

Effect of Synchronizing Starch Sources and Protein (NPN) in the Rumen on Feed Intake, Rumen Microbial Fermentation, Nutrient Utilization and Performance of Lactating Dairy Cows

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ABSTRACT : Eight crossbred (75% Holstein Friesian) cows in mid-lactation were randomly assigned to a switchback design with a 2x2 factorial arrangement to evaluate two nonstructural carbohydrate (NSC) sources (corn meal and cassava chips) with different rumen degradability and used at two levels of NSC (55 vs. 75%) with protein source (supplied by urea in the concentrate mix). The treatments were 1) Low degradable low level of corn (55%) 2) Low degradable high level of corn (75%) 3) High degradable low level of cassava (55%) and 4) High degradable high level of cassava (75%). The cows were offered the treatment concentrate at a ratio to milk yield at 1:2. Urea-treated rice straw was offered *ad libitum* as the roughage and supplement with 1 kg/hd/d cassava hay. The results revealed that total DM intake, BW and digestion coefficients of DM were not affected by either level or source of energy. Rumen fermentation parameters; NH₃-N, blood urea nitrogen and milk urea nitrogen were unaffected by source of energy, but were dramatically increased by level of NSC. Rumen microorganism populations were not affected ($p>0.05$) by source of energy, but fungal zoospores were greater for cassava-based concentrate than corn-based concentrate. Milk production and milk composition were not affected significantly by diets containing either source or level of NSC, however milk fat percentage showed a trend towards higher levels for cassava-based concentrate than corn-based concentrate averaging (4.4 and 4.2, respectively). Likewise, income over feed, as estimated from 3.5% FCM, was higher on cassava-based concentrate than corn-based concentrate averaging (54.0 and 51.4 US\$/mo, respectively). These results indicate that feeding diets containing either cassava-based diets and/or a higher of concentrates up to 75% of DM with NPN (supplied by urea up to 4.5% of DM) can be used in dairy rations without altering rumen ecology or animal performance compared with corn-based concentrate. (*Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 10 : 1400-1410*)

Key Words : Synchronizing, Protein, Starch Sources, Cassava Chip, Corn, Rumen Degradability, Ruminal Microbial Fermentation, Dairy Cows

INTRODUCTION

It has been clearly shown that rumen fermentation can supply 70-100% of a ruminant animal's amino acid supply (AFRC, 1992) and 70-85% of the energy supply can be absorbed as volatile fatty acids, the main end-products of microbial fermentation (Dewhurst et al., 1986). As microbial fermentation is such a major component of digestion in ruminants, optimizing microbial growth is obviously important. Synchronizing the rate of supply of nitrogen and energy sources to rumen micro-organisms has been proposed in order to maximize the capture of rumen degradable protein (RDP) and to optimize microbial growth rate and efficiency. Therefore, in order to enhance microbial growth rates and efficiency of nutrient utilization, nitrogen and energy must be simultaneously available for utilization (Sniffen and Robinson, 1987), excess ammonia will be

carried via the portal blood to the liver to form urea of which some will be recycled to the digestive tract, but a large portion will be lost through excretion in urine. On the contrary, when a quantity of highly degradable energy exceeds the nitrogen availability, microbial growth and digestive efficiency decrease (Nocek and Russell, 1988).

A number of studies have demonstrated the effects of synchronization of energy and nitrogen supply on ruminal characteristics *in vivo* or *in vitro* (McCarthy et al., 1989; Stokes et al., 1991; Aldrich et al., 1993; Casper et al., 1999). However, the results are not consistent between these studies indicating that the responses to synchronization of energy and protein availability in the rumen have been variable. In most of these studies, different sources of feed ingredients were used to formulate diets with high or low degradation rates. Therefore, treatment effects may have been confounded with ingredient source and with different total amounts and/or ratios of nutrients released in the rumen. Furthermore, roughage and feeding frequency also contributed to the ruminal available energy.

In an earlier *in situ* experiment (Chanjula et al., 2003) large differences were observed in rate of energy degradability from several sources particularly corn and cassava chip which are major starch sources fed to dairy

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Table 1. Ingredient and chemical composition of dairy rations (% DM basis)

Composition	Dietary treatments ¹			
	CM		CC	
	LLCM	LHCM	HLCC	HHCC
Ingredients, %				
Corn meal, CM	55	75	-	-
Cassava chip, CC	-	-	55	75
Urea ²	2.2	3.0	3.3	4.5
Whole cottonseed meal, WSC	17.8	11.0	18.7	11.0
Molasses	3.0	3.0	3.0	3.0
Rice bran, RB	17.0	3.0	15.0	1.5
Dicalcium phosphate	1.0	1.0	1.0	1.0
Minerals and vitamins ³	1.0	1.0	1.0	1.0
Limestone	1.0	1.0	1.0	1.0
Salt	1.0	1.0	1.0	1.0
Tallow	0.5	0.5	0.5	0.5
Sulfur	0.5	0.5	0.5	0.5
Estimated nutrients (%)				
CP	17.3	17.5	17.3	17.4
EE	5.5	4.2	3.1	1.2
Ca	0.8	0.8	0.8	0.9
P	0.5	0.5	0.5	0.4
TDN	81.2	82.5	76.9	76.4
ME, Mcal/kg, DM ⁴	2.9	2.9	2.8	2.8
NE _L , Mcal/kg, DM ⁵	1.7	1.8	1.7	1.6
Feed cost, US\$/kg	0.11	0.11	0.10	0.09

