Effects of Urea Level and Sodium DL-malate in Concentrate Containing High Cassava Chip on Ruminal Fermentation Efficiency, Microbial Protein Synthesis in Lactating Dairy Cows Raised under Tropical Condition

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ABSTRACT : Four, lactating dairy cows were randomly assigned according to a 2×2 Factorial arrangement in a 4×4 Latin square design to study supplementation of urea level (U) at 2 and 4% and sodium dl-malate (M) at 10 and 20 g/hd/d in concentrate. The treatments were as follows U2M10, U2M20, U4M10 and U4M20, respectively. The cows were offered the treatment concentrate at a ratio to milk yield at 1:2.5 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 urea level and sodium dl-malate. In addition, the viable bacteria were similar for amylolytic and proteolytic bacteria. Cellulolytic bacteria were significantly affected by level of sodium dl-malate especially *Selenomonas ruminantium* and *Megasphaera elsdenii* while *Butyrivibrio fibrisolvens* was significantly affected by level of urea supplementation. In conclusion, the combined use of concentrate containing high level of cassava chip at 75% DM with urea at 4% in concentrate and sodium dl-malate at 20 g/hd/d with UTS as a roughage could improv rumen ecology and microbial protein synthesis efficiency in lactating dairy cows. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 837-844)***

Key Words : Urea, Sodium DL-malate, Rumen Fermentation, Cassava Chip, Urea-treated Rice Straw, Tropical Dairying

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 et al., 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 et al., 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 (Gottschalk, 1986). Both malate and fumalate are key intermediates in the succinate propionate pathway, and *S. ruminantium* uses this pathway (Gottschalk, 1986). The fact that dicarboxylic acids, especially malate and fumalate, stimulate lactate utilization is consistent with the presence of this pathway by 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 sodium dl-malate in cassava based-diets has not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of urea level and sodium dl-malate supplementation in concentrates containing high level of cassava chip with urea-treated rice straw as a basal roughage on ruminal fermentation, microbial protein synthesis efficiency and milk production in lactating dairy cows.

MATERIALSS AND METHODS

Animals, diets and experimental design

Four, Holstein-Friesian crossbred cows (75%) in the first lactation were used in experiment. Milk yield pre-experiment was 10±2 kg/day and the body weight were 390±10 kg. Cows were randomly assigned according to a 2 ×2 Factorial arrangement in a 4×4 Latin square design to study two levels of urea with sodium dl-malate supplementation on ruminal fermentation efficiency,

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Table 1. Chemical composition of concentrates and UTS used in the experiment (% DM basis)

Item	Dietary treatments		
	Concentrate I	Concentrate II	UTS
Ingredient (% DM)			
Cassava chip	70	75	
Palm meal	1.5	4	
Soybean meal	17	3.5	
Molasses	1	5	
Coconut oil	4	4	
Urea	2	4	
Sulfur	1	1	
Salt	1	1	
Limestone	1	1	
Mineral mix	1.5	1.5	
Chemical compositions (%)			
DM	88.7	89.1	55.8
OM	91.1	91.2	88.9
CP	16.1	16.0	8.0
NDF	13.1	12.1	73.2
ADF	8.1	7.2	52.3
NSC[1]	58.1	59.0	6.7
Ash	8.9	8.8	11.1
EE	4.0	3.9	1.2
Ca	1.7	1.8	-
P	0.7	0.6	-
TDN	80.1	80.2	55.1
ME (Mcal/kg, DM)[2]	2.9	2.9	1.9
NE_L (Mcal/kg, DM)[3]	1.8	1.8	1.2
Feed cost (US$/kg)	0.21	0.11	0.09

[1] Estimated: NSC = 100-((NDF-NDF protein)+protein+EE+Ash).

[2] Estimated: Metabolizable energy (ME, Mcal/kg, DM) = TDN×0.04409 ×0.82.

