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Supplementation of Yeast Fermented Cassava Chip as a Replacement Concentrate on Rumen Fermentation Efficiency and Digestibility of Nutrients in Cattle

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Abstract: Ten, one year old male cattles with initial body weight of 150±10 kg were randomly divided into two groups and received concentrate at 14% CP (T_1) and Yeast Fermented Cassava Chip (YFCC) (T_2). The cows were offered the treatment concentrate at 1% BW and urea-treated rice straw was fed *ad libitum.* Means were compared using t-test. All animals were kept in individual pens and received free access to water. The results have revealed that replacement of YFCC on feed intake was non-significantly different, while Average Daily Gain (ADG) and digestibility of nutrients were higher ($p<0.05$) in cattle fed YFCC (T_2) treatments than received concentrate at 14% CP (T_1) (235 and 203 g day^{-1}). In addition, the ruminal pH, ammonia-nitrogen and blood urea nitrogen concentration were significantly different ($p<0.05$). The concentration of volatile fatty acid was significantly different especially the concentration of propionic acid was slightly higher in cattle receiving T_2 than T_1. Supplementation of YFCC (T_2) could improve population of bacteria and fungal zoospore, but decreased populations of *Holotrich* and *Entodiniomorph* protozoa in rumen ($p<0.05$). The results indicate that supplementation of Yeast-Malate Fermented Cassava Chip (YFCC) as a replacement concentrate at 14% CP could improve rumen fermentation efficiency and digestibility of nutrients in cattle.

Key words: *Saccharomyces cerevisae*, urea-treated rice straw, cassava chip, rumen ecology, ruminants

INTRODUCTION

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). 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). In addition, supplementing diets with yeast (*Saccharomyces cerevisiae*) increases milk production of dairy cows and weight gain of growing cattle (Brossard *et al*., 2006). Production

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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 yeast fermenting cassava as a replacement for concentrate not yet been investigated. Therefore, the objective of this experiment was to investigate the supplementation of yeast fermenting cassava with urea-treated rice straw as a basal roughage on rumen fermentation efficiency and growth in cattle.

MATERIALS AND METHODS

Preparation of Yeast Fermented Cassava Chip (YFCC)

This technique is based on the method developed by Oboh (2006) and Boonnop *et al*. (2008), which enriching nutritive value of cassava chip with yeast (*Saccharomyces cerevisiae*) fermentation. The method for synthesis of YFCC is as follows:

- Weigh 20 g of yeast in to a flask and add with sugar 20 g and distill water 100 mL then mixed and incubated at room temperature for 1 h (A)
- Preparation of medium by weigh 20 g of molasses directly into a warring blender vessel flushed with O_2, add distill water 100 mL and urea 48 g then pour solution and incubated at room temperature for 10 min (B)
- Adjusting pH media solution by 70% H_2SO_4 between 3.5-3.7 and continue mix with incubated for 1 h
- Remove yeast-malate media solution in a flask from (A) into a medium (B) and continue flush O_2 for 60 h
- After 60 h, then transfer yeast-malate media solution 50 mL mix with cassava chip 100 g and then covered by plastic bag for a minimum of 72 h
- Drying of Yeast Fermented Cassava Chip (YFCC) at 30°C for 24 h before feeding to animals

Animals, Diets and Experimental Design

Ten, one-year old of male cattles weighing about 150±10 kg were randomly divided into two groups according to receive two groups of supplemental feeds by receiving concentrate at 14% CP (T_1) and Yeast Fermented Cassava Chip (YFCC). The composition of dietary treatments and urea-treated rice straw (UTS) used are shown in Table 1 and 2.

Cows were housed in individual pens and individually fed concentrate at 1% BW. All cows 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 for 120 days, the first 15 days for treatment adaptation and for feed intake measurements whist the last 7 days were for

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

Ingredients (% DM)	Concentrate
Cassava chip	65.0
Fine rice bran	6.0
Brewer's grain	10.0
Palm meal	10.0
Urea	2.0
Molasses	5.0
Sulfur	0.5
Salt	0.5
Mineral mix[1]	1.0

[1]Minerals and vitamins (each kg contains). 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

sample collections of feces, urine and rumen fluid. Body weights were measured each 30 days 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 animals (Wanapat, 1990).

Data Collection and Sampling Procedures

UTS, YFCC and concentrate diets were sampled each 30 days and were composted by period prior to analyses. Feed, fecal and urine samples were collected by rectal sampling whist urine samples were collected by spot 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, 1990), NDF, ADF and ADL (Van Soest *et al*., 1991) and AIA. AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

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 (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, 1990) and Volatile Fatty Acids (VFAs) analyses using a HPLC according to Zinn and Owens (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).

Statistic Analysis

The mean values of each parameter measured in the digestibility studies and internal parasitic egg counts were analyzed by the analysis of variance procedure of SAS (1998) and means were compared using t-test.

RESULTS AND DISCUSSION

Chemical Composition of Feeds

Crude proteins of concentrate, YFCC and UTS were at 14.2, 29.1 and 7.9%, respectively. 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 UTS is presented in Table 2. Similar values for UTS have been similar to those reported by Wanapat (2000).

Effect on Feed Intake and Digestibility of Nutrients

Feed intake were non-significantly different among treatments and was higher in cattle receiving T_2 than T_1 (2.6 and 2.5% BW) (Table 3). This result was in agreement with earlier study by Sommart *et al*. (2000) and Khampa *et al*. (2006), 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.

