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Effect of Disodium Fumarate on *In vitro* Rumen Fermentation of Different Substrates and Rumen Bacterial Communities as Revealed by Denaturing Gradient Gel Electrophoresis Analysis of 16S Ribosomal DNA

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ABSTRACT : Two experiments were conducted to investigate the effects of disodium fumarate on the *in vitro* rumen fermentation profiles of different substrates and microbial communities. In experiment 1, nine diets (high-forage diet (forage:concentrate, e.g. F:C = 7:3, DM basis), medium-forage diet (F:C = 5:5, DM basis), low-forage diet(F:C = 1:9, DM basis), cracked corn, cracked wheat, soluble starch, tall elata (*Festuca elata*), perennial ryegrass and rice straw) were fermented *in vitro* by rumen microorganisms from local goats. The results showed that during 24 h incubations, for all substrates, disodium fumarate increased ($p<0.05$) the gas production, and tended to increase ($p<0.10$) the acetate, propionate and total VFA concentration and decrease the ratio of acetate to propionate, whereas no treatment effect was observed for the lactate concentration. The apparent DM loss for tall elata, perennial ryegrass and rice straw increased ($p<0.05$) with the addition of disodium fumarate. With the exception of tall elata, perennial ryegrass and rice straw, disodium fumarate addition increased the final pH ($p<0.05$) for all substrates. In experiment 2, three substrates (a high-forage diet, a medium-forage diet and a high concentrate diet) were fermented by mixed rumen microbes *in vitro*. A polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique was applied to compare microbial DNA fingerprints between substrates at the end of 24 h incubation. The results showed that when *Festuca elata* was used as substrate, the control and disodium fumarate treatments had similar DGGE profiles, with their similarities higher than 96%. As the ratio of concentrate increased, however, the similarities in DGGE profiles decreased between the control and disodium fumarate treatment. Overall, these results suggest that disodium fumarate is effective in increasing the pH and gas production for the diets differing in forage: concentrate ratio, grain cereals and soluble starch, and in increasing dry matter loss for the forages (tall elata, perennial ryegrass and rice straw) *in vitro*, whereas its effect on changes of ruminal microbial community may largely depend on the general nature of the substrate. (**Key Words** : Disodium Fumarate, *In vitro* Fermentation, Denaturing Gradient Gel Electrophoresis)

INTRODUCTION

Antimicrobial compounds are routinely incorporated into ruminant diets to improve production efficiency (Phipps et al., 2000; Singh and Debasis, 2005). However, in recent years there has been an increasing concern regarding the use of antibiotics in ruminant feeding. In 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. Organic acids have been widely regarded as alternatives to currently used antimicrobial compounds in livestock production. In ruminants, fumarate and malate have been shown to be potent in improving rumen fermentation and animal production (Martin, 1998; Khampa et al., 2006). Fumarate and malate, salts of the four-carbon dicarboxylic acids, are commonly found in biological tissues as intermediates of the citric acid cycle. 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. Subsequently, much research has been conducted on the effects of fumarate on rumen fermentation. Asanuma et al. (1999) found that the addition of fumarate not only reduced CH_4 production but also increased propionate, succinate or both and slightly increased acetate and butyrate. Carro and Ranilla (2003) showed that fumarate had a beneficial effect on *in vitro* rumen fermentation of concentrate feeds by increasing final pH and the production of acetate and propionate, while L-

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lactate and NH_3-N concentrations in the cultures were not affected. Although many studies have shown that fumarate and its sodium salts favorably alter ruminal fermentation, little information is available for detailed effects of fumarate on dietary factors such as forage: concentrate ratio, and forage or cereal grain type (Castillo et al., 2004). Yet, no research has been reported about the effect of fumarate on the changes in the rumen bacterial-community structure. Therefore, the aim of this study was to evaluate the effects of disodium fumarate on the *in vitro* fermentation profiles of different substrates, such as forage-concentration combinations, forage or cereal grain, and on the fluctuation of ruminal bacterial community.