[3] Estimated: Net energy for lactation (NE_L, Mcal/kg) = (0.0245×TDN)-0.12.

digestibility of nutrients, ruminal microbial protein synthesis and milk production. The dietary treatments were as follows: T1 = supplementation of urea at 2% with sodium dl-malate at 10 g/hd/d in concentrate (U2M10); T2 = supplementation of urea at 2% with sodium dl-malate at 20 g/hd/d in concentrate (U2M20); T3 = supplementation of urea at 4% with sodium dl-malate at 10 g/hd/d in concentrate (U4M10); T4 = supplementation of urea at 2% with sodium dl-malate at 10 g/hd/d in concentrate (U4M20), respectively. The composition of dietary treatments and urea-treated rice straw (UTS) used are shown in Table 1.

Cows were housed in individual pens and individually fed concentrate at a ration to milk yield of 1:2.5, twice daily at 0600 a.m. and 1600 p.m. 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 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. 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% (W/W) 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 whist 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 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).

Cows were milked twice daily, and milk weights were recorded at each milking of each period. Milk samples were composited 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). Moreover, milk samples were homogenized using a T1500 homogeniser (Ystral, Dottingen, Germany). Five hundred µl aliquots of milk were diluted with 4.5 ml of HPLC grade acetonitrile and vortex-mixed for 15 s. A 2 ml aliquot of this mixture was passed through a 13 mm disposable syring filter containing a 0.45 µm PTFE menbrane (HPLC Technology, Cheshire, UK) into a HPLC vial (Shingfield and Offer, 1998). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (HPLC) as described by Chen and Gomes (1992).

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

Table 2. Influence of urea level and sodium dl-malate on feed-intake and digestibility of nutrients in lactating cows

Item	Treatments[1]				SEM	Contrast[2]		
	U2M10	U2M20	U4M10	U4M20		U	M	U×M
DM intake (%BW)								
UTS	1.5	1.6	1.5	1.5	0.08	NS	NS	NS
Conc.	1.3	1.3	1.3	1.4	0.03	NS	NS	NS
Total	2.8	2.9	2.8	2.9	0.03	NS	NS	NS
Apparent total-tract digestibility (%)								
DM	72.3^{ab}	77.8^{a}	70.5^{b}	75.4^{ab}	1.81	*	NS	NS
OM	77.8^{a}	81.2^{b}	77.0^{a}	79.6^{ab}	1.05	NS	*	NS
CP	73.0^{ab}	76.3^{a}	70.5^{b}	74.6^{a}	1.23	NS	*	NS
EE	76.9	77.4	75.8	76.2	0.79	NS	NS	NS
NDF	60.0^{a}	65.9^{b}	57.0^{c}	61.0^{a}	0.59	*	*	NS
ADF	52.0^{a}	56.9^{b}	51.1^{a}	56.0^{b}	1.11	NS	*	NS

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

[1] U2M10 = Urea at 2% with sodium dl-malate at 10 g/hd/d; U2M20 = Urea at 2% with sodium dl-malate at 20 g/hd/d; U4M10 = Urea at 4% with sodium dl-malate at 10 g/hd/d; U4M20 = Urea at 2% with sodium dl-malate at 20 g/hd/d.

[2] Probability of main effects of level urea in concentrates (2 vs. 4%), levels of sodium dl-malate (10 vs. 20 g/hd/d), or the U×M interaction.

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

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 minute and the supernatant stored at -20°C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985) and volatile fatty acids (VFAs) analyses using a HPLC according to Zinn and Owens (1986). Another portion was fixed with10% 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) and culture groups of bacteria using the roll-tube method described by Hungate (1969), for identifying of bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria). In addition, specific bacteria namely *Butyrivibrio fibrisolvens* was grown anaerobically at 39^0C in basal medium containing (per liter) 292 mg of K_2HPO_4, 292 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, 600 mg of cysteine hydrochloride, 10 g of Trypticase (BBL Microbiology Systems, Cockeysville, MD.), 2.5 g of yeast extract, and branched-chain volatile fatty acids (1 mmol each of isobutyrate, isovalerate, and 2-methylbutyrate), plus hemin, vitamins, and trace minerals. *Megasphaera elsdenii* was grown in a basal medium that was prepared anaerobiaclly under O_2-free CO_2 (per liter) K_2HPO_4, 292 mg; KH_2PO_4, 292 mg; $(NH_4)_2SO_4$, 480 mg; NaCl, 480 mg; $MgSO_4 \cdot 7H_2O$, 100 mg; $CaCl_2 \cdot 2H_2O$, 64 mg; Na_2CO_3, 4,000 mg; cystein hydrochloride, 600 mg; vitamins and micromineral mixture (Cotta and Russell, 1982).