Table 2: Chemical composition of concentrate, Yeast Fermented Cassava Chip (YFCC) and urea-treated rice straw (UTS)

Analyzed composition (%)	T_1	T_2	UTS
DM	91.50	89.10	55.80
OM	90.30	89.50	88.90
CP	14.20	29.10	7.90
TDN^1	78.30	78.90	55.10
NDF	25.70	17.50	73.20
ADF	14.60	6.10	52.30
ME (Mcal kg^{-1})	3.10	3.30	1.90
Price (US$ kg^{-1})	0.28	0.23	0.05

T_1: Concentrate; T_2: YMFCC; TDN^1: Dig CP+dig CF+dig EE×2.25+dig NFE (NRC, 1989)

Table 3: Effects of supplementation of Yeast Fermented Cassava Chip (YFCC) as a replacement concentrate on feed intake, digestibility of nutrients and average daily gain (ADG) in cattle

Item	T_1	T_2	p-value
DM intake (% BW)			
Concentrate	1.00	-	-
YFCC	-	1.0	-
Rice straw	1.50	1.6	0.7732^{NS}
Total	2.50	2.6	0.6841^{NS}
Apparent digestibility (%)			
DM	65.70	67.1	0.521^{NS}
OM	68.50	71.2	0.987^{NS}
CP	74.30	76.3	0.536^{NS}
NDF	62.40	64.9	0.742^{NS}
ADF	47.20	49.1	0.856^{NS}
ADG (g day^{-1})	203.00	235.0	0.0278*
Cost production (US$/kg BW)	2.94	2.4	0.0351*

T_1: Supplementation of concentrate at 14% CP, T_2: Supplementation of Yeast Fermented Cassava Chip (YFCC) NS: Non significant at $p>0.05$; *: Significant at $p<0.05$

Apparent digestibility of DM, OM, CP, NDF and ADF were non-significant different ($p<0.05$) for all diets, however, digestible of nutrient intake tended to be higher in cattle fed YFCC (T_2) than T_1. The slightly lower NDF digestibility of the cassava-based diets may have contributed to higher degradation in substantial decrease in fiber digestibility. Furthermore, in the experiment by Hoover (1986) reported that 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.

Characteristics of Ruminal Fermentation and Blood Metabolism

Rumen ecology parameters were measured for pH, NH_3-N and VFA (Table 4). 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 was changed by dietary treatments, however, the values were quite stable at 6.6-6.9, 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 altered by YFCC (T_2) supplement which containing high cassava-based diets. 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^{-1} (Wanapat and Pimpa, 1999; Chanjula *et al*., 2003, 2004) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages.

The influence of supplementation of Yeast Fermented Cassava Chip (YFCC) as a replacement concentrate on 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 significantly different by increased with receiving YFCC (T_2) than T_1 (117.6 and 102.4 mM). However, it was found that total VFA concentration in all diets ranged from

Table 4: Effects of supplementation of Yeast Fermented Cassava Chip (YFCC) as a replacement concentrate on rumen fermentation and blood metabolites in cattle

Item	T_1	T_2	p-value
Ruminal pH	6.6	6.9	0.0372*
NH_3-N (mg dL^{-1})	17.2	21.4	0.0432*
BUN (mg dL^{-1})	8.6	13.4	0.0457*
Total VFA (mmol L^{-1})	102.4	117.6	0.0351*
Molar proportion of VFA (mol/100 mol)			
Acetate (C2)	72.4	66.8	0.0481*
Propionate (C3)	17.8	23.9	0.0531*
Butyrate (C4)	9.8	9.3	0.0842^{NS}
C2:C3 ratio	4.1	2.7	0.0412*
C2+C4:C3 ratio	4.6	3.1	0.0429*

T_1: Supplementation of concentrate at 14% CP, T_2: Supplementation of Yeast Fermented Cassava Chip (YFCC), NS: Non significant at $p>0.05$, *: Significant at $p<0.05$

Table 5: Effects of supplementation of Yeast Fermented Cassava Chip (YFCC) as a replacement concentrate on rumen microorganisms in cattle

Item	T_1	T_2	p-value
Total direct counts (cell mL^{-1})			
Bacteria ($\times 10^{11}$)	6.8	8.4	0.0452*
Protozoa			
Holotric ($\times 10^{3}$)	6.5	4.6	0.0463*
Entodiniomorph ($\times 10^{5}$)	5.1	2.7	0.0374*
Fungal zoospores ($\times 10^{6}$)	4.9	6.8	0.0472*

T_1: Supplementation of concentrate at 14% CP, T_2: Supplementation of Yeast Fermented Cassava Chip (YFCC), NS: Non significant ($p>0.05$), *: Significant ($p<0.05$)

70 to 130 mM. Especially, the acetate to propionate ratio was decreased by receiving YFCC (T_2) than T_1 (2.7 and 4.1) but the supplementation of YFCC (T_2) increased the daily output of propionate without decreasing the production of acetate (23.9 and 17.8 mol/100 mol) and it was in agreement with the results reported by Callaway and Martin (1996) and Khampa *et al*. (2006).

Rumen Microorganisms Populations

The populations of fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria had higher numbers in cattle receiving diets YFCC (T_2) than T_1 (Table 5). In contrast, the present number of protozoa in the rumen was decreased by YFCC supplementation in high cassava-based 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.

CONCLUSION

Based on this experiment, it could be concluded that supplementation of Yeast Fermented Cassava chip (YFCC) as a replacement concentrate at 14% CP could improved ruminal fermentation efficiency, digestibility of nutrients and increasing propionate production, but decreased acetate to propionate ratio. In addition, supplementation of YFCC increase populations of bacteria, but decreased protozoal populations.

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