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Effects of Pelleted Cassava Chip and Raw Banana (Cass-Bann) on Rumen Fermentation and Utilization in Lactating Dairy Cows

R. Lunsin, M. Wanapat, C. Wachirapakorn and C. Navanukraw
Tropical Feed Resources Research and Development Center (TROFREC),
Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

Abstract: Six, multiparous early-lactation Holstein-Friesai and Thai native crossbred dairy cows were randomly assigned according to Switch back design to study the effects of cass-bann feed on rumen fermentation, milk yield and milk composition in lactating dairy cows. The dietary treatments were as follows; T1-non-supplementation of cass-bann pellet, T2-supplementation of cass-bann pellet I in concentrate, T3-supplementation of cass-bann pellet II in concentrate. The results showed that dietary treatments had no effect on voluntary dry matter intake ($p>0.05$) while digestion coefficients of organic matter and neutral detergent fiber in T2 and T3 were significantly higher than those in T1 ($p<0.05$) but T2 and T3 were similar ($p>0.05$). Total direct count of bacteria and fungal zoospores in T3 tended to be higher than those in other treatments and total direct count of protozoa in T3 tended to be lower than those in other treatments. Total viable bacteria counts in T3 were significantly higher than those in T1 and T2 ($p<0.05$). Total Volatile Fatty Acids (TVFA), acetic acid (C2), propionic acid (C3) and butyric acid (C4) in T3 tended to be higher than those in other treatments but were not significantly different among treatments ($p>0.05$). Milk yield and milk compositions were not significantly different among treatments ($p>0.05$). Therefore, the results indicate that cass-bann pellet can be used in TMR. However, Feed intake, end-products of ruminal fermentation, milk yield and quality with supplementation of cass-bann pellet II (T3). Based on this study, the new feed (cass-bann) could be processed and used as an alternative quality feed source (energy and protein source) in dairy rations.

Key words: Cass-bann, dairy cows, milk yield, compositions, ruminal fermentation, volatile fatty acids

INTRODUCTION

Dairy farming for smallholders has been currently promoted and encouraged as a means to produce milk supply, increase income and improve standards of living (Chantalakhana, 1994). Feed and feeding could attribute to the production efficiency and success of dairy farming production as well as other associated dimensions (Wanapat, 1999). The main problems encountered by smallholder farmers were high cost of production particularly the cost of concentrate and shortage of good quality roughage. Cassava (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropical areas. It can easily thrives in sandy-loam soils with low organic matter, receiving low rainfall and high temperature. It is therefore a cash crop cultivated by small-holder farmers within the existing farming systems in many countries (Wanapat, 1999). Cassava chip contained high levels of non-structural carbohydrate and were highly degradable in the rumen as compared with other energy sources including corn meal (Chanjula *et al*., 2003) and have been used as readily fermentable energy in ruminant rations (Wanapat, 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 (Chanjula *et al*., 2004; Khampa *et al*., 2006). Khampa and Wanapat (2006) reported that supplementation of concentrate containing a high level of cassava chip with urea-treated rice straw in dairy steers could improve rumen fermentation efficiency and rumen microbial protein synthesis. Furthermore, supplementation of urea and sodium dl-malate in concentrate containing high level of cassava chip not only to improve rumen fermentation but also increase microbial protein synthesis in rumen (Wanapat and Khampa, 2007). However, a remarkable drop of rumen pH was found as cassava chip level increased in concentrate (6.7-5.7) and resulted in rumen fermentation (Wanapat, 2005). Improving of cassava chip together with other local feed resources in concentrate aims to reduce rate of degradation in the rumen such as banana fruits as a pellet (cass-bann) could be processed and manipulated effectively. Because of the

Corresponding Author: Metha Wanapat, Tropical Feed Resources Research and Development Center (TROFREC), Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

high energy content unripe bananas are suitable energy feeds for ruminants in tropical and subtropical zones. Banana fruits contained 20.9% DM and their average composition of OM, EE, CP and NDF was 92.2, 2.1, 6.0 and 16.6% DM, respectively.

Degradable and digestible OM content of bananas was 62.8 and 78.3% DM, respectively. Degradability of CP was 74.1%. The good high energy content make unripe banana fruits a suitable feed for ruminants. However, bananas must be complemented with protein feeds because of their low protein content (Pieltain *et al*., 1998).

