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Enrichment Value of Yeast-malate Fermented Cassava Pulp and Cassava Hay as Protein Source Replace Soybean Meal in Concentrate on Rumen Ecology in Crossbred Native Cattle

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Abstract: The objectives of this study was to evaluate the influence supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal in concentrate on feed-intake, Average Dairy Gain (ADG), rumen ecology and blood metabolites in crossbred native cattle. Five, two years of female crossbred native cattles weighing at 250±10 kg were randomly assigned according to a 5 x 5 Latin square design. The dietary treatments were as follows: T1 = supplementation of YMFCP replace soybean ratio at 0:100%; T2 = supplementation of YMFCP replace soybean ratio at 25:75%; T3 = supplementation of YMFCP replace soybean ratio at 50:50%; T4 = supplementation of YMFCP replace soybean ratio at 75:25%, T5 = supplementation of YMFCP replace soybean ratio at 100:0%, respectively. The cows were offered the treatment concentrate at 1.0%BW. All cows were fed *ad libitum* of rice straw with water and a mineral salt block. The results have revealed that rumen fermentation, blood metabolites and average daily gain were significantly different for all treatments. The populations of protozoa and fungal zoospores were significantly different as affected by supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal in concentrate. Moreover, supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal ratio at 50:50% in concentrate with rice straw as roughage source could highest improved ruminal fermentation efficiency, average daily gain including increase populations of bacteria and fungi zoospores, but decreased protozoal populations in rumen of crossbred native cattle.

Key words: Yeast, cassava pulp, soybean meal, cassava hay, rumen ecology, crossbred native cattle

INTRODUCTION

Most farmers in Thailand are smallholder, with more than 60% of the cost for cattle production is the cost of feeds, particularly concentrate and roughages. Feed is the major cost associated with cattle production. Increases in the cost of feeds inevitably cause in creases in the cost of cattle production. In addition, the increase in demand for renewable energy has affected the price of livestock feeds such as corn meal and cassava chip. There are many attempts to reduce cost of feeds through the utilization of cheap raw materials, such as agro-industrial by productions. Although cassava pulp, the residue obtained after the extraction of starch from cassava roots, is low in crude protein (Lounglawan *et al.*, 2010). Cassava pulp offers an alternative to high-starch grains and can be used as an energy source in the diets. 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 source carbohydrates are fed and maintain (Wohlt *et al.*, 1978). However, efficiency utilization of protein and Non-Protein Nitrogen (NPN) in ruminants depends upon knowledge of the basic principles under underlying ruminant microbial N metabolism (Fernandez *et al.*, 1997).

The development and utilization of cassava hay (cassava whole crop at a young growth stage, 3-4 months, harvested about 30-45 cm above ground and sun-dried for 1-2 days until having a final dry matter of at least 85%, as an on-farm feed has been recommended as a possible solution to the lack of good-quality roughages during the dry season in the tropics. The cassava hay contains high protein, 20-27% CP and condensed tannins, 1.5-4%. The use of cassava hay has been successfully implemented in several ways by either direct feeding or as a protein source in concentrate mixtures (Wanapat, 2003; Wanapat and Khampa, 2006; Promkot and Wanapat, 2009).

Some authors (Callaway and Martin, 1996) have suggested that organic acids (aspartate, fumarate, malate) could potentially provide an alternative to currently used antimicrobial compounds. Thus, malate supplementation 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 (Sanson and Stallcup, 1984). Moreover, malic

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acid is a key intermediate in the production of succinate or propionate in some ruminal bacteria and therefore could stimulate propionate production. In fact, propionate production increased by adding malate to *in vitro* cultures with malate might act as an electron sink for hydrogen (Nisbet and Martin, 1991).

In addition, dietary yeast can be used as a ruminant feed especially *Saccharomyces cerevisiae* because the yeast cell contained useful nutrients for ruminant feed especially with high lysine composition (8.0 g/100 g of protein) (Yamada and Sgarbieri, 2005). Fermentation of cassava peels by pure culture *S. cerevisiae* could increase its protein content from 2.4% in nonfermented cassava to 14.1% in fermented products (Antai and Mbongo, 1994). The fermented cassava flour with *S. cerevisiae* enhanced the protein level (from 4.4-10.9%) and decreased the amount of cyanide content (Oboh and Akindahunsi, 2005). Previous study from Boonnop *et al.* (2009) reported that cassava chip can be nutritionally improved with *S. cerevisiae* call yeast fermented-cassava chip (YEFECAP) and could be used for animal feeding as well as increase productivity in dairy heifer (Khampa *et al.*, 2009).

