Effect of rumen acid load on i*n vitro* ruminal total bacteria and *Fibrobacter succinogenes* populations determined by real-time PCR

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Introduction One consequence of feeding excessive amounts of rapidly fermentable carbohydrates in conjunction with inadequate fibre to ruminants is subacute ruminal acidosis, characterized by periods of low ruminal pH. Fibrolytic bacteria are unable to maintain the pH inside their cells when ruminal pH is low (Russell and Wilson, 1996). Rustomo *et al.* (2006) evaluated the acidogenicity value (AV) of feeds using an *in vitro* laboratory technique. Danesh Mesgaran *et al.* (2009) demonstrated that various kinds of dairy diets with different NFC contents had different acidogenicity values. The aim of the present study was to evaluate the effect of rumen acid load on ruminal total anaerobic bacteria and *Fibrobacter succinogenes* populations determined by real-time PCR.

Material and methods Various commercial dairy diets (forage:concentrate as 1:1) containing different levels of non-fibre carbohydrates (NFC) were provided as low (LNFC), medium (MNFC) and high (LNFC) to obtain different AV (Danesh Mesgaran *et al.*, 2009). The concentration of NFC in LNFC, MNFC and HNFC was 336, 366 and 371 g/kg DM. The acidogenicity values of the diets were determined using the procedure as described by Wadhwa *et al*. (2001). One-gram (DM) of each diet was weighed and incubated, in triplicate, with 30 ml of buffered rumen liquor comprising 60% buffer and 40% rumen liquor. The buffer was made up at 20% of the strength of the Tilley-Terry (1963) buffer. Cysteine hydrochloride monohydrate (0.025% wt/vol) was added just prior to incubations. The incubations were carried out in 100 ml bottles held in a water bath at 38.7 °C. After the incubation, samples were taken for DNA extraction (0.2 ml). Then, 2 ml of the each bottle content were located into micro tubes containing 50 mg (excess) of $CaCO_3$ powder. The mixture was shaken manually for 5 s, then centrifuged at 4000 rpm for 10 min before analysis of Ca content of the supernatant using Atomic Absorption. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30 ml) divided by the sample weight. DNA was extracted from the samples using the Bioneer Accuprep Genomic DNA Extraction Kit. The 16s rRNA gene-targeted primer sets used in the present study were forward: GTTCGGAATTACTGGGCGTAAA and reverse: CGCCTGCCCCTGAACTATC. Cycling conditions were 95 °C for 5 min, forty cycles of 95 °C for 15 s, 61 °C for 1 min and 72 °C for 30 s; 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. Total bacteria concentration was determined relative to bacterial standard. *Fibrobacter succinogenes* population was expressed relative to quantification of the total bacterial population. Data were analyzed as a complete randomized design using GLM procedure of SAS (2003). Model was: Y = Mean + Treatment + residual.

Results The AV of LNFC, MNFC and HNFC was 10.7, 11.2 and 11.9, respectively. Data on Table 2 showed that the *Fibrobacter succinogenes* population in LNFC were significantly higher than the other diets ($P< 0.05$).

Table 1 *In vitro* DNA concentration of total bacteria and the population of the *Fibrobacter succinogenes* relative to total bacteria in commercial dairy diets containing different levels of NFC.

Bacteria	Treatments			s.e.d	P
	LNFC	MNFC	HNFC		
Total bacteria (ng/µl)	34.55	44.27	44.65	5.72	> 0.05
Fibrobacter succinogenes	0.1281	0.0310	0.0298	0.03	< 0.05

Conclusions The results of the present study demonstrated that increasing in NFC in commercial diets caused a decrease in ruminal AV and the population of *Fibrobacter succinogenes*. It was previously indicated that the rate at which rumen fluid pH changed followed a pattern similar to changes in the AV (Rustomo *et al*., 2006), and the differences in AV and pH changes likely were associated with the fermentability of the feeds. Therefore, higher NFC content in HNFC led to higher available nutrients for bacterial growth, and consequently lower pH. This situation made a condition in which a rise in total bacteria might be assumed, with a decline in relative fibrolitic bacteria population, as demonstrated by the present results.

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