Effect of Levels of Sodium DL-malate Supplementation on Ruminal Fermentation Efficiency of Concentrates Containing High Levels of Cassava Chip in Dairy Steers

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ABSTRACT : Four rumen-fistulated dairy steers were randomly assigned according to a 4×4 Latin square design to investigate effects of supplementation levels of sodium dl-malate in concentrates on rumen ecology, ruminal fermentation, nitrogen balance, feed intake and digestibility of nutrients and ruminal microbial protein synthesis. The dietary treatments were cassava concentrate-based, containing sodium dl-malate supplementation at 0, 9, 18 and 27 g/hd/d with urea-treated rice straw (UTS) fed *ad libitum*. The experiment was conducted for four periods, each period lasting 21 days. Ruminal pH increased with incremental addition of malate ($p<0.05$). Additionally, molar proportions of propionate were higher in supplemented groups and was highest at 18 g/hd/d of malate supplement ($p<0.05$). Microbial protein synthesis tended to be higher in dairy steers receiving sodium dl-malate supplements and also was the highest at 18 g/hd/d. Variable bacterial populations, such as amylolytic, proteolytic and cellulolytic species were increased ($p<0.05$). Furthermore, protozoal populations were decreased significantly ($p<0.05$), while fungal zoospores were dramatically increased in dairy steers receiving sodium dl-malate supplement ($p<0.05$). These results suggested that supplementation of concentrate containing a high level of cassava chip at 18 g/hd/d with UTS in dairy steers could improve rumen fermentation efficiency and rumen microbial protein synthesis. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 368-375)***

Key Words : Sodium DL-malate, Rumen Fermentation, Microbial Protein Synthesis, Urea-treated Rice Straw, Dairy Steers, Ruminant

INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) is an important cash crop widely grown in sandy loam soils receiving low fertilizer application in the dry season. Cassava production in tropical areas has a potential for increased use in ruminant nutrition. In addition, cassava root contains high levels of energy but minimal levels of crude protein which has been used as an energy source in ruminant diets (Wanapat et al., 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005).

Some authors (Callaway and Martin, 1996) have suggested that organic acids (aspartate, fumarate, malate) could potentially provide an alternative to currently used antimicrobial compounds. Thus, malate supplementation in ruminant diets has been shown to increase nitrogen retention in sheep and steers, and to improve average daily gain and feed efficiency in bull calves (Sanson and Stallcup, 1984). Moreover, malic acid is a key intermediate in the production of succinate or propionate in some ruminal bacteria and therefore could stimulate propionate production. In fact, propionate production increased by adding malate to *in vitro* cultures (Martin and Streeter, 1995). Nisbet and Martin (1991) hypothesized that malate might act as an electron sink for hydrogen. However, the mechanism of action is not yet well understood. The objective of this experiment to investigate the use of levels of malate supplementation with concentrate containing a high level of cassava chip on ruminal fermentation efficiency of dairy steers fed urea-treated rice straw (UTS).

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MATERIALS AND METHODS

Animals, diets and experimental design

Four-fistulated dairy steers (Holstein Friesian-based, 250±10 kg BW) were randomly assigned according to a 4×4 Latin Square Design to investigate the supplementation levels of sodium dl-malate in concentrates on rumen ecology, ruminal fermentation, nitrogen balance, feed intake and digestibility of nutrients and ruminal microbial protein synthesis. The dietary treatments were as follows: supplementation with sodium dl-malate at 0, 9, 18 and 27 g/hd/d, respectively.

Urea-treated rice straw (UTS) was prepared by using 5% (w/w) urea mixed with 100 kg of water in 10 kg of rice straw (RS) batches (50:50, water to straw) and poured over a stack of straw and then covered with a plastic sheet for a minimum of 10 days before feeding to animals (Wanapat, 1990).

Table 1. Ingredients composition of concentrate and urea-treated rice straw (UTS) used in the experiment (%DM basis)

Item	Concentrate	Urea-treated rice straw (UTS)
Ingredient (% DM)		
Cassava chips	70.0	
Cottonseed meal	8.5	
Brewer's grains	4.0	
Molasses	5.0	
Urea	4.0	
Tallow	4.5	
Sulphur	1.0	
Salt	1.0	
Limestone	1.0	
Mineral mix	1.0	
Analyzed composition (%)		
OM	90.9	91.0
CP	17.9	7.0
TDN[a]	80.0	54.1
NDF	37.5	71.1
ADF	11.5	53.3

[a] TDN = dig CP+dig CF+dig NFE+dig EE (2.25).

