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Supplementation Levels of Palm Oil in Yeast (*Saccharomyces cerevisiae*) Culture Fermented Cassava Pulp on Rumen Fermentation and Average Daily Gain in Crossbred Native Cattle

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Abstract: Nine, two-years old of crossbred native cattle were used to examine the effects of supplementation levels of palm oil in yeast culture fermented cassava pulp on rumen fermentation and average daily gain. The cows were randomly allocated in a complete randomized design and three replicates (animals) per treatment. The first group (control) was fed on a yeast fermented cassava pulp without palm oil (YFCP0), The second group was fed yeast fermented cassava pulp + palm oil at 1% (YFCP1)and third groups was fed yeast fermented cassava pulp + palm oil at 2% (YFCP2), respectively. The cows were offered the treatment diets at 2%BW and rice straw was fed *ad libitum*. The results have revealed that supplementation of dietary treatment on feed intake, ruminal pH, ammonia-nitrogen and blood urea nitrogen concentration were non-significantly different, while Average Daily Gain (ADG) and cost production were significantly different and had highest in cattle receiving YFCP2 than those fed YFCP1, YFCP0 diets (633.1, 614.5 and 511.1 g/day of ADG and 0.92, 0.81, 0.73 US$/kgBW of cost production, respectively). The populations of bacteria and fungal zoospores were significantly different as affected by levels of palm oil supplementation. Especially, supplementation of YFCP2 in cattle had highest increase populations of bacteria and fungi zoospore than those fed YFCP1 and YFCP0 but decreased protozoal populations. Therefore, supplementation levels of palm oil at 2% in Yeast Culture Fermented Cassava Pulp (YFCP1) as supplement diets 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: *Saccharomyces cerevisiae*, cassava pulp, palm oil, rumen fermentation, crossbred native cattle

INTRODUCTION

Cassava is widely cultivated in tropical areas and used as food and animal fodder. In Thailand, approximately 10 million tons of fresh cassava tubers are consumed annually as a starch staple. When starch is extracted from cassava tubers during manufacturing, grated cassava tubers are separated into starch granules and fibrous residual materials by water extraction followed by centrifugation. The fibrous residual material, called cassava pulp, accounts for approximately 10-30% by weight (wet) of the original tubers (Kosugi *et al*., 2009). Therefore, the tapioca starch industry in Thailand is estimated to generate at least one million ton of cassava pulp annually from 10 million tons of fresh tubers. Processing practices in Thailand, a large amount of starch (up to 60%, on a dry weight basis) together with cellulosic fiber is contained in the cassava pulp which could be used as diets to feeding animals. In addition, palm oil is an important energy component in the diet of ruminants and fat supplementation has been become a common practice to increase the energy density of the diets. Dietary fats have been identified as efficient means of decreasing ruminal methanogenesis.

Utilization of local feed especially 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). Furthermore, 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. The use of yeast culture to improve livestock productivity, and the underlying mechanisms for such improvement, have attracted increasing attention during recent years (Williams and Newbold, 1990). Yeast cells are known to be a rich source of vitamins, enzymes and some

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unidentified cofactors that are helpful in increasing microbial activity in the rumen (Dawson *et al*., 1990; Williams *et al*., 1991). Hence, yeast culture supplementation has been shown to improve the growth rate and feed conversion efficiency (Rouzbehan *et al*., 1994). Several benefits of yeast product supplementation to ruminant nutrition have been demonstrated: an increase in nutrient digestibility, reduction in ruminal ammonia and increase of ruminal microorganism population (Chaucheyras-Durand *et al*., 2008). However, the mechanism of action of yeast products is not completely described. Yeast culture provides various growth factors, pro-vitamins and other stimulants to bacteria growth in the rumen (Miller-Webster *et al*., 2002). Especially, *Saccharomyces cerevisiae*, have been used in animal diets for several decades and are considered sources high quality proteins and B-complex vitamins, selenium and zince (Queiroz *et al*., 2004).