Therefore, combination of cassava chip, raw banana fruit and urea or cass-bann could be potentially used to increase rumen fermentation efficiency and suitable rumen degradation and subsequent productivity for ruminants (Lunsin *et al*., 2006). Hence, the objectives of this study were to investigate effects of pelleted cassava chip and raw banana (cass-bann) on rumen fermentation process and utilization in lactating dairy cows.

MATERIALS AND METHODS

Animals, diets and management: About 6, multiparous early-lactation Holstein-Friesian and Thai native crossbred cows, 36±10 day in milk and initial weight of 399±55 kg were used in this experiment. The cows were randomly allocated to dietary treatments according to a Switch back design. Cows were housed in individual pens and fed with Total Mixed Ration (TMR) containing 60% concentrate and 40% roughage (urea-treated rice straw, UTS as a roughage source).

The dietary treatment s were as follows; T1-non-supplementation of cass-bann pellet, T2-supplementation of cass-bann pellet I (cassava chip, CC 60: raw banana BN, 40: urea 4% in cass-bann 2 feed), T3-supplementation of cass-bann pellet II (cassava chip, CC 60: raw banana, BN 40: urea 6% in cass-bann feed) in concentrate mixtures. The cass-bann pellets in concentrate mixtures containing CC, BN and urea are shown in Table 1. Ingredient composition of concentrate mixtures feed are shown in Table 2.

Cows were housed in individual pens and individually fed in Total Mixed Ration (TMR) containing concentrate 60% and roughage 40%. All cows were fed *ad libitum* with water and a mineral-salt block. The experiment was run in 3 periods, each experimental period lasted for 21 days, the first 14 days as a period for treatment adaptation and for feed intake measurements with the last 7 days for sample collections. Milk yield were recorded daily.

Table 1: Ingredients and Crude Protein (CP) content of cass-bann pellet containing cassava chip 60%, raw banana 40% and urea at 4% (cann-bann I) and 6% (cass-bann II)

Type	Cassava Chip (CC)	Raw Banana (BN)	Urea	Sulphur	Salt	CP%
Cass-bann I	57.0	38.0	4	0.5	0.5	14.9
Cass-bann II	55.8	37.2	6	0.5	0.5	23.7

Table 2: Ingredients mixtures (%) of experimental diets

Items	T1	T2	T3
Cassava chip	49.50	16.50	30.50
Fine rice bran	5.00	5.00	5.00
Soybean meal	25.50	14.00	14.00
Brewers grain	15.50	16.50	15.50
Cass-bann pellet I	-	43.50	-
Cass-bann pellet II	-	-	30.50
Molasses	2.00	2.00	2.00
Coconut oil	1.00	1.00	1.00
Salt	0.50	0.50	0.50
Mineral mixture	1.00	1.00	1.00
Estimated nutrients (%)			
CP	18.10	18.00	18.10
TDN	81.90	79.30	80.60
ME (Mcal kg^{-1}, DM)	3.00	2.90	2.90
NE_l (Mcal kg^{-1}, DM)	1.90	1.80	1.90
Feed cost (US $ kg^{-1})	0.19	0.22	0.21

T1 = non-supplementation of cass-bann in concentrate, T2 = supplementation of cass-bann I in concentrate, T3 = supplementation of cass-bann II in concentrate, CP = Crude Protein, TDN= Total Digestible Nutrients; Official rate of exchange: 1 US $ = 38 baht

Data collection, sampling procedures and chemical composition analysis: Feed intake were measured and refusals recorded. Body weights 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 analyses. Feed and fecal samples were collected during the last 7 days of each period. Fecal samples were collected by rectal sampling. Composited samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analysed for DM, EE, ash and CP content (AOAC, 1985) NDF and ADF (Goering and Van Soest, 1970) and Acid Insoluble Ash (AIA). AIA was used to estimate digestibility of nutrients (van Keulen and Young, 1977).

Cows were milked twice daily and milk yield were recorded at each milking of each period. Milk samples were composted daily according to yield for both the meaning and evening milking, preserved with 2-bromo-2 nitropropane-1, 3-dial and stored at 4°C until analysis for fat, protein, lactose, total solids and solid-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark). Milk Urea N (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO) (Valadares *et al*., 1999).

