Feed intake, digestibility and blood parameters as influenced by Aspergillus Niger or Saccharomyces Cerevisiae fermented napier grass (Pennisetum purpureum) mixed with fresh cassva root in beef cattle

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ABSTRACT: This experiment was to investigate the effect of *Aspergillus niger* or *Saccharomyces cerevisiae* or fermented Napier grass mixed with fresh cassva root (NC) on blood biochemistry, blood enzymes, hematological and nutrient digestibility in beef cattle. Four female beef cattle (350±14 kg) were randomly assigned according to a 4×4 Latin square design, in which the cattle were fed with four dietary treatments with Napier grass (Control), non-microbial fermented NC (FNC), *A. niger* fermented NC (AFNC) and *S. cerevisiae* fermented NC (SFNC). All animals were respective treatments given *ad libitum*. The results revealed that dry matter (DM) intake and nutrient intake were increased (P<0.01) by AFNC and SFNC. Nutrient digestibility was significantly affected by AFNC and SFNC (P<0.01). Moreover, NH_3-N and glucose concentrations increased (P<0.05) when cattle were fed AFNC whereas the blood enzymes and hematological parameters were not altered among all treatments (P>0.05). Based on this experiment, it could be concluded that *A. niger* and *S. cerevisiae* fermented NC could improve feed intake, nutrient digestibility and blood biochemistry in beef cattle.

Keywords: Fresh cassava chip, *Saccharomyces cerevisiae*, *Aspergillus niger*, blood biochemistry, beef cattle

Introduction

In Thailand, Napier grass (*Pennisetum purpureum*) (NG) is among the major feed resources for beef cattle in the medium and high rainfall areas. The NG is an important forage species in tropical areas due to its large biomass production and if harvest at the right moment can supply a high amount of nutrients (Córdoba et al., 2013). Since feeding a NG only diet at the recommended stage of maturity does not support high ruminant performance. Improvements in growth performance from concomitant improvements in roughage to gain ratios would be a significant advancement upon current management practices (Rouquette et al., 1980).

Cassava (*Manihot esculenta,* Crantz) is an annual tropical tuber crop grown widely in tropical

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and sub-tropical areas (Wanapat, 2009). This plant is well easily grown under minimal management and it adapts to poor soil condition, high temperature and low rainfall (Chanjula et al., 2007). Cassava root contains high levels of energy and has been used as a source of readily fermentable energy and has been used with non-protein nitrogen (NPN), especially urea in ruminants (Wanapat et al., 2011a).

Microbial products claiming to improve ruminant production by modulating rumen function, feed intake, fiber digestibility and the activities of its microflora, are based either on yeast (*Saccharomyces cerevisiae*) or on spent culture medium from growth of *Aspergillus niger*. The process of protein enrichment of ruminant feed using microorganisms in a semi-solid culture to improve the nutritional value of forage for ruminants has been evaluated (Wanapat et al., 2011b). Wanapat et al. (2011b) reported that cassava chips fermented with *S. cerevisiae* (yeast-fermented cassava chips) significantly increase crude protein (CP) content (29.7%) and enhancing dry matter (DM) intake, nutrient digestibility and overall animal performance in ruminants. Moreover, Oboh et al. (2002) also reported that *Aspergillus niger* fermentation of cassava increased the protein contents (12.2%), as well as the fat content (5.7%) and lower cyanide content (9.1 mg/kg). However, limited data has been available regarding *S. cerevisiae* or *A. niger* fermented napier grass mixed with fresh cassava root (NC) on feed intake, digestibility, blood biochemistry and hematology. Therefore, the objective of the current experiment was to investigate the effect of different microbial fermented NC on feed intake, nutrient digestibility, blood biochemistry and hematology in beef cattle.

Materials and methods

Preparation of microbial fermented NC

Napier grass was harvested at the maturing stage after 60 d of re-growth and chopped with a forage cutter into pieces of 4 cm. Microbial fermented NC was prepared by using *A. niger* ($1x10^6$ cell/ml.) or *S. cerevisiae* ($1x10^6$ cell/ml.) mixed with Napier grass 65.8 kg, Fresh cassava root 30 kg, Urea 4 kg and Sulphur 0.2 kg and then covered up for 21 days before directly feeding to the animals.