Concentrates were offered at 1.5% of body weight/hd/d and UTS 5% was offered *ad libitum* as a roughage source. All animals were kept in individual pens and received free choice of water. The experiment was conducted for four periods, each period was lasted 21 days. During the first 14 days, all animals were fed their respective diets, while during the last 7 days, the animals were in metabolism crates for total collection during which they were restricted to 90% of the previous voluntary feed intake of straw. Chemical and composition of concentrate and UTS used are shown in Table 1.

Data collection, sampling procedures and statistical analysis

Feeds were regularly sampled and faecal samples were collected from the total collection of each individual cow on each treatment during the last 7 days of each period. Composites samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ether extract, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Goering and Van Soest, 1970) and AIA. AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Rumen fluid and jugular blood samples were collected at 0, 1, 2, 4, 6 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by using a 60-ml hand syringe at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. The samples were divided into three portions. The first portion was used for ammonia-nitrogen (NH_3-N) analysis where 5 ml of H_2SO_4 solution (1 M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000×g for 15 minutes (Table Top Centrifuge PLC-02, USA) and supernatant was stored at -20°C prior to NH_3-N and volatile fatty acid (VFAs) analyses using a HPLC (Instruments by controller water model 600 E; water model 484 UV detector; column novapak C_{18}; column size 4 mm× 150 mm; mobile phase 10 mM H_2PO_4 (pH 2.5)) according to Zinn and Owens (1986). Second portion was fixed with 10% formalin solution in normal saline (0.9% NaCl, Galyean, 1989). The total direct count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco). Third portion was taken to study cultured groups of viable bacteria using roll-tube technique (Hungate, 1969), for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 500×g for 10 minutes (Table Top Centrifuge PLC-02, USA) and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (HPLC) (Instruments by controller water model 600 E; water model 484 UV detector; column novapak C_{18}; column size 4 mm×150 mm; mobile phase 10 mM H_2PO_4 (pH 2.5)) as described by Chen et al. (1993).

Statistical analyses were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Means with a significant *F* ($p<0.05$) for treatment were statistically compared using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Apparent digestibility and feed intake

Intake of UTS by dairy steers was higher in the treatment receiving 18 g/hd/d which was significantly higher than those receiving 0 or 27 g/hd/d, respectively (Table 2). The concentrate containing a high level of cassava chip is highly degradable in the rumen and could decrease ruminal pH. When ruminal pH was reduced below 6.3 in dairy cows, ADF digestion could be decreased at 3.6% unit per 0.1 pH and may result in depressed feed intake (Erdman, 1998). Apparent digestibilities (%) of DM, OM, CP and NDF were not significantly different ($p>0.05$) between treatments; however, ADF digestibilities in all the malate treatments tended to be higher but were not significantly different ($p>0.05$). It is possible that the high fibrous fraction (ADL) could have attributed to a lower digestibility (Hart and Wanapat, 1992; Wanapat et al.,

Table 2. Apparent digestibility and feed-intake of dairy steer fed different level of sodium dl-malate supplementation

Item	Levels of sodium dl-malate supplementation (g/hd/day)				SEM[g]	Contrast[f]		
	0	9	18	27		L	Q	C
DM intake (kg/hd/d[e])								
UTS	2.2[a]	2.3[b]	2.5[b]	2.2[a]	0.15	NS	**	**
Conc.	2.9[a]	3.1[b]	2.6[c]	3.1[b]	0.04	**	**	**
Total DM intake/d								
% BW	2.6	2.6	2.8	2.6	0.04	NS	NS	NS
g/kg $BW^{0.75}$	96.9	101.6	101.1	98.3	3.42	NS	NS	NS
Apparent total-tract digestibility (%)								
DM	78.8	79.6	77.1	78.1	1.86	NS	NS	NS
OM	79.9	81.2	80.1	81.1	2.63	NS	NS	NS
CP	63.5	66.3	67.9	67.8	0.95	NS	NS	NS
NDF	72.9	74.2	72.5	73.6	2.34	NS	NS	NS
ADF	60.2	68.5	67.4	68.9	3.11	NS	NS	NS

[a, b, c, d] Values on the same row with different superscripts differ ($p<0.05$).
[e] UTS = Urea-treated rice straw; Conc. = Concentrate.
[f] Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects of levels sodium dl-malate.
[g] Standard error of the mean.
* $p<0.05$; ** $p<0.01$; NS: $p>0.05$.