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%); HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² Estimated: based on 30 g N/kg DOMR (Preston and Leng, 1987) Equation= $(N \times 0.46 \times 1,000) / (1 \times ED \times \% OMI)$ (Chanjula et al., 2003).

³ Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50g; Zn: 40g; Mn: 40g; Co: 0.1g; Cu: 10g; Se: 0.1g; I: 0.5g.

⁴ Estimated: Metabolizable energy (ME, Mcal/kg, DM)=TDN \times 0.04409 \times 0.82.

⁵ Estimated: Net energy for lactation (NE_L, Mcal/kg)= (0.0245 \times TDN)-0.12 (NRC, 1989).

cows. These two starch sources were shown to have widely varying rumen degradabilities.

The objective of the present study was to examine the synchronization of nitrogen and energy using different levels and sources of starch with their degradation rates in the rumen on feed intake, ruminal microbial fermentation, nutrient utilization and performance of lactating dairy cows fed on urea-treated rice straw as a basal roughage.

MATERIALS AND METHODS

Animals, diets and experimental designs

Eight, multiparous mid-lactation (153 DIM: ± 6 SD) Holstein-Friesian crossbred cows (75%) were used in the experiment. Milk yield pre-experiment was 9 ± 2 kg/day and the body weight were 383 ± 9 kg. Two cows were allocated to each of four experimental diets. All cows within each

group were randomly allocated to one of the four experimental diets accordingly to a Switchback design with a 2 \times 2 factorial arrangement of treatments involving two nonstructural carbohydrate (NSC) sources (corn and cassava) with two levels of NSC (55 and 75%) in diets.

The dietary treatments were as follows: 1) LLCM=Low degradability low level corn meal (55%), 2) LHCM=Low degradability high level corn meal (75%), 3) HLCC=High degradability low level cassava chips (55%) and 4) HHCC=High degradability high level cassava chips (75%). Ingredient and composition of each diet is shown in Table 1. The concentrate mixed diets were formulated to be isonitrogenous at 17% CP (2.72% N).

Cows were housed in individual pens and individually fed concentrate at a ratio to milk yield of 1:2, twice daily at 0600 and 1600 after milking. All cows were fed *ad libitum* with water and a mineral-salt block. Urea-treated rice straw was given *ad libitum*. In addition, cassava hay (CH) was supplemented at 1 kg/hd/d as a roughage source. During the preliminary period, cows received a control diet containing 30 percent of cassava and corn meal in the concentrate with urea-treated rice straw as a roughage. Cows were then fed one-half of the control diet and one-half of their respective experimental diet for 3 d during a transitional. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run in three period, each experimental period lasted for 21 days, the first 14 days as a period for treatment adaptation and for feed intake measurements while the last 7 days were for sample collections of rumen fluid and faeces. Body weights were measured daily during the sampling period prior to feeding. Milk yield were recorded during the 21 day-period and samples were collected during the last 7 d of each period.

Feed management

Urea-treated rice straw (UTRS) 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).

The cassava crop was planted at 30 \times 40 cm between plants and rows in a well-prepared soil, which was fertilized with cow manure at 700 kg (fresh) per hectare. Cassava Hay (CH) made from the aerial parts of the whole cassava crop were harvested at 3 month maturity after planting, and chopped into small pieces (about 2-4 cm) and then sun-dried for 2-3 days to obtain CH, enrichment storage and feeding. Sun drying consisted of spreading the CH on the ground and turning whilst exposed to the sun during a 1-2 day period and samples were taken for subsequent chemical analysis (Wanapat, 2003).

Table 2. Chemical composition (%DM basis) of cassava hay (CH), urea-treated rice straw (UTRS) and experimental diets

Item	CH	UTRS	Dietary treatments ¹			
			CM		CC	
			LLCM	LHCM	HLCC	HHCC
DM ²	85.9	52.8	89.5	88.9	89.9	89.0
Ash	7.7	12.1	7.8	6.2	8.7	8.7
OM	92.3	87.9	92.2	93.8	91.3	91.3
CP	19.6	7.4	18.8	19.3	18.6	19.1
EE	5.4	1.3	9.4	8.3	8.1	3.8
NSC ³	20.7	6.6	32.5	35.6	48.8	54.9
NDF	46.6	76.2	31.5	30.6	15.8	13.5
ADF	28.6	54.4	7.9	7.5	9.5	8.7
CT	3.5	ND ⁴	ND	ND	ND	ND

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%); HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NSC: nonstructural carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; CT: condensed tannins.

