The effect of non-fibre carbohydrates supplementation on methanogenesis bacteria and protozoa populations in rumen fluid as determined by real-time polymerase chain reaction

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Introduction Methane produced during ruminal fermentation represents a loss of 2–12% of the gross energy consumed by ruminants, and it is also a greenhouse gas that has been implicated as a contributor to the global warming (Johnson and Johnson, 1995). Thus, much research has been carried out on manipulation of the rumen fermentation to inhibit rumen methanogenesis with a view to increase energetic efficiency. The present experiment was conducted to determine the effects of diets containing different non-fibre carbohydrates (NFC, sucrose or starch) on rumen methanogenesis bacteria and protozoa populations in Holstein steers by real time PCR.

Material and methods Four Holstein steers (280± 15 kg, body weight) with rumen fistulae were assigned to a 4×4 Latin square design with 21 day periods; 17 days diet adjustment and 4 days sample collection. The basal diet contained lucerne hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/ kg, respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+Su) was added to the basal diet at the rate of 70g/kg DM. Diets were offered at 2-2.5 times maintenance requirements (7kg DM/day). The samples of rumen fluid taken before the morning feed, and 4 h post feeding were stored in liquid N2 until used for bacterial and 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. Methanogenesis and protozoa 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 for methanogenesis bacteria were forward: TTCGGTGGATCDCARAGRGC and reverse: GBARGTCGWAWCCGTAGAATCC. Cycling conditions were 95 °C for 5 min, forty six cycles of 95 °C for 15 s, 61 °C for 15 s and 72 °C for 15 s. The 18s rRNA gene-targeted primer sets used in the present study for protozoa were forward: GCTTTCGWTGGTAGTGTATT and reverse: CTTGCCCTCYAATCGTWCT. Cycling conditions were 95 °C for 5 min, fifty cycles of 95 °C for 15 s, 55 °C for 20 s 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. Data are express relative to quantification of the total bacterial population using the primers described by Maeda *et al* (2003). Data were analyzed 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 Ribosomal DNA (rDNA) concentration of methanogenesis bacteria and protozoa in rumen fluid is shown in Figure1. Population of methanogenesis bacteria and protozoa in the ruminal fluid decreased, when basal diet was supplemented by either Sucrose or Starch ($P < 0.05$). In addition St+Su has a higher significant decreasing effect on ruminal protozoa populations.

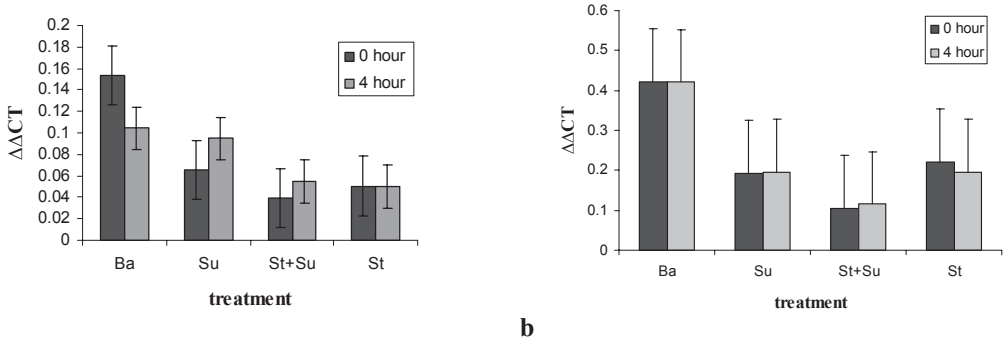

Figure 1 Methanogenesis bacteria(a) and protozoa (b) (mean ± SD) in rumen fluid before and 4 h after morning feeding

Conclusions The results of the present study demonstrated that supplementation of ruminant diets with NFC caused a decrease on population of methanogenesis bacteria and protozoa in the free rumen fluid. In addition, results concluded that NFC types have different effect on rumen methanogenesis bacteria and protozoa populations. Type of supplemental carbohydrate provided in ruminant diets has been suggested to be a factor that may impact on ruminal microbiota populations. Among the NFC types, starch has the greatest potential to suppress rumen methanogenesis. 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

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