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Effects of Malate and Cassava Hay in High-Quality Feed Block on Ruminal Fermentation Efficiency and Digestibility of Nutrients in Dairy Heifer

[1]Sittisak Khampa, [1]Pala Chaowarat, [2]Rungson Singhalert and [3]Metha Wanapat
[1]Faculty of Agricultural Technology, [2]Faculty of Humanities and Social Sciences,
Rajabhat Mahasarakham University, Maha Sarakham, 44000, Thailand
[3]Department of Animal Science, Faculty of Agriculture,
Tropical Feed Resources Research and Development Center,
Khon Kaen University, Khon Kaen, 40002, Thailand

Abstract: Four, one-year old of dairy heifers were randomly assigned according to a 2×2 Factorial arrangement in a 4×4 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 concentrate at 1.3% BW and urea-treated rice straw was fed *ad libitum*. The results have revealed that feed-intake, rumen fermentation and blood metabolites were similar for all treatments. Apparent digestibility of nutrients were significant ($p<0.05$) for all diets. The populations of protozoa and fungal zoospores were significantly different as affected by malate level and cassava hay supplementation. 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 in dairy heifers.

Key words: Malate, cassava hay, high-quality feed block, rumen fermentation, dairy heifers

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 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. 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, digestibility of nutrients in dairy heifers.

Corresponding Author: Sittisak Khampa, Faculty of Agricultural Technology, Rajabhat Mahasarakham University, Maha Sarakham, 44000, Thailand

MATERIALS AND METHODS

Animals, diets and experimental design: Four, one-year old of dairy heifers weighing at 150±10 kg. Cows were randomly assigned according to a 2×2 Factorial arrangement in a 4×4 Latin square design to study two levels dl-malate with cassava hay in high-quality feed block supplementation on ruminal fermentation efficiency and digestibility of nutrients. 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 1,000g, 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 1.3% BW. 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 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.

UTS was prepared by using 5% (w w^{-1}) 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 and fecal were collected 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 Dry Matter (DM), ether extract, ash and Crude Protein (CP) content (AOAC, 1990), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) (Van Soest *et al*., 1991) and Acid Insoluble Ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

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^{-1})	500	1,000	500	1,000

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

Chemical composition (%)	T1	T2	T3	T4	Conc.[1]	UTS
DM (%)	79.8	79.2	79.1	79.8	89.1	55.8
OM	75.7	75.3	76.7	76.1	91.2	88.9
CP	36.6	36.1	37.5	37.3	16.0	8.1
NDF	24.1	24.8	33.5	33.2	12.1	73.2
ADF	18.6	18.2	15.9	15.2	7.2	52.3
Condensed Tannins (CT)	-	-	1.6	1.5	-	-

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, UTRS = Urea-Treated Rice Straw. ([1]Ingredients = Concentrate compost 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

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 (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_3-N analysis using the micro Kjeldahl methods (AOAC, 1990). 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 4×4 Latin square design with 2×2 Factorial arrangement of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998).

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical composition of dietary treatments and concentrate diets fed in dairy heifers 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 (2000).

Effect on feed intake and digestibility: The effects of malate level with cassava hay in high-quality feed block on feed-intake of dairy heifers are presented in Table 3. Feed intakes were not significantly affected by malate level with cassava hay in high-quality feed block supplementation (3.3-3.5% BW). This data indicated that malate level with cassava hay in high-quality feed block supplementation had no effect on feed-intake in dairy heifers. These results was in agreement with earlier research by Sommart *et al.* (2000) 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 (1998) 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_3-N (Table 4). In addition, BUN was determined to investigate their relationships with rumen NH_3-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 6.5-6.7, 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_3-N and BUN 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_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 between at 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 dairy heifers receiving at 1,000 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 fumalate (another intermediate in the

Table 3: Effect of supplementation of malate and cassava hay in High-Quality Feed Block (HQFB) on feed-intake and digestibility of nutrients in dairy heifers

	Treatments					Contrast[1]		
Item	T1	T2	T3	T4	SEM	CH	M	CH×M
DM intake (BW%)								
HQFB	0.5	0.5	0.5	0.5	-	NS	NS	NS
Concentrate	1.3	1.3	1.3	1.3	-	NS	NS	NS
UTS	1.5	1.6	1.6	1.7	0.28	NS	NS	NS
Total	3.3	3.4	3.4	3.5	0.16	NS	NS	NS
Apparent digestibility (%)								
DM	70.5^{a}	72.3^{ab}	75.4^{ab}	77.8^{b}	1.82	*	NS	NS
OM	77.0^{a}	77.8^{a}	79.6^{ab}	81.2^{b}	1.03	NS	*	NS
CP	70.5^{a}	73.0^{ab}	74.6^{a}	76.3^{b}	1.21	NS	*	NS
NDF	57.0^{a}	60.0^{b}	61.0^{b}	65.9^{c}	0.54	*	*	NS
ADF	51.1^{a}	52.0^{a}	56.0^{b}	56.9^{b}	1.13	NS	*	NS

^{a,b,c}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, [1]Probability of main effects of supplementation of cassava hay in HQFB (with vs without), levels of malate (500 vs 1,000 g 100 kg^{-1}), or the CH x M interaction. * = $p<0.05$, ** = $p<0.01$, NS = $p>0.05$

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 dairy heifers

	Treatments					Contrast[1]		
Item	T1	T2	T3	T4	SEM	CH	M	CH×M
Ruminal temperature (°C)	39.7	39.5	39.8	40.1	0.21	NS	NS	NS
Ruminal pH	6.6	6.5	6.7	6.6	0.52	NS	NS	NS
NH_3-N (mg dL^{-1})	16.8	17.1	18.6	19.1	2.95	NS	NS	NS
BUN (mg dL^{-1})	11.7	12.5	12.1	15.5	2.69	NS	NS	NS
Blood glucose (mg dL^{-1})	52.2	52.6	56.3	57.1	3.73	NS	NS	NS
Total direct counts (cell mL^{-1})								
Bacteria ($\times 10^{11}$)	5.6^{a}	6.3^{a}	9.3^{b}	8.3^{ab}	0.93	NS	**	NS
Protozoa								
Holotrich ($\times 10^{4}$)	2.0^{a}	2.1^{a}	1.5^{b}	1.1^{ab}	0.21	NS	**	NS
Entodiniomorph ($\times 10^{5}$)	11.3^{a}	7.8^{b}	5.1^{c}	3.7^{c}	0.73	*	**	NS
Fungal zoospores ($\times 10^{4}$)	2.5^{a}	3.7^{a}	5.6^{b}	7.1^{b}	0.52	*	**	NS

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

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*.

CONCLUSION

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.

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