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 minutes and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Furthermore, the blood samples was analyzed for plasma allantoin by centrifuging at 3,000 g at 4°C; plasma was preserved at -20°C and prepared for HPLC analysis according to Giesecke et al. (1994).

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). 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 roughage and concentrate diets fed in dairy cows are presented in Table 1. Concentrate diets contained similar concentrations of DM, OM, CP, NDF, ADF, EE and non-structural carbohydrate (NSC). 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 1. Similar values for UTS has been found to those reported by Wanapat et al. (2000).

Effect on feed intake and digestibility

The effects of urea level with sodium dl-malate on feed-intake of lactating dairy cows are presented in Table 2. Feed intakes were not significantly affected by urea level and sodium dl-malate supplementation (2.8-2.9% BW). This data indicated that urea level with sodium dl-malate

Table 3. Influence of urea level and sodium dl-malate on rumen fermentation blood metabolites and VFA characteristics in lactating cows

Item	Treatments				SEM	Contrast		
	U2M10	U2M20	U4M10	U4M20		U	M	U×M
Ruminal temperature (°C)	39.6	40.0	40.1	39.5	0.22	NS	NS	NS
Ruminal pH	6.5	6.5	6.6	6.6	0.13	NS	NS	NS
CH_4 (mmol/L)	10.6	11.4	13.0	11.2	3.77	NS	NS	NS
Lactic acid (mg/ml)	24.7[a]	21.7[b]	26.7[a]	22.1[b]	0.67	NS	**	NS
NH_3-N (mg/dl)	15.8	14.7	16.2	16.1	1.96	NS	NS	NS
BUN (mg/dl)	11.5	11.0	16.7	14.7	2.66	NS	NS	NS
Glucose (mg/dl)	50.5	55.2	51.7	56.0	1.05	NS	NS	NS
Plasma allantoin (μmol/ml)	126.2	139.5	128.6	138.5	6.44	NS	NS	NS
Total VFA (mmol/L)	114.1	116.1	121.4	123.2	6.88	NS	NS	NS
Molar proportion of VFA (mol/100 mol)								
Acetate (C2)	64.3	62.7	64.2	63.5	1.59	NS	NS	NS
Propionate (C3)	26.9	27.8	27.7	27.6	1.40	NS	NS	NS
Butyrate (C4)	8.7	9.4	8.0	8.8	0.57	NS	NS	NS
C2:C3 ratio	2.4	2.3	2.3	2.4	0.19	NS	NS	NS
C2+C4:C3 ratio	2.7	2.6	2.6	2.7	0.20	NS	NS	NS

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

supplementation had no effect on feed intake in lactating dairy cows. These results were in agreement with earlier work by (Sommart et al., 2000) who 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, even though digestible nutrient intake of ether extract (EE) tended to be slightly greater for cows fed cassava-based diets with U2M20 than U2M10, U4M20 and U4M10 (77.4, 76.9, 76.2 and 75.8%, respectively) (Table 2). 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, CH_4, lactic acid, NH_3-N, VFA (Table 3). In addition, BUN and MUN were 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 6.5-6.6, 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). The concentrations of lactic acid in rumen were significant ($p<0.05$) when dairy cows received different level of sodium dl-malate supplement with high cassava-based diets. However, the values of lactic acid in rumen for dairy cows received high cassava-based diets were not exceeding levels (range between at 21.7-26.7 mM). As reported, the concentrations of lactic acid in rumen exceeding 40 mM were indicative of severe acidosis in cattle (Owen et al., 1998).

The concentrations of methane production in rumen were not affected ($p>0.05$) by dairy cows receiving different urea level and sodium dl-malate supplementation with concentrates containing high cassava-based diets. In previous studies by Asanuma et al. (1999) who reported that the use of malate and fumalate as feed additives could reduce methanogenesis and increase propionate production in the rumen.

