusarium,* and *Penicillium* (Alonso et al., 2013). As grass silages can form up to 60–80% of the ration of an adult dairy cow, herd performance and health may be severely impacted upon long-term consumption of mycotoxin-contaminated grass silages (Korosteleva et al., 2009). This survey aimed to characterise the mycotoxin profile of various grass silages on commercial GB dairy farms, and determine the relationship with management factors and nutritive content that may be influential in predicting mycotoxin occurrence.

#### Materials and methods

Thirty-six dairy farms across six regions of Great Britain (South West, West Midlands, East Midlands and North West England, Southern Scotland and Northern Wales) were sampled between March to April 2022 and March 2023. Information on harvest and clamp management was collected via a questionnaire during the farm visit. Thirty-seven grass silage clamps were sampled via a 5 cm diameter steel corer at least 15 cm deep into the open face of the clamp in four locations; three at the top of the clamp (top left shoulder, top centre, and top right shoulder, which were bulked to provide a "top" sample), and a "centre" sample, yielding 74 samples in total. Clamp temperature at 50 cm depth from the face, and silage density were recorded for each core site (n = 148). Samples were sent for silage analysis by near-infrared spectroscopy (Trouw Nutrition, Derbyshire, United Kingdom), and the mycotoxin concentration analysed by liquid-chromatography mass spectrometry (Alltech 37+, Dublin, Ireland). The "Risk Equivalent Quantity (ppb)" value for each silage sample was calculated based on different weightings assigned to individual mycotoxins with respect to their severity. Summary statistics were performed using R (Table 1; R Core, 2023).

#### Table 1

Clamp characteristics, silage chemistry and mycotoxin content for 148 cored samples obtained from thirty-seven grass silage clamps across Great Britain.

	n <sup>1</sup>	%n <sup>2</sup>	Mean	Median	Min <sup>3</sup>	Max <sup>4</sup>	s.d. <sup>5</sup>
Clamp characteristics							
Silage density (kg FM/m <sup>3</sup> )	148		559	590	211	935	177
Temp at 50cm depth (°C)	148		23.1	22.9	9.40	51.2	7.59
Silage chemistry, g/kg DM							
Dry matter (g/kg)	74		351	349	238	485	62.7
Neutral detergent fibre	74		444	441	390	500	25.9
Crude protein	74		152	150	94.0	215	26.4
рН	74		4.23	4.20	3.50	4.90	0.386
Silage mycotoxin concentration, $\mu g/l$	kg						
Deoxynivalenol	11	14.9	53.7	44.2	37.5	112	22.1
Fumonisin B1	7	9.46	207	151	78.6	357	104
Fusaric acid	56	75.7	69.6	25.6	7.88	297	78.2
Mycophenolic acid	2	2.70	222	222	36.9	407	262
Penicillic acid	53	71.6	1361	710	24.5	10541	2025
Roquefortine C	2	2.70	53.1	53.1	17.9	88.3	49.8
Zearalenone	9	12.2	75.6	91.8	9.05	157	51.5

<sup>1</sup> n=number of samples.

<sup>2</sup> %n=number of samples as a percentage of total clamps sampled.

<sup>3</sup> Min=minimum value.

<sup>4</sup> Max=maximum value.

<sup>5</sup> s.d.=standard deviation.

#### Results

In the 148 silage samples collected the density ranged from 211 to 935 kg fresh matter/m<sup>3</sup>, with a mean of 559 kg fresh matter/m<sup>3</sup>, and the temperature ranged from 9 to 51°C, with a mean of 23°C (Table 1). The mean DM, crude protein and pH was 351 g/kg, 152 g/kg DM and pH 4.2, although there was a large range in all values. The mycotoxins identified in the samples were consistent with those synthesised by *Aspergillus, Fusarium, Penicillium* and *Claviceps* fungal species (Table 1) with fusaric acid and penicillic acid present in 76 and 72% of the samples, irrespective of the cut number of the grass silage. The highest mycotoxin risk equivalent quantities were recorded in silages with a lower DM content, but there was no relationship between the clamp density and mycotoxin concentration (Fig. 1A and B).



Fig. 1. A and B. Silage dry matter content, g/kg (A) and density, kg /m<sup>3</sup> (B) against the mycotoxin Risk Equivalent Quantity (ppb), for 148 cored samples obtained from thirtyseven grass silage clamps across Great Britain.

#### Conclusions

Mycotoxin contamination was widespread in the grass silages sampled, particularly with respect to penicillic acid. The risk equivalent quantity of mycotoxins was higher with lower DM silages, however silage compaction had little effect.

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#### 139. Measuring seasonal and agro-ecological effects on nutritional status in tropical ranging dairy cows

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#### Application

The findings of this study can be used to develop targeted interventions to improve the nutrition and productivity of ranging dairy cows. This could include providing supplementary feed during the dry season, grazing management strategies to improve pasture quality, and the use of breed selection to improve resistance to nutritional stress.

#### Introduction

Ranging dairy cows in tropical regions face nutritional challenges due to seasonal variations in pasture quality and availability. This study aimed to evaluate the nutritional status of ranging dairy cows in Ethiopia in relation to season and agro-ecology.

#### Methods

Blood samples were collected from 170 ranging dairy cows in six different districts, along a transect extending from the lowlands to the highlands, in both dry and rainy seasons. Serum urea, creatinine, triglyceride, and non-esterified fatty acid concentrations were quantified spectrophotometrically. Dried serum spots were subject to quantitative electrospray tandem mass spectrometry to estimate changes in nutrient metabolism based on selected free amino acids (AA) and carnitine esters. Body condition score and milk yield of all cows were also determined for both seasons. Statistical analysis was performed using SPSS 21 (IBM Corp., Armonk, NY). Repeated measures variance

analysis was used to evaluate the effects of season, agro-ecological zone, and their interactions on blood metabolites, BCS, and milk yields of dairy cows. Posthoc differences between agro-ecological zones were evaluated using independent t-tests. Pearson correlation analysis and principal component analysis were performed to establish the relationship between pairs of variables and groups of variables. Significance was accepted at the 5% probability level.

#### Results

Serum urea and creatinine concentrations were significantly higher in the dry season compared to the rainy season. This suggests that cows were catabolizing body protein for energy during the dry season. Triglyceride concentrations were significantly lower in the dry season, indicating reduced fat intake. Non-esterified fatty acid concentrations were significantly higher in the dry season, suggesting increased mobilization of body fat for energy. Mass spectrometry analysis showed significant changes in the concentrations of several AA and carnitine esters, indicating alterations in energy and protein metabolism. Body condition score and milk yield were significantly lower in the dry season.

#### Conclusion

Nutritional status varied with season and geographical region. Cows were in a state of negative energy balance during the dry season, with increased protein catabolism and mobilization of body fat. This resulted in reduced milk yield and body condition score. The results of this study suggest that nutritional interventions are needed to improve the performance of ranging dairy cows in tropical regions.

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#### 140. Quantifying the digestibility of feedstuffs using the in-sacco technique in horses

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#### Application

Knowledge of the digestibility of a feedstuff is essential when evaluating the potential energy value in practice. Total collection of feces is considered as the gold standard method for measuring digestibility of the total diet. However, techniques for estimating the digestibility of individual feedstuffs fed to horses are needed for accurate feedstuff evaluation. Furthermore, it is beneficial if these techniques are relatively cheap, less labor intensive and has limited influence on normal behavior of animals compared to traditional techniques.

#### Introduction

The *in-sacco* technique is commonly used in feedstuff evaluation systems for ruminants, and it provides information on both the digestibility and digestion kinetics of a feedstuff. However, few studies have used this technique to estimate feed digestibility in horses. The objective of this study was thus to adopt the *in-sacco* technique to estimate the digestibility of a wide range of feedstuffs used for horses. It was hypothesized that the *in-sacco* technique can be used to rank feedstuffs according to their expected dry matter (DM) digestibility.

#### Materials and methods

Three cecum cannulated Norwegian cold-blooded trotters (body weight: ~550kg) fed a basal diet of timothy hay supplemented with a mineral-vitamin feed were used in the study. Twelve feedstuffs were tested: oats, barley, oat bran, wheat bran, faba beans, rapeseed meal, sunflower meal, soybean meal, carrots, sugar beet pulp, alfalfa pellets, and straw. Feedstuffs were dried and ground to 1 mm and 1 g was added to each *in-sacco* bag (bag size  $6 \times 15$  cm, 5.13 mg feed/cm<sup>2</sup>, pore size of  $36 \mu$ m). Four bags with 4 different feedstuffs were fastened to a 78 cm long nylon tube with rubber bands and incubated in the cecum of each horse for 2, 4, 8, 16 and 24 h. In each horse, 4 bags containing 4 different feedstuffs were incubated for the specified time points (3 rounds were needed at each time point before all 12 feedstuffs were tested in each horse). Then all bags (including bags not incubated, 0 h bags) were washed in a washing machine in cold water for 35 min without spinning, dried for 48 h, and weighed to determine DM loss after incubation. The *in-sacco* digestibility was modelled in GraphPad Prism (version 8.0.1, GraphPad Software, San Diego, USA) according to the equation  $D=A+B^*(1-e^{-C*t})$ , where D is the digestibility through time, t, A is the soluble fraction of the feedstuff and curve interception, B is the potential digestibility of the insoluble fraction, and C is the digestion rate of B (Ørskov and McDonald, 1979). The effective degradability (ED) was then calculated according to the equation  $ED=A+B^*C/(C+k)$ , where A, B and C are as described above, and k is the outflow rate (0.04% per h).