MATERIAL AND METHODS

Inocula

Rumen contents were obtained from four rumen-cannulated goats fed forage (medium-quality lucerne hay) *ad libitum* and 200 g concentrate per day administered in two equal portions at 08.00 and 16.00 h. Concentrate was based on maize-soybean meal (70:30, dry matter (DM) basis). Ruminal contents were obtained at 2 h after morning feeding and squeezed through four layers of cheesecloth into a flask with an O_2-free headspace. The flask was not disturbed for 20 min (39°C), allowing feed particles to rise to the top of the flask. The upper portion containing the particles was removed. The resultant lower mixture was used as inocula.

Experiment design

Experiment 1 : Effect of disodium fumarate on *in vitro* fermentation by rumen micro-organisms

Nine diets were used in the present study: a high-forage diet (tall elata-soybean meal-maize grain, 70:21:9, DM basis), a medium-forage diet (tall elata-soybean meal-maize grain, 50:35:15, DM basis), a low-forage diet (tall elata-soybean meal-maize grain, 10:63:27, DM basis), cracked maize, cracked wheat, soluble starch (Nanjing Chemical Company, China), tall elata, perennial ryegrass and rice straw. With the exception of soluble starch, samples of each diet were ground through a 1 mm screen.

Particle-free rumen fluid as an inoculum was anaerobically transferred (20% vol/vol) to a medium (pH 6.7) containing 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4$-$7H_2O$, 764 mg of $CaCl_2$·$2H_2O$, 4,000 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter (Russell and Van Soest, 1984; Russell and Strobel, 1988). After mixing, 50 ml of the buffered rumen fluid was transferred anaerobically to 160 ml serum bottles that contained either no substrate or 0.5 g of the diet described earlier. Disodium fumarate was added to achieve final fumarate concentrations of 7 mmol/L. Control bottles had the same ingredients except for disodium fumarate. Both disodium fumarate treatment and the control had four replicate bottles. Bottles were sealed with rubber stoppers and aluminum caps and incubated at 39°C for 24 h. Gas production was measured at 2, 4, 6, 9, 12, 16, 24 h using a pressure transducer technique (Theodorou et al., 1994). After 24 h of incubation, pH value was measured immediately after each bottle was uncapped, and the fermentation was stopped by swirling the bottles on ice. Bottles were emptied into centrifuge tubes, and the solid residue remaining at the end of fermentation was separated by centrifugation at 12,000×g for 10 min. Supernatant fluid (5 ml) was added to 1 ml deproteinizing solution (metaphosphoric acid, (100 ml/L)) for volatile fatty acid (VFA) analysis and another 5 ml was added to 5 ml 0.5 mol/L-HCl for ammonia-N (NH_3-N) analysis. A sample of the supernatant fraction was taken to analyze concentration of lactate. For the tall elata, perennial ryegrass and rice straw, the solid residues were transferred to pre-weighed filter crucibles, dried at 50°C for 48 h and the apparent disappearance of substrate was calculated.

Experiment 2 : Effect of disodium fumarate on changes of rumen bacterial communities as revealed by denaturing gradient gel electrophoresis analysis

Three substrates, namely (a) a forage diets (*Festuca elata* only), (b) a medium-forages diet (maize grain-soybean meal-tall elata, 30:20:50, DM basis), (c) a concentrate diet (maize grain-soybean meal, 70:30, DM basis), were used in experiment 2. Each substrate was accurately weighed (0.5 g) into a 160 ml serum bottle. Disodium fumarate (7 mmol/L, in medium) was then added to treatment bottles and withheld from controls. Each treatment and its corresponding control had four replicates. The bottles containing 50 ml buffer as used in experiment 1, substrate and disodium fumarate were autoclaved. After cooling, the bottles were pre-warmed (39°C), inoculated with 10 ml rumen contents, and incubated at 39°C. Bottles were withdrawn after 24 h incubation and the fermentation was stopped by swirling the bottles on ice. Solid residues were obtained by centrifugation at 12,000×g for 10 min and re-suspended in phosphate buffer solution (PBS, pH 7.2) and stored at -20°C for further analysis. From the stored solution, 1ml was used for bacterial DNA fingerprint analysis by the PCR technique (Vaughan et al., 1999). DNA was extracted according to a bead-beating method using a mini-bead beater (Biospec Products, USA) and followed by phenol-chloroform extraction (Zoetendal et al., 1998). The solution was then precipitated with ethanol and pellets were suspended in 50 μl of TE.