Ruminal NH_3-N, BUN and MUN concentrations were not altered by urea level and sodium dl-malate supplement in diets containing high cassava-based diets. As NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen (Bryant, 1974). In addition, the result obtained was closer to optimal ruminal NH_3-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. Milk allantoin concentrations were found to range between 82.9-103.4 μmol/L depending upon diets and production level, which were lower than those values (159-237 μmol/L) reported by Giesecke et al. (1994). Allantoin concentrations in bovine milk were similar to those in plasma but were much lower than urinary concentrations, which typically ranged between 0.7 and 29.4 mM (Shingfield and Offer, 1998).

Table 4. Influence of urea level and sodium dl-malate on rumen microorganisms in lactating cows

Item	Treatments				SEM	Contrast		
	U2M10	U2M20	U4M10	U4M20		U	M	U×M
Total direct counts								
Bacteria ($\times10^{11}$ cell/ml)	6.3[a]	9.3[b]	5.6[a]	8.3[ab]	0.91	NS	**	NS
Protozoa								
Holotric ($\times10^{4}$ cell/ml)	2.1[a]	1.0[b]	2.0[a]	1.5[ab]	0.26	NS	**	NS
Entodiniomorph ($\times10^{5}$ cell/ml)	7.8[a]	3.4[b]	11.3[c]	5.0[b]	0.71	*	**	NS
Fungal zoospores ($\times10^{4}$ cell/ml)	3.7[a]	7.0[b]	2.5[a]	5.6[b]	0.51	*	**	NS
Roll tube techniques								
Total viable bacteria ($\times10^{7}$ CFU/ml)	31.2[a]	54.1[b]	27.6[c]	52.7[b]	0.73	*	**	NS
Amylolytic ($\times10^{6}$ CFU/ml)	12.6	15.9	12.6	9.2	4.00	NS	NS	NS
Proteolytic ($\times10^{6}$ CFU/ml)	6.8	8.6	9.9	11.5	3.99	NS	NS	NS
Cellulolytic ($\times10^{7}$ CFU/ml)	10.6[a]	27.3[b]	28.6[b]	5.6[a]	7.15	NS	*	NS
Selenomonas ruminantium ($\times10^{4}$ CFU/ml)	8.7[a]	13.7[b]	7.1[a]	14.1[b]	1.41	NS	**	NS
Megasphaera elsdenii ($\times10^{4}$ CFU/ml)	10.4[a]	17.3[b]	10.3[a]	17.0[b]	1.69	NS	**	NS
Butyrivibrio fibrisolvens ($\times10^{4}$ CFU/ml)	7.7[a]	7.0[a]	14.0[b]	15.6[b]	1.75	*	NS	NS

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

The influence of urea level with sodium dl-malate 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 urea level and sodium dl-malate in the diet. However, the concentration of propionic acid was slightly higher in U2M20 than U4M10, U4M20 and U2M10, respectively. However, it was found that total VFA concentration in all diets ranged from 70 to 130 mM. Although the acetate to propionate ratio was decreased by the level of sodium dl-malate, but the supplementation of urea level with sodium dl-malate increased the daily output of propionate without decreasing the production of acetate, and it was in agreement with the results reported by other authors (Callaway and Martin, 1996; Khampa et al., 2006).

Rumen microorganisms populations

Table 4 presents rumen microorganism populations. Total viable bacteria counts cellulolytic, *Selenomonas ruminantium*, *Megasphaera elsdenii* and *Butyrivibrio fibrisovens* bacteria were significantly different, whilst populations of amylolytic and proteolytic bacteria were similar by urea level and sodium dl-malate supplementation in diets containing high cassava-based diets. Nevertheless, fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria were higher numbers in lactating cows receiving at 20 than 10 g/hd/d of sodium dl-malate. In contrast, the present number of protozoa in the rumen was decreased by urea level and sodium dl-malate supplementation in high 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 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 urea 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*.