However, the use of Yeast-Malate Fermented Cassava Pulp (YMFCP) and cassava hay as protein source replace soybean meal in concentrate not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation levels of YMFCP and cassava hay as protein source replace soybean meal in concentrate with rice straw as basal roughage in crossbred native cattle.

MATERIALS AND METHODS

Preparation of Yeast-malate Fermented Cassava Pulp (YMFCP): This technique is based on the method developed by Oboh (2006) and Boonnop *et al.* (2009), which enriching nutritive value of cassava pulp fermented by yeast-malate (*Saccharomyces cerevisiae*). The method for synthesis of Yeast-Malate Fermented Cassava Pulp (YMFCP) is as follows:

I. Weighing of yeast at 20 g + sugar at 20 g + malate 10 g + distill water at 100 ml into flask, then mixed and incubated at room temperature for 1 h. (A)
II. Preparation of medium by weigh at 20 g of molasses directly into a warring blender vessel flushed with O_2, add distill water at 100 ml and urea at 30 g then pour solution and incubated at room temperature for 10 min. (B)
III. Adjusting pH media solution by 70% of H_2SO_4 between 3.5-3.8 and continue mix with incubated for 1 h.
IV. Remove yeast media solution in a flask from (A) into a medium (B) and continue flush O_2 for 60 h.
V. After 60 hs, then transfer yeast media solution about 50 ml mix with cassava pulp at 100 g and then covered by plastic bag for a minimum at least 10 days before feeding to animals.

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

	Treatments				
Item	T1	T2	T3	T4	T5
Ingredients (%)					
Cassava chip	65.0	65.0	65.0	65.0	65.0
Cassava hay	5.0	5.0	5.0	5.0	5.0
Rice bran	4.0	4.0	4.0	4.0	4.0
Molasses	5.0	5.0	5.0	5.0	5.0
YMFCP[1]	0.0	3.5	7.5	11.5	15.0
Soybean meal	15.0	11.5	7.5	3.5	0.0
Urea	2.5	2.5	2.5	2.5	2.5
Premix	1.0	1.0	1.0	1.0	1.0
Sulfur	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Sunflower oil	1.0	1.0	1.0	1.0	1.0
Limestone	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0

[1]YMFCP = Yeast-malate fermented cassava pulp. T1 = YMFCP replace soybean meal ratio at 0:100%, T2 = YMFCP replace soybean meal ratio at 25:75%, T3 = YMFCP replace soybean meal ratio at 50:50%, T4 = YMFCP replace soybean meal ratio at 75:25%, T5 = YMFCP replace soybean meal ratio at 100:0%

Animals, diets and experimental design: Five, two years of female crossbred native cattles weighing at 250±10 kg were randomly assigned according to a 5x5 Latin square design to study supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) and cassava hay as protein source replace soybean meal in concentrate on rum ecology and average daily gain. The dietary treatments were as follows: T1 = supplementation of YMFCP replace soybean ratio at 0:100%; T2 = supplementation of YMFCP replace soybean ratio at 25:75%; T3 = supplementation of YMFCP replace soybean ratio at 50:50%; T4 = supplementation of YMFCP replace soybean ratio at 75:25%, T5 = supplementation of YMFCP replace soybean ratio at 100:0%, respectively. The compositions of dietary treatments and rice straw used are shown in Table 1.

Cows were housed in individually pens and individually fed concentrate at 1.0 %BW. All cows were fed *ad libitum* of rice straw with water and a mineral salt block. Feed intake of concentrate and roughage were measured separately and refusals recorded.

The experiment was run in five 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 blood. Body weights were measured daily during the sampling period to feeding.

Data collection and sampling procedures: Concentrate, Yeast-Malate Fermented Cassava Pulp (YMFCP) and roughage were sampled daily during the collection period and were composted by period prior to analyses. Composites samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, OM and CP content (AOAC, 1985), NDF and ADF (Goering and Van Soest, 1970).

Rumen fluid samples were collected at 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 at 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, 1985). Another portion was fixed with 10% of 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 withdrawal 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).

Statistic analysis: All data obtained from the experiment were subjected to ANOVA for a 4 x 4 Latin square design with using the General Liner Model (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment mean was compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical compositions of concentrate diets, Yeast-Malate Fermented Cassava Pulp (YMFCP) and rice straw fed in crossbred native cattle are shown in Table 2. The crude proteins of concentrate, YMFCP and rice straw were at 14.2, 14.3, 14.5, 14.7, 14.8 and 72.1%, respectively. Diets containing high level of Organic Matter (OM) and lower NDF.