Primers U968-GC (5'CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC) and L1401 (5'CGG TGT GTA CAA GAC

Table 1. Effects of disodium fumarate on *in vitro* fermentation of diets with a high (HF), medium (MF) and low (LF) forage content by mixed rumen microorganisms

Fumarate (mmol/L)	HF		MF		LF	
	0	7	0	7	0	7
Total gas production (ml/g)	92±3	108±2**	99±4	106±5*	118±6	130±2*
pH	6.21±0.03	6.28±0.01**	6.16±0.03	6.35±0.03**	6.07±0.02	6.17±0.01**
Acetate (mmol/L)	29.68±4.27	32.51±6.06	36.18±4.69	37.72±5.22	44.33±6.38	45.47±4.81
Propionate (mmol/L)	10.36±1.45	17.58±2.16**	15.35±2.51	19.94±3.74**	18.08±4.11	26.35±1.27**
Butyrate (mmol/L)	4.97±0.51	6.47±0.85*	7.63±1.35	6.51±1.66	10.21±2.73	12.23±0.34
TVFA (mmol/L)	45.02±6.03	56.56±8.99**	59.16±8.37	64.16±10.31	72.62±12.45	84.04±6.15
A/P ratio	2.87±0.18	1.84±0.16**	2.37±0.14	1.91±0.20**	2.51±0.38	1.72±0.13**
Lactate (mmol/L)	0.43±0.20	0.36±0.13	0.34±0.08	0.28±0.02	0.38±0.06	0.29±0.04
NH_3-N (mmol/L)	4.95±0.64	6.48±0.56*	5.78±0.69	6.60±0.26*	7.47±1.16	7.11±1.49

For each substrate. * $p<0.05$; ** $p<0.01$.

CC) (Nübel et al., 1996) were used to amplify the V6-V8 regions of the bacterial 16S rDNA. PCR was performed with a *Taq* DNA polymerase kit (Promega, USA). The samples were amplified in a thermocycler (T1 Whatman Biometra, Göttingen, Germany) using the following program: 94°C for 5 min, and 35 cycles of 94°C for 30 s, 56°C for 20 s, 68°C for 40 s, and 68°C for 7 min last extension. Aliquots (5 μl) were analyzed by electrophoresis on 1.2% agarose gel (w/v) containing ethidium bromide to check the sizes and amounts of the amplicons (Zhu et al., 2003).

Amplicons of V6-V8 regions of 16S rDNA were used for sequence-specific separation by DGGE according to the specifications of Muyzer et al. (1993), using a Dcode DGGE system (Bio-Rad, USA). Denaturing gradient gel electrophoresis was performed in 8% polyacrylamide gels containing 37.5:1 acrylamide-biacrylamide and a denaturing gradient of 38-53% of urea. The electrophoresis was initiated by pre-running for 10 min at a voltage of 200 V, and subsequently run at a fixed voltage of 85 V for 12 h at 60°C. The gel was stained with $AgNO_3$ after completion of electrophoresis (Sanguinetti et al., 1994).

Denaturing gradient gel electrophoresis profiles were analyzed for similarities between the samples by a computer program (Molecular Analyst version 1.6, Bio-Rad, California, USA). Similarities between DGGE profiles were determined by calculating band similarity coefficients (SD) (Dice: SD = 2nAB/(nA+nB), where nA is the number of DGGE bands in lane 1, nB represents the number of DGGE bands in lane 2, and nAB is the number of common DGGE bands (Gillan et al., 1998; Simpson et al., 1999).

