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## Supplementation of Malate Level and Cassava Hay in High-Quality Feed Block on Ruminal Fermentation Efficiency and Digestibility of Nutrients in Lactating Dairy Cows

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**Abstract:** Four, lactating dairy cows 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 and cassava hay in high-quality feed block. The treatments were as follows: T1 = supplementation of high-quality feed block without cassava hay + malate at 500 g; T2 = supplementation of high-quality feed block without cassava hay + malate at 1,000 g; T3 = supplementation of high-quality feed block with cassava hay + malate at 500 g; T4 = supplementation of high-quality feed block with cassava hay + malate at 1,000 g, respectively. The cows were offered the treatment concentrate at a ratio to milk yield at 1:2 and urea-treated rice straw was fed *ad libitum*. The results have revealed that rumen fermentation and blood metabolites were similar for all treatments. The populations of protozoa and fungal zoospores were significantly different as affected by malate level and cassava hay supplementation. In conclusion, the combined use of cassava hay and malate at 1,000 g in high-quality feed block with concentrates containing high levels of cassava chip at 65% DM could highest improved rumen ecology and nutrients digestibility in lactating dairy cows.

**Key words:** Malate, cassava hay, high-quality feed block, rumen fermentation, lactating cows

### INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) production in tropical areas has a potential use in ruminant livestock nutrition and feeding. Cassava root contains high levels of energy and has been used as a source of readily fermentable energy in ruminant rations (Wanapat, 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005). One strategy for using high degradable carbohydrates is to use in combination with readily available NPN sources such as urea. Urea is commonly used as N source when highly soluble carbohydrates are fed and maintained (Wohlt *et al.*, 1978). However, efficient utilization of protein and Non-Protein Nitrogen (NPN) in ruminants depends upon knowledge of the basic principles underlying ruminal microbial N metabolism (Fernandez *et al.*, 1997). Moreover, ruminal pH has great impact on rumen fermentation efficiency (Wanapat, 2003).

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 fumarate are key intermediates in the succinate propionate pathway and *S. ruminantium* uses this pathway (Gottschalk, 1986). The fact dicarboxylic acids, especially malate and fumarate, 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. However, the use of dl-malate and cassava hay in high quality feed block with cassava based-diets has not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of malate levels and cassava hay in high quality feed block with urea-treated rice straw as a basal roughage on ruminal fermentation, nutrients digestibility and milk production in lactating dairy cows.

### MATERIALS AND METHODS

**Animals, diets and experimental design:** Four, Holstein-Friesian crossbred cows (75%) in the second lactation were used in experiment. Milk yield pre-experiment was 10±2 kg/day and the body weight were 350±10 kg. Cows were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study 2 levels dl-malate with cassava hay in high quality feed block supplementation on ruminal fermentation efficiency, digestibility of nutrients and milk production. The dietary treatments were as follows: T1 = supplementation of high-quality feed block without cassava hay + malate at 500 g; T2 = supplementation of

high-quality feed block without cassava hay + malate at 1,000 g; T3 = supplementation of high-quality feed block with cassava hay + malate at 500 g T4 = supplementation of high-quality feed block with cassava hay + malate at 1,000 g, respectively. The composition of dietary treatments, concentrate and Urea-Treated rice Straw (UTS) used are shown in Table 1 and 2.

Cows were housed in individual pens and individually fed concentrate at a ration to milk yield of 1:2, twice daily at 0600 am and 1600 pm after milking. All cows 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 4 periods, each experimental period lasted for 21 days, the first 14 days for treatment adaptation and for feed intake measurements whilst 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. Milk yield was recorded during the 21 day-period and samples were collected during the last 7 day of each period.

UTS was prepared by using 5% (WW) urea mixed with 100 kg of water in 100 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).