However, the use of levels palm oil with in yeast culture fermented cassava pulp not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation levels of palm oil in yeast culture fermented cassava pulp as supplement diets with rice straw as basal roughage in crossbred native cattle.

MATERIALS AND METHODS

Preparation of yeast culture plus palm oil fermented cassava pulp: This technique is based on the method developed by Oboh (2006), Boonnop *et al*. (2009) and Khampa *et al*. (2010) which enriching nutritive value of cassava pulp fermented by yeast (*Saccharomyces cerevisiae*). The method for synthesis of yeast fermented cassava pulp is as follows:

I. Weighing of yeast at 20 g + sugar at 20 g + distill water at 100 ml into flask, then mixed and incubated at room temperature for 1 hour. (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, urea at 30 g and palm oil at 1, 2 ml, respectively then pour solution and incubated at room temperature for 10 minutes. (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 2 h.
V. After 2 h, then transfer yeast media solution about 50 ml mix with yeast fermented cassava pulp (YFCP0), yeast fermented cassava pulp + palm oil at 1% (YFCP1) and yeast fermented cassava pulp + palm oil at 2% (YFCP2) and then covered by plastic bag for a minimum at least 15 days before feeding to animals.

Table 1: Chemical composition of treatments and Rice Straw (RS) used in the experiment

CC (%)	YFCP0	YFCP1	YFCP2	RS
DM (%)	22.3	20.1	22.4	87.8
OM	87.8	85.6	86.9	74.4
CP	12.1	15.3	17.6	2.1
NDF	22.1	23.1	25.3	77.2
ADF	15.3	16.4	17.4	54.3
Ash	3.4	3.5	3.7	13.1
ME (Mcal/kg)	3.4	3.5	3.5	1.5

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, RS = Rice Straw, CC = Chemical Composition (%). YFCP0 = Yeast fermented cassava pulp, YFCP1 = Yeast fermented cassava pulp + palm oil at 1%, YFCP2 = Yeast fermented cassava pulp + palm oil at 2%

Animals, diets and experimental design: Nine, two-years old of female crossbred native cattle weighing about at 250±20 kg were randomly allocated in a complete randomized design and 3 replicates (animals) per treatment to one of three treatment groups. The first group (control) was fed on a yeast fermented cassava pulp without palm oil (YFCP0), The second group was fed yeast fermented cassava pulp + palm oil at 1% (YFCP1)and third groups was fed yeast fermented cassava pulp + palm oil at 2% (YFCP2), respectively. The composition of dietary treatments and Rice Straw (RS) used are shown in Table 1.

Cows were housed in individual pens and individually dietary treatments. The cows were offered the treatment diets at 2%BW and rice straw was fed *ad libitum* with water and a mineral-salt block. Feed intake of dietary treatments and roughage were measured separately and refusals recorded. The experiment was run for 120 days, the first 15 days for treatment adaptation for feed intake measurements whist the last 30 days were for sample collections of faeces and rumen fluid. Body weights were measured each 30 days during the sampling period prior to feeding.

Data collection and sampling procedures: Rice Straw (RS) and treatment diets were sampled each 30 days and were composted by period prior to analyses. Feed and fecal samples were collected by grab sampling during the last seven 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 DM, ether extract, ash and CP content (AOAC, 1985), NDF, ADF and ADL (Van Soest *et al*., 1991) and AIA.

Rumen fluid and blood samples were collected at 0, 2 and 4 h post-feeding on last period. 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 5,000 g for 15 min and the supernatant stored at -20°C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985).

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).

Statistic analysis: All data obtained from the experiment were subjected to ANOVA for a complete randomized design a complete randomized design and 3 replicates (animals) per treatment 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 yeast fermented cassava pulp without palm oil (YFCP0), yeast fermented cassava pulp + palm oil at 1% (YFCP1)and yeast fermented cassava pulp + palm oil at 2% (YFCP2) and Rice Straw (RS) fed in crossbred native cattle are shown in Table 1. Crude proteins of dietary treatments such as YFCP0, YFCP1, YFCP2 and RS were at 12.1, 15.3, 17.6 and 2.1%, respectively. Similar values for rice straw have been similar to those reported by Wanapat (2000).