Analysis of fermentation end products

The VFA concentrations were analyzed according to the method of Qin(1983) by gas chromatography (GC-14A, Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (ULBON HR-52, 0.53 mm i.d.×30 m, 3.0 μm; column temperature = 120°C, injector temperature = 180°C, detector temperature = 180°C). Values were calculated using a Chromatopac data processing system (C-R 4A, Shimadzu). Ammonia-N was measured by the indophenol method (Weatherburn, 1967). Absorbance was measured using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan) at 625 nm. Reaction mixtures contained 1 ml of phenate reagent consisting of 1.1% (v/v) phenol, 0.005% (w/v) sodium nitroprusside and 1 ml alkaline hypochlorite (0.8% (v/v) (sodium hypochlorite (Clorox) and 0.6% (w/v) sodium hydroxide). Concentration of lactic acid was analyzed following the method described by Baker and Summerson (1941). Absorbance was measured using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan) at 560 nm.

Statistical analysis

Data were analyzed by hypothesis tests procedure (SAS). Differences between treatments were tested for significance using a two-tailed t-test at the 5% level.

RESULTS

Experiment 1

Effects of disodium fumarate on fermentation of diets differing in forage: concentrate ratio : As shown in Table 1, for all substrates disodium fumarate treatment increased ($p<0.05$) the gas production, pH and propionate production, and reduced ($p<0.05$) the ratio of acetate to propionate, whereas no significant effect ($p>0.10$) was observed on lactate concentration. The addition of disodium fumarate tended ($p<0.10$) to increase the acetate and total VFA (TVFA) production. Ammonia-N (NH_3-N) concentration was increased ($p<0.05$) with fumarate addition when the HF and MF diets were incubated.

Effect of disodium fumarate on fermentation of maize, wheat or soluble starch : When mixed ruminal microorganisms were incubated with wheat, maize and soluble starch (Table 2), for all substrates the addition of

Table 2. Effect of disodium fumarate on the fermentation of wheat, maize and soluble starch by mixed ruminal microorganisms

Fumarate (mmol/L)	Wheat		Maize		Soluble starch	
	0	7	0	7	0	7
Total gas production (ml/g)	171±5	186±5**	175±6	198±5**	116±4	131±4**
pH	5.18±0.04	5.34±0.01**	5.32±0.02	5.40±0.02**	5.69±0.03	5.88±0.13**
Acetate (mmol/L)	49.60±7.22	68.74±7.77*	49.21±4.53	55.64±7.39	50.28±6.36	51.73±8.71
Propionate (mmol/L)	31.93±4.27	49.91±3.71*	26.45±2.89	29.51±2.00	25.74±4.27	29.24±4.35
Butyrate (mmol/L)	39.90±4.29	41.77±4.07	28.40±3.84	20.08±1.18*	11.60±2.09	7.52±1.21
TVFA (mmol/L)	121.42±11.72	160.42±14.15**	104.06±9.07	105.23±9.31	87.62±7.37	88.48±13.98
A/P ratio	1.56±0.17	1.38±0.05	1.87±0.20	1.89±0.20	2.01±0.47	1.77±0.13
Lactate (mmol/L)	0.38±0.08	0.33±0.01	0.47±0.10	0.41±0.04	0.50±0.18	0.42±0.22
NH_3-N (mmol/L)	10.93±0.23	10.94±0.08	10.70±1.06	10.09±0.48	11.10±0.16	11.86±2.47

For each substrate, * $p<0.05$; ** $p<0.01$.

