

The effect of fluctuations in rumen pH on protozoa populations in rumen fluid as determined by real-time polymerase chain reaction

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Introduction Ruminal ciliated protozoa are similar to bacteria in that their numbers increase in response to increased substrate availability. Moderate increases in grain intake result in large increases in the numbers of ciliated protozoa (Dennis *et al.*, 1983). In contrast, high- or all grain diets have variable effects, including total elimination of protozoa in some animals (Franzolin and Dehority, 1996). Ciliated protozoa are believed to be much more sensitive than bacteria to fluctuations and reductions in ruminal pH; hence, ruminal pH is a critical factor in the maintenance of ciliated protozoa in the rumen. The objective of the present experiment was to investigate the effect of fluctuation in ruminal pH on the population of protozoa in rumen fluid.

Materials and methods Four Holstein steers (300 ± 15 kg, body weight) with rumen fistulae were fed experimental diets (7 kg of DM/d) differing in their concentrate (155 g CP/kg DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCO₃, 0.5% mineral and vitamin premix, 0.2% salt) to forage (lucerne hay; 155 g CP/kg DM) ratio (60:40, 70:30, 80:20, and 90:10) in a 4×4 Latin square design (28-day periods). Steers were housed in individual pens, and fed the experimental diets as a total mixed ration at 0800 and 2000h. Animals had access to drinking water at all times. Ruminal fluid was taken, by suction, via rumen fistula on days 24 to 28 of each period. The pH of the ruminal fluid samples was measured immediately with a portable pH meter (Metrohm 744, Switzerland) before the morning feeding (0.0h) until 8h post feeding at 15 min intervals. The samples of rumen fluid taken before the morning feed, and 4h post feeding were stored in liquid N₂ until used for protozoa quantitation by qPCR. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. Protozoa rDNA concentrations were measured by real time PCR relative to total bacteria amplification ($\Delta\Delta Ct$). The 18s rRNA gene-targeted primer sets used in the present study were forward: GCTTTCGWTGGTAGTGTATT and reverse: CTTGCCCTCYAATCGTWCT. Cycling conditions were 95°C for 10 min, forty five cycles of 94°C for 10s, 55°C for 20s and 72°C for 15s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 45 to 94°C, with fluorescence collection at 0.1°C at intervals. Data are expressed relative to quantification of the total bacterial population. Data were analyzed using the GLM procedure of SAS

($y = \text{Mean} + \text{Treatment} + \text{Animal} + \text{Period} + \text{Time} + \text{Time} \times \text{Treatment} + \text{Animal} \times \text{Time} + \text{residual}$) and the means compared by the Tukey test ($P < 0.05$).

Results Ruminal pH decreased, from before the morning feeding 6.91 (60:40), 6.76 (70:30), 6.52 (80:20) and 6.50 (90:10) to 4h after feeding 6.30 (60:40), 6.20 (70:30), 5.94 (80:20) and 5.50 (90:10), respectively, ($P < 0.05$). rDNA concentration of protozoa in rumen fluid is shown in Figure 1. The population of protozoa in the ruminal fluid increased, when level of concentrate was increased ($P < 0.05$).

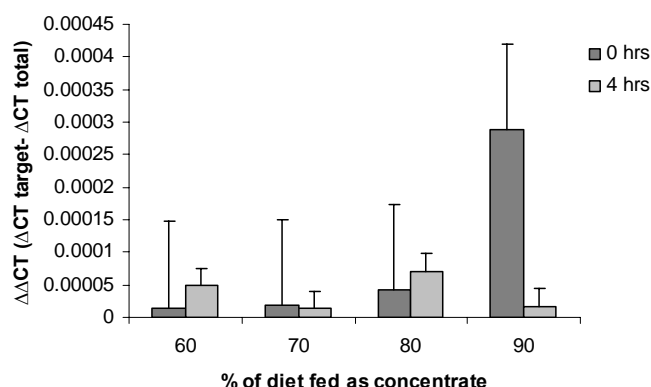


Figure 1 Protozoa (± SD) in rumen fluid before and 4h after morning feeding

Conclusions The results of the present study demonstrated that increasing the inclusion of concentrate in diets caused a decrease in rumen pH and an increase in the population of protozoa in the free rumen fluid. These results are in contradiction with some studies that suggest that reduction in ruminal pH is a method for experimental defaunation in ruminants (Franzolin and Dehority, 1996). The reason for this contradiction is the protozoa numbers in the liquid phase are not determined solely by rumen pH, several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. These include composition of diet, pH, turnover rate, frequency of feeding, and feed level (Franzolin and Dehority, 1996).

References

- Dennis, S. M., Arambel, M. J., Bartley E. E., and Dayton A. D. 1983. Journal of Dairy Science. 66, 1248–1254.
 Franzolin, R., and Dehority, B. A. 1996. Journal of Animal. Science. 74, 2803–2809.