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Effect of Levels of Supplementation of Concentrate Containing High Levels of Cassava Chip on Rumen Ecology, Microbial N Supply and Digestibility of Nutrients in Beef Cattle

M. Wanapat* and S. Khampa

Tropical Feed Resources Research and Development Center (TROFREC),
Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

ABSTRACT : The object of this study was to determine the influence of supplementation of concentrate containing high levels of cassava chip on rumen ecology, microbial protein and digestibility of nutrients. Four, rumen fistulated crossbred beef steers with initial body weight of 400±10 kg were randomly assigned according to a 4×4 Latin square design. The dietary treatments were concentrate cassava chip based offering at 0, 1, 2 and 3% BW with urea-treated rice straw fed *ad libitum*. It was found that ruminal pH was significantly decreased with increase of concentrate. Volatile fatty acids (VFA) concentration in the rumen was significantly different among treatments. In addition, a molar proportion of propionate was higher in supplemented groups at 2 and 3% BW ($p<0.05$), leading to significantly decreased acetate:propionate ratio. Furthermore, microbial N supply was significantly improved and was highest at 2% BW supplementation. The efficiency of rumen microbial-N synthesis based on organic matter (OM) truly digested in the rumen was highest in level of concentrate supplementation at 2% BW (80% of cassava chip in diets). Moreover, bacterial populations such as amylolytic bacteria was linearly increased, while cellulolytic bacteria was linearly decreased ($p<0.01$) when cattle received concentrate supplementation in all levels. The total protozoal counts were significantly increased, while fungal zoospores were dramatically decreased in cattle receiving increased levels of concentrate. In conclusion, cassava chip can be use as energy source at 80% in concentrate and supplementation of concentrate at 2% BW with urea-treated rice straw as roughage could improve rumen fermentation efficiency in beef cattle. (**Key Words :** Concentrate, Cassava Chip, Urea, Rumen Ecology, Microbial Protein Synthesis, Beef Cattle, Ruminants)

INTRODUCTION

Cassava (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropical regions of Africa, Asia and Latin America. It thrives in sandy-loam soils with low organic matter, and in climate with low rainfall and high temperature (Hong et al., 2003; Wanapat et al., 2004). Cassava can be grow to produce cassava foliage as a protein feed sources (Wanapt, 2003 ; Khang et al., 2005) and tuber is a energy source for animal feed. Cassava tubers contain high levels of energy and minimal levels of crude protein and have been used as readily fermentable energy in ruminant rations and has been used extensively as a feed for livestock (Wanapat, 2003; Kiyothong and Wanapat, 2004; Promkot and Wanapat, 2005).

Cassava chip (CC) or pellet contained high level of non-structural carbohydrate and were highly degradable in the rumen as compared with other energy sources including corn meal (Sommart et al., 2000; Chanjula et al., 2003). In addition, higher level of non-protein nitrogen (NPN) particularly urea could be incorporated in concentrate due to cassava chip's high rate of ruminal degradation. Current research work using high CC and urea levels in dairy steers (80% CC with 4% urea; Khampa et al., 2006a; Kampa et al., 2006b), in lactating dairy cows resulted in good milk yield and quality (75% CC with 4.5% urea). Most importantly CC could completely replace corn meal in concentrate and resulted in more lucrative productivity (Chanjula et al., 2004). Therefore, this present study was conducted to determine the influence of levels of supplementation of concentrate containing high levels of cassava chip on rumen

* Corresponding Author: Metha Wanapat. Department of Animal Science, Faculty of Agriculture, Khon Kaen University, P.O. Box 40002, Thailand. Tel: +66-43-202368, Fax: +66-43-202368, E-mail: metha@kku.ac.th
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Table 1. Composition of concentrate diet and urea-treated rice straw (UTS) used in the experiment (% DM basis)

Item	Concentrate	Urea-treated rice straw (UTS)
Ingredient (%DM)		
Cassava chip	80	
Fine rice bran	6	
Whole cotton seed	5	
Urea	4	
Molasses	3	
Sulfur	0.5	
Salt	0.5	
Mineral mix	1	
Analyzed composition (%)		
DM	92.8	52.4
OM	91.2	84.7
Ash	8.9	15.3
CP	15.7	8.9
TDN	79.1	54.1
NDF	14.5	89.8
ADF	10.1	57.0
NDF protein	1.5	-
Fat	2.5	1.2
NSC[1]	59.9	-

[1] NSC = 100-((NDF-NDF protein)+protein+fat+ash), DM = Dry matter, CP = Crude protein, OM = Organic matter, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, TDN = Total digestible nutrient, NSC = Non-structural carbohydrate.

ecology, microbial protein and digestibility of nutrients in beef cattle.

