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Effects of Malate and Yeast Supplementation in Concentrate Containing High Cassava Chip on Rumen Ecology and Digestibility of Nutrients in Beef Cattle

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ABSTRACT

Four, 1-year old beef cattle 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 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 is supplementation of malate at 500 g with yeast at 1,000 g; T2 is supplementation of malate at 500 g with yeast at 2,000 g; T3 is supplementation of malate at 1,000 g with yeast at 1,000 g; T4 is 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 dry matter basis and urea-treated rice straw. The results revealed that rumen fermentation and blood metabolites were similar for all treatments. However, the digestibility of nutrients were significantly different for the diets, especially digestible nutrient intake of crude protein (CP) which was higher for cows fed cassava-based diets with T4 rather than T3, T2 and T1 (74.3, 72.5, 71.1 and 68.9 %, respectively). In addition, the concentration of volatile fatty acid was significantly different especially the concentration of propionic acid which 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 and yeast levels. In conclusion, the combined use of concentrate containing high levels 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 improved rumen fermentation and digestibility of nutrients in beef cattle.

Keywords: Malate, yeast, *Saccharomyces cerevisiae*, cassava chip, cattle, rumen ecology

INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) production in tropical areas has a potential use in ruminant feed. Cassava roots contain high levels of energy and have been used as a source of readily fermentable energy in ruminant rations [1-3]. One strategy for using highly degradable carbohydrates is to use it in combination with readily available non-protein nitrogen (NPN) sources such as urea. Urea is commonly used as a nitrogen source when highly soluble carbohydrates are fed and maintained [4]. However, efficient utilization of protein and NPN in ruminants depends upon knowledge of the basic principles underlying ruminal microbial N metabolism [5]. Moreover, ruminal pH has a great impact on rumen fermentation efficiency [1].

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 *Selenomonas ruminantium* uses this pathway [6]. The fact that dicarboxylic acids, especially malate and fumalate, stimulate lactate utilization is consistent with the presence of this pathway in this ruminal anaerobe [7]. Previous studies have found that malate supplements in ruminant diets have been shown to increase nitrogen retention in sheep and steers and to improve average daily gain and feed efficiency in bull calves [8]. In addition, supplementing diets with yeast (*Saccharomyces cerevisiae*) increases milk production in dairy cows and weight gain in growing cattle [9]. 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 [10]. 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 malate and yeast supplement levels in concentrates containing high levels of cassava chip with urea-treated rice straw as basal roughage on ruminal fermentation and nutrients digestibility in beef cattle.

MATERIALS AND METHODS

Animals, Diets and Experimental Design

Four, 1-year old beef cattle weighing 150 ± 10 kg were used in this study. Cows were randomly assigned according to a 2 × 2 Factorial arrangement in a 4 × 4 Latin square design to study 2 malate levels (500 and 1,000 g) with yeast *(S. cerevisiae)* (1,000 and 2,000 g) in concentrate supplements on the ruminal fermentation efficiency and digestibility of nutrients. The dietary treatments were as follows: T1 is a supplement containing 500 g of malate with 1,000 g of yeast; T2 is a supplement containing 500 g of malate with 2,000 g of yeast; T3 is a supplement containing 1,000 g of malate with 1,000 g of yeast; T4 is a supplement containing 1,000 g of malate with 2,000 g of yeast,

respectively. The composition of dietary treatments and urea-treated rice straw (UTS) used are shown in **Tables 1** and **2**.

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

Items	Dietary treatments[1]			
	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[2]	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. is concentrate
[2] Minerals and vitamins (per kg): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g

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

Chemical compositions (%)	Dietary treatments[1]				UTS
	Conc. I	Conc. II	Conc. III	Conc. IV	
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
TDN	79.5	79.7	79.5	79.7	55.1
ME, Mcal/kg (DM)[2]	2.9	2.9	2.9	2.9	1.9
Feed cost (US$/kg)	0.25	0.28	0.28	0.30	0.05

[1] Conc. is concentrate
[2] Estimated: Metabolizable energy (ME, Mcal/kg, DM) is TDN × 0.04409 × 0.82.
DM is dry matter, OM is organic matter, CP is crude protein, NDF is neutral detergent fiber, ADF is acid detergent fiber, TDN is total digestible nutrients

Animals were housed in individual pens and individually fed concentrate at 1 % body weight (BW) of DM basis. 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 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.

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 the animals [11].

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 samples were collected by rectal sampling 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 (EE), ash and crude protein (CP) content, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid insoluble ash (AIA) [12]. AIA was used to estimate digestibility of nutrients [13].

Rumen fluid samples were collected at 0, 2 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 each time at the end of each period. Rumen fluid was immediately measured for pH and temperature (HANNA instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through 4 layers of cheesecloth. Samples were divided into 2 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 °C prior to NH_3-N analysis using the micro Kjeldahl method and volatile fatty acids (VFAs) were analysized using HPLC [12,13]. Another portion was fixed with a 10 % formalin solution in normal saline [14].