**Data collection and sampling procedures:** UTS and concentrate were sampled daily during the collection period and were composted by period prior to analyses. Feed, fecal and urine samples were collected during the last seven days of each period. Fecal samples were collected by rectal sampling whilst urine samples were collected by spot sampling. Composites samples were dried at 60°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) and Acid Insoluble Ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Cows were milked twice daily and milk weights were recorded at each milking of each period. Milk samples were composite daily, according to yield, for both the a.m. and p.m. milking, preserved with 2-bromo-2 nitropropane-1, 3-dial and stored at 4°C until analysis for fat, protein, lactose, totals solids and solids-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO) (Valladares *et al.*, 1999).

Rumen fluid samples were collected at 0 and 4 h post-feeding. Approximately 200 mL of rumen fluid was taken

Table 1: Ingredients of high-quality feed block used in the experiment (DM% basis)

Ingredients (DM%)	T1	T2	T3	T4
Coarse rice bran	30	30	-	-
Cassava hay	-	-	30	30
Molasses	40	40	42	42
Urea	13	13	11	11
Limestone	12	12	12	12
Sulfur	1	1	1	1
Mineral mix	1	1	1	1
Salt	1	1	1	1
Tallow	2	2	2	2
DL-malate (g/100 kg)	500	1,000	500	1,000

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 2 portions. One portion was used for NH<sub>3</sub>-N analyses where 5 mL of H<sub>2</sub>SO<sub>4</sub> solution (1 M) 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°C prior to NH<sub>3</sub>-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). A blood sample (about 10 mL) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5,000 g for 10 min and stored at -20°C until analysis of Blood Urea Nitrogen (BUN) according to the method of Crocker (1967).

**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).

## RESULTS AND DISCUSSION

**Chemical composition of feeds:** The chemical composition of dietary treatments and concentrate diets fed in dairy cows are presented in Table 2. Concentrate diets containing high levels of cassava chip based diets had a slightly higher NSC and lower NDF due to increased level of cassava chip in the diets. Furthermore, the chemical composition of UTS is presented in Table 2. A similar value for UTS has been similar to those reported by Wanapat *et al.* (2000).

Table 2: Chemical composition of high-quality feed block, concentrates and UTS used in the experiment

Chemical composition (%)	T1	T2	T3	T4	Concentrate <sup>1</sup>	UTS
DM (%)	79.2	79.2	79.8	79.8	89.1	55.8
OM	75.3	75.3	76.1	76.1	91.2	88.9
CP	36.1	36.1	37.3	37.3	16.0	8.0
NDF	24.8	24.8	33.2	33.2	12.1	73.2
ADF	18.2	18.2	15.2	15.2	7.2	52.3
Condensed tannins (CT)	-	-	1.5	1.5	-	-

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, UTS = Urea-Treated rice Straw. (<sup>1</sup>Ingredients = concentrate composed of cassava chips 65, palm meal 2.5, soybean meal 17, urea 3, molasses 5, coconut oil 4, sulfur 1, salt 1, mineral mix 1.5%) as dry weight

Table 3: Effect of supplementation of malate and cassava hay in high quality feed block (HQFB) on feed-intake and digestibility of nutrients in lactating cows

Item	Treatments					SEM	Contrast <sup>1</sup>		
	T1	T2	T3	T4	CH		M	CH x M	
<b>DM intake (BW%)</b>									
HQFB	0.5	0.5	0.5	0.5	-	NS	NS	NS	
UTS	1.5	1.6	1.5	1.6	0.08	NS	NS	NS	
Concentrate	1.3	1.3	1.4	1.3	0.03	NS	NS	NS	
Total	3.3	3.4	3.4	3.4	0.06	NS	NS	NS	
<b>Apparent digestibility (%)</b>									
DM	70.5 <sup>a</sup>	72.3 <sup>ab</sup>	75.4 <sup>ab</sup>	77.8 <sup>b</sup>	1.82	*	NS	NS	
OM	77.0 <sup>a</sup>	77.8 <sup>a</sup>	79.6 <sup>ab</sup>	81.2 <sup>b</sup>	1.03	NS	*	NS	
CP	70.5 <sup>a</sup>	73.0 <sup>ab</sup>	74.6 <sup>a</sup>	76.3 <sup>b</sup>	1.21	NS	*	NS	
NDF	57.0 <sup>a</sup>	60.0 <sup>b</sup>	61.0 <sup>b</sup>	65.9 <sup>c</sup>	0.54	*	*	NS	
ADF	51.1 <sup>a</sup>	52.0 <sup>a</sup>	56.0 <sup>b</sup>	56.9 <sup>b</sup>	1.13	NS	*	NS	

