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Full Paper

Effects of alternative protein sources on rumen microbes and productivity of dairy cows

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Abstract: This experiment was conducted to investigate the effect of various protein sources on digestibility, rumen fermentation, milk yield and milk composition in dairy cows. Four Holstein Friesian native crossbred cows in early lactating were randomly assigned according to a 4x4 Latin square design. The dietary treatments containing different protein sources in concentrate diets were soybean meal (SBM), cassava hay (CH), *Leucaena leucocephala* (LL) and yeast-fermented cassava chips (YEFECAP), with ad libitum intake of urea-treated rice straw. Digestibility of DM, OM, NDF and ADF was not different among treatments ($P>0.05$) while CP digestibility was highest ($P<0.05$) in CH and YEFECAP supplemented groups. Ruminal NH_3-N and BUN concentrations varied among protein sources and were highest in SBM and LL fed groups ($P<0.05$). Ruminal total volatile fatty acid (VFA) and propionic acid were found highest in cows receiving CH and YEFECAP ($P<0.05$). Ruminal fungi, proteolytic and cellulolytic bacteria were highest when YEFECAP was supplemented. Milk fat and milk protein were significantly increased ($P<0.05$) in cows fed with CH and YEFECAP. Based on this study, it was concluded that providing CH or YEFECAP as protein source in concentrate diets could improve rumen fermentation and milk production in lactating dairy cows fed on rice straw.

Keywords: yeast-fermented cassava chips, cassava hay, rumen microorganism, milk production, lactating dairy cows

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INTRODUCTION

The requirement for nutrients to support high milk production during early lactation is great. Cows in early lactation often suffer from a shortage of energy and protein because maximal DM intake does not occur until after the peak of milk production. Complex interrelationships exist between dietary protein, energy and the amount of protein that will be utilised by the dairy cow [1]. These interrelationships have important ramifications on overall N efficiency of the dairy farm. Dietary protein supplies metabolisable protein by providing both rumen degradable protein (RDP) utilised for microbial protein formation and rumen undegradable protein (RUP) that is digested directly by the cow.

The process of protein enrichment of animal feed using microorganisms in a semi-solid culture to improve the nutritional value of forage for ruminants has been evaluated [2-3]. Incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition. Boonnop et al. [4] reported that cassava chips fermented with *S. cerevisiae* (yeast-fermented cassava chips) significantly increase crude protein (300 g/kg DM) and lysine contents as well as reduce cyanide level.

Grown in tropical areas in large scale, Cassava (*Manihot esculenta*, Crantz) has a potential use in ruminant livestock nutrition and feeding. Cassava root contains a high level of energy and has been used as a source of readily fermentable energy in ruminant rations [5-7]. Whole cassava crop (cassava hay) was introduced by Wanapat [8] into a dry-season feeding system for ruminants by managing cassava crop growth in order to obtain optimal yield and good protein quality. Cassava hay is high in protein (200-250 g/kg DM) and contains condensed tannins (15-40 g/kg DM). It has proved to be an excellent ruminant protein feed and its use has been successfully implemented in several ways either by direct feeding or as a protein source in concentrated mixtures and high-quality feed blocks [8-9].

However, a comparative study of various protein sources in feed for ruminants has not yet been substantiated. It is therefore the objective of this investigation to determine the effects of yeast-fermented cassava chips, soybean meal, cassava hay and *Leucaena leucocephala* as protein sources in concentrated diets on feed intake, digestibility of nutrients, rumen fermentation, milk yield and milk composition of lactating crossbred dairy cows.

MATERIALS AND METHODS

Animals, Treatments and Experimental Design

Each of four crossbred (75% Holstein Friesian x 25% Thai native) early-lactating dairy cows with an average weight of 410±12.5 kg and 18±11 days in milk (DIM) was randomly assigned according to a 4×4 Latin square design to receive one of the four concentrated diets with different protein sources [soybean meal (SBM), cassava hay (CH), *Leucaena leucocephala* leaves (LL) and yeast-fermented cassava chips (YEFECAP)]. The composition of the feed concentrates is shown in Table 1. Cows were housed in individual pens and fed with the concentrated diets (ratio of concentrate to milk yield = 1: 2) twice daily at 6.00 a.m. and 16.00 p.m. after milking. All cows were additionally fed with urea-treated rice straw (UTRS) ad libitum as a roughage source while allowing for 10% refusal. UTRS (composition shown in Table 1) was made by pouring urea solution over a stack of straw

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(urea : water : straw = 5 : 100 : 100 by weight), which was then covered with a plastic sheet for a minimum of 10 days before feeding directly to the animals [7].