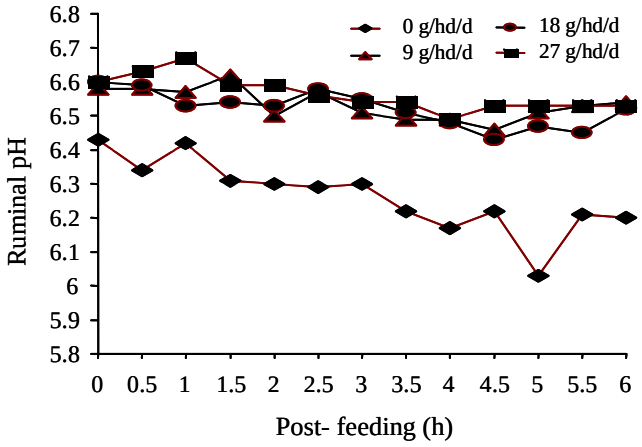

Figure 1. Effect of levels of sodium dl-malate supplementation in concentrate containing high level of cassava chip on ruminal pH in dairy steers.

2000), especially the large proportion of lignified cell walls with low fermentation rate and digestibility, leading to a low rate of disappearance through digestion or passage and limited feed intake. Mertens (1977) concluded that changes in the composition of cell wall involving lignin and possibly silica limited the potential extent of digestion whereas the rate of digestion is limited by the chemical entities other than by crystalline or physical nature of fiber.

Rumen fermentation parameters

Rumen temperature, pH, NH_3-N and BUN concentrations are presented in Table 3. Ruminal pH was significantly affected ($p<0.05$) by treatment (Figure 1). As shown, incorporation of concentrates containing a high level of cassava chip with sodium dl-malate supplementation at 9, 18 and 27 g/hd/d lead to as higher and more stable pH values than on the control treatment. All were within the normal range (6.54 to 6.56), which range has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.5 to 7.0) (Firkins, 1996). It has been suggested that the presence of organic acids such as malate or fumarate may increase the inter-species hydrogen transfer by enhancing the disposal of reducing equivalents produced by carbohydrate-fermenting bacteria (Rogers and Withman, 1991). As hydrogen is removed and used to convert these organic acids into propionate, the feedback effect on the glycolysis pathway is neutralized and the fermentation process might be significantly enhanced. This would result in an increased carbon turnover, an increased growth of microorganisms and an increased efficiency of energy extraction from the fermented organic matter in the rumen (Rogers and Withman, 1991). Moreover, Nisbet and Martin (1993) showed that adding malate to *in vitro* cultures stimulated the growth of the predominant ruminal bacteria *Selenomonas ruminantium*, which can account for up to 51% of the total viable bacteria in the rumen. These authors reported that malate increased lactate utilization by *S. ruminantium* and CO_2 production, thus buffering the ruminal contents. Therefore, malate treatment may act to increase the pH of ruminal contents by dual mechanism of increased lactate utilization and CO_2 production by *S. ruminantium*.

The results from the present experiment are in agreement with this suggestion, as the addition of malate could result in a decrease ($p<0.05$) of the daily production of lactate and an increase in the production of carbon dioxide. Increased ruminal NH_3-N levels were found ($p>0.05$) as level of sodium dl-malate increased in the diets and were closer to the optimal ruminal NH_3-N (15 to 30 mg%, Perdok and Leng, 1990; Wanapat and Pimpa, 1999; Chanjula et al., 2003, 2004) for increasing microbial protein

Table 3. Rumen temperature, NH_3-N, BUN and volatile fatty acid (VFA) concentrations after feeding of dairy steer fed different level of sodium dl-malate supplementation

