

# Biplot analysis to describe the relationships between plant and microbial fatty acids in ingested herbage

E. J. Kim, R. Sanderson, M. S. Dhanoa and R. J. Dewhurst

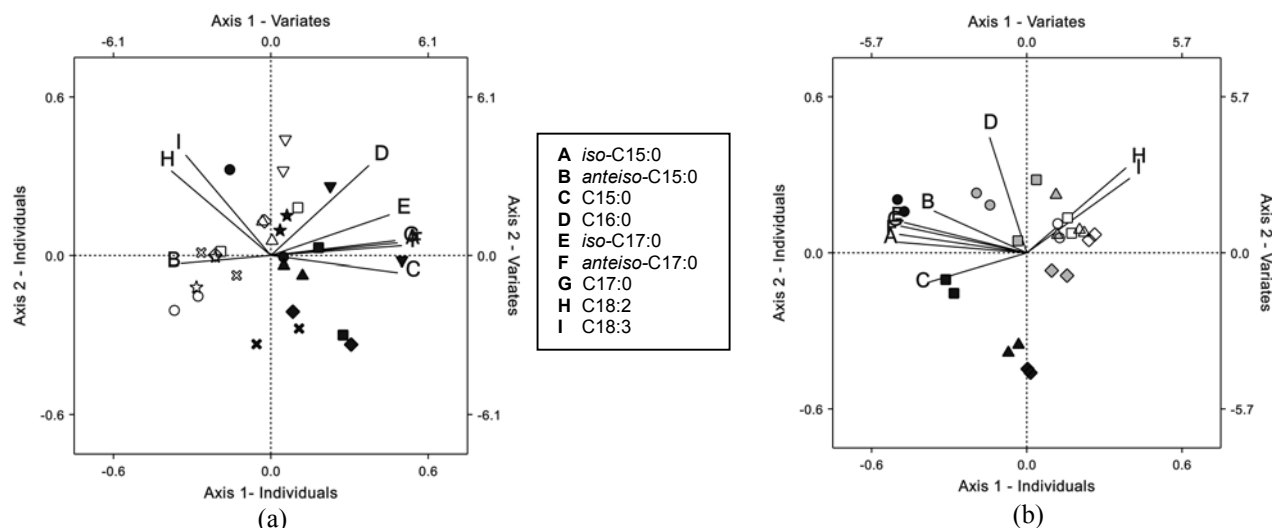
*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, U.K.*

*Email: eun-joong.kim@bbsrc.ac.uk*

**Introduction** The Dacron bag technique has been widely used to estimate degradation in the rumen. The drawbacks, such as variation in rinsing losses and inability to correct for microbial contamination, are well known. However, these effects also suggest the potential to use the technique in studies of microbial colonisation. Other studies in our laboratory have investigated the use of odd-chain fatty acids (C15:0 and C17:0) as markers of rumen microbial activity (Fievez *et al.*, 2003) because they are generally rare or absent from feeds. The objective of this work was to use multivariate statistical analysis to explore the relationships between plant and microbial fatty acids in ingested herbage.

**Materials and methods** Two *in situ* experiments were conducted using two dry Holstein-Friesian cows, fitted with rumen cannulae, grazing perennial ryegrass pasture. In experiment 1, the effects of sample preparation method were investigated with perennial ryegrass: (M1) no further processing: grass was gently folded and placed into Dacron bags; (M2) chopping into approximately 1 cm lengths using scissors; (M3) crushing with a metal roller, but not chopping; (M4) chopping and crushing- a combination of (M2) and (M3); (M5) mechanical chopping (Lynhacker) for 30 sec; (M6) ingested bolus material, and (M7) freeze-dried and ground. Duplicate bags were incubated in each of two cows for both 2 and 7 hour periods. Experiment 2 investigated the effects of different washing procedures after removal of Dacron bags from cows. Approximately 7 g of DM was weighed into Dacron bags, which were incubated in each of two cows for 2, 8 and 24 hours. On removal of bags from the rumen, duplicate bags from each cow were washed according to one of four washing procedures: (W1) squeezing of the bag and its contents, so that no more liquid ran out; (W2) gentle hand washing by agitation in a sink of cold water: repeating until there was no further visible loss of bag contents; (W3) hand washing under a continuous stream of cold water: until the water ran clear; and (W4) machine washing in cold water for 50 minutes. Fatty acid methyl esters were prepared (methanolic HCl, 5%), extracted and determined by gas chromatography using tricosanoic acid (C23:0) as an internal standard. Biplot analysis (GenStat® 7) was used to examine the variation in the major plant and microbial fatty acids in these samples.

**Results** In both experiments, the concentration of odd-chain fatty acids increased with time of incubation while that of C18:2 and C18:3 decreased (data not shown). The biplot procedure (Figure 1) simultaneously showed variation in fatty acids and the effects of treatments on that variation. Generally, C18:2 and C18:3 behaved similarly, whilst the odd-chain fatty acids varied in a similar but opposite direction. The effect of the incubation periods, sample processing methods and washing procedures were clearly separated, indicating different degrees of microbial colonisation/contamination.



**Figure 1** Biplot showing the relationship between treatments and selected fatty acids for experiment 1 (a: white and black coloured symbols for 2 and 7 hr incubation, respectively; for symbols M1 (●), M2 (★), M3 (■), M4 (◆), M5 (π), M6 (θ) and M7 (×)) and experiment 2 (b; white, grey and black coloured symbols for 2, 8 and 24 hr incubation, respectively; For symbols W1 (●), W2 (■), W3 (π) and W4 (◆))

**Conclusions** Biplot analysis was successful in describing variation (86.1 and 86.8% for experiments 1 and 2) in, and treatment effects on, fatty acids profiles of ingested herbage. Concentrations of plant-derived fatty acids (C18:2 and C18:3) decreased over time, while microbially-derived odd-chain fatty acids increased in concentration with time.

## References

Fievez, V., Vlaeminck, B., Dhanoa, M. S. and Dewhurst, R. J. 2003. Use of principal component analysis to investigate the origin of heptadecenoic and conjugated linoleic acids in milk. *Journal of Dairy Science* **86**: 4047-4053.