Table 1. Ingredients and nutritional composition (g/kg DM basis) of feed concentrates (SBM, CH, LL and YEFECAP) and urea-treated rice straw (UTRS)

Ingredient (g/kg DM)	Protein source				UTRS
	SBM	CH	LL	YEFECAP	
Cassava chips	651	602	603	600	
Rice bran	80	80	76	67	
Molasses	20	19	19	16	
Soybean meal	189	-	-	-	
Cassava hay	-	231	-	-	
Leucaena leucocephala	-	-	237	-	
YEFECAP	-	-	-	255	
Urea	25	31	30	27	
Tallow	10	11	11	10	
Salt	10	10	10	10	
Mineral pre-mix	10	10	10	10	
Sulphur	5	5	5	5	
Nutritional composition					
Organic matter	938	945	929	941	905
Crude protein	183	181	180	182	79
Neutral detergent fibre	161	175	163	168	705
Acid detergent fibre	113	123	115	118	406

YEFECAP used in this study were described by Boonnop et al. [4]. In brief, cassava chips were washed and grated, and the processed pulp (100 g) was spread in a tray (about 50 cm diameter) to an average layer thickness of 2 cm. Commercial baker yeast (*Sacchromyces cerevisiae*, manufactured by Berly Speciality Industries Co., Bangkok) was used in the fermentation proceses. A nutrient solution was prepared by adding distilled water (100 mL), and then urea (48 g), to molasses (24 g) placed in a warm blender vessel flushed with O_2, and incubating the mixture at room temperature for 10 minutes. The resulting nutrient solution (250 mL) along with the yeast (20 g) was then inoculated into 0.5 kg of the processed pulp above and fermentation was conducted for 132 hours at 25°C. The fermented pulp was sun-dried for 3 days at an average temperature of 30°C and milled to give the YEFECAP (containing 300 g/kg DM).

All animals were kept in individual pens (4×6 m) and mineral block and water were freely available. The experiment was conducted in 4 periods according to 4x4 Latin square design (4 treatments and 4 periods), each period lasting 21 days. During the last 7 days of each period, samples were collected (diets, feces, milk, blood and rumen fluid).

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Data Collection, Sampling Procedures and Methods of Analysis

Feed, refusal and fecal sample (grab sampling) were randomly collected (2 samples/day/cow) from each individual cow during the last 7 days of each period. Combined samples were dried at 60ºC and ground (1-mm screen, Cyclotech mill, Teactor, Sweden) and then analysed for DM, OM, ash, CP content [10], NDF, ADF [11] and acid-insoluble ash (AIA). The AIA was used to estimate digestibility of nutrients as described by Van Keulen and Young [12].

Cows were milked twice daily by a bucket-type milking system and milk was weighed at each milking of each period. Milk samples from both the morning and afternoon milking were combined daily, preserved with 2-bromo-2-nitropropane-1,3-diol and stored at 4°C until analysis of milk composition (fat, protein, lactose, total solids and solids-not-fat) by infrared method using Milko-Scan 33 (Foss Electric, Hillerod, Denmark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, USA).

Rumen fluid was collected by a stomach tube connected with a vacuum pump and jugular blood samples were collected at 0 and 4 h post-feeding on the last day of each period. Approximately 200 mL of rumen fluid were taken from the rumen using a 60-mL hand syringe at the end of each period. The pH and temperature of the rumen fluid were immediately measured by means of a portable pH and temperature meter (Hanna HI 8424, Singapore). Rumen fluid samples were then filtered through two layers of cheesecloth and divided into three portions.

The first portion was used for analysis of volatile fatty acids (VFA) and NH_3-N. 1M H_2SO_4 solution (5 mL) was added to 45 mL of rumen fluid. The mixture was centrifuged at 16,000×*g* for 15 minutes and the supernatant was stored at -20°C prior to VFA analysis by HPLC (Waters, model 600E with a UV detector; Novapak C_{18} column, column size: 4 mm x 150 mm; mobile phase: 10 mM H_2SO_4, pH 2.5) according to Samuel et al. [13]. NH_3-N analysis was done by micro-Kjeldahl method [10].

The second portion was used for a total direct count of bacteria, protozoa and fungal zoospores with a haemacytometer (Hausser Scientific, USA) by the methods of Galyean [14]. The third portion was taken for the study of cultured groups of viable bacteria by roll-tube technique [15] for identifying rumen bacterial groups (cellulolytic, proteolytic, amylolytic and total viable bacteria).

