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Research Article

Influence of *Aspergillus niger* or *Saccharomyces cerevisiae*-Fermented Napier Grass (*Pennisetum purpureum*) Mixed with Fresh Cassava Root on Blood Parameters and Nutrient Digestibility in Growing Beef Cattle

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Abstract

Objective: The current study was designed to determine the effect of *Aspergillus niger* or *Saccharomyces cerevisiae* fermented napier grass (NG) mixed with fresh cassava root (CR) on the blood biochemistry, blood enzymes, hematological parameters and nutrient digestibility in growing beef cattle. **Materials and Methods:** Four male beef cattle (150±10 kg) were randomly assigned according to a 4×4 Latin square design, to receive four dietary treatments: Napier grass (Control), non-microbial-fermented NG mixed with CR (F-NGCR), *A. niger*-fermented NG mixed with CR (AF-NGCR) or *S. cerevisiae*-fermented NG mixed with CR (SF-NGCR). **Results:** The results revealed the dry matter (DM) intake was similar among the treatments (p>0.05). The intake of organic matter (OM), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were not significantly different among the treatments (p>0.05). However, the intake of crude protein (CP) was affected by AF-NGCR and SF-NGCR compared with the control (p<0.05). The digestibility of DM, OM, CP, EE and NDF were increased in the beef cattle that consumed AF-NGCR and SF-NGCR (p<0.05). The blood biochemistry, blood enzymes and hematological parameters did not differ among the treatments (p>0.05). **Conclusion:** *Aspergillus niger* and *S. cerevisiae*-fermented NG mixed with CR could improve the CP intake and nutrient digestibility and had no effect on the blood biochemistry, blood enzymes and hematological parameters in growing beef cattle.

Key words: Fresh cassava root, napier grass, *Aspergillus niger*, *Saccharomyces cerevisiae*, digestibility, blood biochemistry, hematological parameters, cattle

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Beef cattle are an essential part of most smallholder farming systems in Southeast Asia, providing draft power, manure, meat for home consumption and cash income. The increasing consumption of meat in Southeast Asia is related to increasing household income and rapid urbanization. This rising demand for beef presents poor livestock producers with significant opportunities to increase the benefits gained from their livestock and raise income through increasing livestock sales[1].

Napier grass (*Pennisetum purpureum*) is an important forage species in tropical areas due to its large biomass production and when harvested at the right time, it can provide a high amount of nutrients[2]. However, feeding forages usually cannot meet cattle nutritional requirements due to limited energy and protein content. Supplementation of energy and protein has been shown to improve the nutrient intake, digestibility and performance of cattle.

Cassava (*Manihot esculenta* Crantz) is an annual crop widely grown in tropical and subtropical regions. It thrives in sandy-loam soils with low organic matter and in climates with low rainfall and high temperature[3,4]. Cassava roots have high levels of energy (75-85% soluble carbohydrates) and minimal levels of crude protein (2-3% CP)[5,6]. However, cassava root easily deteriorates a few days after harvesting and contains hydrocyanic acid (HCN). Ensiling is an effective way of decreasing the cyanide concentration in cassava root[7].

Aspergillus niger and *Saccharomyces cerevisiae* has been used to enhance protein levels and improve the digestibility of feedstuffs and forage in ruminants. Microbial products have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance. A new method of yeast supplementation for ruminants, which uses cassava chips fermented with a pure culture of microbial products, has been examined[8]. Wanapat *et al*.[6], reported that yeast-fermented cassava chips could be an alternate protein source for soybean meal in a concentrate diet, providing improved nutrient digestibility and rumen fermentation efficiency in ruminants. *Aspergillus niger* would efficiently increase the protein content of cassava root and reduce the level of cyanide[9]. Belewu and Yahaya[10] reported that *A. niger*-treated shea butter cake improved weight gain and fiber digestibility and thus resulted in an enhanced performance of goats compared to untreated shea butter cake.

Blood was examined as a screening procedure to monitor and evaluate the health and nutritional status of ruminants[11,12]. Increased blood urea nitrogen (BUN) concentrations alter hepatic metabolism by increasing ureagenesis and may also affect glucose metabolism in the liver and peripheral tissues[13]. Moreover, hematological indices have been used to monitor and evaluate the health and nutritional status of ruminants[11]. However, limited data are available regarding the effect of *S. cerevisiae* or *A. niger*-fermented Napier grass (NG) mixed with cassava root (CR) on digestibility, blood biochemistry and hematology. Therefore, the objective of the current study was to investigate the effect of different microbial fermented-NG mixed with CR on feed intake, nutrient digestibility, blood biochemistry and hematology in beef cattle.

