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The effect of fluctuation in rumen pH on attachment of *Ruminococcus flavefaciens* to dietary substrates as determined by real-time PCR

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Introduction Rumen pH, together with the microbial population, nature of substrates, environmental factors such as temperature and existence of cations and soluble carbohydrates have been suggested as factors governing bacterial attachment to ingested feed particles (Miron *et al*., 2001). Ruminal pH is one of the most important of these factors, because fibrolytic bacteria are very sensitive to pH change. Fibre digestion decreases at low rumen pH, especially below pH 6.0, as observed previously in studies using continuous cultures of mixed ruminal micro organisms, *in vitro* rumen, and *in situ* techniques. The objective of the present experiment was to investigate the effect of fluctuation in rumen pH on the attachment *of Ruminococcus flavefaciens* to feed particles incubated in the rumen.

Materials and methods Four Holstein steers (300 ± 15kg, body weight) with rumen fistulae, were fed experimental diets (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% $CaCO_3$, 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 0800h and 2000h. Animals had access to drinking water at all times. Ruminal fluid was taken, by suction, via the 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 feed (0.0h) until 8h post feeding at 15min intervals. Feed samples (lucerne hay and barley grain) were dried using a forced-air oven at 96 ºC for 48 h, and ground to pass through a 2mm screen. Approximately 1.2g DM of each sample was placed in polyester nylon bags, 3 × 6cm; 48µm pore size, (4 bags per each feed) and incubated in the rumen of each steer for 12h. After removal from the rumen, DNA was extracted from the incubated samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. *R. flavefaciens* rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: CGAACGGAGATAATTTGAGTTTACTTAGG and reverse: CGGTCTCTGTATGTTATGAGGTATTACC. Cycling conditions were 95°C for 5min, forty cycles of 95°C for 15s, 61°C for 1min and 72°C for 30s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 65 to 95°C, with fluorescence collection at 0.1°C at intervals. Data were expressed relative to quantification of the total bacterial population quantified using the primers described by Maeda *et al*. (2003). Data were analysed using the GLM procedure of SAS (y = Mean + Treatment + Animal + Period + Time + Time × Treatment + residual) and the means compared by the Duncan test (P <0.05).

Results Rumen pH at different sampling times is shown in Figure 1. Rumen pH decreased, when the level of concentrate was increased (P <0.05). Concentrations of rDNA in *R. flavefaciens* attached to lucerne hay and barley are shown in Figure 2. Bacterial attachment to both lucerne and barley decreased as the level of concentrate in the diet increased (P <0.05).

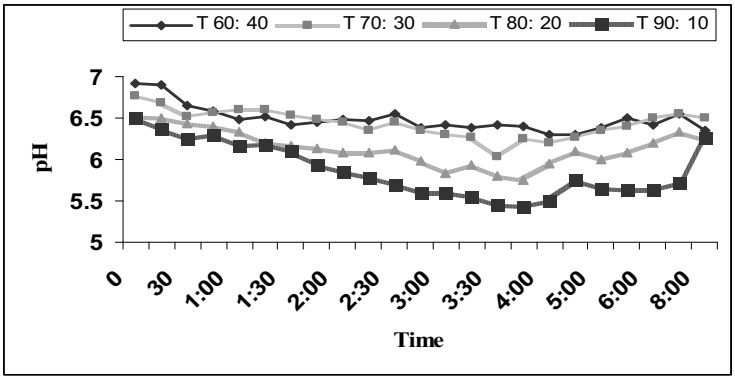

Figure1 Rumen pH in Holstein steers fed diets differing in concentrate: forage ratios

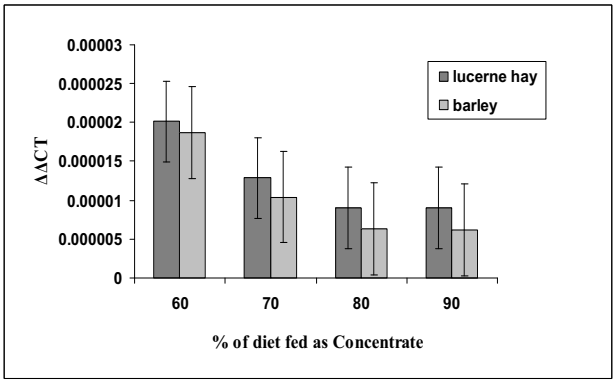

Figure 2 *R. flavefaciens* (± SD) attached to lucerne hay and barley after 12h incubation in the rumen

Conclusions The results of the present study demonstrated that increasing the inclusion of concentrate in diets caused a decrease in ruminal pH and the population of *R. flavefaciens* attached to substrates. The exact mechanism involved in reduced attachment at low pH is not known. However, Russell and Wilson (1996) suggested that as pH declines, the increase in the transmembrane pH gradient in the bacterium causes a logarithmic accumulation of intracellular fermentation acid anions and hence leads to anion toxicity and product inhibition. .

References

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