The populations of *Megasphaera elsdenii* were significantly ($p<0.05$) affected by different level of sodium dl-malate supplementation with high cassava-based diets (Table 4). In contrast, the populations of *Butyrivibrio fibrisolvens* were affected when receiving different urea level supplementation in concentrate containing high cassava-based diets. In the experiment by Kim et al. (2002) who reported that only a few ruminal bacteria can utilize lactate (e.g. *M. elsdenii* and *S. ruminantium*), in which *M. elsdenii* was the most important predominant ruminal bacteria that produces *trans*-10, *cis*-12 conjugated linoleic acid (CLA) from linoleic acid. The use of sodium dl-malate may enhance in these regards.

Table 5. Effects of urea and sodium dl-malate supplementation on nitrogen balance, excretion of purine derivatives (PD) and microbial nitrogen supply in lactating cows

Item	Treatments				SEM	Contrast		
	U2M10	U2M20	U4M10	U4M20		U	M	U×M
Nitrogen balance (g/d)								
N intake	211.6	209.0	205.0	206.4	2.23	NS	NS	NS
Fecal N	46.7[a]	38.5[b]	47.0[a]	34.4[b]	2.62	NS	*	NS
N absorption	61.9[a]	68.2[b]	88.7[c]	69.6[b]	1.74	**	*	*
N retention	12.8[a]	29.9[b]	42.9[c]	35.7[d]	1.21	**	*	**
Allantoin absorption (mmol/d)	544.7 [a]	674.0[b]	553.4[a]	1,000.3[c]	5.47	*	*	*
Urine N (g/d)[1]	149.5	140.5	115.3	136.1	10.60	NS	NS	NS
Urine creatinine (mg/dl)	35.4	44.7	40.4	36.6	3.88	NS	NS	NS
Urine allantoin (μmol/ml)	24.3	27.1	25.8	40.1	10.02	NS	NS	NS
Microbial N supply (g N/d) [2]	62.0[a]	76.4[b]	64.0[a]	116.3[c]	1.27	*	*	*

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

[1] Urinary nitrogen (g/d) = 0.0259×BW (kg)×MUN (mg/dl).

[2] Microbial N (g N/day) = (X×70)/(0.116×0.83×1,000) = 0.727×X (Where, X = total absorption of purine derivatives).

Table 6. Effects of urea and sodium dl-malate supplementation on milk yield and composition in lactating dairy cows

Item	Treatments				SEM	Contrast		
	U2M10	U2M20	U4M10	U4M20		U	M	U×M
Production								
Milk yield (kg/d)	11.6	12.0	10.8	11.6	1.35	NS	NS	NS
3.5% FCM (kg/d)	12.5	13.6	11.2	12.9	1.37	NS	NS	NS
Milk composition (%)								
Milk fat	3.8	4.0	3.6	3.9	0.37	NS	NS	NS
Milk protein	3.1	3.0	3.0	3.1	0.09	NS	NS	NS
Lactose	4.7	4.6	4.7	4.3	0.23	NS	NS	NS
Solids not fat	9.2	8.6	8.8	8.4	0.28	NS	NS	NS
Total solids	13.2	12.5	13.3	12.0	0.73	NS	NS	NS
Milk urea nitrogen (mg/dl)	17.0	16.4	14.7	16.0	1.19	NS	NS	NS
Milk allantoin (μmol/L)	87.5[ac]	103.4[b]	82.9[a]	96.6[bc]	3.51	NS	**	NS

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

Nitrogen balance and efficiency of microbial protein synthesis

As shown in Table 5, N balance in terms of N absorption and retention were significantly different among treatments. The excretions of nitrogen, creatinine and allantoin concentrations in urine were not affected in all treatments. In this regard, the positive N balance observed in this study indicated the positive influence of the different urea level with sodium dl-malate supplements and UTS based feeding systems of lactating dairy cows.

Excretion of allantoin in the urine was non-significantly different among treatments and was higher in dairy cows receiving sodium dl-malate supplement at 20 g/hd/d than 10 g/hd/d (40.1, 27.1 and 25.8, 24.3 μmol/ml, respectively). The microbial nitrogen supply as calculated from purine derivative excretion using the equation of Chen and Gomes (1992) ranged from 62.0 to 116.3 g N/day. However, as indicated that 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.

