BRIEF COMMUNICATION: Differential passage of rumen bacterial populations to the abomasum in sheep

J. LAPORTE-URIBE and S.J. GIBBS*

Faculty of Agricultural and Life Sciences, P.O. Box 84, Lincoln University, Lincoln 7647, New Zealand
*Corresponding author

Keywords: rumen; rumen microflora; abomasum.

INTRODUCTION

Ruminant microflora and fauna enable the degradation of otherwise poorly digestible feeds and are a principal source of metabolisable protein (Faichney, 1996). Rumen outflow, which is dependent on many factors including the physical characteristics of the diet and the level of feed intake, carries both nutrients and microorganisms to the abomasum. The proportional contribution of the various sub-populations of microorganisms to the total pool of abomasal protein is still poorly defined (Dewhurst *et al*., 2000). It is therefore possible that differences in motility or microhabitat between sub-populations may generate differential retention of certain groups.

Contemporary molecular identification and characterisation of rumen microbiota is now routinely done by the use of polymerase chain reaction based techniques. Microbial community profiling, using 16S ribosomal RNA and denaturing gradient gel electrophoresis (DGGE), has enabled rapid assessment and comparison of sub-population shifts (Zoetendal *et al*., 2004).

In this study the simultaneous, diurnal, bacterial population profiles of rumen and abomasal contents of sheep were compared using DGGE to determine if there was differential rumen outflow of some groups of bacteria.

MATERIALS AND METHODS

Fourteen twin bearing ewes were surgically fitted with abomasal and rumen cannulae at 100 days of gestation. The experiment was a 2 x 19 day crossover design. In early lactation the ewes were randomly divided into two diet treatment groups: pasture (clover-ryegrass mix) only (PO) or pasture plus 500g of a high protein (fish meal) nuts (PN). For 19 days the groups were co-grazed and yarded each morning at 09:00 h when the PN group was individually fed the nuts. From day 15 to day 19 all ewes were also yarded for sampling with 100 ml of rumen digesta and 100 ml of abomasal fluid taken from each ewe every six hours over five consecutive days. This allowed the generation of a time series with a sample every three hours across the 24 hour diurnal period. The groups were then switched and the process repeated.

Rumen and abomasal samples were immediately frozen in liquid nitrogen and then stored at -20^{o}C until analysed. Extraction of DNA from rumen digesta and abomasal samples was done by a modified method of Chomczynski and Sacchi (1978).

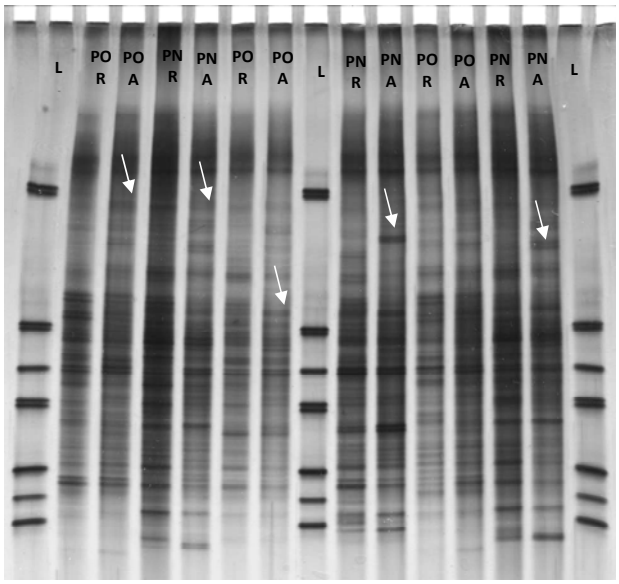
RESULTS AND DISCUSSION

A large number of bands consisting of fragments of the extracted 16S RNA were visible in the DGGE gels of both the rumen and abomasal samples at all times (Figure 1). Direct comparisons of the bands demonstrated generally strong similarities between rumen and abomasal samples from the same time of the day, and this was consistent in both PO and PN groups. The strong similarities are in contrast to results reported for cattle using DGGE that suggested significant differences between rumen and omasal content (Karnati *et al*., 2007). This finding could be related to the use of rumen fluid in that experiment, rather than the rumen digesta that was used in this experiment, as solid-associated bacteria may have been underestimated due to removal of some digesta from the rumen fluid.

