Effect of Level of Crude Protein and Use of Cottonseed Meal in Diets Containing Cassava Chips and Rice Straw for Lactating Dairy Cows

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ABSTRACT : The effects of different levels of crude protein (CP) and cottonseed meal replacing for soybean meal in cassava chips and rice straw-based diets for mid-lactating cows (100-150 day in milk [DIM]), were studied using 32 multiparous Holstein Fresian crossbred dairy cows. Diets containing 10.5, 12.5, 13.7, 14.4% CP of the rations and 0, 12.1, 14.9, 17.8% cottonseed meal were fed to cows for 60 days. Thirty two cows were randomly divided into four dietary treatments using a Randomized complete block design. Four dietary treatments were offered in the form of total mixed ration (TMR) with concentrate to roughage (chopped rice straw) at 60:40 and offered *ad libitum*. Dry matter (DM) and neutral detergent fiber (NDF) intakes tended to linearly increase with increasing dietary CP levels. Intakes and digestibility of crude protein increased linearly with increasing dietary CP level ($p<0.01$). Crude protein digestibility of the 10.5% CP diet was lower ($p<0.05$) than that in diets with higher levels of CP, while there were no significant differences among the other three levels of CP (12.5, 13.7 and 14.4%). Daily milk yield tended to increase with increased CP from 10.5 to 14.4%. Income over feed in terms of US$/kg of milk increased with increased CP from 10.5 to 13.7% and decreased when the CP level was higher than 13.7% (quadratic effect $p<0.09$). Milk composition was not significantly affected by increasing level of CP, however there were relatively high contents of protein and fat among treatments. The proportion of milk-urea N (MUN), ammonia-N (NH_3-N) and blood-urea N (BUN) were closely correlated and increased linearly with increasing CP levels ($p<0.01$). Balanced diet was found in diet containing 12.5 and 13.7% CP of the rations when BUN and MUN were used as indicators of the protein to energy ratio in the diet. Conclusions can be made that increasing dietary CP levels from 10.5 to 13.7% using cottonseed meal as the main source to completely replace soybean meal was beneficial to cows consuming rice straw and cassava chips based-diets. Increasing the CP level above 13.7% of total ration did not additionally improve milk yield and composition or net income. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 502-511)***

Key Words : Protein, Cottonseed Meal, Lactating Dairy Cow, MUN, BUN, Ammonia-N, Dry Matter Intake, Milk Yield and Composition, Rice Straw, Urea-treated Rice Straw

INTRODUCTION

Feeding of dairy cattle in the tropics is often difficult because of deficiencies in feed supply, in both quality and quantity (Wanapat and Devendra, 1992). The use of rice straw as a feed in the dry season, in spite of its low nutritive value, has been a common feeding system, generally practiced by dairy farmers in the tropics when green forages are often scarce (Leng and Preston 1983; Wanapat, 1994).

Cottonseed meal (CSM) is a by-product from oil extraction and is a source of rumen by-pass protein supplement in dairy cattle feeding (Grings et al., 1991; Wanapat et al., 1996). Several studies have been devoted to study the potential value of CSM in ruminants (Erdman et al., 1987; Cunha et al., 1998; Sampaio et al., 2000). According to NRC (1989), the degradability of CSM is 57% and for crude protein (CP) and total digestible nutrients (TDN), 44.3% and 78%, respectively (Kearl, 1982). Cottonseed meal has been used at 8.6% of a total mixed ration in dairy cattle diets, and could increase feed intake, economical benefits and milk yield (Grings et al., 1991). Paengkoum (1998) reported that in lactating cows voluntary roughage intake of a ration containing cottonseed meal, cassava chips and urea-treated rice straw was higher than of the ration without cottonseed meal. Wanapat et al. (1996) reported that milk yield of crossbred Holstein-Zebu cows fed a low protein basal diet of rice straw and cassava chips was markedly improved when the CSM supplement was increased from 2 to 4 kg/day, while Blackwelder et al. (1998) suggested that cottonseed meal in the diet can be substituted for soybean meal, resulting in similar milk yield and composition. This feeding system is an economically attractive alternative to farmers who traditionally use commercial concentrates. Cassava is an annual crop grown wildly in the tropical area not only for tuber but for biomass to produce hay for dairy cattle (Wanapat, 2003; Kiyothong and Wanapat, 2004ab) as well. So cassava chip is locally available in Thailand,and the price is relatively low. Cassava chip is known to be a good energy source, with 80% TDN (Kearl, 1982) and is highly degraded in the rumen (94% DM) (Sathapanasiri et al., 1990). Cassava chip contains 88.3% DM, 2.1% CP, 1.5% CF (Kearl, 1982). Several studies have been carried out on the effects of

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Table 1. Ingredients and compositions of experimental concentrates