Milk production and composition

The influences of urea level and sodium dl-malate supplementation in lactating dairy cows receiving high cassava-based diets are shown in Table 6. All cows were able to maintain levels of milk yield during the 80 days of experiment. Yield of milk was greatest in cows fed cassava-based diets with U2M20, but were lowest ($p>0.05$) when receiving U4M10 in diets. In addition, production of 3.5% FCM exhibited similar results for all treatments ($p>0.05$). The supplementation of urea level and sodium dl-malate in high cassava-based diets fed to lactating dairy cows and UTS as roughage sources did not affect on milk compositions (Table 6).

CONCLUSIONS AND RECOMMENDATIONS

Based on this experiment, it could be concluded that supplementation of urea level with sodium dl-malate in concentrate containing high level of cassava chip could

improved ruminal fermentation efficiency, increasing propionate production and decreased acetate to propionate ratio. Moreover, high level of cassava chip in diet resulted in increased populations of bacteria, but decreased protozoal populations and improving microbial nitrogen supply in rumen. These results suggest that the combined use of concentrates containing high level of cassava chip at 75% DM with urea at 4% DM in concentrate and sodium dl-malate at 20 g/hd/d could improve rumen ecology and microbial protein synthesis in rumen. 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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REFERENCES

AOAC. 1985. Official Methods of Analysis. Association of Official Analysis Chemists, DC, USA.

Asanuma, N., M. Iwamoto and T. Hino. 1999. Effect of the addition of fumalate on methane production by ruminal microorganisms *in vitro*. J. Dairy Sci. 82:780-787.

Callaway, T. R. and S. A. Martin. 1996. Effects of organic acid and monensin treatment on *in vitro* mixed ruminal microorganism fermentation of cracked corn. J. Anim. Sci. 74:1982-1989.

Chanjula, P., M. Wanapat, C. Wachirapakorn, S. Uriyapongson and P. Rowlinson. 2003. Ruminal degradability of tropical feeds and their potential use in ruminant diets. Asian-Aust. J. Anim. Sci. 16:211-216.

Chanjula, P., M. Wanapat, C. Wachirapakorn, S. Uriyapongson and P. Rowlinson. 2004. Effect of synchronizing starch sources and protein (NPN) in the rumen on feed intake, rumen microbial fermentation, nutrient utilization and performance of lactating dairy cows. Asian-Aust. J. Anim. Sci. 17:1400-1410.

Chen, X. B. and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-an overview of the technical details. International Feed Resources Unit, Rowel Research Institute, Aberdeen, UK.

Cotta, M. A. and J. B. Russell. 1982. Effect of peptides and amino acids on efficiency of rumen bacteria protein synthesis in continuous culture. J. Dairy Sci. 65:226-234.

Crocker, C. L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinzation. Am. J. Medical Techn. 33:361-365.

Erdman, R. A. 1988. Dietary buffering requirements of the lactating dairy cows. A review. J. Dairy Sci. 71:3246-3266.

Fernandez, J. M., T. Sahulu, C. Lu, D. Ivey and M. J. Potchoiba. 1987. Production and metabolic aspects of non-protein nitrogen incorporation in lactation rations of dairy goats. Small Rum. Res. 26:105-107.

France, J. and R. C. Siddons. 1993. Volatile fatty acid production. In: Quantilitive Aspects Ruminant Digestion and Metabolisim (Ed. J. M. Forbes and J. France). CAB International, Willingford, UK.

Galyean, M. 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science. New Mexico states University, USA.

Giesecke, D., L. Ehrentrich and M. Stangassinger. 1994. Mammary and renal excretion of purine metabolites in relation to energy intake and milk yield in dairy cows. J. Dairy Sci. 77:2376-2381.

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some application). Agric. Handbook No. 379, ARS, USDA, Washington, DC.

Gottschalk, G. 1986. Bateria metabolism (2nd Ed.). Sparinger-Verlag. New York.

Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.

