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Manipulation of Rumen Ecology by Malate and Yeast in Native Cattle

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Abstract: Four, one-year old of native cattle were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study supplementation of malate level at 500 and 1,000 g with yeast *(Saccharomyces cerevisiae)* at 1,000 and 2,000 g in concentrate containing high levels of cassava chip. The treatments were as follows: T1 = supplementation of malate at 500 g with yeast at 1,000 g; T2 = supplementation of malate at 500 g with yeast at 2,000 g; T3 = supplementation of malate at 1,000 g with yeast at 1,000 g; T4 = supplementation of malate at 1,000 g with yeast at 2,000 g in concentrate, respectively. The animals were offered the treatment concentrate at 1% BW of DM and urea-treated rice straw was fed *ad libitum*. The results revealed that concentration of volatile fatty acid was significantly different especially the concentration of propionic acid was slightly higher in cattle receiving T4 than T3, T2 and T1 (23.3, 21.9, 20.9 and 18.0%, respectively). The populations of protozoa and fungal zoospores were significantly different as affected by malate level and yeast. In conclusion, the combined use of concentrate containing high level of cassava chip at 70% DM with malate at 1,000 g and yeast at 2,000 g in concentrate with urea-treated rice straw as a roughage could improved rumen ecology in native cattle.

Key words: Malate, yeast, *Saccharomyces cerevisiae*, cassava chip, rumen ecology, native cattle

INTRODUCTION

The rumen has been well recognized as an essential fermentation that is capable of preparing end-products particularly Volatile Fatty Acids (VFAs) and microbial protein synthesis as major energy and protein for the ruminant host, hence, the more efficient the rumen is, the optimum the fermentation end-products are being synthesized. In recent years, there have been increasing interests, researches conducted as well as reviews in relation to rumen studies, rumen ecology and rumen manipulation (Martin *et al*., 1999; Wanapat, 2003; Khampa *et al*., 2006). In the tropics, most of ruminants have been fed on low-quality roughages, agricultural crop-residues, industrial by-products which basically contained high levels of lingo-celluloses materials, low level of fermentable carbohydrate as well as low level of good-quality protein.

Some strictly anaerobic bacteria use a reductive or reverse citric acid cycle known as the succinate-propionate pathway to synthesize succinate and (or) propionate. Both malate and fumalate are key intermediates in the succinate propionate pathway and *S. ruminantium* uses this pathway (Gottschalk, 1986). The fact dicarboxylic acids, especially malate and fumalate, stimulate lactate utilization is consistent with the presence of this pathway in this ruminal anaerobe (Callaway and Martin, 1996). Previous studies by Sanson and Stallcup (1984) reported that supplementation of malate 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. In addition, supplementing diets with yeast *(Saccharomyces cerevisiae)* increases milk production of dairy cows and weight gain of growing cattle (Brossard *et al*., 2006). Production responses attributed to yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes *et al*., 2007). However, the use of malate and yeast in cassava based-diets has not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of malate and yeast in concentrates containing high level of cassava chip with urea-treated rice straw as a basal roughage on rumen ecology in native cattle.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, one-year old of native cattle weighing at 150±10 kg. Animals were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study two levels malate (500 and 1,000 g) with yeast *(Saccharomyces cerevisiae)* (1,000 and 2,000 g), respectively. The dietary treatments were as follows: T1 = supplementation of malate at 500 g with yeast at 1,000 g; T2 = supplementation of malate at 500 g with yeast at

2,000 g; T3 = supplementation of malate at 1,000 g with yeast at 1,000 g; T4 = supplementation of malate at 1,000 g with yeast at 2,000 g in concentrate, respectively. The composition of dietary treatments and Urea-treated Rice Straw (UTS) used are shown in Table 1, 2.

Animals were housed in individual pens and individually fed concentrate at 1% BW of DM. All animals were fed *ad libitum* of UTS with water and a mineral-salt block. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run in four periods, each experimental period lasted for 21 days, the first 14 days for treatment adaptation and for feed intake measurements whist the last 7 days were for sample collections of rumen fluid and faeces. Body weights were measured daily during the sampling period prior to feeding.

Data collection and sampling procedures: Urea-treated rice straw and concentrate were sampled daily during the collection period and were composted by period prior to analyses. Composites samples were dried at 60 ^{0}C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for Dry Matter (DM), Ether Extract (EE), ash and Crude Protein (CP) content (AOAC, 1985), Neutral-detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) (Goering and Van Soest, 1970).