Animals, diets and experimental design

Four, female crossbred beef cattle (50% Brahman × 50% Thai Native breed) with 350±14 kg of body weight (BW) were randomly assigned to receive four dietary treatments according to a 4×4 Latin square design. Dietary treatments were as follows: T1=Napier grass (Control), T2= non-microbial fermented NC (FNC), T3=*A. niger* fermented NC (AFNC), T4=*S. cerevisiae* fermented NC (SFNC). All treatments were fed *ad libitum*. Animals were housed individually and fed the experimental diets twice daily at 08.00 hrs. and 16.00 hrs. 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. The chemical composition of experimental diets is shown in **Table 1**.

Data collection, sampling procedures

Feed intakes were measured and refusals recorded. BW were measured daily during the sampling period prior to feeding. Feeds were

sampled daily during the collection period and were composited by period prior to chemical analyses. Feeds and fecal were collected during the last 7 days of each period. 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 analysed using the standard methods of AOAC (1995) for DM, CP, EE and ash while NDF and ADF were analysed according to Van Soest et al. (1991) and acid-insoluble ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

At the end of each period, blood samples (10 ml) were collected from the jugular vein at 3 hrs. after feeding into tubes containing 12 mg of EDTA as anticoagulant and plasma was separated by centrifugation at 500×g for 10 min at 4°C and stored at -20°C until used. Concentrations of BUN, blood glucose (BGlu) was 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 Roche Hitachi 912 Plus automatic analyzer (Indianapolis, IN). The commercial kits were used in determination the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) with using specific spectrophotometer (Apple 302, USA). Blood hematocrit (Hct) and hemoglobin (Hb) were determined as described by Kume and Tanabe (1993).

Statistical analysis

Data were analysed as a 4×4 Latin square design using the PROC GLM procedure of SAS (1996). Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980) and differences among means with $P<0.05$ were accepted as representing statistically significant differences.

Results and Discussion

Chemical composition of feeds

The chemical compositions of diets fed in beef cattle are presented in **Table 1**. The FNC was greater in CP and was the greatest in the AFNC and SFNC while it was lower in NDF and ADF than control group. The increase in the CP content of the cassava root could be attributed to the possible secretion of some extracellular enzymes into the cassava mash in an attempt to make use of the cassava starch as a source of carbon (Oboh et al., 2002). Wanapat and Khampa (2007) reported that cassava chip was a good source of rumen fermentable carbohydrate and was efficient when used with urea for efficient microbial protein synthesis in the rumen and nutrient digestibility. Similarily, Wanapat et al. (2011b) reported that high level of CP of yeast-fermented cassava chip and good amino acid profile, especially lysine. Oboh et al. (2002) reported that *A. niger* fermentation of cassava increased the protein contents of cassava products.

Feed intake, nutrient intake and digestibility coefficients

The effect of microbial fermented NC on feed intake, nutrient intake and digestibility in beef cattle are presented in **Table 2**. DM intake, nutrient intake and nutrient digestibility are influenced by AFNC and SFNC ($p<0.05$). Greater

feed intakes could be attributed by greater digestibility. Increased digestibility of fiber was concurrent with increased number and proportion of cellulolytic organism (Wiedmeier et al., 1987). The fungi and yeast may have provided both stimulating factors and cellulolytic organisms and fortified with vitamins and minerals that may have stimulated cellulolytic bacteria (Bryant, 1973). In addition, digestibility of CP increased with microbial fermented NC. These results could be due to *A. niger* and *S. cerevisiae* were stimulatory and actively proteolytic bacteria (Boing, 1983). Wanapat et al. (2011b) reported that yeast-fermented cassava chip increased the digestibility of CP, EE and NDF. Weidmeier et al. (1987) reported that digestibility of CP and ADF increased with *S. cerevisiae* and *A. oryzae* supplementation. Di Francia et al. (2008) found that combination of *A. oryzae* and *S. cerevisiae* supplementation could improve digestibility of NDF. On the basis of these previous results and our own results, we propose that the microbial fermented NC may increase fiber and other nutrient digestibility, which could increase the rate of passage and therefore improve feed intake in beef cattle.