Hoover, W. H. and S. R. Stokes. 1991. Balancing carbohydrate and proteins for optimum rumen microbial yield. J. Dairy Sci. 74:3640-3644.

Hungate, R. E. 1969. A roll tube method for cultivation of stric anaerobes. In: Methods in Microbiology. (Ed. J. R. Norris and D. W. Ribbons). New York.

IAEA. 1997. Estimation of the rumen microbial protein production from purine derivatives in rumen. Animal production and healt section. Vienna, Austria.

Khampa, S., M. Wanapat, C. Wachirapakorn, N. Nontaso and M. Wattiaux. 2006. Effect of levels of sodium dl-malate supplementation on ruminal fermentation efficiency in concentrates containing high levels of cassava chip in dairy steers. Aisan-Aust. J. Anim. Sci. (In Press).

Kim, Y. J., R. H. Liu, L. L. Rychlik and J. B. Russell. 2002. The enrichment of a ruminal bacterium (*Megasphaere elsdenii* Y J-4) that produces the *trans*-10, *cis*-12 isomer of conjugated linoleic acid. J. Appl. Environ. Microbiol. 92:976-982.

Kiyothong, K. and M. Wanapat. 2004. Growth, hay yield and chemical composition of cassava and Stylo 184 grown under intercropping. Aisan-Aust. J. Anim. Sci. 17:799-807.

Lopez, S., C. Newbold and R. J. Wallace. 1999. Influence of sodium fumarate addition on rumen fermentation *in vitro*. Br. J. Nutr. 81:59-64.

Martin, S. A., M. N. Streeter, D. J. Nisbet, G. M. Hill and E. E. Williams. 1999. Effect of DL-malate on ruminal metabolism and performance of cattle fed a high concentrate diets. J. Anim. Sci. 77:1008-1015.

Newbold, C. J., R. J. Wallace and F. M. McIntosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr. 76:249-261.

Owens, F. N., D. S. Secrist, W. J. Hill and D. R. Gill. 1998. Acidosis in cattle: a review. J. Anim. Sci. 76:275-286.

Promkot, C. and M. Wanapat. 2005. Effect of level of crude protein and use of cottonseed meal in diets containg cassava chips and rice straw for lactating dairy cows. Asian-Aust. J. Anim. Sci. 18:502-511.

Sanson, D. W. and O. T. Stallcup. 1984. Growth response and serum constituents of Holstein bulls fed malic acid. Nut. Rep. Int. 30:1261-1267.

SAS. 1998. SAS/STAT User's Guide. Version 6.12. SAS Inst. Inc., Cary, NC, USA.

Shingfield, K. J. and N. W. Offer. 1998. Determination of allantoin in bovine milk by high-performance liquid chromatography. J. Chromatogr. B. 706:342-346.

Sommart, K., M. Wanapat, D. S. Parker and P. Rowlinson. 2000. Cassava chip as an energy source for lactating dairy cows fed rice straw. Asian-Aust. J. Anim. Sci. 13:1094-1101.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedure of Statistics. McGraw-Hill Publishing Co., New York.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686-2696.

Van Keulen, J. and B. A. Young. 1977. Evaluation of acid insoluble ash as a neutral marker in ruminant digestibility studies. J. Anim. Sci. 44:282-287.

Wanapat, M. 1990. Nutritional Aspects of Ruminant Production in Southeast Asia with Special Reference to Thailand. Funny Press, Ltd., Bangkok, Thailand.

Wanapat, M. 2000. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. Asian-Aust. J. Anim. Sci. 13:59-67.

Wanapat, M. 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. Asian-Aust. J. Anim. Sci. 16:463-472.

Wanapat, M. and O. Pimpa. 1999. Effect of ruminal NH_3-N levels on ruminal fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. Asian-Aust. J. Anim. Sci. 12:904-907.

Wohlt, J. E., J. H. Clark and F. S. Blaisdell. 1978. Nutritional value of urea versus preformed protein for ruminants. II. Nitrogen utilization by dairy cows fed corn based diets containing supplemental nitrogen from urea and/or soybean meal. J. Dairy Sci. 61:916-931.

Zinn, A. R. and F. N. Owen. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-163.