#### Results

The horses tolerated the technique well and did not have to wear collection harnesses or be confined to stalls as they must when measuring the digestibility using total collection of feces. The ED of the feedstuffs tested varied, and the feedstuffs were ranked as expected (from lowest to highest ED): Straw, oat bran, wheat bran, alfalfa, rapeseed meal, oats, sugar beet pulp, field beans, soybean meal, barley, sunflower meal, and carrots (Table 1)

#### Table 1

The effective degradability (ED, k=0.04% per h), the soluble fraction of the feedstuff and curve interception (A), the potential digestibility of the insoluble fraction (B), and the digestion rate of B (C) (Ørskov and McDonald, 1979).

	Oats	Oatbran	Weaht bran	Barley	Rapeseed meal	Sunflower meal	Soyabean meal	Faba beans	Carrots	Sugar beet pulp	Alfalfa	Straw
ED	79.1	43.8	63.0	87.4	76.0	88.7	86.0	84.9	96.7	80.8	69.3	32.7
Α	54.1	34.6	38.9	33.1	34.9	35.8	37.7	49.7	77.0	16.9	37.5	16.9
В	26.0	10.1	26.5	57.9	47.9	56.7	60.4	46.7	22.2	83.4	38.1	65.3
С	1.044	0.532	0.417	0.617	0.254	0.581	0.170	0.132	0.319	0.138	0.214	0.015

#### Conclusions

The *in-sacco* technique showed promising results when evaluating the digestibility of individual feedstuffs for horses. Further validation of the technique against *in-vivo* digestibility studies are required. Even though cannulated horses are needed, the *in-sacco* technique has limited effects on the normal behavior of the animals used.

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141. Pasture productivity impacts of transitioning to perennial ryegrass white clover swards on wetland intensive dairy grazing systems

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#### Application

This study documents the pasture and animal performance impacts of transitioning from perennial ryegrass to perennial ryegrass clover swards on a wetland mineral soil type managed under an intensive dairy grazing system.

#### Introduction

In an effort to achieve climate neutrality in the European Union (EU) by 2050, Ireland has developed a climate action plan which includes a target to reduce chemical nitrogen (N) fertiliser applications to agricultural land by 25% by 2030 (DAFM, 2020). Legumes such as *Trifolium repens* (White clover; WC) within grazing swards have received much attention to reduce dependence on chemical Nitrogen (N) fertilizer application. White clover inclusion within traditional *Lolium perenne L*. (perennial ryegrass; PRG) swards has also been shown to improve milk and milk solids yields (Dineen et al., 2018). The majority of the research undertaken on WC inclusion has been conducted on freely draining soils primarily in the south of Ireland (Hennessy et al., 2012; Egan et al., 2018) with minimal evaluation within poorly drained soils which experience a shorter growing season. Moreover, there has been limited research evaluating the transition from traditional PRG to PRG/WC systems. On that basis, the objective of this study was to investigate the transition from high chemical N PRG only swards to lower chemical N PRG/WC swards on a wetland soil type in the border midland and western region of Ireland.

#### Material and methods

This study was undertaken at Teagasc, Ballyhaise College, Co. Cavan, Ireland (54°051'N, 07°031'W) over a 3 yr period from 2021 to 2023. The study was conducted on 104, 120, and 144 cows during 2021, 2022, and 2023, respectively, divided into one of 2 treatment sward systems. In 2021, 2022 and 2023, 30, 20 and 20%, respectively of each grazing platform was reseeded with either PRG only (PRrsed) or PRG with WC (PRWCrsed) swards. In addition, a further 20% of the PRWC grazing platform was oversown with WC (PRWCover) during both 2021 and 2022, respectively so that the entire farmlet area had some clover establishment. Following establishment, PRG only swards received 225 kg N/ha of chemical N per year, whereas chemical N application was reduced for PRWC swards from May each year based on prevailing sward clover contents. All treatments were managed similarly at grazing in terms of pre-grazing herbage mass, post-grazing sward height, and residency time. Total net herbage production, botanical composition and sward chemical composition were measured over the three years (2021 to 2023, inclusive) and analysed for sward, season and year effects using mixed models (Proc Mixed, SAS Institute, 2006).

#### Results

There was no significant sward system effects on grazing characteristics. Pre-grazing and post-grazing heights and pre-grazing pasture masses were on average 106.3 and 43.9 mm and 1,538 kg DM/ha, respectively. In accordance with the project objectives, the establishment of PRWCrsed and PRWCover resulted in a significant reduction in chemical N fertiliser application from 222 and 187 kg for PRold and PRrsed, to 145 kg for PRWCover and 69 kg for PRWCrsed. Total pasture production reduced (*P* < 0.001) from 16,376 kg DM/ha in PR pasture prior to reseeding to 9,462 and 9,339 kg DM/ha for PRrsed and PRWCrsed swards. Where permanent pasture was oversown with clover (PRWCover), total pasture production was also reduced (14,478 kg DM/ha). The establishment of WC within PRWC was highly effective, with mean annual sward clover contents of 34, 32 and 19% during 2021, 2022 and 2023, respectively. Establishment method had a significant effect on sward WC content with 21.7% WC present in PRWCrsed compared to 3.9% within PRWCover during the study. There was no significant effect of grassland management on sward nutritive parameters (CP, NDF, ADF and OMD contents were on average 197.2, 416.1, 232.4 and 822.6 g/kg DM, respectively). There was also no significant differences in milk production or milk composition between sward systems. Mean annual milk and milk fat plus protein yield was 5,255 and 462 kg per cow, respectively while milk fat, protein or lactose contents were 50.8, 38.7 and 46.9 g/kg, respectively.

#### Conclusion

The results from this study should give pasture-based dairy farmers on wetland soils confidence to include WC in PR swards to substantially reduce requirements for chemical N fertiliser application while maintaining pasture and animal productivity. The results also indicate that, although complete sward renewal is the most consistent method to effectively transition to PRWC swards, the significant reduction in DM production during reseeding requires that substantial winter feed reserves are present to meet animal requirements during the transition.

#### Acknowledgements

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# 142. Growth performance and blood indices of pigs fed diets containing graded levels of supplementary defatted Black Soldier fly (*Hermetia illucens* L.) larvae meal

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#### Application

Insect meal inclusion up to 120 g/kg can be considered a suitable replacement for soyabean meal in growing pig diets.

#### Introduction

Insect meal is a novel feedstock for future livestock production. However, a review on the literature suggests that dietary inclusion of insect meal over 100 g/kg may have negative impact on nutrient availability and growth of pigs (Meyer et al., 2020) and poultry (Moula and Detilleux, 2019). Although dietary insect meal did not affect animal health, Biasato et al. (2019) found some modulation of blood profile variables. The aim of this study was to assess the impact of graded levels of dietary insect meal on the growth performance and blood profile variables of growing pigs.

#### Materials and methods

The study was approved by the Ethics Committee of the Agricultural Academy, Shumen, Bulgaria. A total of forty Danube White pigs with a starting body weight of 31.9 kg (STDEV ± 3.93) were used. The mixed sex pigs were fed for 38 days one of five isocaloric (13.40 MJ/kg DE) and isonitrogenic (176 g/kg CP) diets. Diet 1 contained 228 g/kg soyabean meal. In the other diets soyabean meal was replaced with either 30 (diet 2), 60 (diet 3), 90 (diet 4) or 120 (diet 5) g of defatted Black Soldier fly (*Hermetia illucens* L.) larvae meal. Data were analysed by one-way ANOVA, followed by orthogonal polynomials contrasts to test for linear (L) and quadratic (Q) relationships.

#### Results

There were no differences in growth performance or blood variables of pigs fed different levels of insect meal (P > 0.05), as reported in Table 1. No clinical adverse effects were observed in animals fed over 100 g/kg insect meal.

Table 1
Growth performance and blood variables of pigs fed graded levels of dietary insect meal.

Insect Meal (g/kg)	0	30	60	90	120	SEM	Р	L	Q
LW end (kg) FI (g/d/p) WG (g/d/p) FCR RBC ( $10^{12}/L$ ) WDC ( $40^{9}/L$ )	60.80 1.89 0.76 2.525 7.4	59.48 1.85 0.72 2.581 7.3	60.40 1.92 0.75 2.576 7.4	58.74 1.87 0.71 2.654 7.0	58.89 1.82 0.71 2.566 7.1	2.034 0.035 0.029 0.0878 0.19	0.935 0.278 0.638 0.888 0.580	0.481 0.238 0.241 0.584 0.213 0.200	0.962 0.245 0.932 0.537 0.731
PLT $(10^{9}/L)$	503	404	433	504	522	36.6	0.120	0.239	0.043

#### Conclusions

Insect meal inclusion up to 120 g/kg can be considered a suitable replacement for soyabean meal in growing pig diets, since growth performance and blood variables were unaffected in this study. Greater inclusion levels of insect meal may be possible, although further research is needed.

#### Acknowledgements

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#### 143. Optimising in vitro fermentation models for accurate representation of the pre-weaning piglet hindgut environment

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#### Application

*In vitro* simulation of a pre-wean piglet's hindgut provides a cost-effective, high-throughput method for assessing gut fermentation and health parameters. This model offers a quicker, more accessible alternative to *in vivo* trials, supporting industry efforts to efficiently study and optimise piglet gut health while addressing ethical and resource challenges.

#### Introduction

*In vivo* animal science trials are costly, time-consuming, and ethically challenging. Gold standard *in vitro* fermentation systems, though dynamic, often rely on anaerobic chambers and continuous reagent usage, making them impractical and costly. Batch *in vitro* fermentation models present an accessible and high-throughput alternative for studying hindgut environments and feed fermentation patterns, however, many traditional methods are derived from rumen studies, raising concerns about their validity for the porcine gut. Key variables, such as the buffer, media preparation, and incubation period, vary across studies. This study systematically assessed common variables across batch fermentation models to identify the optimal protocol for an accessible *in vitro* model mirroring the pre-weaning piglet colon environment.