Blood biochemistry and hematology

The effect of microbial fermented NC on blood biochemistry and hematology in beef cattle are presented in **Table 3**. Concentration of blood components was used to monitor nutrient status (e.g. blood glucose) and blood urea nitrogen (BUN) and associated muscle mass (e.g. creatinine) (Turner et al., 2005). Concentration of BUN was significantly affected by FNC and AFNC ($P<0.01$). Metabolism of creatine in muscle results in urinary creatinine, creatinine production has been used as an index of total muscle mass or the turnover of the nitrogen pool in the body (Xue et al., 1988). Bcre levels in ruminants have been used to reflect muscle protein mass and it was not significantly different among treatments ($P>0.05$). In addition, BGlu were also not altered when FNC or microbial fermented NC ($P>0.05$). Observed BCre (1.0 mg/dl) and BGlu (74.6 mg/dl) concentrations were similar to those reported by Cherdthong et al. (2014). Blood enzyme ALT was not affected by treatments ($P>0.05$). Enzyme AST catalyzes the transfer of α-amino group from an amino acid to an α-keto acid and is widely distributed in ruminant tissues (Doornenbal et al. 1988). In the present study, blood enzyme AST was not affected by microbial fermented NC ($P>0.05$). Moreover, the Hct and Hb were unaffected ($P>0.05$) as FNC or microbial fermented NC. This result indicates that *A. niger* and *S. cerevisiae* fermented NC positively related to health in ruminants.

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

Item	Dietary treatments[1]			
	Control	FNC	AFNC	SFNC
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
		% of DM		
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

[1]Control, napier grass; FNC, Non-microbial fermented NC; AFNC, *A. niger* fermented NC; SFNC, *S. cerevisiae* fermented NC.

Table 2 Effects of microbial fermented NC on feed intake, nutrient intake and digestibility coefficientin beef cattle

Item	Dietary treatments[1]				SEM
	Control	FNC	AFNC	SFNC	
DM intake					
kg/d	8.1^{ab}	7.7^{a}	8.5^{b}	8.5^{b}	0.16
%BW	2.7^{ab}	2.6^{a}	2.8^{b}	2.8^{b}	0.05
Estimated energy intake					
ME, Mcal/d	17.4	17.1	18.7	17.8	0.57
Nutrients intake, kg/d					
OM	7.4^{a}	7.0^{b}	7.7^{c}	7.8^{c}	0.15
CP	0.7^{a}	0.8^{b}	1.0^{c}	0.9^{c}	0.02
EE	0.2^{a}	0.2^{b}	0.3^{c}	0.2^{b}	0.01
NDF	4.3^{a}	4.6^{b}	5.1^{c}	5.1^{c}	0.09
ADF	2.7^{a}	3.1^{b}	3.2^{b}	3.3^{b}	0.06
Digestibility coefficients, %					
DM	49.0^{a}	55.6^{b}	55.1^{b}	56.4^{b}	1.85
OM	54.2^{a}	60.1^{b}	64.3^{c}	64.7^{c}	1.12
CP	47.8^{a}	55.1^{b}	63.3^{c}	64.0^{c}	1.92
EE	75.3^{a}	79.6^{b}	79.7^{b}	81.8^{b}	1.20
NDF	49.4^{a}	55.3^{b}	57.1^{c}	58.4^{c}	0.54
ADF	37.1^{a}	39.9^{b}	41.2^{b}	39.8^{b}	0.54

[1]Control, napier grass; FNC, Non-microbial fermented NC; AFNC, *A. niger* fermented NC; SFNC, *S. cerevisiae* fermented NC.

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

Item	Dietary treatments[1]				SEM
	Control	FNC	AFNC	SFNC	
Blood metabolites, mg/dl					
BUN	11.6[a]	13.8[b]	14.5[b]	11.8[a]	0.60
Creatinine	1.0	1.0	1.0	1.0	0.05
Glucose	74.0	78.0	82.0	80.5	3.31
Blood enzymes (IU/L)					
Alanine aminotransferase	8.8	9.8	9.3	9.0	0.36
Aspartate aminotransferase	31.0	32.7	33.2	31.5	1.19
Hematology, %					
Hematocrit	33.7	33.5	32.0	32.5	1.40
Hemoglobin	11.2	11.1	10.6	10.8	0.46

[1]Control, napier grass; FNC, Non-microbial fermented NC; AFNC, *A. niger* fermented NC; SFNC, *S. cerevisiae* fermented NC.

Conclusion

Based on this study, it could be concluded that *A. niger* and *S. cerevisae* fermented NC resulted in an improved DM intake, nutrient intake and nutrient digestibility while they did not effect on blood biochemistry and hematological in beef cattle.

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