Table 3. Effect of disodium fumarate on the fermentation of tall elata, perennial ryegrass and rice straw by mixed ruminal microorganisms

Fumarate (mmol/L)	Tall elata		Perennial ryegrass		Rice straw	
	0	7	0	7	0	7
Total gas production (mL/g)	57±4	70±2**	78±2	86±2**	57±3	78±3**
pH	6.77±0.02	6.73±0.02	6.60±0.02	6.62±0.03	6.79±0.04	6.78±0.03
Acetate (mmol/L)	32.88±7.04	42.34±7.6	31.81±1.31	36.72±4.37	35.64±3.80	44.76±2.75
Propionate (mmol/L)	10.13±1.85	16.95±5.31**	12.13±0.63	15.49±0.45**	14.55±2.60	13.54±2.40
Butyrate (mmol/L)	5.14±0.80	5.82±1.83	5.35±0.57	5.20±0.55	5.04±0.81	5.57±0.41
TVFA (mmol/L)	48.14±9.58	65.12±4.55**	49.29±2.03	57.42±4.10**	55.24±7.21	63.87±4.76
A/P ratio	3.23±0.15	2.44±0.26**	2.62±0.07	2.37±0.29**	3.35±0.39	2.48±0.20**
lactate (mmol/L)	0.22±0.08	0.20±0.06	0.16±0.01	0.21±0.01	0.15±0.03	0.18±0.01
NH_3-N (mmol/L)	12.73±1.27	11.55±0.71	14.27±0.32	14.00±0.64	9.49±0.34	9.39±0.61
Dry matter loss (%)	40.47±4.15	51.08±3.14*	47.24±1.68	53.93±2.84*	32.16±2.02	41.34±2.86**

For each substrate, * $p<0.05$; ** $p<0.01$.

disodium fumarate increased ($p<0.05$) gas production and pH, whereas no treatment effects ($p<0.05$) were observed for lactate, NH_3-N concentration and the ratio of acetate to propionate. Disodium fumarate treatment tended to ($p<0.10$) increase the acetate, propionate and TVFA production, although no significant effect ($p>0.05$) was observed for the maize and soluble starch.

Effect of disodium fumarate on fermentation of tall elata, perennial ryegrass and rice straw : When mixed ruminal microorganisms were incubated with tall elata, preminal ryegrass or rice straw (Table 3), for all substrates disodium fumarate treatment increased ($p<0.05$) the gas production and the apparent DM loss, and decreased ($p<0.01$) the ratio of acetate to propionate, whereas no treatment effects ($p>0.10$) were observed for pH, lactate, NH_3-N and butyrate concentrations. There was no significant change ($p>0.05$) in propionate production with added disodium fumarate for rice straw, but disodium fumarate tended to increase ($p<0.10$) the acetate and TVFA concentration for all substrates.

Experiment 2

Effect of disodium fumarate on changes of rumen bacterial communities : As shown in Figure 1 and 2, after 24 h fermentation, DGGE profiles showed that some bands (such as indicated as B, C and D) became predominant, and, some dominant bands (such as indicated as A) apparently disappeared in the treatment as compared to its corresponding control. Similarity analysis indicated that with substrates of forage only or low concentrate, the control and treatments had similar DGGE profiles, with similarities higher than 96%. But, as the ratio of concentrate increased, the similarities between the treatment and the control decreased, with 91% for concentrate diet (70% maize+30% soybean combination) versus 96.7% for tall elata only.

DISCUSSION

Fumarate is a key intermediate in the succinate-propionate pathway, which is used by microorganisms such as *Selenomonas ruminantium* to synthesize succinate and propionate (Martin, 1998). In this pathway, fumarate is reduced to succinate and succinate is decarboxylated to propionate. In the present study, for most substrates disodium fumarate addition increased the propionate production. Unlike other additives, such as ionophores, which could increase propionate at the expense of acetate (Russell and Strobel, 1989), organic acids, particularly fumarate, can be converted into propionate and acetate