#### Material and methods

The standard protocol involved adding 0.5 g dry matter of piglet milk replacer formula to 200 mL serum bottles with 50 mL of fermentation media containing faecal slurry (50 g/L). After bubbling with  $CO_2$  and crimp sealing, bottles were incubated at 39°C, and gas pressure was recorded and released at 2-h intervals for up to 24 h with a pH reading after fermentations were terminated. In the first experiment, fermentation patterns were assessed using two media solutions; 1) mineral buffer as per Palowski et al. (2021), and 2) phosphate buffered saline (PBS; 20mM). Both were adjusted to pH 6.8, boiled for ~15 minutes, and cooled with  $CO_2$  before faecal inoculation and incubation for 24 h. In the second experiment, mineral buffer was divided into 8 groups, with conditions including L-cysteine addition, boiling, and timing of faecal inoculation (adding day prior to, or day of, fermentation). The third experiment aimed to determine optimal incubation periods where fermentations were terminated at different time points (8, 10, 12, and 24 h). All gas pressure data were fitted to a Gompertz growth curve and slopes compared between treatments using the extra sum of squares F test. End pH was analysed using a Mann-Whitney or ANOVA where appropriate.

#### Results

Significant differences emerged between mineral buffer and PBS in fermentation curves (P < 0.0001; Fig. 1) and end pH ( $6.35 \pm 0.11$  vs  $4.55 \pm 0.21$ ; P < 0.0001).



Fig. 1. Fermentation profile over 24h using a mineral buffer (Buffer) and phosphate buffered saline (PBS)

L-cysteine addition and boiling did not significantly impact the fitted growth curves but addition of slurry the morning of the experiment significantly reduced lag time and increased slope steepness (P < 0.0001; Fig. 2) regardless of cysteine and boiling conditions. End pH was statistically different between non-cysteine groups YY (see Fig. 2 legend) and NY (P < 0.0001) as well as non-cysteine groups YY and NN (P < 0.05), however neither were by a biologically relevant value (6.56 vs 6.22 and 6.56 vs 6.35, respectively).

Fermentation curves were similar across all incubation periods with a clear plateau emerging after 12 h. End pH was statistically different between 8 and 12 h terminations (P < 0.05) as well as 8 and 24 h terminations (P < 0.05), however was again of minimal biological relevance (maximum pH difference of 0.12).



**Fig. 2.** Compertz growth curves in various buffer conditions after an 8h fermentation. A: All conditions containing cysteine; B: All conditions without cysteine (YY: Buffer boiled and slurry added day prior to fermentation; YN: Buffer boiled and slurry added day of fermentation; NY: Not boiled and slurry added day of fermentation).

#### Conclusions

PBS proves inadequate for expected fermentation curves and post-fermentation pH values. A more intricate mineral buffer is advised for microbial fermentation. Faecal slurry addition immediately before the experiment is recommended but the value of incorporating L-cysteine and boiling the buffer warrants further investigation. A suggested incubation time of 24 h ensures comprehensive coverage of the ideal S-shaped fermentation curve. On-going analyses incorporate short chain fatty acid and microbial profiles to further enhance validation of this *in vitro* model.

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#### 144. The impact of various levels of fermented tomato pomace on small intestine morphology in Iranian native poultry

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#### Application

This study contributes to the advancement of sustainable and efficient poultry farming practices, particularly by enhancing small intestine health in indigenous Iranian poultry, and has the potential to mitigate food waste.

#### Introduction

Fermentation is recognized as an effective method for reducing anti-nutritional components and enhancing nutrient bioavailability. This technique employs bacterial species such as *Lactobacillus plantarum*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* yeast to facilitate the fermentation process. Small intestine health plays a vital role in nutrient absorption and overall poultry well-being. The utilization of FTP as a dietary component could offer a sustainable solution for improving poultry performance and reducing food waste, aligning with the growing emphasis on sustainable agricultural practices in Iran. This research seeks to provide valuable insights for the poultry industry and foster the adoption of innovative, environmentally responsible farming techniques.

#### Material and methods

The experiment involved 160 native laying hens, following a completely randomized design (CRD). This experiment was conducted during the pullet period, spanning from the second week to the tenth week. This design comprised four treatments, each with four repetitions, and data were collected over a six-week period, from the 2nd to the 8th week. The treatments comprise different percentages of fermented tomato pomace (0, 5, 10, and 15%) incorporated into a balanced diet. At the conclusion of the experiment, two birds from each replicate were chosen, and samples were collected from the center of the duodenum. Various factors, including villus height, crypt depth, villus thickness, crypt diameter, and goblet cell count, were assessed. The data were analyzed using SPSS, and the differences among means were assessed with Duncan's test. A significance level of P < 0.05 was applied to determine statistical significance.

#### Results

The 10% (FTP) treatment showed the lowest values for villus height and villus thickness, which were statistically significant (P < 0.05). The highest and lowest values for crypt depth were observed in the 5% (FTP) and 15% (FTP) treatments, respectively, and this difference was statistically significant (P < 0.05). The 15% (FTP) treatment exhibited the lowest goblet cell count, and this difference was statistically significant (P < 0.05). No significant differences in goblet cell count were observed among the other treatments. No statistically significant differences were observed in crypt diameter among treatments (P > 0.05).

Evaluating the influence of different Fermented Tomato Pomace (FTP) levels on small intestine morphological parameters.

	Villus Height(µm)	Villus Thickness(µm)	Crypt Depth(µm)	Crypt Diameter(µm)	Goblet Cell
Control	819.2a	88.38b	172.36ab	14.67	16.38a
5%(FTP)	642/0b	84.31b	186.12a	14.48	15.63a
10%(FTP)	530.6c	70.52c	160.80ab	13.97	15.75a
15%(FTP)	518.5c	148.93a	145.86b	14.10	10.38b
P-value	0.001	0.0001	0.041	0.978	0.0001

#### Conclusion

The findings revealed significant variations in villus height, villus thickness, crypt depth, and goblet cell count among the treatments, with the 10% FTP treatment demonstrating notable improvements in villus height and thickness. The use of 10% FTP in the diet resulted in improved performance during the experimental period (data not shown). These results underscore the influence of dietary components on small intestine health, which plays a crucial role in nutrient absorption and poultry well-being. While additional research may be needed to fully elucidate the implications of these morphological changes, this study contributes to the pursuit of sustainable and efficient poultry farming practices, aligning with the global emphasis on environmental responsibility and resource optimization. The utilization of FTP as a dietary component holds promise for enhancing poultry performance while potentially mitigating food waste, offering a path forward for the poultry industry and sustainable agricultural practices in Iran.

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#### 145. Ameliorative effect of turmeric antioxidant extract on performance of finisher broiler during aflatoxicosis

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#### Application

Aflatoxin is known to affect poultry due to its poisonous and cancerous nature. While it has become almost impossible to completely eliminate the scourge of aflatoxin contamination in feeds, its amelioration has become inevitable. Table 1.

#### Table 1

Performance of broilers chicks fed 2 levels of turmeric antioxidant extract during aflatoxicosis.

	Control (no AfB1, no turmeric)	0.5ml/L turmeric	0.5mg/kg Af. B1	0.5mg/kg AfB1+0.5ml/L turmeric	0.5mg/kg AfB1+1.0ml/L turmeric	SEM
Initial Body Weight (g) Final Body Weight (g) Body weight change	975.00 2333.00 <sup>b</sup> 1358.00 <sup>b</sup>	975.00 2403.30ª 1428.33ª	975.00 2282.70 <sup>c</sup> 1307.67 <sup>c</sup>	975.00 2399.30ª 1424.33ª	975.00 2182.00 <sup>d</sup> 1207.00 <sup>d</sup>	0.00 68.19 2.52
(g) Feed intake/bird (g) Feed conversion ratio Mortality (%)	2713.23 <sup>cb</sup> 2.02 0.00 <sup>c</sup>	2604.07 <sup>c</sup> 1.87 5.53 <sup>b</sup>	2824.43 <sup>b</sup> 2.15 16.63 <sup>a</sup>	2619.67 <sup>c</sup> 1.85 5.53 <sup>b</sup>	3326.70 <sup>a</sup> 2.65 16.63 <sup>a</sup>	27.61 0.19 4.07

<sup>a,b,c,d</sup>Means on the same row with different superscripts are significantly different (P<0.05).

#### Introduction

Aflatoxins are the secondary metabolites from toxigenic fungi which are contaminants in foods and feeds, exerting harmful effects as decreased bodyweight, severe immunosuppression, liver cancer on animal and human health (Leslie et al., 2008). Major aflatoxin producing fungi are Aspergillus flavus and Aspergillus parasiticus. Of all types of aflatoxins produced by these fungi, aflatoxin B1 (AfB1) is the most potent and most carcinogenic. Turmeric and its derivatives have proven to have an antimicrobial property based on in vitro and in vivo trials (Jin, 2010). While there are information on the anti-bacterial property of turmeric and its growth promoting ability, the ameliorative effect of turmeric antioxidant extract on aflatoxin-intoxicated broilers has not been well documented. This present study was therefore conducted to determine the ameliorative effect of turmeric extract on broiler chicks during aflatoxicosis.