³ Estimated: NSC=100-(CP+NDF-EE-Ash) (Nocek and Russell, 1988).

⁴ ND: not determined.

Sample collection and chemical analysis

Urea-treated rice straw, cassava hay and concentrate were sampled daily during the collection period and were composited by period prior to analyses. Feeds and fecal samples were collected during the last seven 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, ether extract, ash and CP content (AOAC, 1990), NDF, ADF and ADL (Goering and Van Soest, 1970) and AIA. In addition, condensed tannins was analyzed in cassava hay by the method of Burns (1971). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977).

Cows were milked twice daily, and milk weights were recorded at each milking of each period. Milk samples were composited daily, according to yield, for both the a.m. and p.m. milking, preserved with 2-bromo-2 nitropropane-1,3-dial, and stored at 4°C until analysis for fat, protein, lactose, totals solids and solid not fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Denmark).

Rumen fluid and jugular blood samples were collected at 0, 2, 4 and 6 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 a portable pH and temperature meter (Orion Research portable meter 200 series, USA) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH₃-N analyses where 5 ml of H₂SO₄ solution (1M) was

added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000×g for 15 minute and the supernatant stored at -20°C prior to NH₃-N analyses using the micro kjeldahl method (AOAC, 1990). Another portion was fixed with 10% formalin solution in normal saline (0.9% NaCl) (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 haemocytometer (Boeco) and cultured groups of bacteria using the roll-tube method described by Hungate (1969), for identifying of bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

Samples of jugular blood were drawn into serum separation tubes at the time of rumen fluid sampling and centrifuged for 20 min at 850×g. The supernatant was decanted and frozen (-20°C) until it was analyzed for blood urea nitrogen (BUN) according to the method of Crocker (1967).

Statistical analyses

All data obtained from the experiment were subjected to ANOVA for a Switchback design with a 2×2 factorial arrangement of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1990). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). The following models were used for statistical analysis.

$$\text{Model} = Y_{ijk} = \mu + A_i + P_j + S_k + L_l + SL_{kl} + E_{ijk}$$

Where Y_{ijk} =nutrient intake or rumen fermentation values; μ =overall mean; Co=covariant (pre-treatment of milk yield or body weight); A_i =effect of animal; P_j =effect of period; S_k =effect of NSC sources; L_l =effect of level of NSC; SL_{kl} =effect of interaction of source and level of NSC; E_{ijk} =error of the term. Analysis of covariance was used for the parameters of milk yield, feed intake and animal body weight. When significant differences due to NSC source and level of NSC and the interaction of sources and level were detected, all the analyses followed the procedures of Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Chemical composition of feeds

The chemical composition of roughages and experimental diets fed in the lactation study are presented in Table 2. Experimental diets contained similar concentrations of DM, OM, CP, ADF and ADL. Diets containing cassava-based diets had a slightly higher nonstructural carbohydrate (NSC) (~17.7 percentage unit) and lower NDF than those corn-based diets. NSC was

Table 3. Least square means for intake affected by energy source and level with different degradabilities of lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<.) ²		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
DMI, kg/d								
Total roughage (UTRS+CH)	7.3	7.3	7.3	6.9	0.18	NS	NS	NS
%BW	2.1	2.2	2.1	2.0	0.06	NS	NS	NS
g/kg W ^{0.75}	91.7	93.1	91.0	87.5	2.38	NS	NS	NS
Concentrate	3.7	3.9	3.8	3.8	0.11	NS	NS	NS
%BW	1.1	1.2	1.1	1.1	0.04	NS	NS	NS
g/kg W ^{0.75}	46.6	49.6	48.2	47.4	1.46	NS	NS	NS
Total DMI, kg/d	10.9	11.1	11.1	10.7	0.18	NS	NS	NS
DMI, %BW	3.2	3.3	3.2	3.1	0.07	NS	NS	NS
DMI, g/kg W ^{0.75}	138.3	142.8	139.2	134.9	3.12	NS	NS	NS
OMI, kg/d	9.5	9.8	9.4	9.2	0.22	NS	NS	NS
CPI, kg/d	1.3	1.4	1.4	1.2	0.04	NS	NS	NS
NDFI, kg/d	6.9	7.1	6.9	6.6	0.16	NS	NS	NS
BW change, %	0.5	0.6	0.3	0.4	0.11	NS	NS	NS

^{a,b} Within rows not sharing a common superscripts are significantly different (p<0.05). SEM: standard error of the mean (n=6)

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%);

HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L: interaction of NSC and L source.

increased by level of NSC for both cassava and corn-based diets. The differences among concentrate mixed diets in NSC, ether extract (EE), fiber components and ash concentrations can be related to differences in the ingredients used in diet formulation (Table 1).

