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Supplementation of Cassava and Durian Hull Fermented Yeast (*Saccharomyces cerevisiae*) on Rumen Fermentation and Average Daily Gain in Crossbred Native Cattle

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Abstract: Ten, two-years old of crossbred native cattle weighing about at 250±20 kg were randomly divided into two groups according to receive two groups of supplemental dietary treatments by receiving YFCP1 + YFCP2 (T1) and YFCRR + YFDH (T2). The cows were offered the treatment diets at 2%BW and rice straw was fed *ad libitum*. Means were compared using pair t-test. All animals were kept in individual pens and received free access to water. The results have revealed that supplementation of dietary treatment on feed intake was non-significantly different, while average daily gain (ADG) and rumen microorganisms especially bacteria and fungi zoospores were significant different and cattle in heifer fed YFCRR + YFDH (T2) treatments and received YFCP1 + YFCP2 (T1) (646.4 and 533.2 g/d). In addition, the ruminal pH, ammonia-nitrogen and blood urea nitrogen concentration were non-significantly different. Supplementation of T2 could improve population of bacteria and fungal zoospore higher than those fed T1, but decreased populations of *Holotrich* and *Entodiniomorph* protozoa in rumen. The results indicate that supplementation of yeast fermented cassava and durian hull as supplement diets with rice straw as roughage source could improve ruminal fermentation efficiency, average daily gain in crossbred native cattle.

Key words: *Saccharomyces cerevisiae*, cassava, durian hull, rumen fermentation, crossbred native cattle

INTRODUCTION

The use of yeast products in dairy cattle nutrition has become widespread over the last 20 yr. However, most of the research has focused on yeast culture and less on Live Yeast (LY). 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). 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).

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). 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). Previous study from Kumar *et al*. (1997) reported that when brewer's yeast or live yeast was included in calf diets at levels between 0.001% and 1.00%, DMI, Average Daily Gain (ADG), percentage of Days Scoured (DS), rumen ammonia, rumen lactic acid production and ruminal propionate were either decreased or not affected. However, yeast culture has increased Feed Efficiency (FE), rumen pH, total ruminal VFA concentration and ruminal butyrate and acetate production when included in calf diets. In addition, 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). The fermented cassava flour with *S. cerevisiae* enhanced the protein level (from 4.4% to 10.9%) and decreased the amount of cyanide content (Oboh and Akindahunsi, 2003). Furthermore, Boonnop

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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.

However, the use of yeast fermenting with cassava and durian hull as diets not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of yeast fermented cassava and durian hull with rice straw as basal roughage in crossbred native cattle.

MATERIALS AND METHODS

Preparation of yeast fermented cassava and durian hull: 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 and durian hull fermented by yeast (*Saccharomyces cerevisiae*). The method for synthesis of yeast fermented cassava and durian hull are 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 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 h, then transfer yeast media solution about 50 ml mix with yeast fermented cassava peel (YFCP1), yeast fermented cassava pulp (YFCP2), yeast fermented cassava root raw (YFCRR) and Yeast Fermented Durian Hull (YFDH) at 100 g and then covered by plastic bag for a minimum at least 30 days before feeding to animals.

Animals, diets and experimental design: Ten, two-years old of crossbred native cattle weighing about at 250±20 kg were randomly divided into two groups according to receive two groups of supplemental feeds by receiving T1 = yeast fermented cassava peel (YFCP1) + yeast fermented cassava pulp (YFCP2) ratio at 50:50%; T2 = yeast fermented cassava root raw (YFCRR) + yeast fermented durian hull (YFDH) ratio at 50:50%, 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. All cows were fed *ad libitum* of rice straw 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 and 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.

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

CC (%)	YFCP1	YFCP2	YFCRR	YFDH	RS
DM (%)	45.1	22.3	34.6	40.7	87.8
OM	83.2	88.2	89.5	86.5	88.9
CP	7.4	12.1	13.7	14.7	2.1
NDF	40.1	20.1	17.5	35.4	77.2
ADF	27.3	15.3	6.1	26.1	54.3
Ash	8.1	3.5	3.9	4.1	13.1

DM = Dry Matter, CP = Crude Protein, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, RS = Rice Straw, CC = Chemical Composition (%). YFCP1 = Yeast fermented cassava peel, YFCP2 = Yeast fermented cassava pulp, YFCRR = Yeast fermented cassava root raw, YFDH = Yeast fermented durian hull

Data collection and sampling procedures: Rice Straw (RS) and concentrate 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: The means of each parameter measured were analyzed by the analysis of variance procedure of SAS (1998) and means were compared using pair t-test.