#### Material and methods

Four-week old Abhor acre broiler chicks totalling 90 were allotted to five (5) experimental groups/treatments, 3 replicates and 6 birds per experimental unit in a completely randomized design (CRD). Treatments were: treatment 1 (control, as uncontaminated standard diet, C), treatment 2 (C + 0.5 ml of turmeric extract per litre of water), treatment 3 (C + 0.5 mg/kg aflatoxin), treatment 4 (C + 0.5 mg/kg aflatoxin + 0.5 ml turmeric per litre of water), treatment 5 (C + 0.5 mg/kg aflatoxin + 1.0 ml turmeric per litre of water). The feeding trial lasted for four weeks. Aflatoxin contaminated maize was prepared by the method of Shotwell et al. (1966) while aflatoxin levels was confirmed in treatments 3, 4 and 5 by gas liquid chromatographic analysis. Turmeric antioxidant extract in aqueous solution was obtained through extraction in a method described by Ahmadi et al. (2007) with slight modification. The effects of treatments on feed intake (FI), body weight change (BWC), feed conversion ratio (FCR) and mortality were subjected to analysis of variance and means were separated based on Duncan multiple range test (5%  $\alpha$ -value). All animals procedures were approved by the animal welfare regulations unit and conducted in accordance with the guide for the care and use of agricultural animals in research and teaching.

#### Results

Dietary aflatoxin ingestion was detrimental to feed intake and body weight change (P < 0.05). These parameters however were improved following the diet supplementation with turmeric anti-oxidant extract. Aflatoxicosis resulted in mortality in all groups that were treated with aflatoxin as supplementation of the diets with turmeric at 1 ml/L of water did not show potency against mortality, whereas 0.5 ml/L of turmeric anti-oxidant extract did not show any effect on the feed conversion ratio at the concentration of aflatoxin and turmeric administered on the birds.

#### Conclusion

Supplementation of broiler feed with turmeric anti-oxidant extract can improve the feed intake and body weight gain but not necessarily reduce mortality especially at level up to 1.0 ml turmeric per litre of water.

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#### 146. Assessment of six poultry probiotics for bacterial composition

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Mostafa Taha. M.W.T.Taha2@newcastle.ac.uk KeywordsPoultry probiotics; Probiotics composition; Probiotics labels

#### Application

Six poultry probiotics commercially available in the UK were in line with their label claims regarding their live bacterial composition and identity.

#### Introduction

The probiotics are defined by FAO/WHO as "Live microorganisms which when consumed in adequate amounts as part of a diet confer a health benefit on the host". Thus, the existence of live microorganisms is crucial for probiotics to show their health benefits on the host (Berreta et al., 2021). However, several investigations conducted worldwide, found that the bacterial content and identity in most human probiotics was significantly lower than the corresponding label claims (Korona-Glowniak et al., 2019, Kesavelu et al., 2020). It appears that there is no recent research conducted to assess the label claims of poultry probiotics. Therefore, this study examined the bacterial contents of six poultry probiotics available in the UK.

#### Material and methods

Six commercial poultry probiotics were evaluated for their live bacterial composition and identity. These probiotic products were chosen based on their availability at online sources for the users in the UK and codified as P1 (Probiotic 1) to P6 (Probiotic 6) by the authors of this summary. The bacterial compositions of probiotics 1, 2, 3, and 4, as indicated on their labels, were 2.5 \* 10<sup>7</sup> of Bacillus Subtilis C-3102 DSM 15544, 3 \* 10<sup>5</sup> of Bacillus subtilis C-3102 DSM 15544; 2 \* 10<sup>9</sup> of Bacillus subtilis ATCC PTA-6737, and 2 \* 10<sup>8</sup> of Bacillus subtilis ATCC PTA-6737 Colony forming units per gram (CFU/g) respectively. Probiotic 5 contained 2 \* 10<sup>10</sup> CFU/g of Enterococcus faecium NCIMB 10415 and Probiotic 6 contained 1 \* 10<sup>9</sup> (CFU/g) of a blend of Lactobacillus salivarius, Bifidobacterium animalis and Enterococcus faecium DSM 21913. The mentioned numbers are the minimum per gram for (P3,4,5,6) as mentioned on the labels of each of them.

All products were stored according to their manufacturer's storage instructions. In a sterile environment, one gram was taken from each probiotic and mixed well with 20 ml of phosphate buffered saline (pH 7.4) while vertexing in a sterile plastic tube. Up to 9 serial dilutions were made from each probiotic and spread on the agar containing plates, for cultivation and enumeration. Tryptic soy agar (for single strain Bacillus spp. probiotics), MRS agar + vancomycin 20 mg/l (to selectively grow Lactobacillus salivarius in P6), BSM Agar (Sigma) + Lithium mupirocin supplement (to selectively grow Bifidobacterium animalis) and Enterococcus faecium ChromoSelect Agar (Sigma) (for P5 enumeration and identification). The temperature of the serial dilutions related to Bacillus bacteria was raised to 80°C. The inoculated plates were incubated at 37°C anaerobically for 72 h for Lactobacillus salivarius and Bifidobacterium animalis, and aerobically at 35 or 37 °C for up to 24 h for Bacillus subtills and Enterococcus faecium respectively. Colonies were counted at the end of each incubation. The number of viable bacteria present in each agar plate was expressed as log10 colony forming units per gram (log CFU/g) of each probiotic. Each experiment was replicated 4 times, and the results were presented as means with standard deviations. Additionally, 95% confidence intervals for the means were included.

For further identification of each product (besides, the observation of colony morphology and the use of selective agars), at least 4 colonies for each bacterial species were picked on the same day of enumeration for the identification using Matrix-assisted laser desorption/ion-ization time-of-flight mass spectrometry (MALDI-TOF MS), according to the NHS protocols.

#### Results

Table 1 shows total enumeration values of each poultry probiotic. As it can be seen, all the CFU log numbers were within the declared bacterial numbers on the product labels, with some acceptable variability of no more than log 0.2 decrease in numbers for P1 and P2. This could be due to the limitation of the culture plate method for enumeration. While the laboratory log CFU numbers were higher for probiotics 3, 4, 5, and 6, they were also in line with their respective label claims. This is because the label CFU numbers represent the minimum per gram, as indicated on the labels of each one of them.

Table 1 also shows the bacterial identification results obtained by using MALDI-TOF MS. It appeared that the bacterial identification results in all the listed probiotic products were in line with their respective label claims.

Table 1	1
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Laboratory results vs Label claims of the total bacteria numbers (log CFU/g) and bacterial identity in different poultry probiotics.

Probiotic code	MALDI-TOF MS identification results	Total bacterial numbers (log CFU/g) <sup>4</sup>	Laboratory total bacteria numbers		
			Means (log CFU/g±Standard deviation) n4	95% CI for the Means	
P1	Bacillus amyloliquefaciens <sup>1</sup>	7.40	7.13±0.067	(7.067, 7.200)	
P2	Bacillus velezensis <sup>2</sup>	8.48	8.28±0.042	(8.235, 8.318)	
P3	Bacillus amyloliquefaciens	9.30	10.14±0.081	(10.060, 10.219)	
P4	Bacillus amyloliquefaciens	8.30	8.77±0.023	(8.7510, 8.7965)	
P5	Enterococcus faecium	10.30	10.76±0.033	(10.728, 10.795)	
P6	Ligilactobacillus salivarius <sup>3</sup> Bifidobacterium animalis Enterococcus faecium	9.00	9.08±0.015	(9.068, 9.100)	

<sup>1,2</sup> Bacillus velezensis and Bacillus amyloliquefaciens are members of Bacillus subtilis group.

<sup>3</sup> Ligilactobacillus salivarius is a synonym of Lactobacillus salivarius.

<sup>4</sup> Derived from product CFU/g label claim.

#### Conclusion

It is concluded that the tested poultry probiotic bacterial numbers and identity were in line with their respective label claims and so these products appeared to be reliable. This is in contrast with most human probiotics enumeration tests in the literature, where significantly lower bacterial numbers were commonly reported in their tested products. Better encapsulation methods for P5 and P6 and bacterial species with a better viability (*Bacillus spp.* in P1,2,3,5) might be the cause of the results of this study.

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147. Hatchability and physiological performance of broilers chickens given inovo administration of lycopene under heat-stressed environment

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#### Application

*Inovo* feeding is a viable technology that facilitates extra nutrient deposition into chickens between the late-term embryo stage to early post-hatch period, meant to deliberately promote development of gastrointestinal tract and strengthen the immune system. Table 1

#### Table 1

Hatching Performance of heat-stressed broilers given in-ovo injection of lycopene.

Parameters	Control (untreated) (T1)	S/W(0.5mil/egg) (T2)	Lyc(0.125mg/0.5/egg) (T3)	Lyc(0.25mg/0.5/egg) (T4)	Vit.C. (0.25/0.5/egg) (T5)	SEM	P value
Hatched eggs Un-hatch eggs Dead embryo Mortality after Hatched	12.33 <sup>bc</sup> 4.33 <sup>b</sup> 1.00 <sup>b</sup> 0.67 <sup>aa</sup>	15.67 <sup>a</sup> 0.33 <sup>c</sup> 0.00 <sup>b</sup> 0.00 <sup>a</sup>	14.67 <sup>a</sup> 1.33 <sup>c</sup> 0.33 <sup>b</sup> 0.33 <sup>a</sup>	13.33 <sup>ba</sup> 3.33 1.33 <sup>b</sup> 0.667 <sup>a</sup>	10.67 <sup>c</sup> 6.33 <sup>a</sup> 4.67 <sup>a</sup> 1.00 <sup>a</sup>	0.6 0.53 0.89 0.47	0.0004 0.0008 0.0777 0.7570
Livability	11.67 <sup>b</sup>	15.67 <sup>a</sup>	14.67 <sup>a</sup>	12.67 <sup>b</sup>	9.67 <sup>c</sup>	0.49	0.0004

#### Introduction

Continous changes in climatic condition globally has caused an out-play of extreme weather conditions, culminating into heat-stress, experienced by man and livestock. Heat stress (HS) results into an increase in production and activity of reactive oxygen species, which negatively impact the physiological mechanism and hatchability of chickens. *Inovo* feeding of chickens with phytonutrients and plant products has been reported to improve their defenses against various diseases like contagious bursal disease, influenza virus and new castle disease. Restricting chicken embryos the access to extra nutrient supply, at the later stage of development may prevent freshly hatched chicks from growing and developing into their full potential. Lycopene is a phytochemical, known for its powerful free radical-scavenging qualities and thermoregulatory potential, according to its thermotolerance effect on heat-stressed animals (Han et al. 2020). Antioxidant activity (peroxyl-radical scavenging) of lycopene has been noted to be twice as efficient as beta-carotene and 10 times higher than alpha-tocopherol in extinguishing ability. Revealation shows that lycopene supplementation could improve antioxidant capacity, immune function and regulate lipid metabolism in chicks, especially at hatching (Lee et al., 2016). Although lycopene has been proven to have ROS scavenging abilities, however, *in-ovo* injection of lycopene administration in HS broilers has not been well documented. This research was conducted to evaluate the hatchability and physiological performance of broilers chickens given *in-ovo* administration of lycopene under heat-stressed environment.