Chemical composition of urea-treated rice straw (UTRS) and cassava hay (CH) are presented in Table 2. Similar values for UTRS and CH have been previously reported by Ravindran (1993); Wanapat et al. (2000a); Hong et al. (2003) when CH was harvested at a younger stage of growth (3-4 months) or regrowth (2-3 months). With regards to CP and CT percentages of CH, they were found to be in similar range to those reported by Wanapat et al. (2000a, 2000b). Nevertheless, the nutritive value of CH may depend on cultivar, age of plant, plant density, the plant part, soil fertility, harvesting frequency, season and climate (Wanapat et al., 2000a).

Effect on feed intake

The effects of NSC sources and level (L) on feed intake of mid-lactating dairy cows are presented in Table 3. Feed intakes were not affected by energy source or level. Overall mean feed intakes for the four diets in terms of total DMI, %BW g/kgBW^{0.75}, OMI, CPI, and NDFI were similar for all dietary treatments, average total DMI of cassava-based diets compared with corn-based diets were 10.9 and 11.0 kg/d, respectively. The data indicate that NSC source and L had no effect on feed intake in mid-lactating dairy cows. These data support earlier work (Brigstocke et al., 1981; Zinn and DePeters, 1991; Sommart et al., 2000) which have reported that inclusion of cassava in diets results in satisfactory animal performance and no negative effects on animal health in finishing beef cattle and

lactating dairy cows. Other studies (Lebzien and Engling, 1995) have undertaken a comparison of cassava, corn, barley and wheat as sources of starch in non-lactating dairy cow diets, while Kanjanaputhipong et al. (2001) found no difference in DMI, ruminal pH and Total VFA concentration in rumen fluid to varying levels in dairy cow diets. However, feeding corn diets tended to increase the DMI and OMI as the level of corn increased as compared to cassava. Previous reports (Hoover, 1986) have suggested that providing a source of more degradable NSC can result in a substantial decrease in ruminal pH and fiber digestibility thus reducing feed intake.

No significant (p>0.05) interaction of NSC source and L were detected for BW change, indicating that cows attained positive energy balance before the study conclusion, but BW change shown altered to be slightly greater for cows fed diets containing corn-based diets compared with cassava-based diets. This trend may be related to the higher DMI and OMI of corn-based diets compared with cassava-based diets.

Effect of rumen fermentation

Rumen ecology parameters were measured for pH, NH₃-N, BUN and MUN. In addition, BUN and MUN were determined to investigate their relationship with rumen NH₃-N and protein utilization. The pattern of ruminal fermentation at 0, 2, 4 and 6 h post feeding and overall means are given in Table 4. Rumen fluid pH at 0, 2, 4 and 6 h post feeding were unchanged by dietary treatments and the values were quite stable at 6.8-7.0, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber (Hoover, 1986) and also digestion of protein (6.0-7.0) (Hoover, 1986).

Table 4. Least square means for rumen pH, ammonia nitrogen (NH₃-N), blood urea nitrogen (BUN), milk urea nitrogen (MUN) affected energy level and source with different degradabilities of lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ²		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Ruminal pH								
0 h post feeding	7.2	7.3	7.0	7.0	0.09	NS	NS	NS
2	6.9	7.0	6.9	6.8	0.04	NS	NS	NS
4	6.9	6.8	6.5	6.8	0.09	NS	NS	NS
6	7.1	6.8	6.9	7.3	0.11	NS	NS	NS
Mean	7.0	6.9	6.8	6.9	0.13	NS	NS	NS
NH₃-N, mg/dl								
0 h post feeding	7.5	13.1	8.2	8.8	1.73	NS	NS	NS
2	19.5 ^a	29.8 ^b	23.8 ^{ab}	24.5 ^{ab}	2.40	NS	0.05	NS
4	20.3	22.3	19.9	19.6	2.41	NS	NS	NS
6	12.7	15.4	14.5	19.4	2.28	NS	NS	NS
Mean	15.6 ^a	20.0 ^b	16.6 ^{ab}	18.1 ^{ab}	1.04	NS	0.01	NS
BUN, mg/dl								
0 h post feeding	10.2	10.3	11.0	12.5	0.80	NS	NS	NS
2	14.1 ^b	13.5 ^b	15.6 ^{ab}	18.2 ^a	0.89	0.04	NS	NS
4	16.6 ^b	15.1 ^b	16.6 ^{ab}	22.0 ^a	1.10	0.06	NS	NS
6	15.1	17.2	15.5	18.2	1.08	NS	NS	NS
Mean	13.8 ^b	14.4 ^{ab}	14.4 ^{ab}	17.0 ^a	0.78	NS	0.08	NS
MUN, mg/dl								
	13.6	16.9	17.5	17.7	1.91	NS	NS	NS

^{a,b} Within rows not sharing a common superscripts are significantly different (p<0.05). SEM: standard error of the mean (n=6).

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%);

HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L=interaction of NSC and L source.