Rumen fluid samples were collected at 0 and 4 h post feeding. 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 portable pH and temperature meter. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into 3 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, 1985) and VFA analyses using a HPLC (Samuel *et al*., 1997). Second 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) and third portion was taken to study culture groups of viable bacteria using roll-tube technique described by Hungate (1969) for identifying of bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

Sample of jugular blood (about 10 mL) were drawn into serum separation tube at the same time of rumen fluid sampling and centrifuged for 10 min at 5,000×g. The supernatant was decanted and frozen (-20°C) until it was analyzed of Blood Urea Nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA according to a Switch back design arrangement of treatments using the procedure of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). The following models were used for statistical analysis:

$$\text{Model: } Y_{ijk} = \mu + \alpha_j + \delta_{k(i)} + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = Observation
μ = Overall mean
α_j = Effect of sequence
β_j = Effect of period
$\alpha\beta_{ij}$ = Effect of sequence and period combination
$\delta_{k(i)}$ = Main plot error or animal within sequence error
ε_{ijk} = Sub-plot error

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical composition of experimental diets are shown in Table 3. Experimental diets contained similar concentrations of DM, OM, CP, ADF and NDF. Chemical composition of Urea Treated Rice Straw (UTRS), cass-bann pellet I and cass-bann pellet II are shown in Table 3.

Feed intake and digestibility of nutrients: The effects of cass-bann feed on feed intakes and digestibility of nutrients are shown in Table 4. Feed intakes were not significantly affected by cass-bann feed but total DMI, kg day^{-1}, %BW and g kg^{-1} $W^{0.75}$ tended to be higher with supplementation of cass-bann pellet II in dietary treatments (T3) ($p>0.05$). Digestibilities of organic matter and neutral detergent fiber were higher ($p<0.05$) in cows fed dietary T2 and T3 than tose in T1. Moreover, digestible nutrient intakes tended to be higher in cow fed dietary T3 ($p>0.05$). Nevertheless, ME (Mcal day^{-1}) and ME (Mcal kg^{-1} DM) were not affected by cass-bann pellet supplementation.

Rumen fermentation and blood metabolite: Rumen ecology parameters were measured for temperature, pH, NH_3-N and VFA (Table 5). Rumen pH and temperature were unchanged by dietary treatments and the values were relatively stable at 6.5-6.7 and 38.3-39.0°C. The concentrations of NH_3-N, BUN and MUN were not changed ($p>0.05$). However, concentrations of NH_3-N and MUN tended to be higher in cow fed dietary T1.

Total VFA concentration, proportion of acetic acid, propionic acid, butyric acid and acetic and propionic ratio were not significantly ($p>0.05$) influenced by cass-bann pellet (Table 5). Total of VFA concentrations in the rumen were increased from 98.7-103.7 mM L^{-1} and proportion of acetic acid, propionic acid and butyric acid ranged from 68.4-71.0; 21.2-22.9 and 9.3-10.3 mol/100 mol, respectively. Methane production in rumen (calculated from VFA) were not different ($p>0.05$) between the 2 cass-bann pellets.

Table 3: Chemical composition of experimental diets, Urea-treated Rice Straw (UTRS), cass-bann I, II, Cassava Chip (CC) and raw Banana (BN) (%DM basis)

Items	T1	T2	T3	UTRS	Cass-bann pellet I	Cass-bann pellet II	CC	BN
DM	87.7	89.0	88.2	54.3	90.9	92.9	88.2	83.7
OM	91.8	93.3	92.6	87.8	95.0	95.0	87.4	89.1
Ash	5.2	6.7	4.4	12.2	5.0	5.0	2.6	2.9
CP	17.6	17.9	17.8	8.5	14.1	19.8	2.6	3.5
EE	5.6	4.6	5.2	1.3	2.7	3.2	3.2	2.2
NDF	38.6	36.8	39.9	73.9	28.9	25.0	6.8	21.2
ADF	16.6	19.5	20.3	53.8	10.2	9.5	6.2	11.0
CT	NA	NA	NA	NA	NA	NA	NA	1.5

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, NA = Not Analysis, CT = Condensed Tannins, CC = Cassava Chip, BN = Raw Banana

Table 4: Effect of cass-bann feed on feed intake, digestion coefficients of nutrients and digestible nutrient

Items	T1	T2	T3	SEM
Dry matter intake				
kg day^{-1}	12.90	13.30	13.90	0.55
%BW	3.23	3.36	3.42	0.15
g kg^{-1} W$^{0.75}$	140.10	145.30	150.20	6.15
Digestion coefficient,(%)				
DM	68.10	70.30	69.90	0.55
OM	71.40^{a}	75.10^{b}	75.20^{b}	0.52
CP	59.20	60.70	60.50	1.68
NDF	62.50^{a}	65.20^{b}	66.10^{b}	0.56
ADF	48.50	51.50	49.70	0.87
Digestible nutrient intake (kg day^{-1})				
OM	11.50	11.90	12.10	0.36
DOMI	7.30	7.50	8.50	0.39
CP	1.80	1.90	1.90	0.08
NDF	6.80	7.40	7.40	0.43
ADF	4.40	5.10	5.00	0.32
Estimated energy intake[1/]				
ME (Mcal day^{-1})	27.80	28.40	30.30	1.50
ME kg^{-1} DM (Mcal kg^{-1} DM)	2.10	2.20	2.60	0.22