A blood sample (about 10 mL) was drawn from the jugular vein at the same time as rumen fluid sampling (at 0 and 4 h post-feeding) and centrifuged at 5000×*g* for 10 minutes (Table-top Centrifuge PLC-02, USA). The supernatant was stored at -20ºC until analysis of blood urea nitrogen (BUN) according to the method of Crocker [16].

Statistical Analysis

Statistical analysis was performed using the GLM procedure of SAS (SAS Inst. Inc., USA). Data were analysed using the model $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$, where Y_{ijk} is observation from animal j, receiving diet i in period k; μ is the overall mean; Mi is the mean effect of protein sources (i = 1, 2, 3, 4); A_j is the effect of animal (j = 1, 2, 3, 4); P_k is the effect of period (k = 1, 2, 3, 4); and εijk is the residual effect. The results were presented as mean values and standard error of the means. Significant differences between treatments were determined by Duncan's new multiple range [17]. Differences among means with $P<0.05$ were accepted as statistically significant.

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RESULTS AND DISCUSSION

Effect on the Rumen Ecology and Fermentation Products

The pattern of ruminal fermentation and overall means are presented in Table 2. Ruminal temperature and pH were similar among treatments and the values were quite stable at 39.1-39.4°C and pH 6.2-6.4, which was within the range (pH 6.0-7.0) considered for optimal microbial digestion of fibre and protein [7]. Ruminal NH_3-N, BUN and MUN ranged from 13.7-19.0, 11.3-15.7 and 13.5- 15.9 mg/dL respectively. Ruminal NH_3-N and BUN concentrations were lower in CH and YEFECAP than in SBM and LL. It was reported that ruminal NH_3-N concentration increased linearly with increasing supplemental RDP levels [6]. Therefore, a possible explanation for this could be that SBM and LL contain a high level of RDP, which leads to a high ruminal NH_3-N. Using the in sacco method, Promkot and Wanapat [5] found that effective degradability of CP in SBM and LL was higher than that found in CH. Wanapat [8] also reported that cattle fed on CH (250 g CP/kg) had lowered rumen NH_3-N and BUN concentration, which demonstrated the effect of condensed tannins in CH on the formation of tannin-protein complexes which in turn could enhance the cattle's rumen by-pass protein.

Table 2. Effect of protein source on some ruminal properties in lactating dairy cows (n=4)

Item	Protein source				SEM	P-value
	SBM	CH	LL	YEFECAP		
Ruminal pH	6.2	6.3	6.3	6.4	2.1	0.67
Ruminal temperature	39.2	39.3	39.1	39.4	1.1	1.02
NH_3-N, mg/dL	18.7^a	13.7^b	19.0^a	13.3^b	1.3	0.03
BUN, mg/dL	15.5^a	11.3^b	15.7^a	11.4^b	0.4	0.05
Total VFA, mmol/L	104.1^b	106.2^a	103.6^b	107.3^a	0.8	0.01
Mol % of total VFA						
Acetate (C2)	68.4	65.6	69.0	65.5	5.9	1.32
Propionate (C3)	23.6^b	25.4^a	23.2^b	26.5^a	0.2	0.02
Butyrate (C4)	8.0	9.2	7.8	8.0	2.5	2.22
Acetate to propionate ratio	2.9^a	2.5^b	3.0^a	2.4^b	0.1	0.05

Note: 1) ^{a,b,c} Means in the same row with different superscripts differ significantly ($P<0.05$).

2) SEM = Standard error of mean

The decreasing degradability of feed protein might also be due to an increase in the rumen outflow rate, thus lowering the time available for fermentation. Other authors found increased microbial N flow without changes in dietary N in the duodenum when yeast culture was added to the diet [18]. The other hypothesis could therefore be associated with yeast having a positive influence on ammonia uptake.

As NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen, the rumen pool of NH_3-N should be considered. The result obtained in this study was close

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to optimal ruminal NH_3-N (15-30 mg/dL) [1-2, 6] for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminants fed on low-quality roughage.

The total VFA and propionic acid were significantly different ($P<0.05$) and were highest in CH and YEFECAP (Table 2). These values were similar to those reported by Wanapat et al [19]. The shift in the molar proportion of propionate resulted in a lower acetate:propionate ratio in ruminal fluid of animals receiving YEFECAP and CH. Wanapat et al. [19] reported that total VFA for CH supplementation increased with fermentation time in the rumen. However, recent data suggested that CH and YEFECAP improved rumen efficiency by increasing the C3 (propionate) intermediate and enhancing microbial protein synthesis in in vitro gas fermentation system [20].