According to the reviews by Ørskov (1986), cows with rumen pH above 5.8 are considered normal, while those between 5.0 and 5.8 may be suffering from subclinical acidosis. Furthermore, these values are consistent with previous research on CH supplementation (Wanapat et al., 2000b). Moreover, the ruminal pH is partly regulated by the ammonia concentration in the rumen fluid. The variation in pH may be explained by the urea entering the rumen that is hydrolyzed by microbial ureases to CO₂ and ammonia (2 NH₃) (Van Soest, 1994).

Ruminal NH₃-N, BUN and MUN concentrations were not altered by diets containing cassava-based diets compared with corn-based diets, but were dramatically increased by level of NSC and slightly decreased by diets containing cassava-based diets compared with corn-based diets. Concentrations of NH₃-N in ruminal fluid have been reported to decrease when 1) urea containing diets fed to nonlactating dairy cows were supplemented with molasses and starch (Fadel et al., 1987), 2) the nonstructural carbohydrate content of the diet was increased (MacGregor et al., 1983), or 3) the amount of starch digested in the rumen was increased (McCarthy et al., 1989). Nevertheless, it was closer to optimal ruminal NH₃-N (15-30 mg%, Perdok and Leng, 1990; Wanapat and Pimpa, 1999) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake (Church and Santos, 1981; DelCurto et al., 1990b) of low-quality forages. Meanwhile, no

significant (p>0.05) interaction of NSC source and L was detected for BUN, but, except at 2 and 4 h post feeding, were greater (p<0.05) for cassava-based diets compared with corn-based diets.

The increases in rumen NH₃-N levels also resulted in increasing levels of BUN. Previous studies (Preston et al., 1965; Lewis, 1975) have reported that concentrations of BUN are highly correlated to the level of ammonia production in the rumen. The urea content in the blood has been found to reach a maximum 3 h after feeding (Eggum, 1970b) and is commonly considered to reflect the protein quality in diets. This would indicate that available rumen NH₃-N could be used and/or absorbed in the rumen for further synthesis (Table 5).

No interactions between cassava and corn-based diets were detected on milk urea nitrogen (MUN) (Table 4). Concentrations of MUN were higher than present in the BUN and were a better indicator of N status than BUN concentration because milk samples represented nitrogen metabolism during an entire 24 h period, while BUN concentrations represented N metabolism occurring for a short period immediately after post-feeding. In dairy cows, significant correlations between urea in plasma and milk have been reported (Roseler et al., 1993; Butler et al., 1996). Also, milk urea has been reported to be affected by the content of crude protein in the diet. Overall, MUN concentrations were above the recommended maximum of

Table 5. Least square means for ruminal cellulolytic, proteolytic, amylolytic, total viable bacteria count, ruminal protozoa, fungi and bacteria population affected by energy level and source with different degradabilities in lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ²		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Roll tube techniques								
Total viable bacteria (×10 ¹⁰ CFU/ml)								
0 h-post feeding	10.9	9.4	7.2	4.5	1.70	NS	NS	NS
4	6.2	4.1	7.7	5.8	1.49	NS	NS	NS
Mean	8.5	6.7	7.5	5.2	1.31	NS	NS	NS
Proteolytic bacteria (×10 ⁷ CFU/ml)								
0 h post feeding	3.7	5.2	4.9	4.1	1.62	NS	NS	NS
4	3.5	3.6	6.4	4.4	1.35	NS	NS	NS
Mean	3.6	4.4	5.7	4.3	1.12	NS	NS	NS
Cellulolytic (×10 ⁹ CFU/ml)								
0 h post feeding	8.9	8.0	5.8	5.9	1.80	NS	NS	NS
4	6.7	4.9	9.2	7.7	1.51	NS	NS	NS
Mean	7.8	6.5	7.5	6.8	1.41	NS	NS	NS
Amylolytic (×10 ⁸ CFU/ml)								
0 h-post feeding	2.8	3.6	3.2	2.4	1.56	NS	NS	NS
4	2.7	0.6	3.9	4.4	1.48	NS	NS	NS
Mean	2.8	2.1	3.6	3.4	1.31	NS	NS	NS
Total direct counts								
Bacteria (×10 ¹¹ cell/ml)								
0 h post feeding	0.6	0.4	0.4	0.7	0.08	NS	NS	NS
4	0.9	0.9	1.1	0.8	0.15	NS	NS	NS
Mean	0.7	0.6	0.8	0.7	0.07	NS	NS	NS
Total direct counts								
Protozoa (×10 ⁵ cell/ml)								
0 h-post feeding	4.0	4.2	3.8	4.4	0.90	NS	NS	NS
4	2.0	2.1	2.9	4.2	0.93	NS	NS	NS
Mean	3.0	3.2	3.4	4.3	0.42	NS	NS	NS
Fungal zoospores (×10 ⁶ cell/ml)								
0 h post feeding	0.7 ^a	0.9 ^a	1.4 ^b	0.7 ^a	0.07	0.07	0.001	0.08
4	0.9	1.3	1.2	1.2	0.16	NS	NS	NS
Mean	0.8 ^a	1.1 ^{ab}	1.3 ^b	0.9 ^{ab}	0.10	NS	0.01	NS

^{ab} Within rows not sharing a common superscripts are significantly different (p<0.05). SEM: standard error of the mean (n=6).