Effect on feed intake, average daily gain and cost production: The effects of supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture treated cassava pulp as supplement diets on feed intake, average daily gain and cost production in female crossbred native cattle are presented in Table 2. The dietary treatments intake was non-significant different among treatments and highest in cattle receiving YFCP1 than those fed YFCP2, YFCP0 diets (5.1, 4.8 and 4.6 kgDM/day) as well as rice straw intake was non-significant different and highest in cattle receiving YFCP1 than those fed YFCP2, YFCP0 diets (2.6, 2.6 and 2.5 kgDM/day). In addition, the total intake was similar in all treatments and higher in cattle receiving YFCP1 than those fed YFCP2, YFCP0 diets (7.7, 7.4 and 7.1 kgDM/day). Several benefits of yeast product supplementation to ruminant nutrition have been demonstrated: an increase in nutrient digestibility, alteration of the proportion of volatile fatty acids produced in the rumen, reduction in ruminal ammonia, and increase of ruminal microorganism population (Chaucheyras-Durand *et al*., 2008). In addition, the use of cultures such as *Saccharomyces cerevisiae* or its extracts can improve weight gain, as a result of the response to in creased dry matter intake. Especially, *Saccharomyces cerevisiae*, have been used in animal diets for several decades and are considered sources high quality proteins and B-complex vitamins, selenium and zince (Queiroz *et al*., 2004).

Furthermore, the average daily gain was significantly different and had highest in female crossbred native cattle receiving YFCP2 than those fed YFCP1, YFCP0 diets (633.1, 614.5 and 511.1 g/day), respectively. This data indicated that rate of digestion of carbohydrates was the major factor controlling the energy available for growth of rumen microbes. Furthermore, cassava pulp contains high soluble fractions of starch and protein nitrogen 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 AA. Callaway and Martin (1996) 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

Table 2: Effects of supplementation levels of palm oil in yeast culture treated cassava pulp on feed intake, average daily gain and rumen fermentation in crossbred native cattle

Item	YFCP0	YFCP1	YFCP2	SEM
DM intake (kg/day)				
Treatment	4.60	5.10	4.80	1.037
RS	2.50	2.60	2.60	0.515
Total	7.10	7.70	7.40	0.732
ADG (g/day)	511.10[a]	614.50[b]	633.10[b]	5.071
Cost production (US$/kgBW)	0.73[a]	0.81[b]	0.92[b]	0.024
Ruminal pH	6.60	6.70	6.90	0.337
NH_3-N (mg/dl)	15.80	16.20	17.20	1.543
BUN (mg/dl)	9.60	9.80	10.20	1.156

[a,b]Values on the same row with different superscripts differ ($p<0.05$). YFCP0 = Yeast fermented cassava pulp, YFCP1 = Yeast fermented cassava pulp + palm oil at 1%, YFCP2 = Yeast fermented cassava pulp + palm oil at 2%, SEM = Standard Error of the Means

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. These results suggested that the addition of yeast increased fiber degradation. In the present study, the addition of yeast increased the degradability of forages. The cost production of supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture treated cassava pulp as supplement diets was significantly different and had lower in female crossbred native cattle receiving YFCP0 than those fed YFCP1, YFCP2 diets (0.73, 0.81 and 0.92 US$/kgBW), respectively (Table 2).