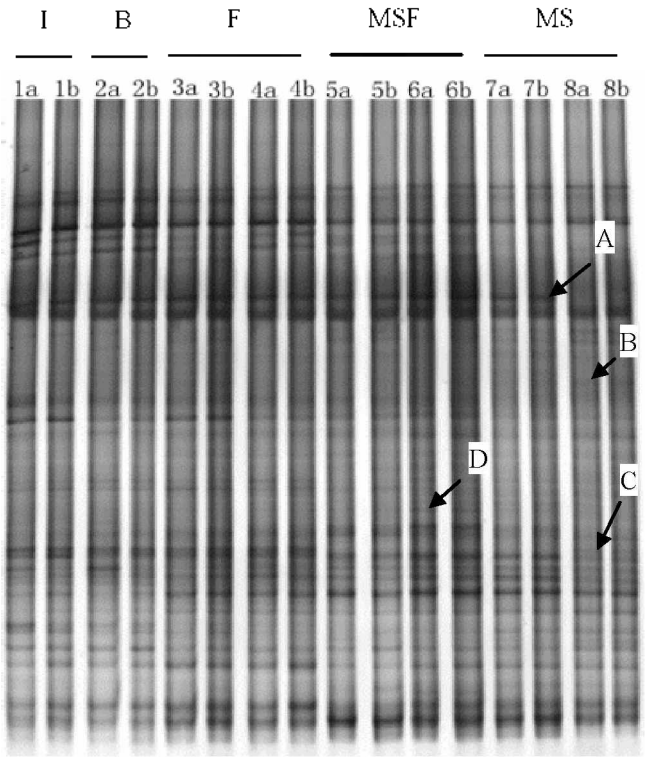

Figure 1. DGGE band pattern of bacteria community profiles of samples. I: inoculum; B: blank; F: *Festuca elata*; MSF: diets based on maize grain, soybean meal and *Festuca elata*, MS: diets based on maize grain and soybean meal; a, b: replicate; DGGE lanes: 1a and 1b, inoculum; 2a and 2b, blank after incubation; 3a and 3b, F; 4a and 4b, F+disodium fumarate; 5a and 5b, MSF; 6a and 6b, MSF+disodium fumarate; 7a and 7b, MS; 8a and 8b, MS +disodium fumarate.

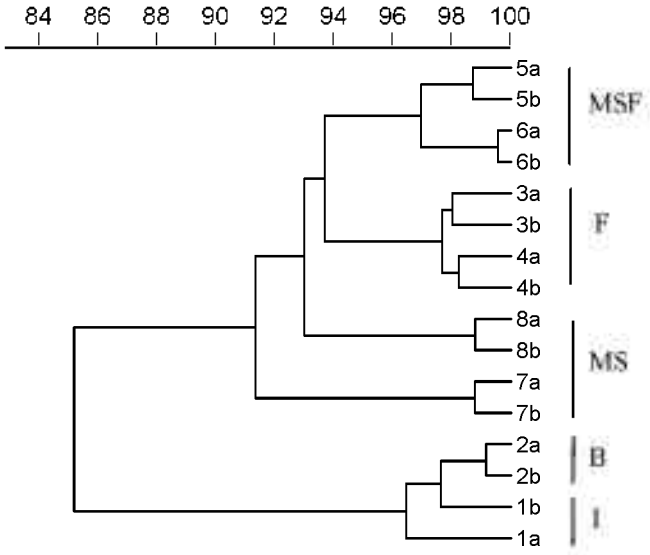

Figure 2. Similarity index of bacterial fingerprints. I: inoculum; B: blank; F: *Festuca elata*; MSF: diets based on maize grain,soybean meal and *Festuca elata*. MS: diets based on maize grain and soybean meal; a, b: replicate; 1a and 1b, inoculum; 2a and 2b, blank after incubation; 3a and 3b, F; 4a and 4b, F+disodium fumarate; 5a and 5b, MSF; 6a and 6b, MSF+disodium fumarate; 7a and 7b, MS; 8a and 8b, MS+disodium fumarate.

following different pathways. Thus, in the present study, although the value of the ratio of acetate to propionate was reduced by the addition of disodium fumarate as compared with control values, for all substrates with the exception of maize, the supplementation with disodium fumarate did not reduce the production of acetate, in agreement with previous reports (López et al., 1999; Carro and Ranilla, 2003).