The total count of bacteria, protozoa and fungal zoospores were made using the Galyean method [14] 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) [15].

Statistical Analysis

All data obtained from the experiment were subjected to ANOVA for a 4×4 Latin square design using the General Linear Model (GLM) procedures of the Statistical Analysis System Institute. Treatment means were compared by Duncan's New Multiple Range Test (DMRT) [16].

RESULTS AND DISCUSSION

Chemical Composition of Feeds

The chemical compositions of roughage and concentrate diets fed to the dairy cows are presented in **Table 2**. Concentrate diets contained high levels of cassava chip and similar concentrations of DM, OM, CP, NDF, ADF and TDN.

Effect on Feed Intake and Digestibility of Nutrients

The effects of malate level with yeast (*S. cerevisiae*) on feed-intake and digestibility of nutrients in beef cattle are presented in **Table 3**. Feed intake was not significantly different among treatments and was higher in beef cattle receiving T4 than in those fed T3, T2 and T1 (2.8, 2.7, 2.7 and 2.6 % BW, respectively). This data indicates that the different malate and yeast levels had no effect on feed intake in beef cattle. This result is 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 dairy cattle and lactating dairy cows [17,18]. However, apparent digestibility of DM, OM, CP, NDF and ADF were significantly different *($p < 0.05$)* for all diets, especially digestible nutrient intake of crude protein which was higher for cows fed cassava-based diets with T4 than T3, T2 and T1, respectively. However, the slightly lower NDF digestibility of the cassava-based diets may have contributed to higher degradation and a substantial decrease in fiber digestibility [19]. Furthermore, 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 [20].

Ruminal NH_3-N and BUN concentrations were not altered by different malate and yeast levels in diets containing high cassava-based diets. 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 the optimal ruminal NH_3-N at 15 - 30 mg for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages [21-23].

The influence of malate and yeast levels in concentrates on volatile fatty acids (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 ($p < 0.05$). In particular, 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 to 130 mM [24]. Although the acetate to propionate ratio decreased by the level of sodium dl-malate. In addition, the malate and yeast supplements increased the daily output of propionate and decreased the production of acetate.

Table 3 Effects of malate level and yeast (*S. cerevisiae*) on feed-intake and digestibility of nutrients in cattle.

Items	Treatments[1]				SEM	Contrast[2]		
	T1	T2	T3	T4		M	Y	M × Y
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
Apparent total-tract digestibility (%)								
DM	70.5^{a}	72.3^{ab}	75.4^{ab}	77.8^{b}	1.81	*	NS	NS
OM	75.1^{a}	75.8^{a}	77.5^{ab}	79.2^{b}	1.05	NS	*	NS
CP	68.9^{a}	71.1^{a}	72.5^{ab}	74.3^{b}	1.23	NS	*	NS
NDF	55.1^{a}	59.2^{b}	60.1^{b}	65.3^{c}	0.59	*	*	NS
ADF	50.1^{a}	50.5^{a}	54.2^{b}	54.4^{b}	1.11	NS	*	NS
ADG (g/d)	269.7^{a}	278.1^{b}	287.5^{c}	293.7^{c}	2.47	*	*	NS

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

[1] T1 is malate at 500 g with yeast at 1,000 g
T2 is malate at 500 g with yeast at 2,000 g
T3 is malate at 1,000 g with yeast at 1,000 g
T4 is 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 × Y interaction

* = $p < 0.05$, NS = $p > 0.05$.

DM is dry matter, OM is organic matter, CP is crude protein, NDF is neutral detergent fiber, ADF is acid detergent fiber, TDN is total digestible of nutrients, ADG is average daily gain.

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 beef cattle receiving diets T4 than T3, T2 and T1. In contrast, the number of protozoa in the rumen decreased as the malate and yeast levels in the supplements increase in high cassava-based diets. Previous studies 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 [25]. 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 [10]. As cassava chip can be readily degraded in the rumen and ruminal pH was decreased, malate could stimulate lactate utilization by *Selenomonas 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 *Selenomonas ruminantium* [26].

Table 4 Effects of malate level and yeast on rumen fermentation and blood metabolites in cattle.

Items	Treatments[1]				SEM	Contrast[2]		
	T1	T2	T3	T4		M	Y	M × Y
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/dl)	17.1	18.3	19.4	19.7	1.99	NS	NS	NS
BUN (mg/dl)	9.5	10.4	12.7	13.1	2.69	NS	NS	NS
Glucose (mg/dl)	55.5	56.1	57.7	58.1	1.95	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/100 mol)								
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
Total direct counts (cell/ml)								
Bacteria ($\times 10^{11}$)	6.1^{a}	7.2^{ab}	8.9^{ab}	10.9^{b}	1.22	*	NS	NS
Protozoa								
Holotric ($\times 10^{4}$)	3.1^{a}	3.0^{a}	2.5^{ab}	2.1^{b}	0.26	*	NS	NS
Entodiniomorph ($\times 10^{5}$)	10.3^{a}	7.8^{b}	4.1^{c}	3.4^{c}	0.71	*	*	NS
Fungal zoospores ($\times 10^{4}$)	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 ($p < 0.05$).
* = $p < 0.05$, NS = $p > 0.05$.

