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Manipulation of Yeast Fermented Cassava Chip Supplementation in Dairy Heifer Raised under Tropical Condition

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Abstract: Four, one-year old of dairy heifers, weighing at 200±10 kg were selected. Cows were randomly assigned according to a 4 x 4 Latin square design to study supplementation levels of Yeast Fermented Cassava Chip (YFCC) replaced concentrate on rumen ecology, cost production and average daily gain. The dietary treatments were as follows: T1 = supplementation of concentrate: YFCC ratio at 100:0; T2 = supplementation of concentrate:YFCC ratio at 75:25; T3 = supplementation of concentrate:YFCC ratio at 50:50; T4 = supplementation of concentrate:YFCC ratio at 25:75, respectively. The animals were offered the treatment concentrate at 1.5 %BW and rice straw was fed *ad libitum*. The results have revealed that feed intake and average daily gain cost productions were significantly different among treatments especially affected the rice straw intake and average daily gain were higher in dairy heifers receiving T3 than T4, T2 and T1. In contrast, the cost productions was lower in dairy heifers receiving T3 than T4, T2 and T1. However, the rumen fermentation and blood metabolites were similar for all treatments. The populations of protozoa and fungal zoospores were significantly different as affected by levels of yeast fermented cassava chip supplementation. These results suggest that supplementation of yeast fermented cassava chip could highest replace at 75% of concentrate in dairy heifers.

Key words: *Saccharomyces cerevisiae*, cassava chip, rumen ecology, dairy heifer

INTRODUCTION

The rumen has been well recognized as an essential fermentation that is capable of preparing end-products particularly Volatile Fatty Acids (VFAs) and microbial protein synthesis as major energy and protein for the ruminant host, hence, the more efficient the rumen is, the optimum the fermentation end-products are being synthesized. In recent years, there have been increasing interests, researches conducted as well as reviews in relation to rumen studies, rumen ecology and rumen manipulation (Martin *et al*., 1999; Khampa *et al*., 2009). 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. In addition, supplementing diets with yeast *(Saccharomyces cerevisiae)* increases milk

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production of dairy cows and weight gain of growing cattle (Brossard *et al*., 2006). 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 (Guedes *et al*., 2007). 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 supplementation levels of yeast fermented cassava chip with rice straw as a basal roughage on rumen ecology in dairy heifers.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, one-year old of dairy heifers weighing at 200±10 kg. Cows were randomly assigned according to a 4 x 4 Latin square design to study supplementation levels of Yeast Fermented Cassava Chip (YFCC) replaced concentrate on rumen ecology, cost production and average daily gain. The dietary treatments were as follows: T1 = supplementation of concentrate:YFCC ratio at 100:0 (control); T2 = supplementation of concentrate:YFCC ratio at 75:25; T3 = supplementation of concentrate: YFCC ratio at 50:50; T4 = supplementation of concentrate:YFCC ratio at 25:75, respectively. The composition of dietary treatments and rice straw are shown in Table 1, 2.

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

Ingredient (DM%)	Concentrates
Cassava chip	65
Rice bran	6
Palm meal	10
Brewer's gain	10
Urea	2
Molasses	5
Sulfer	0.5
Salt	0.5
Mineral mix	1
Total	100

Table 2: Chemical composition of concentrates yeast fermented cassava chip and rice straw used in the experiment

Chemical compositions (%)	Concentrate	Yeast fermented cassava chip	Rice straw
DM	91.5	89.1	91.2
OM	90.3	89.4	86.2
CP	14.2	36.1	3.0
Ash	9.7	10.5	13.8
ADF	35.7	7.5	76.5
NDF	14.6	6.1	54.6
ME (Mcal/kg)	3.1	3.3	1.5
Feed cost (baht/kg)	8.0	6.0	1.0

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

Cows were housed in individual pens and individually fed concentrate at 1.5 %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 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.

Data collection and sampling procedures: Concentrate and rice straw 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, ether extract, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Goering and Van Soest, 1970).

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 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 (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 methods (AOAC, 1985) and Volatile Fatty Acids (VFAs) analyses using a HPLC according to Zinn and Owen (1986). 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 x 4 Latin square design 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 diets, feed-intake average daily gain: The chemical compositions of rice straw and concentrate diets fed in dairy heifers are presented in Table 2. Concentrate diets contained similar concentrations of DM, OM, CP, NDF, ADF and TDN. Diets containing high levels of cassava chip based diets had a slightly higher Non-Structural Carbohydrate (NSC) and lower NDF due to increased level of cassava chip in the diets. Furthermore, the chemical composition of ruzi grass is presented in Table 2.