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%);

HLCC high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L=interaction of NSC and L source.

16 mg/dl, indicating over consumption of CP or RDP, inefficient N use, decreased fertility, and potential loading into environment (Ferguson et al., 1993; Roseler et al., 1993). In contrast, other studies (Butler et al., 1996) reported that concentrations of BUN and MUN above 16 mg/dl, but below 19 mg/dl have not reduced fertility in dairy cows. Based on this study, the present results indicate that synchronization of NSC with a high level of urea in rations (>3% DM) did not affect pregnancy rate after artificial insemination (AI) in lactating dairy cows. All cows had conceived a fetus after rechecking (Table 7). This data is in accordance with reports by Carrol et al. (1988) that high protein (RDP) intake, high BUN and MUN have not always been associated with reduced reproductive efficiency, but BUN and MUN concentrations of greater than 19 to 20 mg/dl has been associated with an altered

uterine environment and decreased fertility (Ferguson et al., 1993; Butler et al., 1996). Other studies, that are well-documented (Butler and Smith, 1989) indicate that a negative energy balance during early lactation is associated with reduced fertility.

Rumen micro-organisms

Table 5 presents rumen microorganism populations. Total viable bacterial counts, proteolytic, cellulolytic and amylolytic bacteria were not affected (p>0.05) by diets containing cassava-based diets compared with corn-based diets. But overall populations tended to be slightly greater from 0 to 4 h post feeding in all treatments. Likewise, fungal zoospores, protozoa and total bacteria direct counts were not affected by the interaction between NSC source and L, but fungal zoospores were greater for cassava-based

Table 6. Least square means for apparent digestibility affected by energy level and source with different degradabilities in lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ²		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Apparent digestibility, %								
DM	53.2	55.5	53.6	56.3	1.10	NS	NS	NS
OM	58.4	60.8	59.8	62.2	1.10	NS	NS	NS
CP	43.8	49.1	49.0	50.9	1.77	NS	NS	NS
NDF	49.6	52.1	46.9	49.9	1.08	NS	NS	NS
ADF	35.5	39.5	34.0	35.6	2.33	NS	NS	NS
Digestible nutrient intake, %								
OM	5.5	5.9	5.6	5.8	0.16	NS	NS	NS
CP	0.5	0.7	0.6	0.6	0.12	NS	NS	NS
NDF	3.4 ^{ab}	3.7 ^a	3.3 ^{ab}	3.2 ^b	0.11	0.07	NS	NS
ADF	1.4	1.5	1.4	1.4	0.10	NS	NS	NS
Estimated energy intake ³								
ME Mcal/d	21.1	22.6	21.4	21.9	0.47	NS	NS	NS
ME Mcal/kg DM	1.9	2.0	1.9	2.1	0.04	NS	NS	NS

^{ab} Within rows not sharing a common superscripts are significantly different (p<0.05). SEM: standard error of the mean (n=6)

¹ LLCM: Low degradability Low level Corn Meal (55%); LHCM: low degradability high level corn meal (75%);

HLCC: high degradability Low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² NSC: Nonstructural carbohydrate source; L: Level of NSC source; NSC×L=interaction of NSC and L source.

³ Estimated: 1 kg DOM=3.8 Mcal ME/kg (Kearl, 1982).

diets than corn-based diets. The high level of cassava-based diets had a higher number of protozoa than corn-based diets. The presence of protozoa in the rumen can also affect rumen fermentation 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. Moreover, if the ration is based on grain, protozoa engulfment of starch grains can modulate pH and protect the animal from acidosis (Russell and Hespell, 1981; McAllister et al., 1993). However, the decrease in protozoal count may attribute the increase in fungal zoospores per ml rumen fluid, as removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981).

In the present study, all treatments were supplemented with 1 kg CH (DM) which could decrease the number of protozoa. This data is in agreement with previous reports Wang et al. (1994). It is possible that condensed tannins (CT) present in the CH may play an important role in decreasing protozoal populations. As previously reported by McSweeney et al. (2000) that condensed tannins altered rumen ecology and increased microbial protein synthesis, however, the mode of action of CT on rumen fermentation is yet to be elucidated. However, at high levels (>6% of dry matter), tannins can adversely affect the microbial activity (Reed, 1995) and also these plants often contain CT (polyphenolics) which inhibit growth of dominant rumen bacteria (*Fibrobacter succinogens*, *Butyrivibrio fibrisolvens* and *Streptococcus bovis*).