<sup>a,b,c</sup>Values on the same row with different superscripts differ (p<0.05). T1 = High quality feed block without cassava hay + malate at 500 g, T2 = High quality feed block without cassava hay + malate at 1,000 g, T3 = High quality feed block with cassava hay + malate at 500 g, T4 = High quality feed block with cassava hay + malate at 1,000 g, <sup>1</sup>Probability of main effects of supplementation of cassava hay in HQFB (with vs without), levels of malate (500 vs 1,000 g/100 kg), or the CH x M interaction. \*p<0.05, \*\*p<0.01, NS = p>0.05

**Effect on feed intake and digestibility:** The effects of malate level with cassava hay in high-quality feed block on feed-intake of lactating dairy cows are presented in Table 3. Feed intakes were not significantly affected by malate level with cassava hay in high-quality feed block supplementation (3.3-3.4% BW). This data indicated that malate level with cassava hay in high-quality feed block supplementation had no effect on feed-intake in lactating dairy cows. These results were in agreement with earlier work by Sommart *et al.* (2000) and Wanapat and Khampa (2006) 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.

Apparent digestibility of DM, OM, CP, NDF and ADF were significant (p<0.05) for all diets (Table 3). However, the slightly lower NDF digestibility of the cassava-based diets may have contributed to higher degradation in substantial decrease in fiber digestibility as reported by Hoover (1986). Furthermore, in the experiment by Erdman (1988) reported that the sources of starch influence the rate of NDF digestion differently at pH 6.8 than 5.5. In addition, 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.

**Characteristics of ruminal fermentation and blood metabolism:**

Rumen ecology parameters were measured for temperature, pH and NH<sub>3</sub>-N (Table 4). In addition, BUN and MUN were determined to investigate their relationships with rumen NH<sub>3</sub>-N and protein utilization. Rumen pH at 0 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.5-6.6, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986). Ruminal NH<sub>3</sub>-N, BUN and MUN concentrations were not altered by malate level with cassava hay supplement in high-quality feed block with concentrate containing high cassava-based diets. As NH<sub>3</sub>-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<sub>3</sub>-N (15-30% mg, Wanapat and Pimpa, 1999; Chanjula *et al.*, 2003, 2004) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages.

**Rumen microorganisms populations:** Table 4 presents rumen microorganism populations. The fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria had higher numbers in lactating cows receiving at 1,000

Table 4: Effect of supplementation of malate and cassava hay in high quality feed block (HQFB) on rumen fermentation, blood metabolites and rumen microorganisms in lactating cows

Item	Treatments					Contrast <sup>1</sup>		
	T1	T2	T3	T4	SEM	CH	M	CH x M
Ruminal Temperature (°C)	40.1	39.6	40.0	39.5	0.21	NS	NS	NS
Ruminal pH	6.6	6.5	6.5	6.6	0.52	NS	NS	NS
NH <sub>3</sub> -N (mg/dl)	15.8	16.2	17.7	18.1	2.95	NS	NS	NS
BUN (mg/dl)	10.7	11.5	11.0	14.7	2.69	NS	NS	NS
Blood glucose (mg/dl)	50.5	51.7	55.2	56.0	2.73	NS	NS	NS
<b>Total direct counts (cell/mL)</b>								
Bacteria (x10 <sup>11</sup> )	5.6 <sup>a</sup>	6.3 <sup>a</sup>	9.3 <sup>b</sup>	8.3 <sup>ab</sup>	0.93	NS	**	NS
Protozoa								
<i>Holotrich</i> (x10 <sup>4</sup> )	2.0 <sup>a</sup>	2.1 <sup>a</sup>	1.5 <sup>b</sup>	1.1 <sup>ab</sup>	0.21	NS	**	NS
<i>Ertodiniomorph</i> (x10 <sup>5</sup> )	11.3 <sup>a</sup>	7.8 <sup>b</sup>	5.1 <sup>c</sup>	3.7 <sup>c</sup>	0.73	*	**	NS
Fungal zoospores (x10 <sup>4</sup> )	2.5 <sup>a</sup>	3.7 <sup>a</sup>	5.6 <sup>b</sup>	7.1 <sup>b</sup>	0.52	*	**	NS