Effect on Feed Intake and Digestibility

The effects of protein source on feed intake of lactating dairy cows are presented in Table 3. Dry matter intake (DMI) of UTRS and total DMI are shown to be similar. Normally, this data indicate that a source of protein has no negative effect on straw intake in dairy cows. This result is in agreement with earlier work by Khampa et al. [21], who reported that inclusion of cassava chips in diets resulted

Table 3. Effect of the main protein source in concentrated feed on voluntary feed intake and nutrient digestibility in lactating dairy cows (n=4)

Item	Protein source				SEM	P-value
	SBM	CH	LL	YEFECAP		
UTRS intake						
kg	5.8	6.0	5.7	6.1	1.9	0.43
g/kg BW	144	145	144	146	1.7	1.22
g/kg $BW^{0.75}$	65.6	66.5	64.9	67.0	2.8	0.67
Total feed intake						
kg	11.4	11.9	10.8	12.3	2.6	0.11
g/kg BW	290	293	288	293	2.4	0.23
g/kg $BW^{0.75}$	129.9	130.2	128.5	131.3	4.6	2.19
Apparent digestibility (g/kg DM)						
Dry matter	620	630	625	631	20.2	1.32
Organic matter	684	703	661	694	32.4	0.09
Crude protein	706 ^{b}	760^{a}	703 ^{b}	750^{a}	10.1	0.02
Neutral detergent fibre	614	632	593	643	25.3	0.55
Acid detergent fibre	562	581	553	584	17.6	0.28

Note: 1) ^{a,b} Means in the same row with different superscripts differ significantly ($P<0.05$).

2) g/kg $BW^{0.75}$ = gram / kilogram of metabolic weight; SEM = Standard error of mean

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in satisfactory animal performance and had no negative effects on the health of lactating dairy cows. Apparent values of digestibility of DM, OM, NDF and ADF were not significantly different (P>0.05) among treatments. Wanapat et al. [19] also found that an increased ratio of CH to SBM in concentrate for dairy cows resulted in similar nutrient digestion coefficients among treatments. The CP digestibility values were significantly different and were highest in CH (760 g/kg DM) and YEFECAP (750 g/kg DM). Miller-Webster et al. [22] reported that protein digestibility and ammonia N were increased by inclusion of yeast culture as compared with control. This protein source could have made the N more available for microbial growth. Wanapat et al. [19] reported that both concentrate and CH were well consumed by cows at all times. However, Onwuka et al. [23] reported that dried cassava leaves contained high level of condensed tannins (30-50 g/kg DM), which adversely affected intake, digestibility and performance of ruminants.

Effect on Microbial Population

Table 4 illustrates data on rumen microbes using a direct count and roll-tube technique. Ruminal microbial count and cellulolytic and proteolytic bacteria were significantly different among treatments (P<0.05); bacteria, fungi zoospores, amylolytic bacteria and cellulolytic bacteria were highest when YEFECAP was supplemented. In contrast, the number of protozoa in the rumen was decreased by YEFECAP and CH supplementation. Although the effect of tannins on ruminal protozoa count is variable in assays carried out in vivo [20], some evidence exists for lower protozoal number in the presence of tannins [8-9]. Therefore, the decrease in protozoa count for CH supplementation could apparently be explained by the presence of condensed tannins in CH [8]. The effect of yeast culture on rumen protozoa is equivocal; whilst Robinson and Erasmus [24] reported that yeast culture exhibited no significant effect on the protozoa count, a trend for the total population to decrease in the presence

Table 4. Effect of the main protein source on microbial population in the rumen of lactating dairy cows (n=4)

Item	Protein source				SEM	P-value
	SBM	CH	LL	YEFECAP		
Total direct count (cells/mL)						
Bacteria, x 10^9	3.6^b	4.8^a	3.1^b	5.3^a	0.2	0.03
Protozoa, x 10^4	8.1^a	5.3^b	8.3^a	4.9^b	0.3	0.05
Fungi zoospores, x 10^3	2.8^b	3.9^{ab}	2.9^b	4.7^a	0.3	0.02
Roll-tube technique (CFU/mL)						
Total viable bacteria, x 10^8	4.8	5.1	4.9	5.2	2.9	1.12
Cellulolytic bacteria, x 10^7	5.2^c	6.0^b	5.1^c	7.5^a	0.2	0.04
Amylolytic bacteria, x 10^6	9.5	9.5	9.8	10.1	1.0	2.12
Proteolytic bacteria, x 10^6	11.0^b	12.1^{ab}	9.2^c	13.3^a	0.3	0.05

Note: 1) ^{a,b,c} Means in the same row with different superscripts differ significantly (P<0.05).