Item	Levels of sodium dl-malate supplementation (g/hd/day)				SEM[f]	Contrast[e]		
	0	9	18	27		L	Q	C
Ruminal temperature (°C)	39.7	40.2	39.9	39.8	0.26	NS	NS	NS
Ruminal pH	6.2[a]	6.5[b]	6.6[b]	6.5[b]	0.04	**	NS	NS
NH_3-N (mg %)	11.4[a]	16.2[b]	17.7[b]	16.2[b]	2.87	**	NS	NS
BUN (mg %)	7.1[a]	10.3[b]	9.0[ab]	9.1[ab]	1.10	**	NS	NS
Total VFA (mM/L)	107.2[a]	118.2[b]	119.2[b]	118.3[b]	1.03	*	NS	NS
Molar proportion of VFA (mol/100 mol)								
Acetate	71.2[a]	68.5[b]	66.7[c]	67.2[b]	0.37	**	NS	NS
Propionate	19.7[a]	22.4[b]	24.4[c]	22.9[b]	0.34	**	NS	NS
Butyrate	9.2	9.1	8.9	9.3	0.26	NS	NS	NS
Acetate:propionate ratio	3.6[a]	3.1[b]	2.7[c]	3.0[b]	0.04	**	NS	NS
Acetate+butyrate:propionate ratio	4.1[a]	3.5[b]	3.1[c]	3.4[b]	0.05	*	NS	NS

[a, b, c, d] Values on the same row with different superscripts differ ($p<0.05$).

[e] Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects of levels sodium dl-malate.

[f] Standard error of the mean.

* $p<0.05$; ** $p<0.01$; NS: $p>0.05$.

synthesis, feed digestibility and voluntary feed intake lowest in 18 g of treatment. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN and the values were linearly increased as levels of sodium dl-malate increased in the diets. The differences in NH_3-N and BUN concentrations among treatments may have been directly related to the CP levels of the concentrate. Concentrations of BUN were reported to be highly correlated to protein intake and reflected the level of ammonia production in the rumen (Preston et al., 1965). This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis (Table 3). This study revealed that inclusion of malate in the concentrate has increased NH_3-N concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation (Satter and Slyter, 1974). Therefore, increasing ruminal NH_3-N concentration by dietary additions of concentrate containing high level of cassava chip with sodium dl-malate should improve efficiency of digestibility by ruminants, resulting in maximum intake and digestibility of low quality roughage. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN. The differences in NH_3-N and BUN concentrations among treatments may have been directly related to CP levels in concentrate.

The influence of sodium dl-malate level supplementation on total VFA concentrate, production of total VFA, acetic acid proportion, propionic acid proportion, butyric acid proportion and acetic to propionic ratio are shown in Table 3. Mean total VFAs and propionate concentrations in the rumen were increased with increasing levels of malate in the diet. The observed reduction in pH associated with increased concentrate feeding was associated with increased VFA concentrations. In general, increasing concentration of VFA and lactic acid in rumen was effected on ruminal pH lower; however, these experiment as shown ruminal pH quite stable values in optimal range. It may be due to some microorganisms in rumen especially *S. ruminantium* can using lactic acid to produce propionic acid which could maintain pH in rumen. In addition, when ruminal pH increased which can stimulation of chewing roughage and increasing nitrogen return into rumen via salivation also increase ammonia-nitrogen in rumen. Martin et al. (1994) have reported that increasing the starch content of the concentrate causes a depression in rumen pH. Moreover, increasing the readily degradable starch content of the concentrate resulted in higher rumen propionate concentrations in accordance with the results of Sutton et al. (1993) and decreased rumen acetate concentration. However, this study found that total VFA concentration in all diets ranged from 70 to 130 mM, the range suggested by France and Siddons (1993). Thus, although the acetate to propionate ratio was decreased ($p<0.05$) by the addition of malate compared to control (Table 3), the supplementation with malate increased the daily output of propionate without decreasing the production of acetate, in agreement with the results reported by other authors (Callaway and Martin, 1996). Moreover, Demeyer and Henderickx (1967) have proposed that the balance between acetate and propionate production from organic acids depends on the relative proportions of both fumarate (formed from malate) and pyruvate.