<sup>a,b,c</sup>Values on the same row with different superscripts differ (p<0.05). T1 = High quality feed block without cassava hay + malate at 500 g, T2 = High quality feed block without cassava hay + malate at 1,000 g, T3 = High quality feed block with cassava hay + malate at 500 g, T4 = High quality feed block with cassava hay + malate at 1,000 g, <sup>1</sup>Probability of main effects of supplementation of cassava hay in HQFB (with vs without), levels of malate (500 vs 1,000 g/100 kg), or the CH x M interaction. \*p<0.05, \*\*p<0.01, NS = p>0.05

Table 5: Effect of supplementation of malate and cassava hay in high quality feed block (HQFB) on milk yield and composition in lactating dairy cows

Production	Treatments					Contrast		
	T1	T2	T3	T4	SEM	CH	M	CH x M
Milk yield (kg/d)	10.8	11.6	11.6	12.1	1.33	NS	NS	NS
3.5%FCM (kg/d)	11.2	12.2	12.9	14.1	2.35	NS	NS	NS
Milk composition (%)								
Milk fat	3.6	3.7	3.9	4.1	1.75	NS	NS	NS
Milk protein	3.2	3.3	3.3	3.4	1.97	NS	NS	NS
Lactose	4.4	4.4	4.5	4.6	1.71	NS	NS	NS
Solids not fat	8.1	8.2	8.4	8.6	1.85	NS	NS	NS
Total solids	13.3	13.2	13.5	13.6	1.71	NS	NS	NS
MUN (mg/dL)	15.7	15.3	16.1	16.4	1.17	NS	NS	NS

T1 = High quality feed block without cassava hay + malate at 500 g, T2 = High quality feed block without cassava hay + malate at 1,000 g, T3 = High quality feed block with cassava hay + malate at 500 g, T4 = High quality feed block with cassava hay + malate at 1,000 g, <sup>1</sup>Probability of main effects of supplementation of cassava hay in HQFB (with vs without), levels of malate (500 vs 1,000 g/100 kg), or the CH x M interaction

than 500 g of dl-malate. In contrast, the present number of protozoa in the rumen was decreased by cassava hay and malate supplementation in high-quality feed block with concentrate contained cassava-based diets. In the experiment by Newbold *et al.* (1996) has shown 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. 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. 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 and cassava hay in high quality feed block 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*.

**Milk production and composition:** The influences of malate and cassava hay in high-quality feed block supplementation in lactating dairy cows receiving high cassava-based diets are shown in Table 5. All cows were able to maintain levels of milk yield during the 80 days of experiment. Yield of milk was greatest in cows fed dietary treatments and cassava-based diets with T4, but were lowest (p>0.05) when receiving T1 in diets. In addition, production of 3.5%FCM exhibited similar results for all treatments (p>0.05). The supplementation of malate and cassava hay in high-quality feed block with concentrate containing high cassava-based diets fed in lactating dairy cows and UTS as roughage sources did not affect on milk compositions (Table 5).

**Conclusions and Recommendations:** Based on this experiment, it could be concluded that supplementation of malate and cassava hay in high-quality feed block with

concentrate containing high cassava-based diets could improved ruminal fermentation efficiency and increase populations of bacteria, but decreased protozoal populations. These results suggest that the combined use of cassava hay and malate at 1,000 g in high-quality feed block with concentrates containing high levels of cassava chip at 65% DM could highest improved rumen ecology and digestibility of nutrients. However, further studies should be conducted, particularly in concentrate containing high level of cassava on milk compositions especially on Conjugated Linoleic Acid (CLA) in lactating cows fed straw based-diets.

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