MATERIALS AND METHODS

Animals, treatments and experimental design

Four-fistulated crossbred (Brahman×Native) beef steers with initial body weight of 400±10 kg were randomly assigned according to a 4×4 Latin square design to investigate the effects of levels of supplementation of concentrate containing high level of cassava chip with urea-treated rice straw (UTS) as a roughage source on rumen ecology, ruminal fermentation, nitrogen balance, feed intake and digestibility of nutrients and microbial protein synthesis. The dietary treatments contained concentrate at 0, 1, 2 and 3% BW, respectively.

Urea-treated rice straw (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).

All animals were kept in individual pens and water was available free choice. The experiment was conducted for four periods, and each period lasted for 21 days. During the first 14 days, all animals were fed on respective diets at *ad libitum* basis, while the last 7 days, the animals were kept in metabolism crates for total collection during which they were restricted to 90% of the previous voluntary feed intake of straw. Chemical and composition of concentrate and UTS used are shown in Table 1.

Data collection, sampling procedures and statistic analysis

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 (Goering and Van Soest, 1970) and AIA. AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Rumen fluid and jugular blood samples were collected at 0, 1, 2, 4, 6 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen using a 60-ml hand syringe at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into three portions; one portion was used for NH_3-N analysis 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 minutes and supernatant was stored at -20°C prior to NH_3-N and VFA analyses using a HPLC (Instruments by controller water model 600E; water model 484 UV detector; column novapak C_{18}; column size 4 mm×150 mm; mobile phase 10 mM H_2PO_4 (pH 2.5), ETL Testing Laboratory, Inc., Cortland, New York, 13045, U.S.A.) according to Samuel et al. (1997). Second portion was fixed with 10% formalin solution in sterilized 0.9% saline solution (Galyean, 1989). The total direct count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco). Third portion was taken to study cultured groups of viable bacteria using roll-tube technique (Hungate, 1969), for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 500×g for 10 minutes (Table Top Centrifuge PLC-02, U.S.A.) and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (HPLC) (Instruments by controller water model 600E; water model 484 UV detector; column novapak C_{18}; column size 4 mm×150 mm; mobile phase 10 mM H_2PO_4 (pH 2.5), ETL Testing Laboratory, Inc., Cortland, New York, 13045,

Table 2. Effect of levels of supplementation of concentrate containing high level of cassava chip on feed intake, apparent digestibility and nitrogen balance in beef cattle

Item	Level of concentrate (% BW)				SEM	Contrast	
	0	1	2	3		L	Q
DM intake (% BW)							
UTS	1.9[a]	1.7[ab]	1.2[bc]	1.0[c]	0.20	*	NS
Conc.	0[a]	0.9[b]	1.8[c]	1.9[c]	0.17	**	NS
Total	1.9[a]	2.6[b]	3.0[b]	2.9[b]	0.20	**	NS
Apparent digestibility (%)							
DM	51.4[a]	64.6[b]	66.3[b]	68.7[b]	2.04	**	*
OM	60.3[a]	68.5[b]	71.0[b]	73.1[b]	1.99	**	NS
CP	44.6[a]	77.7[b]	72.0[b]	71.8[b]	2.04	**	**
NDF	57.8[a]	54.2[a]	39.9[b]	44.2[b]	2.04	**	NS
ADF	50.6[a]	48.6[a]	36.7[b]	42.3[c]	0.08	**	**
Nitrogen balance (g/d)							
N intake	96.8[a]	142.8[b]	171.5[c]	181.9[d]	1.17	**	NS
Faecal N	33.0[a]	36.7[a]	50.2[b]	49.9[b]	1.12	**	**
Urinary N	24.1[a]	36.3[b]	34.3[b]	34.8[b]	1.22	**	**
N absorption	60.1[a]	109.1[b]	119.6[b]	133.6[b]	1.18	**	NS
N retention	35.5[a]	68.9[b]	87.3[c]	97.7[d]	2.04	**	NS

[a, b, c] Values on the same row with different superscripts differ ($p<0.05$).
UTS = Urea-treated rice straw, Conc. = Concentrate, DM = Dry matter, CP = Crude protein.
OM = Organic matter, NDF = Neutral -detergent fiber, ADF = Acid -detergent fiber.
L = Linear, Q = Quadratic.
* $p<0.05$, ** $p<0.01$, NS: $p>0.05$.