^{a,b}The values on the same row with different superscripts differ ($p<0.05$), DM = Dry Matter, OM = Organic Matter, DOMI = Digestible Organic Matter Intake, CP = Crude Protein, NDF = Nneutral Detergent Fiber, ADF = Acid Detergent Fiber, [1/]Estimated: 1 kg DOMI = 3.8 Mcal ME kg^{-1} DM; SEM = Standard Error of the Means

Table 5: Effect of cass-bann feed on rumen fermentation, Blood Urea-Nitrogen (BUN) and Volatile Fatty Acids (VFAs)

Items	T1	T2	T3	SEM[2/]
Temperature (°C)	39.0	38.3	38.9	0.14
Ruminal pH	6.7	6.6	6.5	0.09
NH_3-N (mg %)	16.2	15.2	13.7	0.85
BUN (mg %)	15.4	14.0	12.9	0.77
MUN (mg %)	14.1	13.7	12.7	0.76
Total VFA (mM L^{-1})	98.7	100.2	103.7	2.03
Molar proportion of VFA (mol/100 mol)				
Acetic acid (C2)	69.3	68.6	68.1	0.94
Propionic acid (C3)	21.4	21.8	22.0	1.16
Butyric acid (C4)	9.4	9.6	10.0	0.61
C2:C3 ratio	3.2	3.1	3.1	0.20
CH_4 (mol %[1/])	29.0	28.6	28.7	0.79

[1/]Estimated: CH_4 = (0.45×acetic acid)-(0.275×propionic acid) + (0.40× butyric acid) (Moss *et al*., 2000), [2/]SEM = Standard Error of the means

Rumen microorganism population: Effect of cass-bann feed on rumen microorganism population are shown in Table 6. Total viable bacteria counts were significantly different ($p<0.05$), whilst population of cellulolytic, proteolytic and amylolytic bacteria were not significantly different among treatments ($p>0.05$). Total populations, not amylolytic bacteria tended to be highest in dietary T3 with supplementation of cass-bann pellet II. Moreover, total bacteria and fungal zoospores tended to be higher in dietary T3 with supplementation of cass-bann pellet II while protozoa tended to be lower ($p<0.05$) as compared to these.

Milk production and composition: Milk yield and milk composition in lactating dairy cows were not significantly different among treatments ($p>0.05$) (Table 7). Yield of milk and production of 3.5% FCM was greatest in cows fed

Table 6: Effect of cass-bann feed on rumen microorganism

Items	T1	T2	T3	SEM
Total direct counts (cells mL^{-1})				
Bacteria (×10^{9})	6.5	5.6	7.9	0.90
Protozoa (×10^{5})	5.7	4.8	4.1	0.69
Fungal zoospore (×10^{5})	2.6	2.7	3.3	0.35
Total viable counts (CFU mL^{-1})				
Total bacteria (×10^{7})	3.9^{a}	4.5^{ab}	5.7^{b}	0.30
Cellulolytic (×10^{7})	3.0	3.9	4.6	0.86
Proteolytic (×10^{6})	4.6	4.5	4.7	0.59
Amylolytic (×10^{6})	4.6	4.0	4.3	0.36

^{a,b}The values on the same row with different superscripts differ ($p<0.05$)

Table 7: Effect of cass-bann feed on milk yield and milk composition

Items	T1	T2	T3	SEM
Milk yield (kg day^{-1})	12.7	13.2	13.8	0.37
3.5% FCM (kg day^{-1})	12.4	13.1	14.0	0.57
Fat (kg day^{-1})	0.40	0.50	0.50	0.03
Protein (kg day^{-1})	0.40	0.40	0.50	0.03
Milk composition (%)				
Fat	3.30	3.50	3.60	0.12
Protein	2.80	3.10	3.20	0.10
Lactose	4.60	4.70	5.00	0.09
Solids-not-fat	8.20	8.10	8.50	0.17
Total solids	11.5	11.6	12.1	0.29
MUN (mg %)	14.1	13.7	12.7	0.76

SEM = Standard Error of the Means, MUN = Milk Urea Nitrogen

supplementation of cass-bann pellet II (T3). Supplementation of cass-bann pellet did not affect on percentage of fat, protein lactose, solids-not-fat and total solids. Overall milk composition tended to be higher in cows fed on supplementation of cass-bann pellet II (T3).