MATERIALS AND METHODS

The study was conducted under the control and advice of the Phusing Research and Training Center, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Isan, Kalasin Campus, Sahatsakhan, Kalasin, Thailand. Animals involved in this study were approved by the Animal Ethics Committee of Rajamangala University of Technology Isan, based on the Ethic of Animal Experimentation of National Research Council of Thailand. Napier grass was harvested during the maturing stage after 60 days of re-growth and chopped with a forage cutter into pieces of 2 cm. Fresh cassava root was chopped by machine. Microbial-fermented NG mixed with CR was prepared by mixing 200 g of *S. cerevisiae* or *A. niger* culture with 100 kg NG mixed with CR (30% Napier grass, 65.8% fresh cassava root, 4% urea and 0.2% sulfur) (Table 1). The mixture was covered with a plastic sheet for a minimum of 21 days before being fed directly to the animals.

Four, male, crossbred beef cattle (50% Brahman×50% Thai Native breed) of 150±10 kg body weight (BW) were randomly assigned to receive four dietary treatments

Table 1: Ingredients and chemical composition of dietary treatments used in the experiment

	Dietary treatments			
Items	Control	F-NGCR	AF-NGCR	SF-NGCR
Ingredients (%) DM				
Napier grass	100	30	30	30
Cassava root	-	65.8	65.8	65.8
Urea	-	4	4	4
Sulphur	-	0.2	0.2	0.2
Chemical composition				
DM (%)	26.4	30.6	30.8	30.5
DM of (%)				
OM	91.4	91.4	91.4	91.4
CP	8.2	10.6	11.9	11.5
EE	2.4	2.9	3.0	2.8
Ash	8.6	8.7	8.8	8.7
NDF	63.7	26.8	26.3	27.0
ADF	33.7	14.1	13.7	13.4

were as follows: Napier grass (Control), Non-microbial-fermented NG mixed with CR (F-NGCR), *A. niger*-fermented NG mixed with CR (AF-NGCR) and *S. cerevisiae*-fermented NG mixed with CR (SF- NGCR). All treatments were fed *ad libitum*. Animals were housed individually and fed the experimental diets twice daily at 08:00 and 16:00 h. Clean fresh water and mineral blocks were available *ad libitum*. The experiment was conducted over four periods, each lasting for 21 days: The first 14 days were used for feed intake measurements and the remaining 7 days for fecal collection.

Feed intakes were measured and refusals were recorded. Body weights were measured daily during the sampling period prior to feeding. Fecal samples were collected by rectal sampling. Feed, refusals and fecal samples were dried at 60°C, ground (1 mm screen using a Cyclotech Mill, Tecator) and analyzed using the standard methods of the Association of Official Analytical Chemists (AOAC)[14] for DM, CP, EE and ash, whereas NDF and ADF were analyzed according to Van Soest *et al.*[15]. Acid-insoluble ash (AIA) was analyzed and used to estimate the digestibility of the nutrients[16].

At the end of each period and at 3 h after feeding, blood samples (10 mL) were collected from the jugular vein into tubes containing 12 mg of EDTA as an anticoagulant. Plasma was separated by centrifugation at 500 rpm for 10 min at 4°C and stored at -20°C until analysis. Concentrations of BUN and blood glucose (BGlu) were determined using a diagnostic kit (Albumin-HRII, L type Wako UN, Glucose-HRII Wako and NEFA-HR, Tokyo, Japan). Blood creatinine (BCre) was measured by the Hitachi 912 (Roche Diagnostic System, Basel, Switzerland). Commercial kits were used to determine the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with a specific spectrophotometer (Apple 302, USA). Blood hematocrit (Hct) and hemoglobin (Hb) were determined as described by Kume and Tanabe[17].

Statistical analysis: The data were analyzed using statistical analysis system (SAS)[18] with a 4×4 Latin square design. Data were analyzed using the model:

$$Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk18}$$

Where:

Y_{ijk} = Observation from treatment i, animal j and period k

μ = Overall mean

M_i = Mean effect of treatments (i = 1-4)

A_j = Mean effect of animals (j = 1-4)

P_k = Mean effect of periods (k = 1-4)

ϵ_{ijk} = Residual error. Differences among the treatment means were determined by Duncan's new multiple range test[19] and the differences among means with a p<0.05 were considered statistically significant. Comparisons among the treatments were tested by orthogonal contrast

RESULTS AND DISCUSSION

The chemical compositions of the beef cattle diets are presented in Table 1. The CP was greater in the F-NGCR and was greatest in the AF-NGCR and SF-NGCR, whereas the NDF and ADF were lower in all three groups than those in the napier grass (control). Wanapat and Khampa[20] reported that cassava chips are a good source of rumen fermentable carbohydrates and are efficient when used with urea as a non-protein nitrogen source for efficient nutrient digestibility and microbial protein synthesis. In addition, microbial-fermented cassava chips produced the highest increase in CP content. Similar results have been reported by Iyayi[21] and Wanapat *et al.*[6], when a yeast- or *A. niger*-fermented by-product or cassava chip was used.