U.S.A.) as described by Chen et al. (1993).

The amount of microbial purines absorbed (X mmol/day) corresponding to the purine derivatives excreted (PD) (Y mmol/day) was calculated based on the relationship derived by Chen and Gomes (1995).

$$Y = 0.85X+(0.385\ W^{0.75})$$

where Y is the excretion of purine derivatives (mmol/day); X the microbial purines absorbed (mmol/day).

The supply of microbial N in gram per day was estimated as follows:

$$\text{Microbial N (g/day)} = \frac{X\times 70}{0.116\times 0.83\times 1{,}000} = 0.727\times X$$

with X being the absorption of purine derivatives in mmol per day, following the assumptions made by Chen and Gomes (1995).

- Digestibility of microbial purine is 0.83.
- The N content of purines is 70 mg N/mmol.
- The ratio of purine-N: total N in mixed rumen microbes is 11.6:100

The efficiency of microbial protein supply (EMNS) to denote the microbial N supplied to the animal per unit of DOMR was calculated using the following formula:

$$\text{EMNS} = \frac{\text{MN (g/day)}\times 1{,}000\ \text{(g)}}{\text{DOMR (g)}}$$

Where DOMR = DOMI×0.65 (ARC, 1990), DOMR = digestible organic matter apparently fermented in the rumen and DOMI = digestible organic matter intake.

Statistical analyses were performed using the GLM procedure of SAS (1998). Data were analyzed using the model $Y_{ijk} = \mu+M_i+A_j+P_k+\varepsilon_{ijk}$. Where Y_{ijk} observation from animal *j*, receiving diet *i*, in period *k*; μ, the overall of mean, M_i,the mean effect of level concentrate (i = 1, 2, 3, 4), A_j, the effect of animal (j = 1, 2, 3, 4), P_k, the effect of period(k = 1, 2, 3, 4), ε_{ijk} the residual effect. Mean separations with a significant F ($p<0.05$) for treatment were statistically compared using the Duncan's New Multiple Rang Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSION

Apparent digestibility, feed intake and nitrogen balance

Intake of UTS had linearly decreased ($p<0.05$) when cattle received concentrates supplementation. Additionally, apparent digestibilities (%) of DM, OM, CP, NDF and ADF were significantly different in all treatments (Table 2). In addition, the mean DM digestibility in cattle received supplementation of concentrate at 3% BW was higher than those fed 2, 1 and 0% BW of concentrate supplemented (66.3, 64.4 and 51.4%), respectively. This data indicated

Table 3. Effect of levels of supplementation of concentrate containing high level of cassava chip on ruminal pH, ammonia-nitrogen (NH_3-N), blood urea nitrogen (BUN) and volatile fatty acid (VFAs) in beef cattle

Item	Level of concentrate (% BW)				SEM	Contrast	
	0	1	2	3		L	Q
Ruminal pH	6.7[a]	6.6[a]	6.3[b]	5.7[c]	0.08	*	NS
NH_3-N (mg/dl)	12.8[a]	15.4[b]	16.1[b]	17.1[b]	2.04	*	NS
BUN (mg/dl)	12.3	12.5	14.1	14.5	0.81	NS	NS
Total VFA (mmol/L)	107.2[a]	118.2[b]	119.2[b]	118.8[b]	3.76	*	NS
VFA (mol/100 mol)							
Acetate	71.1[a]	67.9[ab]	64.8[ab]	63.4[b]	2.09	*	NS
Propionate	17.5[a]	21.5[b]	24.9[c]	26.1[c]	0.81	**	NS
Butyrate	10.9	10.6	10.3	10.5	0.82	NS	NS
Acetate:propionate ratio	4.0[a]	3.1[ab]	2.6[b]	2.4[b]	0.04	*	NS
Acetate+butyrate: propionate ratio	4.6[a]	3.6[b]	3.0[bc]	2.8[c]	0.20	**	NS

[a, b, c] Values on the same row with different superscripts differ ($p<0.05$).
L = Linear, Q = Quadratic, C = Cubic.
* $p<0.05$, ** $p<0.01$, NS: $p>0.05$.

that rate of digestion of carbohydrates was the major factor controlling the energy available for growth of rumen microbes. Furthermore, cassava chip contain high soluble fractions of starch and sugar and can be to added to diets to increase utilization of ruminal ammonia-N for microbial protein synthesis. Although, previous reports (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, 1988).