#### Material and methods

The pre-hatching, hatching data collection was carried out at Broncho hatchery, Oluyole, Ibadan, Nigeria. Three hundred Arbor-Acre broiler breeder hatching eggs were obtained from Farmer's Agro, Ibadan, with mass average of 53.5 g. Eggs were incubated according to standard hatchery practices for 21 days using Chick Master, single stage setter and hatcher (Ohio, USA). Incubation temperature, relative humidity used was 37.7°C and 70–75% relative humidity from 1 to 18 days of incubation; while 36.66°C and 75–82% was used from 19 to 20 days of incubation. On the 18th day of incubation, eggs were removed from incubator and candled to separate unfertilized ones or dead embryos. Fertile eggs (N = 240) were selected from set eggs and sanitized using alcoholic antiseptic solution, prior to experimental injection of lycopene. The eggs were divided into 5 experimental groups with 48 fertile eggs per group, replicated thrice with 16 fertile eggs per replicate. The frozen lycopene was thawed and reconstituted using sterile water to form varying concentration of sterile water-lycopene homogenized solution. Eggs were cleaned prior to injection to ensure a sterilized in-ovo injection procedure. Thereafter, a pin head-sized hole was made on each egg, just below air cell, injections were performed using insulin syringe (0.25mm  $\times$  8mm) on the 18th day of incubation. Experimental groupings were; Negative Control: Eggs not injected (T1); Positive Control: Eggs injected with 0.5 ml of sterile water (SW) (T2); Lyc 0.125 Group: Eggs injected with 0.125 mg of lycopene + 0.5 ml of SW (T3); Lyc 0.25 Group: Eggs injected with 0.25 mg of lycopene + 0.5 ml of SW (T4); Vitamin C Group: Eggs injected with 0.25 mg of Vitamin C + 0.5 ml of SW (T5). Immediately after injection, site was sealed using a sterile sealant and eggs were returned to the incubator. On the 21st day of incubation, temperature was decreased gradually (hour by hour) from 36.66 to 36.11°C till onset of pipping. The hatched chicks in each group were transferred into different units within the brooding pen for post-hatch data collection. The pre-hatching mortality (18-21 day), hatchability, liveability, and physiological performance at post hatch were subjected to statistical analysis. All data collected were statistically analysed with SAS software, using Oneway analysis of variance (ANOVA). Means were separated using Duncan Multiple Range Test.

#### Result

Results recorded statistically (P < 0.05) highest values of unhatched chicks, dead embryo and mortality after hatch in T5 injected with 0.25 vitamin C/0.5mls SW). Statistically (P < 0.05) elevated number of hatched eggs was noted in T2 (0.5 mls SW/egg) and T3 Lyc(0.125 mg/0.5/egg), while the significantly (P < 0.05) depressed livability value was observed in T5 (0.25 vitamin C/0.5 mls SW). Rectal and body temperatures were within the normal range of 41–42°C.

#### Conclusion

In conclusion, *in-ovo* injection of either saline water (SW) at 0.5 mls or lycopene at 0.25 mg 1.25 mg of lycopene/ 0.5 mls saline water (SW) is recommendable

#### Acknowledgements

NIL

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#### 148. Estimating the cattle herd biomass and associated herd stock value over time

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#### Introduction

We have generated estimates of the livestock biomass and its associated stock value of the Irish cattle population across ten years, using monthly and annual data from the animal health systems in Ireland. The aims of this study were to 1) develop liveweight and valuation models following method three outlined by Rothman-Ostrow et al. (2020), 2) apply these models to the Irish cattle AIM database to better estimate biomass and economic stock value in the Irish cattle sector, and 3) to explore trends in these metrics over time and according to herd categories.

#### Materials and methods

This study used bTB-positive reactor slaughter data collected as part of the bovine TB eradication scheme, movement data, and livestock mart data obtained from the Department of Agriculture, Food and the Marine Ireland's Animal Health Computer System, Animal Identification and Movement (AIM) database, and Live Cattle Pricing database, respectively. The AIM data consisted of the herd that each animal was located once a month from 2011 to 2021. The bTB-positive reactor slaughter data contained the liveweight and value of cattle at a given age, and the results were cross-checked against the livestock mart data. Two animal-level linear models were created using the bTB-positive reactor slaughter records, including age, breed type, sex, liveweight, and value, and adjusted for month and year. The models were run via the AIM data, giving each animal a biomass (kg) and stock value ( $\in$ ) figure. The estimates were then aggregated at herd level. The herd types were classified based on the Brock et al. (2021) herd classification tree model.

#### Results

The total number of herds included in the final dataset was 129,940, involving 29,927,470 animals (890,809,644 records). From 2011 to 2021, there were, on average, 55,856 herds classified as beef, 16,552 as fattening, 15,995 as store/rearing, 13,101 as dairy, 5,238 as mixed production, 4,316 as seasonal, and 797 as trading each year. The average median herd size from 2011 to 2021 was 138 for dairy herds, 110 for mixed production herds, 31 for beef herds, 31 for fattening herds, 25 for trading herds, 17 for store/rearing herds, and 7 for seasonal herds. Overall, the Irish cattle sector biomass increased from 2,890,400 tonnes in 2011 to 3,185,000 tonnes in 2021, and the cattle sector stock value increased from  $\notin$ 5,280.1m in 2011 to  $\notin$ 7,567.2m in 2021 (Table 1).

#### Table 1

At a glance are the biomass and value estimates at sector level in 2011 and 2021.

	20	11	202	21
Sector	Biomass ('000 Tonnes)	Value (€ Million)	Biomass ('000 Tonnes)	Value (€ Million)
Beef	1,234.6	2,389.9	1,043.3	2,613.5
Dairy	869.9	1,418.8	1,127.1	2,446.5
Fattening	341.9	674.0	417.7	1,053.5
Mixed Production	271.7	462.7	327.6	745.1
Store/Rearing	125.9	247.3	228.8	607.2
Trader (Dealer)	18.7	35.0	19.9	49.1
Seasonal/Non-Permanent	27.7	52.5	20.6	52.3
Total	2,890.4	5,280.1	3,185.0	7,567.2

The most substantial changes in sector-level biomass and stock value were observed in 2012, 2015, and 2021. Further, this study demonstrates the biomass and stock value at herd level within year and between years.

#### Conclusions

The herd biomass estimates of this study will contribute to further economic and animal health research. More specifically, a starting point for calculating the animal health losses of the Irish cattle sector and, later, the burden of specific cattle diseases nationally. The livestock biomass findings will be particularly informative to policymakers when planning new agricultural programmes, animal health surveillance, economic assessments, antimicrobial usage estimates, and analysing the structure of GHG emissions across production systems.

#### Acknowledgements

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#### 149. Edible Packaging In ruminant nutritions

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#### Application

Livestock farming must expand to meet the world's demand for food, while respecting environmental preservation and animal welfare. Animal feed packaging, usually made of non-biodegradable plastic, generates polluting waste on farms. Although studies have been carried out to develop biodegradable packaging, this product cannot support a large amount of weight. The implication of this study is to evaluate a resistant packaging made of cellulose and lignin that can be ingested by ruminants as part of their diet and that leaves no residue in the environment.

#### Introduction

In animal nutrition, foods such as mineral salt, feed, wheat and others are transported in non-biodegradable plastic packaging. Improper disposal of this packaging increases the environmental impact on farms. Paper packaging based on lignin and cellulose can replace plastic packaging and contribute to sustainable development (Rosid et al., 2023). Lignin and cellulose are found in the cell walls of plants and are abundant in nature (John et al., 2022). Cellulose and lignin-based packaging for human food is already available in an academic setting, but not yet on a commercial scale (Basbasan et al., 2022). However, lignin and cellulose are part of the diet of ruminants, and at the end of the digestion and absorption process, energy is produced for the animal and, consequently, meat and milk for the population. Therefore, lignin and cellulose could be better utilized globally (Han et al., 2023). The development of packaging based on these compounds and incorporated into the animal's total diet is an important tool for reducing waste on farms and recovering energy from the environment. The aim of this project is to evaluate the influence of the incorporation of edible packaging on the nutrition, metabolism and carcass quality of ruminants.

#### Material and methods

A total of 20 lambs of undefined breed, approximately  $90 \pm 5$  days old, with an average weight of  $23.40 \pm 1$  kg, were equally divided into 2 treatments: CTL (complete diet without packaging) and PACK (complete diet with packaging). They were feedlot in individual metabolic cages for a period of 50 days. The diet consisted of ground Tifton grass hay, ground corn, soybean meal, calcitic limestone, and mineral salt. In the PACK treatment, each 100 kg of diet contained 2 bags of ground packaging. The package weighed 25 grams and had a storage capacity of 30 kg of feed. The packaging was made of cellulose and lignin, produced in the pulp and paper industry, from reforested trees, using wood from Pinus of the Pinaceae family. The chemical composition of the packaging was 100% NDF, with 84.4% cellulose, 9.8% lignin and 5.8% hemicellulose, and no protein. During the confinement period, the feed supply was completely controlled and the lambs were slaughtered at the end of the period. Blood samples were collected from all animals on fiftieth day by jugular vein venipuncture in sterile tubes for analysis of serum levels of total protein, glucose, albumin and urea. After slaughter, cold carcass yield was calculated, and pH, temperature, fat thickness, and loin eye area were measured between the 12th and 13th ribs. The experiment was a completely randomized design in which the statistical data were analyzed using the SAS (SAS Institute Inc., 2013) and the means were compared using the Tukey test (P < 0.05).