Rumen fluid samples were collected at 0 and 4 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using (HANNA instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH_3-N analyses where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 g for 15 min and the supernatant stored at -20^{0}C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985). Another portion was fixed with 10% formalin solution in normal saline (Galyean, 1989).

The total count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haematocytometer (Boeco).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for a 4x4 Latin square design with 2x2 Factorial arrangement of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Table 1: Ingredients of concentrate used in the experiment (% DM basis)

	Dietary treatments[1]			
Items	Conc. I	Conc. II	Conc. III	Conc. IV
Ingredient (%DM)				
Cassava chip	70	70	70	70
Palm meal	3	3.5	3	3.5
Soybean meal	10	10	10	10
Molasses	5	5	5	5
Coconut oil	4	4	4	4
Urea	3.5	3	3.5	3
Sulfur	1	1	1	1
Salt	1	1	1	1
Limestone	1	1	1	1
Mineral mix	1.5	1.5	1.5	1.5
Malate (g)	500	500	1,000	1,000
Yeast (g)	1,000	2,000	1,000	2,000

[1]Conc. = concentrate;

Table 2: Chemical composition of concentrates and urea-treated rice straw (UTS) used in the experiment

	Dietary treatments[1]				
Chemical compositions (%)	Conc. I	Conc. II	Conc. III	Conc. IV	UTS
DM	88.7	89.4	88.7	89.4	55.8
OM	91.1	91.2	91.1	91.2	88.9
CP	16.2	16.1	16.2	16.1	7.9
NDF	13.7	12.9	13.7	12.9	73.2
ADF	8.8	7.9	8.8	7.9	52.3
Feed cost (US$/kg)	0.25	0.28	0.28	0.30	0.05

[1]Conc. = concentrate; UTS = urea-treated rice straw. DM = dry matter, CP = crude protein, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber.

RESULTS AND DISCUSSION

Chemical composition of dietary treatments and feed intake: The chemical compositions of roughage and concentrate diets fed in native cattle are presented in Table 2. Concentrate diets contained similar concentrations of DM, OM, CP, NDF, ADF and TDN. Diets containing high levels of cassava chip based diets had a slightly higher Non-structural Carbohydrate (NSC) and lower NDF due to increased level of cassava chip in the diets.

The effects of malate level with yeast *(Saccharomyces cerevisiae)* on feed intake in native cattle are presented in Table 3. Feed intake were non-significantly different among treatments and was higher in native cattle receiving T4 than in those fed T3, T2, T1 (2.8, 2.7, 2.7 and 2.6% BW, respectively). This data indicated that malate level with yeast supplementation had no effect on feed intake in native cattle. This result was in agreement with earlier work which reported that inclusion of cassava chip in diets resulted in satisfactory animal performance and had no negative effects on animal health in finishing beef cattle and lactating dairy cows (Sommart *et al*., 2000, Khampa *et al*., 2006).

Table 3: Effects of malate level and yeast *(Saccharomyces cerevisiae)* on feed intake and rumen fermentation in native cattle

	Treatments[1]				Contrast			
Items	T1	T2	T3	T4	SEM	M	Y	MxY
DM intake (%BW)								
UTS	1.6	1.7	1.7	1.8	0.08	NS	NS	NS
Concentrate	1.0	1.0	1.0	1.0	-	NS	NS	NS
Total	2.6	2.7	2.7	2.8	0.09	NS	NS	NS
Ruminal Temperature (°C)	39.6	39.4	40.1	39.5	0.52	NS	NS	NS
Ruminal pH	6.6	6.6	6.7	6.8	0.14	NS	NS	NS
NH_3-N (mg%)	17.1	18.3	19.4	19.7	1.99	NS	NS	NS
BUN (mg%)	9.5	10.4	12.7	13.1	2.69	NS	NS	NS
Total VFA (mmol/L)	107.2^{a}	118.2^{b}	119.2^{b}	118.3^{b}	1.03	*	NS	NS
Molar proportion of VFA (mol/100mol)								
Acetate (C2)	72.3^{a}	69.6^{b}	68.3^{b}	67.8^{c}	0.37	*	NS	NS
Propionate (C3)	18.0^{a}	20.9^{b}	21.9^{b}	23.3^{c}	0.34	*	NS	NS
Butyrate (C4)	9.7	9.5	9.8	8.9	0.46	NS	NS	NS
C2:C3 ratio	4.0^{a}	3.3^{b}	3.1^{b}	2.9^{c}	0.04	*	NS	NS
C2+C4:C3 ratio	4.5^{a}	3.7^{b}	3.5^{b}	3.2^{c}	0.05	*	NS	NS