The intake of nitrogen was significantly affected by differences dietary treatments as presented in Table 2, nitrogen balance in term of nitrogen absorption and retention were significantly different among treatments. In addition, nitrogen excreted in faeces in all groups was significantly higher than control group. In this regard, the positive nitrogen balance, which observed in this study indicated that the positive influence of the different treatment feeds as supplements with UTS based feeding of cattle. This result was in agreement with Owens and Zinn (1988) who reported that nitrogen retention was considered as the most common index of the protein nutrition status of ruminants. In addition, Mehrez and Ørskov (1978) also reported that urea supplementation of oat straw diets had little effect on fecal nitrogen excretion but increased urinary nitrogen excretion, which they attributed to the high rumen degradability of urea. This observation can be attributed to the type of protein and its degradability and also possibly due to lack of synchrony between nitrogen release through protein degradation and energy availability.

Rumen fermentation parameters

Concentration of NH_3-N, VFA, BUN and pH in the rumen fluid were used to monitor rumen fermentation pattern (Table 3). The rumen pH was significantly affected by level of concentrate supplementation. The cattle fed UTS with concentrate at 0 and 1% BW supplementation had higher rumen pH (6.7 and 6.6) than those cattle fed UTS with concentrate at 2 and 3% BW (6.3 and 5.7) supplementation. In this experiment, supplementation of concentrate at 3% BW resulted in lowest ruminal pH (5.3) than those received concentrate supplementation at 2, 1 and 0% BW, respectively. It has been suggested that concentrates containing high levels of cassava chip with high levels of nonstructural carbohydrate and readily degradable in rumen could decrease ruminal pH and be lower than optimal values (6.5-7.0) when cattle received high level of concentrate at 3% BW supplementation (Wanapat, 2003).

Other studies Melaku et al. (2004) demonstrated inhibitory effects of rumen pH on cellulolysis only at values below 6.1 while Mould and Ørskov (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. Based on this study, the rumen pH measured in cattle supplemented with any of the treatment feeds, supplementation of concentrate at 3% BW resulted in inhibiting the fermentation of fiber in the rumen as well as microbial protein synthesis.

Ruminal NH_3-N concentrations were significantly different ($p<0.05$) among treatments at each hour of sampling and were in optimal ruminal NH_3-N range (15-30 mg %, Boniface et al., 1986; Perdok and Leng, 1990; Wanapat and Pimpa, 1999) for improving rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake. Furthermore, blood urea-nitrogen concentrations were not significantly different among treatments. 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

Table 4. Effect of levels of supplementation of concentrate containing high level of cassava chip on ruminal bacteria, protozoa, fungi population, total viable, amylolytic, proteolytic and cellulolytic bacteria in beef cattle

Item	Level of concentrate (% BW)				SEM	Contrast	
	0	1	2	3		L	Q
Rumen microbes (cells/g)							
Bacteria ($\times10^{11}$)	1.1^{ab}	1.2^{b}	1.0^{ac}	0.8^{c}	0.13	*	*
Protozoa ($\times10^{5}$)	4.1^{a}	5.3^{ab}	7.6^{bc}	9.6^{c}	0.87	*	NS
Fungal zoospores ($\times10^{5}$)	15.1^{a}	9.6^{ab}	7.0^{c}	5.3^{c}	2.04	*	NS
Viable bacteria (CFU/g)							
Amylolytic ($\times10^{7}$)	5.6^{a}	8.5^{b}	20.2^{c}	3.6^{a}	0.81	**	**
Proteolytic ($\times10^{6}$)	2.8^{a}	7.8^{b}	5.0^{c}	3.6^{a}	0.40	**	**
Cellulolytic ($\times10^{7}$)	23.1^{a}	11.5^{b}	7.0^{c}	6.6^{c}	1.22	**	NS

[a, b, c] Values on the same row with different superscripts differ ($p<0.05$).
L = Linear, Q = Quadratic, C = Cubic.
* $p<0.05$, ** $p<0.01$, NS: $p>0.05$.