Table 2: Effects of supplementation of cassava and durian hull fermented yeast as supplement diets on feed intake, Average Daily Gain (ADG) and rumen fermentation in crossbred native cattle

Item	T1	T2	p-value
DM intake (kg/day)			
Treatment	5.10	5.60	0.4019^{NS}
RS	1.50	1.60	0.3899^{NS}
Total	6.60	7.20	0.0413*
ADG (g/day)	533.20	646.40	0.0047**
Cost production (US$/kgBW)	0.78	1.01	0.0453*
Ruminal pH	6.70	6.80	0.0741^{NS}
NH_3-N (mg/dl)	15.80	16.30	0.0523^{NS}
BUN (mg/dl)	7.80	8.40	0.1374^{NS}

T1 = Supplementation of YFCP1 + YFCP2 ratio at 50:50%, T2 = Supplementation of YFCRR + YFDH ratio at 50:50%, NS = Non significant (p>0.05), * = Significant (p<0.05)

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical compositions of yeast fermented cassava peel (YFCP1) + yeast fermented cassava pulp (YFCP2) ratio at 50:50% (T1) and yeast fermented cassava root raw (YFCRR) + yeast fermented durian hull (YFDH) ratio at 50:50% (T2) and Rice Straw (RS) fed in crossbred native cattle are shown in Table 1. Crude proteins of dietary treatments such as YFCP1, YFCP2, YFCRR and YFDH RS were at 7.4, 12.1, 13.7 and 14.7%, respectively. Furthermore, the chemical composition of rice straw is presented in Table 1. Similar values for rice straw have been similar to those reported by Wanapat (2000).

Effect on feed intake, average daily gain and rumen fermentation: The effects of supplementation of cassava and durian hull fermented yeast as supplement diets on feed intake, average daily gain and rumen fermentation in crossbred native cattle are presented in Table 2. The dietary treatments intake was non-significant different among treatments and higher in cattle receiving T2 than those fed T1 diets (5.6 and 5.1 kgDM/day) as well as rice straw intake was non-significant different and higher in cattle receiving T2 than those fed T1 (1.6 and 1.5 kgDM/day). In contrast, the total intake was significantly differently among treatments and higher in cattle receiving T2 than those fed T1 (7.2 and 6.6 kgDM/day). Furthermore, the average daily gain was significantly different and had higher in crossbred native cattle receiving T2 was higher than those fed T1 (646.4 and 533.2 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 root raw, cassava pulp and durian hull 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 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 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.

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 changed by dietary treatments, however the values were quite stable at 6.7-6.8, 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). 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. In addition, Williams *et al.* (1991) suggested that calf diet supplementation with yeast culture may increase rumen pH regulation via reduced lactic acid production.

Ruminal NH_3-N and BUN concentrations were altered by supplementation of cassava and durian hull fermented yeast as supplement diets which containing high cassava-based diets. 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

Table 3: Effects of supplementation of cassava and durian hull fermented yeast as supplement diets on rumen microorganisms in crossbred native cattle

Item	T1	T2	p-value
Total direct counts (cell/ml)			
Bacteria ($x10^{10}$)	5.9	7.8	0.0473*
Protozoa			
Holotric ($x10^{2}$)	3.9	4.1	0.0843^{NS}
Entodiniomorph ($x10^{6}$)	5.2	4.1	0.0782^{NS}
Fungal zoospores ($x10^{6}$)	4.6	6.3	0.0498*

T1 = Supplementation of YFCP1 + YFCP2 ratio at 50:50%, T2 = Supplementation of YFCRR + YFDH ratio at 50:50%, NS = Non significant ($p>0.05$), * = Significant ($p<0.05$)

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 microorganisms populations: The Effects of supplementation of cassava and durian hull fermented yeast as supplement diets on rumen microorganisms in crossbred native cattle are summarized in Table 3. The supplementation of dietary treatments was significantly different among treatments ($p<0.05$).

The crossbred native cattle received yeast fermented cassava peel (YFCP1) + yeast fermented cassava pulp (YFCP2) ratio at 50:50% (T1) supplementation had highest increased population of bacteria and fungi while protozoal population was decreased than those crossbred native cattle fed yeast fermented cassava root raw (YFCRR) + yeast fermented durian hull (YFDH) ratio at 50:50% (T2) supplementation.

The yeast cells are known to be a source of vitamins, enzyme and some unidentified cofactors 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). 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. The 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 of yeast fermented cassava and durian hull as supplement diets with rice straw as roughage source could 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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