Microbial-fermented NG mixed with CR improved the CP intake and nutrient digestibility in the growing beef cattle. Dry matter intakes and ME intake were not affected by the microbial-fermented NG mixed with CR (p>0.05) (Table 2). This result agrees with a previous study by Polsit *et al.*[22], who reported that DM intake was not influenced by supplementation with yeast-fermented cassava and durian hull in beef cattle. In contrast, Boonnop *et al.*[23] and Wanapat *et al.*[6], reported that using yeast-fermented cassava chip as protein sources in concentrated diets for dairy steers or dairy cows, could improve the DM intake. The present study indicated that microbial-fermented NG mixed with CR had no negative effects on the health of the beef cattle. The nutrient intake of DM and EE were not significantly different among the treatments (p>0.05). In contrast, the intake of CP and the digestibility of DM, OM, CP and EE were increased by F-NGCR and were the highest in the AF-NGCR and SF-NGCR groups (p<0.05). Similarly, Piamphon *et al.*[24], reported that the nutrient digestibility of DM, OM, CP and EE were influenced by yeast or *A. niger*-fermented napier grass mixed with fresh cassava root in beef cattle. Di Francia *et al.*[25], found that supplementation with *S. cerevisiae* increased the digestibility of OM, CP and energy. This digestibility is due to *S. cerevisiae*-fermented NG mixed with CR that may stimulated proteolytic bacteria, whereas *A. niger*-fermented NG mixed with CR may be explained by the growth of protozoa and the related increase in the degradation of proteins. The digestibility of NDF was increased by F-NGCR and was the highest in the

Table 2: Effects of microbial fermented NG on feed intake, nutrient intake and digestibility coefficient in beef cattle

	Dietary treatments					Contrast		
Items	Control	F-NGCR	AF-NGCR	SF-NGCR	SEM	Control vs F-NGCR, AF-NGCR, SF-NGCR	F-NG vs AF-NGCR, SF-NGCR	AF-NGCR vs SF-NGCR
DM intake (kg day^{-1})	4.10	4.20	4.10	4.10	0.15	0.80	0.85	0.83
BW (%)	2.70	2.80	2.70	2.70	0.04	0.59	0.41	0.50
Estimated energy intake ME (Mcal d^{-1})	10.8	11.0	11.0	11.0	0.44	0.78	0.74	0.98
Nutrients intake (kg day^{-1})								
OM	3.70	3.80	3.70	3.80	0.13	0.79	0.84	0.81
CP	0.40^a	0.40^a	0.50^b	0.50^b	0.02	0.04	0.19	0.42
EE	0.10	0.10	0.10	0.10	0.01	0.46	0.59	0.36
NDF	2.60	1.10	1.10	1.10	0.10	0.57	0.51	0.43
ADF	1.40	0.60	0.60	0.60	0.08	0.78	0.71	0.60
Digestibility coefficients (%)								
DM	56.2^a	60.2^b	62.6^b	62.0^b	0.96	0.04	0.67	0.26
OM	62.2^a	64.7^b	67.3^c	66.6^c	0.77	0.02	0.03	0.26
CP	52.2^a	61.4^b	65.5^c	66.1^c	1.34	<0.01	<0.01	0.23
EE	75.3^a	80.9^b	83.0^{bc}	83.9^c	0.95	<0.01	<0.01	0.02
NDF	49.0^a	51.5^b	53.2^c	53.5^c	0.54	<0.01	0.03	0.47
ADF	40.7	40.8	41.2	41.4	0.63	0.75	0.60	0.52

Table 3: Effects of microbial fermented NG on blood biochemistry and hematology in beef cattle