Characteristics of ruminal fermentation and blood metabolism: Rumen ecology parameters were measured for pH, NH_3-N and BUN (Table 2). Especially, 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 was similar in all dietary treatments. The dietary treatments are often claimed to smooth rumen pH fluctuations and increase pH nadir of the rumen especially the values were quite stable at 6.6-6.9. 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). In addition, Williams *et al*. (1991) suggested that calf diet supplementation with yeast culture may increase rumen pH regulation via reduced lactic acid production. However, pH values were high on average and did not indicate a severe acidic condition. This may be due to incorporation of rice straw and cassava pulp is high fiber content in the diet which having a high intrinsic buffering capacity to neutralize fermentation acids.

Ruminal NH_3-N and BUN concentrations were not altered by supplement diets. The mean concentration of rumen ammonia nitrogen was increased after increasing levels of palm oil supplementation (15.8-17.2 mg/dl). As time of blood sampling preceded rumen sampling on the same day, the difference between rumen ammonia and blood urea nitrogen was relatively high. In other works, blood samples were likely taken at the time when urea was at its highest concentration but rumen samples were taken when ammonia concentration had already passed its peak. The reduced concentration of ammonia in the rumen appear to be the result of increased incorporation of ammonia into microbial protein which may, in turn, be the direct result of stimulated microbial activity. However, the result obtained was closer to optimal ruminal NH_3-N between at 15-30 mg/dl for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughage (Wanapat and Pimpa, 1999). 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; 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 microorganisms populations: The effects of supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture treated cassava pulp as supplement diets with rice straw as roughage in female crossbred native cattle on ruminal microorganisms are summarized in Table 3. The supplementation levels of palm oil in yeast (*Saccharomyces cerevisiae*) culture treated cassava pulp was significantly different among treatments ($p<0.05$) on rumen bacteria and fungal zoospores populations. The crossbred native cattle received YFCP2 supplementation had highest increased population of bacteria and fungi than those fed YFCP1 and YFCP0 diets (7.8, 6.6 and 5.9 x 10^{10} cell/ml of rumen bacteria; 6.3, 5.6 and 4.5 x 10^6 cell/ml of rumen fungal zoospores, respectively). The yeast cells are known to be a source of vitamins, enzyme and some unidentified cofactors

Table 3: Effects of supplementation levels of palm oil in yeast culture treated cassava pulp on rumen microorganisms in crossbred native cattle

Item	YFCP0	YFCP1	YFCP2	SEM
Total direct counts (cell/ml)				
Bacteria (x10^{10})	5.9^a	6.6^{ab}	7.8^b	0.427
Protozoa				
Holotric (x10^2)	5.2	4.6	4.2	0.284
Entodiniomorph (x10^5)	3.6	3.2	2.9	0.563
Fungal zoospores (x10^6)	4.5^a	5.6^{ab}	6.3^b	0.362

^{a,b}Values on the same row with different superscripts differ ($p<0.05$). YFCP0 = Yeast fermented cassava pulp, YFCP1 = Yeast fermented cassava pulp + palm oil at 1%, YFCP2 = Yeast fermented cassava pulp + palm oil at 2%, SEM = Standard Error of the Means

which are helpful in increasing the microbial activity in the rumen as well as the beneficial effects of yeast supplementation reported so far include better growth rate, feed conversation efficiency and milk yield (Dawson *et al*., 1990). In addition, 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).

Likewise, protozoal population was lowest decreased in YFCP2 than those receiving YFCP1 and YFCP0 diets (4.2, 4.6 and 5.2 x 10^2 cell/ml of holotrich; 2.9, 3.2 and 3.6 x 10^5 cell/ml of entodiniomorph, respectively). The populations of protozoa were lower when receiving high levels of palm oil in diets. This is probably due to protozoa have a limited ability to take up, assimilate and transform dietary lipids and high dietary lipid concentrations are toxic to protozoa and decreased protozoa numbers due to dietary supplementation with fats and oils especially holotrich were the most susceptible to the toxic effects of the oil, followed by the cellulolytic protozoa. 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 Robinson (1997) 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 palm oil at 2% in yeast (*Saccharomyces cerevisiae*) culture fermented cassava pulp (YFCP1) as supplement diets 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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