Table 5. Effect of levels of supplementation of concentrate containing high level of cassava chip on nitrogen balance (g/d), excretion of purine derivatives (mmol/d) and microbial nitrogen supply in beef cattle

Item	Level of concentrate (%BW)				SEM	Contrast	
	0	1	2	3		L	Q
PD, mmol/d							
Allantoin excretion[1]	143.6^{a}	211.6^{b}	244.6^{c}	130.7^{d}	1.22	**	**
Allantoin absorption	137.8^{a}	215.2^{b}	256.6^{c}	122.7^{d}	2.06	**	**
Microbial N supply, gN/d[2]	98.9^{a}	156.4^{b}	186.6^{c}	89.2^{d}	0.85	**	**
EMNS, gN/kg OMDR[3]	18.3^{a}	20.1^{b}	23.3^{c}	12.1^{d}	0.12	**	**

[a, b] Values on the same row with different superscripts differ ($p<0.05$).
[1] Allantoin in urine cattle was 80-85% of total purine (IAEA, 1997).
[2] Microbial N (gN/day) = $(X\times70)/(0.116\times0.83\times1,000) = 0.727\times X$ (where, X = total absorption of purine derivatives).
[3] EMNS = Efficiency of microbial nitrogen supply (g N/kg OMDR), OMDR (kg) = 65% of organic matter digestible in total tract.

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 % or more for sufficient voluntary intake of low quality roughages.

The influence of levels of supplementation of concentrate containing high levels of cassava chip with UTS as roughage on total VFA concentrate, production of total VFA, acetic acid proportion, propionic acid proportion, butyric acid proportion and acetic to propionic ratio are shown in Table 3. Mean total VFA concentration increased from 107.2 to 119.2 m*M*/L and proportion of propionic acid ranged from 17.5 to 26.1 m*M*/L ($p<0.05$) as linearly as increasing level of concentrate supplementation. The observed reduction in pH associated with increased concentrate feeding was due to increased VFA concentrations and were similar to the results of Robinson et al. (1986) and Sutton et al. (1993) who reported that increasing the starch content of the concentrate resulted in higher rumen propionate concentrations which may cause a depression in rumen pH. However, the results of total VFA concentration in all diets were found in normal concentrations (70 to 130 m*M*/L) and agreed with result of France and Siddons (1993).

Rumen microorganism populations

The effects of supplementation of concentrate with UTS as roughage in cattle on the ruminal microorganisms are summarized in Table 4. The supplementation of concentrate containing high level of cassava chip was significantly different among treatments ($p<0.05$). The higher concentrate supplementation decreased population of bacteria and fungi while protozoal population was decreased ($p<0.05$). In addition, supplementation of levels concentrate on viable bacterial population such as amylolytic and proteolytic bacteria were higher than the control groups while cellulolytic bacteria were linearly decreased ($p<0.05$). However, the populations of protozoa were higher when receiving high levels of concentrate and it could be due to engulfment of starch by protozoa as substrate to produce end-product. Furthermore, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depended on the rate of soluble sugars and starches in the ration and also pH.

Urinary excretion of purine derivatives and microbial nitrogen supply

In ruminants, allantoin is a main product of purine catabolism and the principal purine derivative in urine. The supplementation of level of concentrate on the purine derivative excretion, EMNS and microbial N supply are summarized in Table 5. Excretion of allantoin in urine was linearly and quadratically increased ($p<0.01$) with effects of different level of concentrate supplementation. The microbial nitrogen supplies as calculated from purine derivative excretion were from 89.2 to 186.6 g N/day. Moreover, EMNS ranged from 9.3 to 19.3 g N/kg OMDR. The higher microbial nitrogen supply and EMNS in beef steers fed concentrate at 2% BW may be due to synchronization of the available fermentable energy and degradable nitrogen in the rumen. Hoover and Stokes (1991) reported that the rate of digestion of carbohydrates was a major factor controlling the energy available for microbial growth. In this experiment concentrates with high level of cassava chip at 80% and urea at 4% could be synchronized to produce ruminal NH_3-N and C-skeleton suitable for ruminal microbial protein synthesis.

CONCLUSIONS

Based on this study it was shown that locally available carbohydrate source of cassava chip could be effectively at high level of 80% of concentrate and high level of urea at 4%. Supplementation of concentrate with containing high level of cassava chip at 2% BW was most suitable for rumen ecology, ruminal fermentation and increasing of microbial protein synthesis efficiency in rumen. In addition, this concentrate was inexpensive, easily made by the farmers and could be used for beef cattle as well as dairy cows. Further use of cassava chip in ruminant diets should be widely recommended and advocated both at farm and industrial levels.

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