#### Results

There were no statistical differences between treatments in the performance of the confined lambs for the variables of final weight (36.98 kg), dry matter intake of 1.14 kg, feed efficiency of 0.238 kg/kg. There was no difference in urea (41.49 mg/dL), albumin (3.12 g/dL), globulin (2.82 g/dL), and glucose (85.60 mg/dL). However, there was a difference in total protein, with CTL at 6.12 g/dL and PACK at 5.76 g/dL (P = 0.0492). CTL had a higher cold carcass yield of 45.98% than PACK with 44.43% (P < 0.0434), but there was no difference in pH and temperature 24 hours post mortem, with values of 5.80 and 4.78°C, respectively. The average eye area was 15.32 cm<sup>2</sup> and the fat thickness was 1.73 mm.

#### Conclusion

Based on the analyses, it can be concluded that the inclusion of cellulose- and lignin-based packaging did not have adverse effects on performance, consumption, metabolism and carcass quality parameters in ruminants.

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# 150. Comparison of chemical composition and antioxidant activity of honey from Apis mellifera and stingless bees: an analytical review and meta-analysis

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#### Application

Stingless bees play an important cultural, nutritional and ecological role because they are the main pollinators of many wild and cultivated tropical plants. Their products, such as honey, pollen and cerumen, have been used for medicinal purposes since the Maya culture due to their therapeutic properties.

#### Introduction

The most common bee species used for honey production are European honeybees and stingless bees. European honeybee is classified in the tribe Apini, genera Apis, specie mellífera, while stingless bees are classified in the tribe Meliponini (Rozman et al., 2022). Stingless bees have many genera and species (Kek et al., 2014). They found in tropical and subtropical areas, the majority of these species are in Latin America, Africa, eastern and southern Asia. (Abd Jalil et al., 2017). The different species of stingless bees can impact in the antioxidant activity and flavonoid honey content (Jimenez et al., 2016). The aim of this research was to evaluate and compare the content of phenolic compounds and the antioxidant activity of stingless bee honey and the Apis mellífera bee honey. Table 1.

#### Table 1

Standardized mean difference (SMD) and raw mean difference (RMD) of random effect model with 95% CI and heterogeneity test of reproductive outcomes of *Apis mellifera* and stingless bees honeys.

			Effect size				Hetero	geneity	Bias
Item	Trials (n)	Apis mellífera mean (SD) <sup>a</sup>	RMD (95% CI) <sup>b</sup>	P-value	SMD (95% CI) <sup>c</sup>	P-value	I <sup>2</sup> (%)	P-value	P-value <sup>d</sup>
Phenols (mg GAE/100 g)	34	61.21 (28.30)	+33.69 (+7.11, +60.27)	0.01	+2.45 (-4.78, +9.70)	0.50	90.4	0.0001	0.21
Flavonoids (mg CE/100 g)	33	9.94 (8.7)	3.59 (-0.01, +7.19)	0.05	2.56 (-0.70, +5.83)	0.12	89.3	0.001	0.06
DPPH ( $\mu mol \text{ TE}/100 \text{ g}$ )	30	78.10 (20.21)	37.57 (–13.60, 88.76)	0.15	+2.0 (-3.41, +7.41)	0.46	90.4	0.001	0.19
FRAP ( $\mu mol \ Fe(II)/100 \ g$ )	23	97.34 (7.84)	63.39 (+15.52, +111.26)	0.009	+4.51 (+1.60, +7.42)	0.002	89.7	0.0001	0.08

<sup>a</sup> Mean and standard deviation of Apis m. honey.

<sup>b</sup> RMD is the raw mean difference that estimates the global effect expressed in the original unit measures.

<sup>c</sup> SMD is the standardized mean difference estimated of the random model.

 $^{\rm d}$  P-value of Beggs test; P-values>0.05  $I^2$  is measure of heterogeneity of random model.

#### Material and methods

We conducted a search of scientific articles using the predetermined protocol in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA; Page et al., 2021). To collect the data, we obtained scientific publications from various databases, including Google Scholar, Primo-UAEH, PubMed and DOAJ. We using the follow terms: "honey", "Apis mellifera", "stingless bees", "phenols", "flavonoids" and "antioxidant activity". The first step of the analytical review was to perform a descriptive analysis using the "psych" package version 2.1.9 (Revelle, 2021) in the R statistical computing environment (R Core Team, 2020).

#### Results

The result of the current study revels stingless bee honey showed higher levels of phenols in comparison with *Apis mellífera* honey with a RMD of +33.69 mg GAE/100g (P = 0.01; CI 95% + 7.11, + 60.27 mg GAE/100g). However, the global response of this outcome depicted a considerable heterogeneity (I2 = 90.4 %).

#### Conclusions

The bioactive compounds such as flavonoids and total phenols were higher in stingless bees than *Apis mellífera* honey which promotes their nutraceutical potential and contributes to human health. According to the values of DPPH and FRAP, stingless bee honey has a high antioxidant activity, hence their consumption can be a nutraceutical option to prevent cell damage through the inhibition of free radicals.

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#### 151. Effects of water lily meal supplementation as a natural feed additives in broiler chickens

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#### Application

This research will lead to the commercial production of a cost effective, safe and affordable chicken products using a natural feed additive in an eco-friendly environment.

#### Introduction

The increasing commercialization and intensification of the poultry industry to meet the growing animal protein demand have led to an upsurge in stress and disease outbreaks in the flock with negative economic implications. Therefore, current research is focused on the utilization of suitable feed additives including prebiotics, probiotics, organic acids and herbal products for improved and sustained poultry growth and health (Oko et al., 2014; Kennedy et al., 2020; Jachimowicz et al., 2022; Jyotsana et al., 2023). Significant improvements been observed in the poultry performance and health following several herbal dietary supplementations (Oko and Etukudo, 2011; Lipiński et al., 2019; Jachimowicz et al., 2022). This study was conducted to determine the growth and carcass characteristics of broiler chickens fed water lily (*Nympaea lotus*) as natural feed additives. *Nymphaea lotus*, an indigenous wild crop plant belongs to herbaceous aquatic plant ,which is indigenous to Africa and widely distributed in the stream, rivers and ponds. It is a plant with perennial rhizomes or stock anchored with mud, floating or submerged leaves solitary showy flower (Fulckar, 2005).

#### Materials and methods

A total of 540, One-day old Ross 380 broiler chicks were used in this 42 d study. The birds were randomly assigned into six dietary treatments with six replicates of fifteen birds each in a completely randomized design (CRD). Two basal chick starter (23.00% protein; 3000 kcalME/kg) and finisher (20.00% protein; 3000 kcalME/kg) mashes were formulated and the water lily leaf meal (WLM) was supplemented at the levels; 0g (negative control), 0.02g terramycin (positive control), 1.50, 3.00, 4.50, and 6.00g per kg of basal diet to constitute treatments 1, 2, 3, 4, 5, and 6, respectively. Feed and water were provided *ad libitum* and birds were managed on deep litter system under the same environmental conditions as approved by the University of Calabar Ethics Committee. Data were collected on the growth and carcass characteristics of each treatments and they were subjected to the one-way analysis of variance using the generalized linear model of GENSTAT 2020 version 18 software package (VSN International Limited). The Tukey's test was used for mean separation at a probability level of *P* < 0.05.

#### Results

There were significant improvements in the weight gain (67.35 g/d  $\pm$  0.45, P = 0.04), feed conversion ratio (1.96  $\pm$  0.25, P = 0.01), dressed weight (86.62 %  $\pm$  2.25, P = 0.03) and mortality rate (1.55%  $\pm$  0.25, P = 0.03) between treatments. Birds fed 1.50g per kg diet had 6, 3.5, and 5% improvement in weight gain, feed conversion ratio, respectively with a 1.00% reduction in mortality rate compared to the controls. Similar values were obtained between the control groups and birds fed water lily meal diets for live weight (2850g  $\pm$  6.25, P = 0.08), feed intake (132 g/d  $\pm$  4.55, P = 0.07) and relative organ weights indicative of better feed utilization following supplementation. Immune organs such as the spleen, liver and Bursa fabricus had similar values between birds fed 0.02% terramycin and water lily supplemented diets. These indicated that water lily leaf meal could exert some phytobiotic immunity on broiler chickens as evident in the low mortality recorded for all groups fed water lily leaf meal compared to values obtained in the control groups. At supplementation levels above 1.50g per kg diet, no positive effects were observed for measured traits.

#### Conclusions

It is concluded that up to 1.50g water lily leaf meal supplementation per kg of broiler diet may improve their growth and carcass characteristics. Thus, water lily leaf meal may be use as natural feed additive in broiler production.

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#### 152. Evaluating differences in lipid profiles of lame and non-lame dairy cows using liquid chromatography-mass spectrometry

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#### Application

Differences in lipid profiles may provide the building blocks for the development of tools to predict lameness risk. An unbiased method for early lameness detection enables prompt effective treatment of new cases, which is crucial for lameness reduction programmes with the goal of minimising economic losses, improving cow welfare and sustainability for the dairy industry.