Rumen microorganism populations

Incorporation of sodium dl-malate with concentrate containing high level of cassava chip has shown a trend to increase cellulolytic, proteolytic and amylolytic bacterial populations while total protozoal counts were decreased ($p<0.05$, Table 4). The cassava chip can be readily degraded in the rumen and ruminal pH was decreased, malate could

Table 4. Effect of levels of sodium dl-malate supplementation in concentrate containing high level of cassava chip on ruminal bacteria, protozoa, fungi population, total variable, amylolytic, proteolytic and cellulolytic bacteria in dairy steers

Item	Levels of sodium dl-malate supplementation (g/hd/day)				SEM[e]	Contrast[d]		
	0	9	18	27		L	Q	C
Rumen microbes (cell/g)								
Bacteria ($\times10^{12}$)	6.0[a]	13.2[bc]	11.6[b]	15.2[b]	1.18	**	**	**
Protozoa ($\times0^{6}$)	1.8[a]	1.1[b]	1.5[ab]	1.2[b]	0.23	NS	*	NS
Fungal zoospores ($\times10^{7}$)	0.7[a]	1.1[ab]	1.5[b]	1.4[b]	0.20	**	NS	NS
Variable bacteria (CFU/g)								
Total ($\times10^{9}$)	4.3[a]	6.5[b]	8.2[c]	14.9[d]	0.04	**	**	**
Amylolytic ($\times10^{9}$)	1.6[a]	1.9[a]	5.3[b]	4.7[b]	0.69	*	NS	NS
Proteolytic ($\times10^{9}$)	1.0[a]	1.6[b]	2.0[c]	2.3[d]	4.53	**	NS	NS
Cellulolytic ($\times10^{9}$)	1.7[a]	3.0[b]	2.9[b]	6.9[c]	0.28	*	NS	**

[a, b, c] Values on the same row with different superscripts differ ($p<0.05$).
[d] Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects of levels sodium dl-malate.
[e] Standard error of the mean.
* $p<0.05$; ** $p<0.01$; NS: $p>0.05$.

Table 5. Nitrogen balance, excretion of purine derivatives (PD) and microbial nitrogen supply in dairy steer given levels sodium dl-malate supplementation

Item	Levels of sodium dl-malate supplementation (g/hd/day)				SEM	Contrast[e]		
	0	9	18	27		L	Q	C
Nitrogen balance (g/d)								
N intake	105.6	108.5	108.0	106.1	6.86	NS	NS	NS
Fecal N	20.1[a]	17.2[ab]	15.1[b]	19.0[a]	1.12	**	NS	NS
Urinary N	19.9	19.1	15.3	19.7	2.57	NS	NS	NS
N absorption	85.4[a]	91.2[b]	92.8[b]	87.0[a]	1.12	**	NS	NS
N retention	65.5[a]	72.1[ab]	77.5[b]	67.3[a]	2.07	**	NS	NS
PD (mM/d)								
Allantoin excretion	158.0[a]	230.8[ab]	301.0[b]	180.3[a]	23.98	**	NS	NS
Allantoin absorption	166.9[a]	254.7[ab]	337.7[b]	195.6[a]	27.84	**	NS	NS
Microbial N supply (g N/d[c])	121.3[a]	185.2[ab]	245.5[b]	142.2[a]	20.25	**	NS	NS
EMNS (g N/kg of OMDR[d])	36.2[a]	47.4[a]	73.1[b]	41.3[a]	5.76	**	NS	*

[a, b] Values on the same row with different superscripts differ ($p<0.05$).
[c] Microbial N (g N/day) = $(X\times70)/(0.116\times0.83\times1,000) = 0.727\times X$ (where, X = total absorption of purine derivatives).
[d] Efficiency of microbial protein supply (g N/kg of OMDR), organic matter digestible in the rumen (kg) = 65% of organic matter digestible in total tract.
[e] Orthogonal contrasts included linear (L), quadratic (Q), and cubic (C) effects of levels sodium dl-malate.
* $p<0.05$; ** $p<0.01$; NS: $p>0.05$.

stimulate lactate utilization by *S. ruminantium* and could improve pH in the rumen. It is possible that supplementation with sodium dl-malate may play an important role in increasing bacterial populations. Moreover, Martin et al. (1999) reported that increasing dietary concentrations of malate might help to reduce problems associated with ruminal acidosis by stimulating lactate utilization by *S. ruminantium*. On the other hand, Stallcup (1979) and Kung et al. (1982) reported a positive response in milk production by dairy cows fed diets supplemented with malate. Malate altered the *in vitro* fermentation by mixed ruminal microorganisms of soluble starch (Martin and Streeter, 1995) or cracked corn (Callaway and Martin, 1996).