The effects supplementation levels of yeast *(Saccharomyces cerevisiae)* fermented cassava chip replace concentrate on feed-intake of dairy heifers are presented in Table 3. Feed intake and Average Daily Gain (ADG) were significantly different among treatments especially affected to rice straw intake which higher in dairy heifers receiving T3 than T4, T2, T1 (1.7, 1.6, 1.5 and 1.4 %BW) and (420, 390, 380 and 375 g/day), respectively. Most importantly, the cost productions was significantly differently and lower in dairy heifers receiving T3 than T4, T2, T1 (29.4, 27.4, 30.7 and 32.3 baht/day). This data indicated that yeast fermented cassava chip supplementation replace concentrate had no effect on feed-intake in dairy heifers. This result was in agreement with earlier work by Khampa *et al*. (2009) 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.

Characteristics of ruminal fermentation and blood metabolism: Rumen ecology parameters were measured for temperature, pH and NH_3-N, VFA (Table 3). In addition, BUN was determined to investigate their relationships with rumen NH_3-N and protein utilization. Rumen pH at 0, 2 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.6-6.7, 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 non-significantly different among treatments by levels supplementation of yeast fermented cassava chip replaced concentrate. 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*., 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 populations of fungal zoospores, protozoa and total bacteria direct

Table 3: Effects supplementation levels of Yeast Fermented Cassava Chip (YFCC) on feed-intake and rumen fermentation in dairy heifers

	Treatments[1]					Contrast[2]		
Item	T1	T2	T3	T4	SEM	L	Q	C
DM intake (%BW)								
Concentrate	1.5[a]	1.1[b]	0.7[c]	0.3[d]	0.06	*	NS	NS
YFCC	0[a]	0.3[b]	0.7[c]	1.1[d]	0.07	*	NS	NS
Rice straw	1.4[a]	1.5[ab]	1.7[b]	1.6[ab]	0.07	*	NS	NS
Total	2.9	2.9	3.2	3.1	0.13	NS	NS	NS
ADG (g/day)	375[a]	380[a]	420[b]	390[c]	2.75	*	*	NS
Cost productions (baht/day)	32.3[a]	30.7[ab]	29.4[ab]	27.4[b]	0.65	*	NS	NS
Ruminal pH	6.7	6.6	6.6	6.6	0.09	NS	NS	NS
NH_3-N (mg%)	17.8	18.1	20.4	18.4	1.35	NS	NS	NS
BUN (mg%)	9.2	10.6	11.4	10.2	0.89	NS	NS	NS

[a,b,c]Values on the same row with different superscripts differ ($p<0.05$).
[1]T1 = Supplementation of concentrate:YFCC ratio at 100:0 T2 = Supplementation of concentrate:YFCC ratio at 75:25
T3 = Supplementation of concentrate:YFCC ratio at 50:50 T4 = supplementation of concentrate:YFCC ratio at 25:75
L = linear, Q = quadratic, C = cubic. * = $p<0.05$, NS = $p>0.05$

Table 4: Effects supplementation levels of Yeast Fermented Cassava Chip (YFCC) on rumen microorganisms in dairy heifers

	Treatments[1]					Contrast[2]		
Total direct counts (cell/ml)	T1	T2	T3	T4	SEM	M	Y	M x Y
Bacteria ($x10^{12}$)	4.7[a]	6.2[b]	9.7[c]	7.2[b]	0.37	*	NS	*
Protozoa								
Holotric ($x10^3$)	8.2[a]	5.7[b]	4.4[c]	6.1[b]	0.35	*	NS	NS
Entodiniomorph ($x10^6$)	8.0[a]	6.7[b]	5.8[b]	6.4[b]	0.27	*	NS	NS
Fungal zoospores ($x10^6$)	5.0[a]	6.6[b]	8.7[c]	7.3[d]	0.21	*	NS	*

[a,b,c]Values on the same row with different superscripts differ ($p<0.05$).
[1]T1 = Supplementation of concentrate:YFCC ratio at 100:0 T2 = Supplementation of concentrate:YFCC ratio at 75:25
T3 = Supplementation of concentrate:YFCC ratio at 50:50 T4 = Supplementation of concentrate:YFCC ratio at 25:75
L = linear, Q = quadratic, C = cubic. * = $p<0.05$, NS = $p>0.05$

counts were significantly different and populations of bacteria had higher numbers in heifer receiving diets T3 than T4, T2 and T1. In contrast, the present number of protozoa in the rumen was decreased by yeast fermented cassava chip supplementation replace concentrate diets. In the experiment by Guedes *et al.* (2007) reported that 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. 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 with yeast may play an important role in increasing bacterial populations.

Conclusion: Based on this experiment, it could be concluded that supplementation of yeast *(Saccharomyces cerevisiae)* fermented cassava chip replace concentrate could improve ruminal fermentation efficiency, average daily gain and reduced cost production in daily heifers. Moreover, supplementation of yeast fermented cassava chip in diet resulted increase populations of bacteria, but decreased protozoal populations. These results suggest that supplementation of yeast fermented cassava chip could highest replace at 75% of concentrate in dairy heifers.

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