Effect on digestibility

Apparent digestibilities of DM, OM, CP, NDF and ADF

were similar (p>0.05) for all diets, whilst digestible nutrient intake of NDF (kg/d) tended to be slightly greater (p<0.07) for cows fed corn-based diets compared with cassava-based diets (3.6 and 3.3, respectively) (Table 6). The slightly lower NDF digestibility of the cassava-based diets may have contributed to higher degradation in substantial decrease in fiber digestibility (Hoover, 1986; Ørskov, 1986; Nocek and Russell, 1988). An other study (Grant, 1994) has reported that the sources of starch influence the rate of NDF digestion differently at pH 6.8 than 5.5. Nevertheless, ME (Mcal/d) and ME (Mcal/kgDM) were not affected by NSC source, L, or the interaction between NSC source and L.

Milk production and composition

No significant (p>0.05) interactions of NSC source and L were detected for milk production, efficiency of milk production or milk composition (Table 7). However, milk fat percentages and ECM were greater for cassava-based diets than corn-based diets averaging (4.4 and 4.2, respectively), indicating that NSC degradability influences milk composition when lactating cows are fed diets based on cassava or corn. This data were in accordance with that reported by Kanjanapruthipong et al. (2001), and have shown greater milk fat percentages than in other studies (Grings et al., 1992; Casper et al., 1999). However, milk protein percentages are lower than expected. Why milk protein percentages are lower than expected in this study is not known, but the results could be related to UIP sources or it may be that the degradation rates of both cassava chips and corn are incompatible with that of urea.

Providing a source of NSC in the diet that was more degradable, but not more soluble did increase utilization of

Table 7. Least square means for milk yield and milk composition affected by energy level and source with different degradabilities in lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ⁶		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Production						NS	NS	NS
Milk yield, kg/d	7.3	7.8	7.7	7.4	0.23	NS	NS	NS
3.5%FCM, kg/d ²	8.2	8.7	8.8	8.2	0.28	NS	NS	NS
Fat yield, kg/d	0.3	0.3	0.3	0.3	0.01	NS	NS	NS
Protein yield, kg/d	0.2	0.2	0.2	0.2	0.04	NS	NS	NS
Milk composition								
Milk fat, %	4.2	4.1	4.4	4.3	0.11	NS	NS	NS
Milk protein, %	3.0	3.1	3.0	3.0	0.02	NS	NS	NS
Lactose, %	4.6	4.7	4.6	4.6	0.03	NS	NS	NS
SNF, % ³	8.3	8.4	8.3	8.3	0.05	NS	NS	NS
TS, % ⁴	12.4	12.5	12.7	12.5	0.13	NS	NS	NS
Efficiency of milk production								
ECM, kg/d ⁵	8.1	8.8	9.0	8.6	0.29	NS	NS	NS
3.5% FCM/DMI, kg/kg	0.8	0.8	0.8	0.8	0.03	NS	NS	NS
ECM/NE _L	4.8	4.8	5.3	5.4	0.17	NS	NS	NS
Embryo survival	2	2	2	2	-	-	-	-

SEM: standard error of the mean (n=6).

¹ LLCM: low degradability low level corn meal (55%); LHCM: long=low degradability high level corn meal (75%); HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).² 3.5% FCM (fat collected milk)=0.432 (kg of milk/d)+16.23 (kg of fat).³ SNF: solid not fat. ⁴ TS: total solid.⁵ ECM: energy-corrected milk=7.20×protein (kg/d)–12.95×fat (kg/d)+0.327×milk (kg/d) calculated from Tyrrell and Reid (1965).⁶ NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L: interaction of NSC and L source.

a highly soluble CP source (supplied by urea) for milk production. The data indicate that degradability, but not the solubility, of NSC influences production of milk in mid-lactation dairy cows. However, depending on NSC source fed, these data indicate that diets containing a highly soluble N source together with a degradable NSC source can support milk production at a level equal to cows fed all performed protein sources. This agrees with work of some researchers (Huber and Kung, 1981; Kung and Huber, 1983), but contrasts with that of other (Clark and Davis, 1980) who observed that high producing cows during early lactation often produced less milk than if they were fed only natural protein. A lack of response in milk yield when diets are supplemented with high RUP may be related to either a poor essential AA profile of the RUP sources, low small intestinal digestibility of the RUP sources, decreased microbial protein synthesis in the rumen or control diets already sufficiently high in RUP (NRC, 1989; Clark et al., 1992).

Energy status of the cows are presented in Table 8. No significant (p>0.05) interactions of NSC source and L were detected for energy status of the cows, but cows fed corn based diets tended to higher energy input, these differences reflect the differences in DMI and OMI between these two sources. However, cows fed cassava based diets tended to utilize dietary energy more efficiently than cows offered corn based diets (5.3 and 4.8 ECM/NE_L, respectively). Energy expenditure for maintenance and BW changes were

not affected by dietary treatments. Therefore, total energy output had a similar pattern to milk energy output among the dietary treatments.