CONCLUSIONS

Based on this experiment, it can be concluded that supplementation of malate level with yeast (*S. cerevisiae*) in concentrates containing high levels of cassava chip can improve ruminal fermentation efficiency, increase propionate production and decrease acetate to propionate ratio. Moreover, high levels of cassava chip in the diet resulted in increased populations of bacteria, but decreased protozoal populations. These results suggest that the combined use of concentrates containing high levels of cassava chip (70 % DM) with 1,000 g of malate and 2,000 g of yeast in the concentrate lead to the highest improvement in rumen fermentation efficiency and digestibility of nutrients in beef cattle.

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บทคัดย่อ

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ผลของระดับการเสริมสารมาเลทและยีสต์ในอาหารข้นที่มีมันเส้นเป็นองค์ประกอบในระดับสูงต่อนิเวศวิทยาในกระเพาะหมักและการย่อยได้ของโภชนะในโคเนื้อ

โคเนื้อเพศผู้อายุ 1 ปี จำนวน 4 ตัววางแผนการจัดสิ่งทดลองแบบ 2 × 2 แฟคตอเรียล ตามแผนการทดลองแบบ 4 × 4 จตุรัสละติน ซึ่งมีปัจจัยที่ศึกษาคือสารมาเลท 2 ระดับได้แก่ 500 และ 1,000 กรัม ร่วมกับยีสต์ (*Saccharomyces cerevisiae*) 2 ระดับได้แก่ 1,000 และ 2,000 กรัม โดยมีสิ่งทดลองที่ทดสอบ 4 สิ่งทดลองดังนี้ สิ่งทดลองที่ 1 เสริมมาเลท 500 กรัม ร่วมกับยีสต์ 1,000 กรัม สิ่งทดลองที่ 2 เสริมมาเลท 500 กรัม ร่วมกับยีสต์ 2,000 กรัม สิ่งทดลองที่ 3 เสริมมาเลท 1,000 กรัม ร่วมกับยีสต์ 1,000 กรัม และสิ่งทดลองที่ 4 เสริมมาเลท 1,000 กรัม ร่วมกับยีสต์ 2,000 กรัม ในอาหารข้น ตามลำดับ โดยสัตว์ทดลองทุกตัวได้รับการเสริมอาหารข้น 1 เปอร์เซ็นต์ของน้ำหนักตัว ร่วมกับฟางข้าวหมักยูเรียเป็นแหล่งอาหารหยาบแบบกินเต็มที่ ผลการทดลองพบว่าสิ่งทดลองทั้ง 4 ชนิดที่เสริมมีผลต่อกระบวนการหมักในกระเพาะหมักและเมแทบอลิซึมในกระแสเลือดไม่แตกต่างกัน อย่างไรก็ตามการย่อยได้ของโภชนะในอาหารที่สัตว์ทดลองได้รับพบว่าแตกต่างกันทางสถิติ โดยเฉพาะอย่างยิ่งความสามารถในการย่อยได้ของโปรตีนในอาหารพบว่าในกลุ่มที่ได้รับสิ่งทดลองที่ 4 มีค่าสูงกว่ากลุ่มที่ได้รับสิ่งทดลองที่ 3 2 และ 1 มีค่าเท่ากับ 74.3 72.5 71.1 และ 68.9 เปอร์เซ็นต์ ตามลำดับ นอกจากนี้ความเข้มข้นของกรดไขมันที่ระเหยได้ในของเหลวในกระเพาะหมักโดยเฉพาะอย่างยิ่งกรดโพรพิออนิก พบว่ากลุ่มที่ได้รับการเสริมสิ่งทดลองที่ 4 มีค่าสูงกว่ากลุ่มที่ได้รับการเสริมสิ่งทดลองที่ 3 2 และ 1 มีค่าเท่ากับ 23.3 21.9 20.9 และ 18.0 เปอร์เซ็นต์ ตามลำดับ นอกจากนี้การเสริมสารมาเลทและยีสต์ที่ระดับต่างกันในอาหารข้นมีผลต่อประชากรของโปรโตซัวและซูโอสปอร์ของราในกระเพาะหมักแตกต่างกันทางสถิติ ดังนั้นจากการทดลองสรุปได้ว่าโคเนื้อที่ได้รับการเสริมอาหารข้นที่มีมันเส้นเป็นองค์ประกอบระดับสูง 70 เปอร์เซ็นต์ร่วมกับการเสริมสารมาเลท 1,000 กรัม และยีสต์ 2,000 กรัมในสูตรอาหารข้นสามารถเพิ่มประสิทธิภาพกระบวนการหมักในกระเพาะหมักและความสามารถในการย่อยได้ของโภชนะสูงสุด

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