However, the mode of action of malate appears to be completely different, and in contrast to antimicrobial compounds; it seems to stimulate rather than inhibit some specific ruminal bacterial populations (Nisbet and Martin, 1993). Malic acid is a key intermediate in the production of succinate or propionate in some ruminal bacteria and therefore could stimulate propionate production. In fact, propionate production has been increased by adding malate to *in vitro* cultures (Martin and Streeter, 1995; Callaway and Martin, 1996). Nisbet and Martin (1991) hypothesized that malate might act as an electron sink for hydrogen. However, the mechanism of action is not yet well understood.

The effect of malate addition on *in vitro* ruminal fermentation has been studied in short-term experiments (incubations of up to 24 h), using batch cultures of mixed ruminal microorganisms. However, little is known about longer-term effects of malate on *in vitro* ruminal fermentation (Martin and Streeter, 1995). In addition, The work of Newbold et al. (1996) showed that feeding 100 mg of malate per day to sheep caused an increase in the number of total bacteria and tended to increase the population of

cellulolytic bacteria.In agreement with these observations, Lopez et al. (1999) reported that fumarate (another intermediate in the succinate to propionate pathway) increased the number of cellulolytic bacteria almost three-fold during fermentation in the RUSITEC system. It has been suggested that the presence of organic acids such as malate or fumarate may increase the inter-species hydrogen transfer by enhancing the disposal of reducing equivalents produced by carbohydrate-fermenting bacteria (Rogers and Withman, 1991). As hydrogen is removed and used to convert these organic acids into propionate, the feedback effect on the glycolysis pathway is neutralized and the fermentation process might be significantly enhanced. This would result in an increased carbon turnover, an increased growth of microorganisms and an increased efficiency of energy extraction from the fermented organic matter in the rumen (Rogers and Withman, 1991).

Nitrogen balance, urinary excretion of purine derivatives and EMNS

As shown in Table 5, N balance in terms of N absorption and retention were significantly different among treatments. However, N absorbed, N excreted in feces and N in the urine were all lower in animals supplemented with malate than the control group. N retention is considered as the most common index of the protein nutrition status of ruminants (Owens and Zinn, 1988). In this regard, the positive N balance observed in this study indicates the positive influence of the different level of malate supplements with in UTS based feeding systems of dairy cows. However, the differences in the quantity and routes of N excretion with consequent influences on N retention reflect treatment feed differences in N metabolism.

Excretion of allantoin in the urine was significantly ($p<0.05$) different among treatments and was highest at 18 g/hd/d of sodium dl-malate. These results and microbial nitrogen supply in the rumen are summarized in Table 5. The microbial nitrogen supply as calculated from purine derivative excretion using the equation of Chen and Gomes (1995) ranged from 121.2 to 245.5 g N/day/$BW^{0.75}$. However, the rate of digestion of carbohydrates is a major factor controlling the energy available for microbial growth (Hoover and Stokes, 1991). Therefore, adding malate to the diets of ruminants fed high levels of rapidly fermentable carbohydrates may improve the ability of ruminal microbes, especially *S. ruminantium*, to utilize lactate at low pH. Furthermore, the efficiency of rumen microbial protein synthesis was higher in dairy steers fed sodium dl-malate supplement, where the values ranged from 36.2 to 73.1 g N/kg of OMDR. Non-treatment variability in EMNS may be due to various factors like concentration and sources of nitrogen and carbohydrates.

CONCLUSIONS AND RECOMMENDATIONS

Based on this experiment, it could be concluded that supplementation of sodium dl-malate with concentrates containing a high level of cassava chip increased ruminal pH, and altered rumen fermentation by increasing propionate production and decreasing of acetate to propionate ratio. Moreover, the high level of cassava chip in the diet resulted in increased populations of bacteria and fungi, decreased protozoal populations, and improved rumen microbial N supply and EMNS. These results suggest that the combined use of concentrates containing high level of cassava chip with supplementation of sodium dl-malate at 18 g/hd/d could improve rumen ecology and subsequent performance in ruminants. However, further studies of sodium dl-malate supplementation in concentrate containing a high level of cassava chip with UTS based - roughage on bacterial, protozoal and fungal population changes and their ruminal fermentation and productivity should be conducted, particularly in early lactation dairy cows.

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