#### Introduction

A recent literature review (Thomsen et al., 2023) comparing lameness prevalence studies from around the globe reported 22% prevalence in Europe and 24% in North America. In Great Britain, lameness prevalence was reported to be 30%. Pain and poor welfare, caused by the condition, affect production, and reproduction, and increase veterinary costs. The economic impact of lameness, mostly due to milk loss, was estimated by (Willshire and Bell, 2009) as £323.4 per case on a typical UK farm. Previous research demonstrated that the use of untargeted liquid chromatography-mass spectrometry-based metabolomics results analysed using machine learning algorithms and a stability selection method can predict lameness risk in dairy cows with an accuracy of up to 82% (Randall et al., 2023). In another study by He et al., (2022) to investigate the metabolic alterations in lame cows and assess the suitability of dried milk spots using untargeted metabolomics and machine learning models, the authors were able to successfully differentiate between lame and healthy cows. They found that phosphatidylglycerol (PG 35:4) was the strongest lameness predictor of lameness. The objective of this research is to complement these studies by evaluating differences in the lipid profile of milk from lame and non-lame dairy cows using liquid chromatography-mass spectrometry (LC-MS) on a larger cohort pooled from commercial dairy farms.

#### Materials and methods

Milk samples (214 lame; 211 control) from dairy cows were collected from 10 commercial dairy farms across Derbyshire after cows were mobility scored by visual inspection using 0-3 mobility scoring (AHDB, 2024). Samples (n = 425) were prepared using biphasic extraction for untargeted metabolomics and lipidomics. For this study, only the organic phases were used. LC-MS was performed in 4 batches and pooled quality control (QC) samples were prepared. Pre-processing of the generated LC-MS datasets including lipid identification was performed using Compound Discoverer 3.3 SP2 software (Thermo Fisher Scientific, Hemel Hempstead, UK). Statistical analysis comprised multivariate analysis performed on all the detected peaks in the milk samples using SIMCA 16 software (Umetrics, Sweden). Principal component analysis was used to check the performance of the analytical run and batch-to-batch correction. A node implementing Systematic Error Removal using a Random Forest approach for untargeted metabolomics was included in the workflow used for pre-processing the data. Orthogonal partial least squares - discriminant analysis (OPLS-DA) was used to investigate metabolic differences between the classes. Stability variable selection and triangulation of machine learning models for results validation were performed using the stabiliser R package to reliably identify important discriminative metabolites. The Cox-Battey regression analysis was also employed to assess anomalies and calculate the correlation between all potential variables of importance.

#### Results

Adequate performance of the LC-MS analytical system and batch-to-batch correction was achieved. In comparative class analysis at farm level, separation and clustering was observed in 2 farms using OPLS-DA. However, cross-validation using the leave-one-out method used to evaluate the robustness of the generated PCA and OPLS-DA by monitoring the fitness of model and goodness-of-prediction  $(Q^2)$  showed values for  $Q^2$  under the threshold of 0.5, which is considered to indicate good predictability. On the other 8 farms, an apparent trend of separation was observed but no clustering was identified in the lipid profiles of the samples from lame and non-lame cows using OPLS-DA. Fold change was significant (adj. *P*-value < 0.05) in one of the 10 farms, and 14 metabolites were found significantly altered (VIP>1) across all farms between the 2 different classes (lame and control) in the samples using OPLS-DA. Stability selection found one mass ion predictive of the lameness outcome. Cox-Battey analysis results showed that 25 (*P* value of 0.003) lipids have the strongest associations with lameness risk.

#### Conclusion

This study provides an important contribution to understanding lipidomic differences between lame and non-lame dairy cows using milk samples, which is the easiest biofluid to collect in dairy farms. The untargeted LC-MS-based lipidomics alongside stability selection and Cox-Battey analysis were shown to be adequate in identifying mass ions predictive of lameness compared to conventional regression approaches. Milk lipid analysis has demonstrated potential to be used as an unbiased method for lameness prediction. Further work is required to increase confidence in the annotation of the lipids identified as potential predictors of lameness.

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# 153. Effects of Eucalyptus globulus essential oils on in vitro rumen microbial fermentation and its potential to reduce methane emissions from dairy cows

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#### Application

*Eucalyptus* oils are being investigated as potential modifiers of ruminal fermentation to improve nutrient utilization efficiency and reduce methane production in dairy cows. Table 1.

#### Table 1

Effects of Eucalyptus globulus oil on in vitro DM, CP and NDF degradability parameters of dairy feed.

Parameters	Treatments				SEM
	Control	EO1	EO2	EO3	
Dry matter					
a	19.00 <sup>c</sup>	18.00 <sup>c</sup>	23.94 <sup>b</sup>	29.10 <sup>a</sup>	9.089
b	64.70 <sup>a</sup>	64.90 <sup>a</sup>	58.70 <sup>b</sup>	66.00 <sup>a</sup>	1.11
с	0.09 <sup>b</sup>	0.08 <sup>b</sup>	0.02 <sup>a</sup>	0.03 <sup>a</sup>	0.487
ED1	71.94 <sup>a</sup>	69.91 <sup>a</sup>	52.35.7 <sup>b</sup>	68.60 <sup>a</sup>	7.03
ED2	60.59 <sup>a</sup>	57.93 <sup>b</sup>	39.77 <sup>c</sup>	53.75 <sup>b</sup>	10.51
ED3	53.25 <sup>a</sup>	50.45 <sup>ab</sup>	34.74 <sup>c</sup>	47.00 <sup>b</sup>	14.73
Crude protein					
a	26.35 <sup>a</sup>	35.10 <sup>a</sup>	18.51 <sup>b</sup>	16.10 <sup>b</sup>	9.09
b	57.11 <sup>a</sup>	30.40 <sup>b</sup>	58.70 <sup>a</sup>	50.40 <sup>a</sup>	1.121
с	0.15 <sup>b</sup>	0.17 <sup>b</sup>	0.20 <sup>ab</sup>	0.31 <sup>a</sup>	0.487
ED1	76.48 <sup>a</sup>	62.30 <sup>b</sup>	61.77 <sup>b</sup>	64.56 <sup>b</sup>	7.030
ED2	68.93 <sup>a</sup>	58.59 <sup>b</sup>	55.38 <sup>b</sup>	61.92 <sup>ab</sup>	10.51
ED3	63.34 <sup>a</sup>	55.77 <sup>b</sup>	50.36 <sup>b</sup>	59.55 <sup>ab</sup>	14.73
Neutral detergent fibre					
a	4.5	5.5	6.1	6.9	2.09
b	49.31	50.02	49.74	38.51	2.089
с	0.11 <sup>b</sup>	0.13 <sup>ab</sup>	0.14 <sup>a</sup>	0.14 <sup>a</sup>	0.011
ED1	40.60 <sup>b</sup>	48.83 <sup>a</sup>	49.62 <sup>a</sup>	46.22 <sup>a</sup>	2.203
ED2	38.39 <sup>bc</sup>	41.61 <sup>ab</sup>	42.75 <sup>ª</sup>	35.28 <sup>c</sup>	2.951
ED3	33.04 <sup>b</sup>	36.45 <sup>a</sup>	37.75 <sup>ª</sup>	31.41 <sup>b</sup>	15.57

a: Soluble fraction (%); b: insoluble degradable fraction (%); cetz: Fractional degradation rate (h-1); ED1: Effective degradability assuming kp=0.02 h-1 (% DM); ED2: Effective degradability assuming kp=0.05 h-1 (g kg-1 DM); ED3: Effective degradability assuming kp=0.08 h-1 (g kg-1 DM). <sup>abc</sup>Means in the same row for each nutrient with different superscripts are significantly different at *P*<0.05.

#### Introduction

Ruminant livestock production has been identified as a significant contributor to greenhouse gas emissions (Gerber et al., 2013). Feeding natural feed additives has shown promising results in mitigating methane emissions by modulating ruminal fermentation (Hristov et al., 2013; Knapp et al., 2014). An *in vitro* study suggested that eucalyptus leaves could modify the rumen fermentation and have the potential to mitigate methane emissions in dairy cows, which may be beneficial for improving animal productivity. Increasing forage degradability and digestible forage intake has the potential to decrease enteric methane emission intensity. The study aims to determine the effects of essential oil of leaves of *Eucalyptus globulus* on *in vitro* dry matter (DM), crude protein (CP) and Neutral detergent fibre (NDF) degradability.

#### Materials and methods

The experimental protocol and procedures were approved by the Animal Ethics Committee (MUP141SMOS01) of the University of Fort Hare, Eastern Cape, South Africa. *Eucalyptus globulus* leaf oil was standardized at 79% cineol and evaluated for its effect on dry matter, crude protein and Neutral detergent fibre degradability. The leaves of *Eucalyptus globulus* were harvested at Ga-Masemola village in Makhuduthamaga local municipality in South Africa. The essential oil was extracted using a vertical steam distillation unit (Vineland, NJ) consisting of a hot plate, boiling flask, biomass flask, still head, condenser and receiver. Experimental treatments were control (no additive), TMR + 100 mg/L *Eucalyptus globulus* leaf oil (EO1); TMR + 150 mg/L *Eucalyptus globulus* leaf oil (EO2); TMR + 200 mg/L *Eucalyptus globulus* leaf oil (EO3). The sample size of each treatment used was 0.5 g per bag with 24 bags per incubation jar. Each run contained three replicates by treatment (12 samples) as well as the blank bag. Samples were heat sealed placed in jars, and incubated for 0, 2, 4, 8, 12, 24, 36 and 48 h at 39 °C in a buffer-inoculum solution. The bags were removed from the jars rinsed and air-dried at 60°C for 48 h for the determination of DM. Samples were cooled in a desiccator and weighed and analyses of neutral detergent fibre (NDF) and crude protein (CP).

#### Statistical analysis

The data was subjected to ANOVA using PROC GLM (SAS Institute, 2009). The least squares mean for all treatments were reported, and significance was tested at P < 0.05.