Effect on feed intake and average daily gain: Intake of rice straw had linearly increased when cattle received yeast-malate fermented cassava pulp (YMFCP) supplementation. However, total intake was not significantly different in all treatments. The mean of rice straw in cattle received supplementation of YMFCP replace soybean meal ratio at 50:50% was higher than those fed (T2) 25:75, (T4) 75:25, (T5) 100:0 and (T1) 0:100% of diets supplemented (1.7, 1.6, 1.5 and 1.5% BW), respectively. In addition, the average daily gain was significantly different and had higher in crossbred native cattle receiving YMFCP replace soybean meal ratio at 50:50% was higher than those fed 25:75, 75:25, 0:100 and 100:0% of diets supplemented (520.1, 509.7, 482.5, 480.3 and 478.4 g/d), respectively (Table 3). This data indicated that rate of digestion of carbohydrates was the major factor controlling the energy available for growth of rumen microbes. Furthermore, cassava root contain high soluble fractions of starch and sugar and can be to added in diets to increase utilization of ruminal ammonia-N for microbial protein synthesis. A possible explanation for this effect is that low DMI does not provide the microbial population with enough soluble growth factors, such as organic acids, B vitamins and

Table 2: Chemical composition of treatments concentrate and Rice Straw (RS) used in the experiment

Chemical composition (%)	T1	T2	T3	T4	T5	RS
DM (%)	90.2	90.1	90.4	90.6	90.4	87.8
OM	82.3	82.6	83.1	83.3	83.1	88.9
CP	14.2	14.3	14.5	14.7	14.8	72.1
NDF	15.7	15.5	15.4	15.3	15.1	77.2
ADF	8.7	9.1	9.2	9.4	9.5	54.3
Ash	7.9	7.5	7.5	7.3	7.3	13.1

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, RS = Rice Straw

Table 3: Effect of supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal in concentrate on feed-intake, Average Dairy Gain (ADG), rumen fermentation and blood metabolites in crossbred native cattle

Item	T1	T2	T3	T4	T5	SEM
DM intake (%BW)						
Concentrate	1.0	1.0	1.0	1.0	1.0	-
Rice straw	1.5	1.7	1.8	1.6	1.5	0.161
Total	2.5	2.7	2.8	2.6	2.5	0.173
ADG (g/d)	480.3^{a}	509.7^{b}	520.1^{b}	482.5^{a}	478.4^{a}	4.952
Ruminal temperature (°C)	39.2	39.5	40.1	39.4	39.3	0.514
Ruminal pH	6.6	6.7	6.8	6.5	6.5	0.326
NH_3-N (mg/dl)	16.1^{a}	17.8^{bc}	18.2^{c}	17.3^{abc}	16.5^{ac}	0.425
BUN (mg/dl)	7.5^{a}	8.9^{ab}	11.4^{c}	10.3^{bc}	9.6^{abc}	0.689

^{a,b,c}Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard Error of the Means, T1 = YMFCP replace soybean meal ratio at 0:100%, T2 = YMFCP replace soybean meal ratio at 25:75%, T3 = YMFCP replace soybean meal ratio at 50:50%, T4 = YMFCP replace soybean meal ratio at 75:25%, T5 = YMFCP replace soybean meal ratio at 100:0%

AA. Callaway and Martin (1997) suggested that Yeast culture provides soluble growth factors that stimulate growth of cellulolytic bacteria and cellulose digestion. In addition, supplementing diets with yeast (*S. cerevisiae*) increases milk production of dairy cows and weight gain of growing cattle (Brossard *et al.*, 2006). Boonnop *et al.* (2009) reported that there was a remarkable increase in lysine content in the Yeast (*Saccharomyces cerevisiae*) fermented-cassava chip (YEFECAP) which provide enough soluble growth factors for rumen microbe which leading to increase fiber digestion, which could increase rate of passage and therefore improve feed intake and average daily gain.

Effect on rumen fermentation and blood metabolism: Concentrations of NH_3-N, temperature, ruminal pH and BUN in the rumen fluid were used to monitor rumen fermentation pattern (Table 3). The rumen temperature and pH were non-significantly different in all treatments. However, the cattle received supplementation of YMFCP replace soybean meal ratio at 50:50% was higher on rumen pH than those crossbred native cattle fed 25:75, 0:100, 75:25 and 100:0% of diets supplemented (6.8, 6.7, 6.6, 6.5 and 6.5), respectively. It has been suggested that concentrates containing high levels of cassava chip with high levels of nonstructural carbohydrate and readily degradable in rumen could decrease ruminal pH and be lower than optimal values (6.5-7.0) (Wanapat, 2003). Furthermore, previous reports by Hoover (1986) have suggested that the reduced pH decrease digestion of fibers. In addition, higher degradation rates can result in a substantial decrease in ruminal pH and fiber digestibility thus reducing feed intake. Moreover, when ruminal pH was reduced below 6.3 in dairy cows, ADF digestion could be reduced at 3.6% unit per 0.1 pH and may result in depressed feed intake (Erdman, 1998). Other studies Melaku *et al.* (2004) demonstrated inhibitory effects of rumen pH on cellulolysis only at values below 6.1 while Mould and Orskov (1984) reported that lower pH have a major impact on fiber digestion. In addition, Cheng *et al.* (1984) reported that low ruminal pH appeared to prevent a strong attachment of bacteria to plant cell walls, resulting in lower fiber digestion.