Chemical composition of UTRS similar values with has been reported by Hart and Wanapat (1992) but CP contained (8.5% CP) in this experiment were higher (Hart and Wanapat, 1992) (7.4% CP). It could be due to type and sources of rice straw and time of incubation (Wanapat, 1990). However, chemical treatments of low quality roughage cloud improved CP contained. Chen *et al.* (2007) reported that higher CP concentration of treatment rice straw with sodium hydroxide and sodium bicarbonate and chemical treatments increased digestibility of low quality rice straw.

Goto *et al.* (1993) concluded that general mechanism for improved digestion of straw by alkali is that alkaline treatment partially damages the lignin-polysaccharide bond, solublizes hemicellulose and lignin in straw and hence exposes the cellulose to microbial attack and make microbes more rapidly adhere to feed particles, resulting in higher enzyme activities and improved digestibility of nutrients (Chen *et al*., 2007). Chemical composition of CP in cass-bann feed were different between cass-bann I and cass-bann II due to it's different level of urea in cass-bann feed was 4 and 6% urea, respectively. Condensed Tannins (CT) percentages in raw banana fruit in this experiment were lower than previous study by Lunsin *et al.* (2006). Rumen ecology parameters were not effect by dietary treatments including pH, NH_3-N and VFA

(Table 4). In addition, BUN were determined to investigate their relationships with rumen NH_3-N and protein utilization. Rumen pH and temperature were within the normal range which has been reported as optimal pH for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986).

Concentrations of rumianl NH_3-N was closer to optimal level according to Wanapat *et al.* (1999), ruminal NH_3-N level between 13.6-17.6% mg (15% mg) improved rumen ecology, digestibility and intake. The increases in rumen NH_3-N levels also resulted in increasing levels of BUN. As reported previous studies (Preston *et al.*, 1965; Lewis, 1957) concentrations of BUN are highly correlated to the level of ammonia production in the rumen. Moreover, it is well established that urea equilibrates rapidly with body fluids including milk and this can account for close relationship between Milk Urea Nitrogen (MUN) and BUN (Broderick and Clayton, 1997). MUN can be used as an indicator of the adequacy of protein and the balance between energy and protein in lactating dairy cow diets.

Elevated MUN indicates excess protein has been fed to the dairy cow for her given level of production (Broderick and Clayton, 1997; Wattiaux and Karg, 2004). Jonker *et al.* (1999) reported that optimal MUN in lactating dairy cow averaged 10-16% mg, according to this experiment found that concentrations of MUN were 14.1, 13.7 and 12.9% mg respectively. The total VFA concentrations in all treatments were in the normal concentrations and agreed with values of 70-130 mM L^{-1} (France and Siddons, 1993).

Total viable bacteria counts were highest in dietary T3 ($p<0.05$) due to suitable substrate for bacteria utilization in term of energy and protein source in cass-bann pelleted. Ruminal bacteria play a particularly important role in the biological degradation of dietary fiber, according to digestibilities of organic matter and neutral detergent fiber were highest in cow feed dietary T2 and T3 with supplementation cass-bann I and II ($p<0.05$) in this study. Population of protozoa were lowest in and dietary T3 ($p>0.05$), it could be due to pelleted processing were changed in chemical composition of starch. Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depends on the rate of soluble sugars and starches in the ration and also pH. The decrease in protozoa count may attribute the increase fungal zoospore per mL rumen fluid and removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981) and elimination of protozoa will increase bacteria in liquid pool. Because of protozoa ingest and digest bacteria floating in the rumen and can either compete for nutrients with fungi or reduce fungal growth. Moreover, It is possible that Condensed Tannins (CT) present in raw banana fruit may play an important role in decreasing protozoa populations (Wang *et al.*, 1999).

CONCLUSION

Based on this experiment, it could be concluded that supplementation of cass-bann feed in TMR with UTRS as a roughage source could improve ruminal fermentation efficiency, increasing total VFA production especially propionate production and decreasing of acetate to propionate ratio. Moreover, cass-bann feed could increase populations of bacteria and fungi but decrease protozoal populations. These results suggest that cass-bann feed can be successfully used as good feed source for dairy cows in terms of energy and protein to improve rumen ecology and fermentation by maintaining high pH and increasing propionic acid useable for milk production.

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