#### Results

The ED1 of DM was lower (P < 0.05) for EO2 than other treatments. The ED2 of DM did not differ between EO1 and EO3 and was lower and higher for EO2 and the control than the other treatments, respectively (P < 0.05). The ED3 of DM was lower (P < 0.05) for EO2 than other treatments, lower for EO3 than the control, but did not differ (P > 0.05) between control and EO1 and between EO1 and EO3. The ED1 of CP was lower (P < 0.05) for all EO treatments than the control. The ED2 of CP and ED3 were also lower for EO1 and EO2 than the control. All the ED1 of NDF was higher (P < 0.05) for all EO treatments than the control. At the medium passage rate, the ED2 of NDF was higher in EO2 than the control and EO3, and no differences (P > 0.05) were observed amongst the control, EO1 and EO3, and between EO1 and EO2. At a higher passage rate, ED of NDF was higher for EO1 and EO2 than the control and EO3. The ED did not differ (P > 0.05) between the control and EO3 and between EO1 and EO2.

#### Conclusion

These results suggest that *Eucalyptus globulus essential* oils have the potential to improve ruminal fermentation and fiber degradability in dairy cows. These findings suggest that the *Eucalyptus globulus essential* oils have the potential to mitigate methane production without negatively affecting feed degradability when used as feed additives. Further studies are necessary to better understand of level of doses.

#### Acknowledgements

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#### 154. Commercial farm dairy cattle methane emission prediction: Machine learning vs statistical analysis su

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#### Application

The predictive models developed in this study could be used to identify low methane emitting dairy cattle for selective breeding, which in turn, would lower the greenhouse gas emissions produced on dairy farms, whilst simultaneously increasing their productivity.

#### Introduction

Methane (CH4) emissions, produced by Dairy Cattle (DC), are the result of an energy partition inefficiency within the animal: individually, lowering production levels, and collectively, increasing greenhouse gas emissions. The selective breeding of low CH4 emitting DC is a novel mitigation strategy, where future generations inherit the low CH4 emissions characteristics of their more efficient ancestors. The prediction of DC CH4 emissions, in order to identify optimal candidates for selective breeding, has traditionally been explored from a Statistical (SA) perspective. Yet, due to the smart farming revolution, the scale and variety of complex animal information now available for the prediction of DC CH4 emissions has grown exponentially, and within them are likely to exist non-linear relationships which these traditional SA models may struggle to capture. Instead, this research believes that Machine Learning (ML) models are a more viable alternative for the prediction of DC CH4 emissions, as they can capitalise on these inevitable non-linear relationships present within todays large, heterogenous datasets (Negussie et al., 2022, Shadpour et al., 2022). The aim of this study was to compare the performance of traditional SA methods and original ML models in the prediction of DC CH4 emissions.

#### Materials and methods

The dataset used in this study was made up of a combination of 32 separate DC CH4 emission experiments conducted at the Agri Food and Biosciences Institute (AFBI) in Northern Ireland. Each experiment explored a different strategy in the manipulation of DC CH4 emissions: whether via the effect of an experimental dietary treatment, the impact of a selective breed, or the influence of a certain lactation stage, and each varied in scale and duration. A variety of DC breeds were used in each experiment, including Holstein, Jersey and Norwegian. Respiration Calorimeter Chambers were used in each experiment to record the emission, digestion, and metabolism information of each animal. The physical attributes for each animal, as well as their production level during the experiment, were also available. This resulted in a dataset of 934 observations with 11 available features, made up of 32 experiments and 322 cows. CH4 Production (CH4 g/d) was selected as the target response feature. Explanatory feature significance was determined initially through Pearson's Correlation Coefficient (r) and then validated through Hypothesis Testing (p), with the Experiment and Cow features used as random effects. Only those explanatory features which had (r > 0.25) and (P < 0.05) with CH4 g/d, would be deemed significant and utilised during model development. The models tested in the prediction of DC CH4 emissions included: a Linear Mixed Effects (ME) model, which would represent the traditional SA approach, a Non-linear Random Forest (RF) model, which would represent the original ML approach, and a Mixed Effects Random Forest (MERF) model (Hajjem et al., 2014), a novel, hybrid method, which combined both. The performance of the models was assessed using Root Mean Squared Prediction Error (RMSPE), which was also expressed as a percentage of the response mean (RMSPE%), and Concordance Correlation Coefficient (CCC). The models would be trained on 75% of the dataset using the significant explanatory features identified. After each model was developed, it was then tested on ten random subsets derived from the dataset, 25% each, and the mean of each of their assessment metrics over these ten test subsets was taken.

#### Results

The features Energy Corrected Milk Yield (ECMY) and Live Weight (LWT) were found to have a significant influence over CH4 g/d (P < 0.001). The ML RF model outperformed the SA ME model in the prediction of CH4 g/d, with an RMSPE of 36.25 CH4 g/d and CCC of 0.85, compared to the ME models' 46.15 CH4 g/d and 0.77 (Table 1). When the ME and RF models were combined within a MERF hybrid model, the best performance was obtained, achieving a RMSPE of 34.92 CH4 g/d and CCC or 0.87 (Table 1).

#### Table 1

Model Assessment Metrics.

Model	RMSPE	RMSPE%	CCC
ME RF	46.15 36.25	12.46 10.17	0.77 0.85
MERF	34.92	9.65	0.87

#### Conclusions

Based on the present dataset, it appears that ML is a more viable alternative for the prediction of DC CH4 emissions compared to traditional SA approaches, represented by a RF and ME model respectively. However, the best performance achieved actually came from a combination of both approaches, here encapsulated within a hybrid MERF model. Therefore, whilst ML certainly has the potential to outperform traditional SA methods in the prediction of DC CH4 emissions, instead of acting as direct competitors, it is a combination of the two approaches as complimentary teammates, which secures the best results.

#### Acknowledgments

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# **Author Index**

## AUTHOR NAME

#### ABSTRACT ID

## Δ

Adejumo, R	18
ADELEYE, O	19,20
Ahmad, A	135
Anagnostopoulos, A	136
Angeles Henandez, J	65
Araujo, L	21
Argyriadou, A	9
Ashfield, A	79
Aubry, A	77

## B

Balegi, R	123
Ball, E	102
Barnett, I	67
Beechener, S	47
Bonagurio Gallo, S	149
Boyle, Laura	122
Brans, J	116
Brereton, N	133
Bueno, I	129,130

# С

Cahyo, H	70
Campbell, M	120
Capper, J	111
Cardoso, A	152
Chen, X	15,71
Christodoulou, C	86
Connolly, R	11
Craig, A	16,74
Crawford, P	80
Crawford, P	46
Crawford, P	38
Cristobal-Carballo, O	61
Crump, A	84
Cruywagen, C	134
Cupido, M	102
Cushnahan, A	72

## D

Dabiri, A	
Dong, L	
Dubarry, K	

## Ε

Echeverry Munera, J Eikanger, K Escobar-Bahamondes, P Evans, R Ewing, D
<b>F</b> Folliard, N

AUTHOR NAME	ABSTRACT ID
<b>G</b> Galgano, S Gonzalez, L Gonzalez Ronquillo, M	99 104 87,88
H Haghighat, M Hancock, E Harper, E Henry, N Hewett, E Holohan, C Homer, E. Horan, L	144 41 43 12 101 14 50 81
ldowu, O	132
<b>J</b> Jensen, J Jensen, R Jezequel, A Jiang, W	90 140 13 137
<b>K</b> Kaitholil, S Keady, T Kennedy, O Kliem, K	5 60 151 30
L Lewis, I Lindley, G Little, Mark Lloyd, N	124 56 75 92
M MACARTAN, B Mackintosh, S Macleod, M Madenci, D Makanju, T Malehere, R Mansbridge, S	110 31 23 4 17 55 142
Margerison, J Markland, L McCalman, H McCaughern, J McDermott, K McEvoy, M McEvoy, M	57,58,68 98 82,131 35 125 26 26

Meehan. D.....

Melville, L .....

Mengyuan, W .....

ID

#### AUTHOR NAME ABSTRACT ID Mhlongo, N ..... Morgan, E..... 45,49 Morgan, S..... Moshidi, P..... Mulvenna, C ..... Murray, E ..... Ν Nguyen, T..... Nisbet, H..... O'donovan. S ..... O'Mara. F ..... Ojo, O..... Ong, E..... Oyegunwa, A ..... Ρ Parker-Norman, S..... Prvce. J ..... Pugh, G ..... R Rainey, R..... Richards, S..... Riley, B..... Rivero. J..... 27.28 Ross. S..... Roxborough, C ..... Rutherford, N..... S Sagaye, A ..... Sari, N ..... Savwer. G ..... Scully, S ..... Senanayake, N ..... Sheridan, H..... Sherry, E ..... Shirali, M..... Siachos, N..... 118,119 Smith, G ..... Statham, J..... Stubbings, L..... Sveinbj—rnsson, J ..... т Taha, M ..... Tamer, P ..... Taylor, J ..... Thekkoot, D..... Thomas, E..... Titterington, F..... Trotter, M.....



#### AUTHOR NAME

ABSTRACT ID

51

76

109

44

107

97

#### AUTHOR NAME

ABSTRACT ID

AUTHOR NAME

ABSTRACT ID

## U

Unsal-Polat, G USMAN, M	
V	
Valergakis, G	
Van Winden, S	
Van Zanton	

van za	inten	
Vasa, S	5	

W

Walsh, H	141
Watson, K	64
Watson, K	48
Williams, D	40
Williams, P	83
Williams, T	106
Webster, J	138
Wijesiriwardana, U	115
Worku Bedada, K	139

<b>X</b> Xue, B	85
<b>Y</b> Yousif, M	22
<b>Z</b> Zaldivar-Ortega, A Zulfa, I	150 36



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