Ruminal NH_3-N and BUN concentrations were significantly different ($p<0.05$) among treatments at each hour of sampling between at 16.1-18.2 mg/dl of NH_3-N and 7.5-11.4 mg/dl of BUN, which affected by levels of YMFCP supplementation replace soybean meal in concentrate (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 between at 15-30 mg/dl (Wanapat and Pimpa, 1999) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughage. The differences in NH_3-N and BUN concentrations among treatments may have been related directly to CP levels of concentrate. In addition, Preston *et al.* (1965) reported that concentrations of BUN were highly correlated with protein intake and reflected the level of ammonia production in the rumen. This study revealed that incorporation of concentrate has increased NH_3-N concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation (Satter and Slyter, 1974; Hoover, 1986; Wanapat, 2000). Similarly, Krebs and Leng (1984) suggested requirements for rumen NH_3-N of 20 mg/dl or more for sufficient voluntary intake of low quality roughage.

Rumen microorganism's populations: The effects of Yeast-Malate Fermented Cassava Pulp (YMFCP) supplemented replacements of soybean meal with rice straw as roughage in crossbred native cattle on ruminal microorganisms are summarized in Table 4. The supplementation levels of YMFCP replace soybean meal in concentrate was significantly different among treatments ($p<0.05$) to ruminal microorganism. The crossbred native cattle received concentrate containing YMFCP replace soybean meal ratio at 50:50% had highest increased population of bacteria and fungi while protozoal population was decreased than those crossbred native cattle fed concentrate containing YMFCP replace soybean meal ratio at 75:25, 25:75, 100:0 and 0:100% supplementation, respectively.

However, the populations of protozoa were higher when receiving high levels of concentrate and it could be due to engulfment of starch by protozoa as substrate to produce end-product. In addition, yeast are usually related to stimulation of cellulolytic and lactate-utilizing bacteria in the rumen, increased fiber digestion and

Table 4: Effect of supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal in concentrate on rumen microorganisms in crossbred native cattle

Item	T1	T2	T3	T4	T5	SEM
Total direct counts (cell/ml)						
Bacteria ($x10^{12}$)	3.6^a	5.3^{ab}	7.6^b	5.4^{ab}	5.2^{ab}	0.838
Protozoa						
Holotrich ($x10^2$)	7.4^a	6.5^{ab}	4.3^b	6.9^{ab}	6.8^{ab}	0.864
Entodiniomorph ($x10^3$)	8.4^a	6.7^{ab}	4.6^b	6.6^{ab}	6.8^{ab}	0.933
Fungal zoospores ($x10^6$)	4.6^a	5.2^{ab}	7.4^a	6.7^{ab}	5.9^{ab}	0.691

^{a,b}Values on the same row with different superscripts differ ($p<0.05$). SEM = Standard Error of the Means increased flow of microbial protein from the rumen which may be beneficial for feedlot cattle fed high-grain diets (Guedes *et al*., 2007). These results agreement with Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depended on the rate of soluble sugars and starch in the ration and also pH. Previous study by Rode (2008) reported that entodiniomorph protozoa are predators of rumen bacteria and engulf and digest them just as they engulf starch granules. This is why bacterial numbers are higher when animals are defaunated. Since protozoa tend to stay in the rumen and largely do not pass to the small intestine, they contribute little to the flow of protein and because they digest the bacteria, total protein flow to the small intestine is generally reduced in the presence of protozoa. This is supported by Nguyen *et al*. (2005) who reported the higher bacterial growth efficiency in the absence of the protozoa in the rumen is probably related to the fact that protozoa engulf and digest bacteria. Leng (1990) found that removal of protozoa or a decrease in protozoal density in the rumen can be expected to increase ruminant production under most feeding conditions pertaining to roughage fed ruminants.

Conclusion: Based on this experiment, it could be concluded that supplementation levels of Yeast-Malate Fermented Cassava Pulp (YMFCP) replace soybean meal ratio at 50:50% in concentrate with rice straw as roughage source could highest improved ruminal fermentation efficiency, average daily gain including increase populations of bacteria and fungi zoospores, but decreased protozoal populations in rumen of crossbred native cattle.

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