	Dietary treatments					Contrast		
Items	Control	F-NGCR	AF-NGCR	SF-NGCR	SEM	Control vs F-NGCR, AF-NGCR, SF-NGCR	F-NGCR vs AF-NGCR, SF-NGCR	AF-NGCR vs SF-NGCR
Blood metabolites (mg dL^{-1})								
BUN	12.8	12.9	12.9	12.4	0.72	0.85	0.82	0.67
Creatinine	1.00	1.00	1.00	1.00	0.08	0.80	1.00	0.43
Glucose	73.5	77.2	74.5	73.2	3.34	0.96	0.67	0.41
Blood enzymes (IU L^{-1})								
Alanine aminotransferase	9.30	9.30	9.50	9.80	0.38	0.85	0.60	0.38
Aspartate aminotransferase	30.0	31.7	32.2	31.0	1.20	0.35	0.36	0.66
Hematology (%)								
Hematocrit	34.7	33.0	32.3	32.5	0.96	0.44	0.11	0.72
Hemoglobin	11.5	11.0	10.9	10.8	0.61	0.25	0.10	0.70

AF-NGCR and SF-NGCR (p<0.01). These results were similar to a previous study conducted by Piamphon *et al.*[24], who reported that yeast- or *A. niger*-fermented napier grass mixed with fresh cassava could improve the digestibility of fiber in beef cattle. The mode of action, for *A. niger* is the action of extracellular enzymes remaining in the spent medium and for the yeast, the mode of action is the presence of soluble growth factors or metabolic intermediates that stimulate the growth of the ruminal bacteria that digest cellulose[26-28]. The results of these studies indicate that the stimulation of cellulose degradation by yeast and *A. niger* is associated with a decreased lag time, which results in increased initial rates of digestion.

The effect of microbial-fermented NG mixed with CR on the blood biochemistry and hematology in the beef cattle is presented in Table 3. The concentrations of the ruminant blood components were used to monitor nutrient status (e.g., blood glucose), BUN and associated muscle mass (e.g., creatinine)[29]. The BUN did not differ among the dietary treatments (p>0.05); this supports results presented by Promkot *et al.*[8], who reported that the BUN was not affected by yeast-fermented cassava chip in dairy cows. A decrease in rumen NH_3, N concentrations also decreased the concentration of the BUN[30]. Perhaps more nitrogen is available for ruminal protein synthesis and relatively less NH_3 is available for urea formation in the liver due to feeding with the microbial-fermented NG mixed with CR. Creatinine is an indicator of protein metabolism in ruminants and is positively correlated with muscle mass[31]. The BCre was not significantly different among the treatments (p>0.05). The results of the present study indicate that increasing the CP intake had no effect on the BCre concentrations. Moreover, the BGlu was not altered when the F-NGCR or microbial-fermented NG mixed with CR (p>0.05) was fed. Observed BCre (1.0 mg dL^{-1}) and BGlu (74.6 mg dL^{-1}) concentrations were similar to those reported by Cherdthong *et al.*[12]. The ALT is a cytoplasmic enzyme that catalyzes the transamination of α-ketoglutarate and L-alanine, forming glutamate and pyruvate and may play an important role in protein metabolism[32]. Elevated levels of ALT are associated with skeletal muscle necrosis or injury in ruminants[33]. Blood enzyme ALT and AST did not change in the cattle consuming the microbial-fermented NG mixed with CR ($p>0.05$), indicating that microbial-fermented NG mixed with CR are positively related to health in ruminants. Moreover, hematological indices have been used to monitor and evaluate the nutritional status and health of ruminants because they are correlated to nutritional status[11,12]. The Hct and Hb were unaffected ($p>0.05$) by the non-microbial or microbial-fermented NG mixed with CR. Microbial-fermented NG mixed with CR can be used for feeding beef cattle without negatively affecting the blood biochemistry and hematology. Thus, *A. niger* and *S. cerevisiae*-fermented NG mixed with CR could be used as a good quality roughage and energy source for beef cattle. The use of microbial-fermented NG mixed with CR in feeding trials of lactating dairy cows and fattening beef cattle should be investigated in future studies.

CONCLUSION

The results of the current study indicate that *A. niger* and *S. cerevisiae*-fermented napier grass mixed with fresh cassava root increases CP intake and nutrient digestibility, whereas the mixtures do not adversely affect the blood biochemistry and hematological parameters in the growing beef cattle.

SIGNIFICANCE STATEMENT

This study discovered that *Aspergillus niger* or *Saccharomyces cerevisiae*-fermented Napier grass (*Pennisetum purpureum*) mixed with fresh cassava root improves the intake of crude protein and nutrient digestibility in growing beef cattle. This study provides insight into the critical areas of feed intake, digestibility, blood biochemistry, blood enzymes and hematological parameters that many researchers were not able to explore previously. Thus, a new theory is derived on these microbial-fermented forages mixed with fresh cassava root.

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