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Effect of disodium fumarate on ruminal metabolism and rumen bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA

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Abstract

The aim of the present study was to examine the effects of feeding diets with addition of disodium fumarate (DF) to goats on ruminal metabolism and changes of rumen bacterial communities. Four cannulated goats were used in a 4×4 Latin square design. The results showed that ruminal pH increased linearly ($P<0.01$)as the amount of DF added increased, while lactate production decreased linearly ($P<0.01$). DF addition did not affect the production of acetate, propionate, butyrate, TVFA and NH_3-N. The effect of DF on the changes in rumen bacterial-community structure of goats was analyzed using 16S rDNA-based approaches. Amplicons of the V6-V8 variable regions of bacterial 16S rDNA were analyzed by denaturing gradient gel electrophoresis (DGGE), cloning and sequencing. Differences in rumen bacterial community structure were determined based on the Shannon index of diversity for pairwise comparison of the DGGE fingerprints and revealed significant changes in rumen microbiota after DF addition. As compared with those fed with the control diet, goats fed on the diets with DF addition showed a higher bacterial diversity. The sequences of seven amplicons in total 11 clones showed less than 97% similarity with those of previously identified or unidentified bacteria, suggesting that most bacteria in the gastrointestinal tract have not been cultured or identified. Amplicons related to *Succinivibrio dextrinosolvens* species were found in most DGGE fingerprints

Abbreviations: DF, disodium fumarate; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; aNDF, neutral detergent fiber; ADF, acid detergent fiber; DM, dry matter; BW, body wight; VFA, volatile fatty acids; A:P, acetate to propionate ratio; NH_3-N, ammonia-N

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derived from goats on the diet containing DF, but not in goats on the control diet. These results demonstrated the ability of DF to improve the metabolism of rumen lactate fermentation and to influence the bacterial composition of the rumen in goats.

Keywords: Disodium fumarate (DF); Ruminal metabolism; Goat; Denaturing gradient gel electrophoresis

1. Introduction

Antimicrobial compounds are routinely incorporated into ruminant diets to improve production efficiency (Callaway and Martin, 1996). However, in recent years there has been an increasingly concern regarding the use of antibiotics in ruminant feeding. From January 2006, the European Union banned all antibiotics used as growth promoters in animal feed in the European market. As a consequence, there is an urgent need for the development of alternatives to the use of these feed additives. Some researchers (Callaway and Martin, 1996; Newbold et al., 1996) have suggested that organic acids (aspartate, fumarate, malate) potentially provide an alternative to currently used antimicrobial compounds.

Fumarate and malate, salts of the four-carbon dicarboxylic acids, are commonly found in biological tissues as intermediates of the citric acid cycle. They can be used by microorganisms to produce propionate. Nisbet and Martin (1990) showed that the growth of *Selenomonas ruminantium* HD4 in a medium that contained L-lactate was stimulated approximately two fold by 10 mmol/L L-aspartate, fumarate or L-malate after 24 h of incubation. Afterwards, much research has been conducted on the effects of organic acids on rumen fermentation. Asanuma et al. (1999) found that the addition of fumarate not only reduced methane production but also increased the production of propionate, succinate or both and slightly increased the production of acetate and butyrate. Carro and Ranilla (2003) showed that fumarate could beneficially affect *in vitro* rumen fermentation of concentrate feeds by increasing final pH and the production of acetate and propionate, but had no apparent effect on L-lactate and NH_3-N (ammonia-N) concentrations in the cultures. Although many studies have associated fumarate with favorably altering *in vitro* ruminal fermentation, little research has been conducted to evaluate the effects of fumarate on ruminal metabolism *in vivo*.

The aim of this study was to investigate the effects of DF on the *in vivo* ruminal fermentation, in addition, the changes of rumen bacterial-community structure was evaluated.

2. Materials and methods

2.1. Animals, diets, and sampling

Four goats (25 ± 2.5 kg body wight (BW)) fitted with ruminal cannulae were randomly distributed in a 4×4 Latin square. The animals were maintained in individual pens with free access to water, but received daily 0 g, 5 g, 10 g and 15 g disodium fumarate (DF),