2) SEM = Standard error of mean

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of *Saccharomyces cerevisiae* was observed [4,18]. Some authors reported elevation of total protozoa count when the animals were fed with low-quality diets, but the influence of *Saccharomyces cerevisiae* on the total population was much debated [25].

Guedes et al. [26] found that yeast could stimulate the activity of cellulolytic bacteria and increase lactate utilisation in the rumen, hence increased fibre digestion and flow of microbial protein from the rumen in feedlot cattle fed high-grain diets. Similarly, Erasmus et al. [18] reported that supplementation of yeast culture tended to increase microbial protein synthesis in dairy cows and significantly altered the amino acid profile of the duodenal digesta. When fungal cultures were supplemented in ruminant diets, it was found that microbial protein synthesis increased due to increase in microbial population in the rumen [27].

Effect on Milk Yield and Composition

The influences of protein source in concentrated diets on milk production and milk composition of lactating dairy cows are shown in Table 5. The protein source did not significantly affect milk yield, lactose, solids-not-fat and total solids ($P>0.05$). However, cows fed on CH or YEFECAP had higher milk fat than those supplemented with SBM or LL ($P<0.05$). A greater intake of urea-treated rice straw in the case of cows fed on CH and YEFECAP may partially explain our observed increase in milk fat. Dietary inclusion of yeast culture has shown an improved milk production in early-lactation dairy cattle [18, 24, 26]. All cows were able to maintain levels of milk yield during the days of the experiment. Similarly, Piva et al. [25] observed that milk fat increased significantly for mid-lactating cows fed diets with yeast in the concentrate. Wanapat et al. [19] reported that the fat content of milk was higher in CH-supplemented groups, especially in the ad libitum fed group. CH could have provided additional volatile fatty acids necessary for milk fat synthesis. Higher milk-fat percentage is good for milk price since the sale of milk is based on fat content.

Table 5. Effect of the main protein source on milk production and milk composition of lactating dairy cows (n=4, means of 7 days)

Item	Protein source				SEM	P-value
	SBM	CH	LL	YEFECAP		
Milk yield (kg/day)	15.0	15.6	14.7	15.7	2.2	0.98
Milk composition (g/100 kg of milk)						
Crude protein	3.1^{a}	3.3^{a}	2.2^{b}	3.3^{a}	0.1	0.03
Fat	3.7^{b}	3.8^{ab}	3.5^{c}	3.9^{a}	0.1	0.02
Lactose	4.9	5.0	4.8	5.1	1.2	0.05
Solids-not-fat	8.7	8.8	8.7	8.9	2.5	0.99
Total solid	12.7	12.8	12.5	12.9	1.8	1.22
MUN (mg/dL)	15.9^{a}	13.5^{b}	14.8^{ab}	13.9^{b}	0.3	0.05

Note: 1) ^{a,b,c} Means in the same row with different superscripts differ significantly ($P<0.05$).

2) SEM = Standard error of mean

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Milk crude protein yield was greater in the CH- and YEFECAP-supplemented cows. The increased yield of milk crude protein may possibly be attributed to a greater passage of amino acids to the duodenum when CH or YEFECAP replaced SBM or LL in the diet. Higher ruminal by-pass protein (tannin-protein complex) of CH and higher amino acid content in YEFECAP could have contributed to this improvement [8]. In contrast, Kakengi et al. [28] showed that supplementation of LL to grazing cows significantly increased milk production, weight gain and milk composition, but had no significant effect on milk crude protein and solids-not-fat.

CONCLUSIONS

This study has revealed the importance of various protein sources for lactating dairy cows. Among the protein sources used, cassava hay (CH) and yeast-fermented cassava chips (YEFECAP) resulted in significantly higher rumen bacteria and fungal zoospore population as well as reduced protozoal population. The digestibility of protein also increased. Although milk yield was not different among treatments, milk protein and fat contents were enhanced in CH and YEFECAP supplemented cows. These protein sources could thus be recommended for use by smallholders.

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