Likewise, income over feed, as estimated from 3.5% FCM, was higher on cassava than corn diets averaging (54.0 and 51.4 US\$/mo, respectively) (Table 9). In this study, given the low cost of cassava-based diets compared with corn-based diets, there are likely to be major economic advantages to the use of both urea and cassava chip levels in dairy cow diets. Experimental diets based on cassava were adjusted to be approximately 30 g N/kg DOMR. The price of cassava-based diets was cheaper than corn-based diets. The lower price of cassava resulted in a low cost of the concentrates with the high inclusion rate of cassava. Consequently, there was higher income over feed than corn-based diets. These data were in accordance with those previously reported by Brigstocke et al. (1981), Sommart et al. (2000), Kanjanapruthipong et al. (2001). The results support the conclusion that the efficiency of milk production in dairy cows fed cassava-based diets as a main source of NSC is greater than that in dairy cows fed diets with a similar nutritional specification not containing cassava and our results also clearly showed that this effect did not result in negative effects on voluntary feed intake, rumen ecology, rumen micro-organisms, milk production and composition or animal performance.

Table 8. Least square means for energy status of cow affected by energy level and source with different degradabilities in lactating cows

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ⁶		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Energy intake, Mcal/d								
NE _L ²	14.2	15.5	14.4	13.9	0.42	NS	NS	NS
Energy output, Mcal/d								
Milk ³	5.4	5.8	5.9	5.6	0.18	NS	NS	NS
Maintenance ⁴	6.4	6.3	6.4	6.3	0.04	NS	NS	NS
Body change ⁵	2.5	3.5	1.7	2.1	0.58	NS	NS	NS
Total	14.4	15.7	14.0	14.1	0.55	NS	NS	NS

^{a-d} Within rows not sharing a common superscripts are significantly different (p<0.05). SEM: standard error of the mean (n=6).

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%);

HLCC: high degradability low level cassava chips (55%); HHCC=High degradability high level cassava chips (75%).

² Calculated as DMI×dietary NE concentration.

³ Calculated as milk yield (kg)×(89.584 (%fat)+49.83 (%protein)-226.094)/1000 (Tyrrell and Reid (1965)).

⁴ Calculated as BW^{0.75}×0.08 (NRC, 1989).

⁵ Calculated as 5.12 Mcal/kg of gain or 4.92 Mcal/kg of loss (NRC, 1989).

⁶ NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L=interaction of NSC and L source.

Table 9. Effect of energy level and source with different degradabilities on economical return

Attribute	Dietary treatments ¹				SEM	Main effects (p<) ⁴		
	CM		CC			NSC	L	NSC×L
	LLCM	LHCM	HLCC	HHCC				
Production								
3.5%FCM, kg/d	8.2	8.7	8.8	8.2	0.28	NS	NS	NS
Milk sale, US \$/hd/d ²	2.1	2.2	2.2	2.1	3.04	NS	NS	NS
Milk sale, US \$/hd/mo	62.4	66.2	66.9	62.4	91.38	NS	NS	NS
Concentrate intake, kg/d	0.1	0.1	0.1	0.1	0.11	NS	NS	NS
Concentrate cost, US \$/kg	0.1	0.1	0.1	0.1	-	NS	NS	NS
Concentrate cost, US \$/d	0.4	0.4	0.4	0.3	-	NS	NS	NS
Total feed cost, US \$/d	0.4	0.4	0.4	0.3	-	NS	NS	NS
Income over feed, US \$/hd/d	1.7	1.8	1.9	1.7	15.66	NS	NS	NS
Income over feed, US \$/mo ³	49.9	52.8	56.0	52.0	1.85	NS	NS	NS

SEM: standard error of the mean (n=6).

¹ LLCM: low degradability low level corn meal (55%); LHCM: low degradability high level corn meal (75%); HLCC: high degradability low level cassava chips (55%); HHCC: high degradability high level cassava chips (75%).

² Price:milk=0.3 US \$/kg.

³ Official rate of exchange: 43 Baht=US \$1.

⁴ NSC: nonstructural carbohydrate source; L: level of NSC source; NSC×L=interaction of NSC and L source.

CONCLUSIONS AND RECOMMENDATIONS

Based on this experiment it could be concluded that providing a NSC source from cassava-based diets did not affect feed intake, rumen ecology, rumen micro-organisms, milk production and composition and animal performance compared with corn-based diets. but tended to improve milk fat for cassava-based diets as compared with corn-based diets. In addition, income over feed, as estimated from 3.5% FCM, was higher on cassava than corn diets averaging (54.0 and 51.4 US\$/mo, respectively). These results indicate that feeding diets containing either based on cassava and/ or level greater up to 75% of DM, with NPN supplied by urea >3% DM (3.3-4.5% DM) can be used in dairy rations without altering rumen ecology or animal performance compared with corn-based diets. Given the low cost of cassava compared with corn, based on this data,

it would be desirable to conduct further research on the use of cassava chips at a high level when synchronized with NPN (urea) in practical rations for early to mid-lactation cows as well as using this approach for on farm research.

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