In agreement with previous reports (Asanuma et al., 1999; López et al., 1999; Carro and Ranilla, 2003) with diets of varying composition, disodium fumarate addition increased ($p<0.001$) final pH and gas production for grain cereals, soluble starch and the diets consisting of different forage: concentrate ratio. As suggested by Callaway and Martin (1996), malate may act to buffer rumen contents by a dual mechanism of increased lactate utilization and CO_2 production by *S. ruminantium*. In the present experiment, disodium fumarate addition did not affect ($p<0.05$) lactate concentrations, although it increased the total gas production with all the diets.

In the present study, disodium fumarate addition significantly increased the apparent DM loss for the forages. The reason for this increase is not clear. López et al. (1999) showed that fumarate stimulated the numbers of cellulolytic bacteria threefold in the Rusitec. Asanuma et al. (1999) and López et al. (1999) observed that fumarate could stimulate the growth of *S. ruminantium*, some fumarate-utilizing bacteria such as *Fibrobacter succinogenes*, *Veillonella parvula*, *Wollinella succinogenes*, and cellulolytic bacteria *in vitro*. Thus a probable mechanism is that disodium fumarate provides a substrate for those bacteria that can utilize the fumarate, and those fumarate-utilizing bacteria accelerated the metabolism of the other intermediate products such as hydrogen. It is believed that stimulating the growth of those fumarate-utilizing bacteria could influence the composition of the bacterial community. To gain insight into the effect of disodium fumarate on the different substrates, a PCR-DGGE approach was adopted to monitor the change in the rumen bacterial community. Similarity analysis can illustrate the differences in DGGE profiles between the control and fumarate treatment. With forage only as substrate, the control and the treatments had similar DGGE profiles, with similarity higher than 96%, but as the ratio of concentrate increased, the DGGE similarities between the control and the treatments declined. Thus, it seems that the effect of disodium fumarate was more profound with substrate of high concentrate. This indicated that the effect of disodium fumarate on rumen bacterial-community composition might largely depend on the nature of the fermentative substrate, being more effective on bacterial-community composition with high-concentrate

diets than with forage-based diets.

As an intermediate in the propionate pathway, fumarate can be reduced to succinate by fumarate reductase. Reducing equivalents are needed in this reaction and therefore fumarate may provide an alternative electron sink for hydrogen. Fumarate and other dicarboxylic acids also seem to stimulate the growth and activity of the lactic acid-utilizing rumen bacterium *S. ruminantium* (Nisbet and Martin, 1990), providing an electron sink for this organism (Martin and Park, 1996). Therefore, fumarate may change the rumen fermentation by redirecting the hydrogen produced during rumen fermentation. In addition, Asanuma et al. (1999) showed that 30 mmol/L-fumarate increased the growth of *Fibrobacter succinogenes*, *S. ruminantium*, *Veillonella parvula*, *Selenomonas lactilytica* and *Wolinella succinogenes* in pure cultures. López et al. (1999) found a significant increase in the number of cellulolytic bacteria when 7.35 mmol/L-fumarate was added to semi-continuous fermenters. In this present study, the DGGE profile showed that some predominant bands appeared (such as indicated as B, C and D) or disappeared (such as indicated as A) in the treatment as compared to its corresponding control; this indicated that disodium fumarate may stimulate or inhibit the growth of some rumen bacteria. Therefore, disodium fumarate treatment may act to affect rumen fermentation by three mechanisms, (i) stimulating or inhibit the growth of some rumen bacteria, (ii) redirecting the hydrogen produced during the rumen fermentation, (iii) buffering ruminal pH.

In summary, disodium fumarate was effective in increasing the pH and gas production for the diets differing in forage: concentrate ratio, grain cereals and soluble starch, and increasing the dry matter loss for the forages (tall elata, perennial ryegrass and rice straw) *in vitro*. Disodium fumarate tended to be more effective on rumen bacterial community composition for high-concentrate than for forage-based diets. Nevertheless, these effects need to be confirmed by animal trials and further research is needed to explore the potential of disodium fumarate as an effective alternative to currently used antimicrobial compounds in animals fed high-concentrate diets or forage-based diets.

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