^{a,b,c}Values on the same row with different superscripts differ ($p< 0.05$). [1]T1 = malate at 500 g with yeast at 1,000 g. T2 = malate at 500 g with yeast at 2,000 g. T3 = malate at 1,000 g with yeast at 1,000 g. T4 = malate at 1,000 g with yeast at 2,000 g. [2]Probability of main effects of level malate (M) in concentrates (500 vs 1,000 g), levels of yeast (Y) (1,000 vs 2,000 g), or the M x Y interaction. * = $p<0.05$, NS = $p>0.05$.

Table 4: Effects of malate level and yeast on ruminal microorganisms in native cattle

	Treatments[1]				Contrast[2]			
Items	T1	T2	T3	T4	SEM	M	Y	MxY
Total direct counts (cell/ml)								
Bacteria (x10^{11})	6.1^{a}	7.2^{ab}	8.9^{ab}	10.9^{b}	1.22	*	NS	NS
Protozoa								
Holotric (x10^{4})	3.1^{a}	3.0^{a}	2.5^{ab}	2.1^{b}	0.26	*	NS	NS
Entodiniomorph (x10^{5})	10.3^{a}	7.8^{b}	4.1^{c}	3.4^{c}	0.71	*	*	NS
Fungal zoospores (x10^{4})	2.4^{a}	3.6^{a}	5.5^{b}	7.0^{b}	0.51	*	*	NS

^{a,b,c}Values on the same row with different superscripts differ ($p< 0.05$).

Characteristics of ruminal fermentation and blood metabolism: Ruminal NH_3-N and BUN concentrations were not altered by malate level and yeast supplement in diets containing high cassava-based diets (Table 3). As NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen. In addition, the result obtained was closer to optimal ruminal NH_3-N at 15-30 mg% for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminantfed on low-quality roughage (Wanapat and Pimpa, 1999; Chanjula *et al*., 2003, 2004).

The influence of malate level with yeast supplement in concentrates on Volatile Fatty Acid (VFA) such as production of total VFA, acetic acid proportion, propionic acid proportion, butyric acid proportion and acetic to propionic ratio are shown in Table 4. Mean total VFAs and propionate concentrations in the rumen were increased with increasing malate level and yeast in the diet ($p<0.05$). Especially, the concentration of propionic acid was slightly higher in T4 than in those fed T3, T2 and T1, respectively. However, it was found that total VFA concentration in all diets ranged from 70-130 mM (France and Siddons, 1993). Although the acetate to propionate ratio was decreased by the level of sodium dl-malate, but the supplementation of malate level with yeast increased the daily output of propionate without decreasing the production of acetate.

Rumen microorganisms populations: Table 4 presents rumen microorganism populations. The populations of fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria had higher numbers in native cattle receiving diets T4 than T3, T2, T1. In contrast, the present number of protozoa in the rumen was decreased by malate level and yeast supplementation in high cassava-based diets. Previous study found that feeding 100 mg of malate per day in sheep caused an increase in the number of total bacteria and tended to increase the population of cellulolytic bacteria (Newbold *et al*., 1996). Yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion and increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes *et al*., 2007). As cassava chip can be readily degraded in the rumen and ruminal pH was decreased, malate could stimulate lactate utilization by *S. ruminantium* and could improve pH in the rumen.

It is possible that supplementation of malate with yeast may play an important role in increasing bacterial populations. Moreover, increasing dietary concentrations of malate might help to reduce problems associated with ruminal acidosis by stimulating lactate utilization by *S. ruminantium* (Martin *et al*., 1999).

Conclusions: Based on this experiment, it could be concluded that supplementation of malate with yeast *(Saccharomyces cerevisiae)* in concentrate containing high level of cassava chip maintained could improved ruminal fermentation efficiency, increasing propionate production and decreased acetate to propionate ratio. Moreover, high level of cassava chip in diet resulted increase populations of bacteria, but decreased protozoal populations. These results suggest that the combined use of concentrates containing high level of cassava chip at 70% DM with malate at 1,000 g and yeast at 2,000 g in concentrate could highest improved rumen ecology in native cattle.

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