There was also an increase in the number and intensity of bands of both rumen and abomasal samples from any given time in the PN group. Although caution is required in the interpretation of DGGE to quantify bacterial populations present, this increase suggests an increase in both the number and diversity of bacteria as a result of supplementation, which is consistent with previous work of dietary supplementation with similar protein sources increasing the microbial protein supply in pasture fed sheep (S.J. Gibbs, Unpublished data), and in dairy cows fed cereal based rations (Volden *et al*., 1999).

However, despite the strong similarities in bands overall between contemporaneous rumen and abomasal samples, there were some highly specific, noteworthy differences. Some bands present in the rumen samples were absent, or of markedly reduced intensity, in the simultaneous abomasal sample; a finding that does suggest differential rumen outflow of bacteria. These differences could be related to a selective retention of solid associated bacteria, which if preferentially associated with the rumen

FIGURE 1: Example of a denaturing gradient gel electrophoresis of rumen (R) and abomasal (A) samples of sheep fed pasture (PO) or supplemented (PN) with protein diet. Standard lines (L) were placed between lines. Arrows show abomasal bands not present or in lower concentration than in rumen samples and vice-versa.



'raft', might be less likely to be passed out of the rumen and so be present in lower numbers in the abomasal fluid. In addition, two bands present in the abomasal samples were not found in the corresponding rumen samples, suggesting a resident abomasal microflora. It has been reported that *Escherichia coli*, *Clostridium perfringens* and *Lactobacillus* spp. are present in the abomasum in pre-ruminant lambs (Vatn *et al*., 2000), and Cheng *et al*. (1979) suggested a colonisation of the abomasal epithelia by an un-identified bacteria associated with the degradation of urea. It has also been reported that urease production is the means by which *Heliobacter pylori* is able to colonise the stomach of piglets (Eaton *et al*., 1991). It is possible there is an abomasal microflora in sheep of uncertain function that has not been described to date.

CONCLUSION

This preliminary study has demonstrated that functional community profiles of the microflora of abomasal contents can be achieved using DGGE of 16S DNA. While the microflora profiles of rumen and abomasal samples do have strong similarities, as might be expected, there also appear to be notable differences which have not been described in the literature to date. These differences suggest there are sub-populations of rumen bacteria that are differentially retained in the rumen, but also that there are sub-populations resident within the abomasum. Both findings may have relevance for grass based ruminant nutrition. Further research is required to confirm these findings, identify these sub-populations of bacteria and the mechanisms by which they remain in their respective habitats and determine their significance to ruminant nutrient supply.

REFERENCES

Cheng, K.J.; McCowan, R.P.; Costerton, J.W. 1979: Adherent epithelial bacteria in ruminants and their roles in digestive tract function. *American journal of clinical nutrition* **32**: 139-148.

Chomczynski, P.; Sacchi, N. 1987: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical biochemistry* **162**: 156-159.

Dewhurst, R.J.; Davies, D.R.; Merry, R.J. 2000: Microbial protein supply from the rumen. *Animal feed science and technology* **85**: 1-21.

Eaton, K.A.; Brooks, C.L.; Morgan, D. R.; Krakowka, S. 1991; Essential role of urease in pathogenesis of gastritis induced by Helicobacter pylori in gnotobiotic piglets. *Infection and immunity* **59**: 2470-2475.

Faichney, G.J. 1996: Rumen physiology: the key to understanding the conversion of plants into animal products. *Australian journal of agricultural research* **47**: 163-174.

Karnati, S.K.R.; Sylvester, J.T.; Noftsger, S.M., Yu, Z.; St-Pierre, N.R.; Firkins, J.L. 2007: Assessment of ruminal bacterial populations and protozoal generation time in cows fed different methionine sources. *Journal of dairy science* **90**: 798-809.

Vatn, S.; Tranulis, M.A.; Hofshagen, M. 2000: Sarcina-like bacteria, Clostridium fallax and Clostridium sordellii in lambs with abomasal bloat, haemorrhage and ulcers. *Journal of comparative pathology* **122**: 193-200.

Volden, H.; Harstad, O.M.; Mydland, L.T. 1999: Amino acid content and profile of protozoal and bacterial fractions isolated from ruminal contents of lactating dairy cows fed diets differing in nitrogen supplementation. *Acta agricultura scandinavica. Section A, Animal science* **49**: 245-250.

Zoetendal, E.G.; Collier, C.T.; Koike, S.; Mackie, R.I.; Gaskins, H.R. 2004: Molecular ecological analysis of the gastrointestinal microbiota: a review. *Journal of nutrition* **134**: 465-472.