MOLECULAR CHARACTERISATION OF RUMEN BACTERIAL POPULATIONS IN CATTLE FED MOLASSES DIETS

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Molasses-urea mixtures are widely used in northern Australia as supplements for beef cattle. They supply rumen microorganisms with a rapidly degradable energy source that is thought to affect the profile of the rumen bacterial community. The objectives of this study were 1) to obtain profiles of the predominant bacterial species in the rumen of beef cattle fed diets containing varying proportions of low quality Pangola grass hay and molasses, and 2) to examine the stability of the ruminal microbiota within and between days, through application of denaturing gradient gel electrophoresis (DGGE).

Four rumen-cannulated Brahman-cross steers were fed varying proportions of cane molasses (0, 25, 50 and 75% of the diet) once daily. Urea comprised 3% of the molasses. Steers were allocated to 1 of 4 diets in a 4x4 Latin square design, with periods of 28 days. Each period comprised a 3-week adaptation period and a 1-week measurement period. Following adaptation, rumen fluid samples were taken immediately prior to feeding and 8 h after feeding on 2 consecutive days. Total genomic DNA was extracted from the rumen fluid samples by bead beating. Subsequently, 16S rDNA was amplified by polymerase chain reaction with primers specific for the fragment between variable regions 2 and 3 (V2-V3) of the gene. The amplified V2-V3 products obtained from the samples were analysed using DGGE to profile the complexity of rumen bacterial populations.

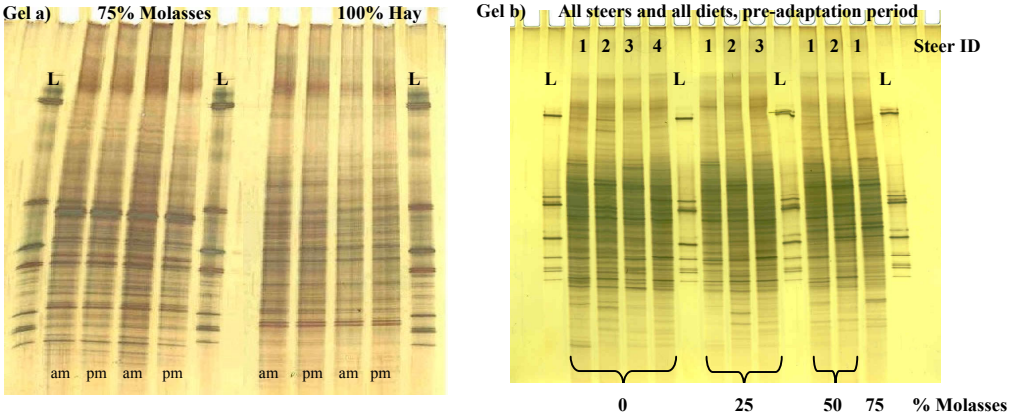

Figure 1. Denaturing gradient gel electrophoresis profiles generated from rumen fluid samples obtained from rumen-cannulated steers. Gel a). Samples from 75:25 molasses-hay and 100% Pangola hay diets. Samples were taken from 2 steers before and after feeding (0800 and 1600 h) on 2 consecutive days. Gel b). Samples from steers gradually fed increasing levels of molasses at the start of the experiment (first set of 4 lanes - all steers received 0% molasses on day 1; second set of 3 lanes - 3 steers received 25% molasses on day 3; third set of 2 lanes - 2 steers received 50% molasses on day 5; last lane - 1 steer received 75% molasses on day 7). L - ladder of DNA marker standards.

Comparison of 2 sets of 4 DNA samples, each set representing the 2 extreme dietary conditions (diets containing 0 and 75% molasses) belonging to 2 different animals (Figure 1, Gel a) revealed the presence of dominant species in the rumen samples from the steer fed the highest molasses treatment that were not present in the samples obtained from the steer fed the control diet. Furthermore, there were no differences in this steer between the DGGE banding pattern derived from the morning and afternoon, and between 2 consecutive days. The similarity among samples belonging to animals receiving gradually increasing levels of molasses during the build up period (Figure 1, Gel b) indicated that it might take more than 2 days for the bacterial community to adapt to a dietary change. It can be concluded that (1) predominant species of the rumen bacterial community appear to be particularly stable during the day, and over at least a 2-day period, in animals fed a given diet, and (2) it takes more than 2 days for the rumen bacterial community to respond to rapid dietary changes.

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