Ingredient	Cottonseed meal concentration (% Dry matter)			
	0	20	25	30
	% Dry matter basis			
Cassava chip	58.5	54.8	52.6	50.0
Cottonseed meal	0	20.1	24.8	29.6
Soyabean meal	14.1	0.0	0.0	0.0
Rice bran	9.4	8.5	8.0	7.5
Broken rice	6.6	6.5	6.1	4.9
Molasses	3.7	3.9	3.9	3.2
Urea	2.5	2.6	2.6	2.6
Sulphur	0.5	0.5	0.5	0.5
Dicalcium[a]	0.7	0.7	0.7	0.7
Salt	0.1	0.1	0.1	0.1
Mineral mix[b]	0.6	0.6	0.6	0.6
Tallow	3.3	1.8	0.3	0.2
Dry matter (%)	89.1	89.4	89.4	89.7
Crude protein (%)	16.6	18.4	20.1	21.9
Total digestible nutrient[d] (%)	76.6	75.1	74.9	74.9
Price per kg of feed[e] (US$)	0.12	0.10	0.11	0.11
Price per kg of CP (US$)	0.75	0.59	0.55	0.52

[a] Dicalcium (each kg contains): Calcium 300 g; Phosphorus 140 g.

[b] Mineral mix (Dailymin®) (each kg contains): Iron 2.14 g; Iodin 0.15 g; sulphur 11.82 g; Copper 0.23 g; Magnesium 0.96 g; Sodium 2.68 g; Manganese 7.21 g; Cobalt 0.03 g; Phosphorus 19.60 g; Selenium 0.003 g; Zing 0.16; Calcium 204.03 g.

[d] By calculation.

[e] Baht: 1 Baht=42 US$.

Table 2. Chemical composition (% of dry matter) of four total mixed rations (TMR) varying in crude protein (CP) concentation fed to cows

Ingredient	CP level (%)			
	10.5	12.5	13.7	14.4
Dry matter	55.4	53.0	54.5	55.9
Organic matter	91.8	91.9	91.3	91.8
Crude protein	10.5	12.5	13.7	14.4
Neutral detergent fiber	48.3	48.7	48.5	48.3
Acid detergent fiber	31.5	31.8	31.2	31.1
Ash	8.2	8.1	8.7	8.2
Total digestible nutrient	63.5	62.6	62.5	62.5

cassava chips replacing from 30 to 50% of corn meal (Pimpa et al., 2000; Sommart et al., 2000). Recently a study by Wanapat and Petlum (2001) reported that a concentrate based on a high proportion of cassava chips (85% of concentrate) and a high urea level could support good milk yield. Rations varying in cassava chips had been studied (Kanjanapruthipong and Buatong, 2004). However, data on different levels of cottonseed meal in concentrate cassava chip-based diets is rather limited. Therefore, the objectives of the experiments were to evaluate the effects of increasing dietary CP by increasing dietary levels of cottonseed meal (CSM) for cows in mid lactation fed cassava chips and rice straw-based diets. The hypotheses are milk yield will increase with increasing of dietary CP concentration by using of cottonseed meal but increases in dietary CP concentration will result in increases in rumen ammonia-N, BUN, MUN and feed cost.

MATERIALS AND METHODS

Experimental design and treatments

Thirty two, multiparous Holstein Fresian crossbred dairy cows, ranging from 100-150 days in milk (DIM) and yielding between 10-15 kg/day were used in a Randomized complete block design (RCBD) to determine the effect of four levels of CP by varying the levels of cottonseed meal (CSM) in the total mixed rations (TMR) on milk yield and milk compositions for 60 days. The experiment was arranged in two periods using 16 cows for each period. Treatments were total mixed rations with varying amount of crude protein and CSM concentrations as follows:

T1: 10.5% CP, no CSM
T2: 12.5% CP, 12.1% CSM
T3: 13.7% CP, 14.9% CSM
T4: 14.4% CP, 17.9% CSM

Experimental feeds and animals management

Ingredient composition and cost of concentrate feeds are shown in Table 1. Concentrate and roughage (chopped rice straw) feeds were provided every day in the form of total mixed ration (TMR) with concentrate to roughage at 60:40 by using a mixing machine. The TMR diets were formulated for varying levels of CP and CSM, and made iso-caloric (TDN). The composition of the TMR diets are shown in Table 2. Cows were housed individually for each treatment and had *ad libitum* access to a TMR (TMR was offered twice a day), fresh water and a mineral block. The cows were acclimatised for the first two weeks, and actual intake and measurements were taken during 60 days.

Data collection, analysis and sampling procedures

Feeds and feces were collected during the last 5 days of each month. Feeds (fresh feed offered and feed residues) were randomly collected (250 g) once a day in the morning before new fresh feed was offered. Fecal samples were collected (250 g) from rectum of individual cow of each treatment once day in the morning before new fresh feed was offered. Collected samples were kept in a refrigerator until analysis. Feed intake and feed composition were analyzed in order to calculate nutrient supply. Feed (both fresh feed offered and feed residues) and fecal samples were analyzed for DM, ash, CP (AOAC, 1990) NDF, ADF (Goering and Van Soest, 1970). Acid insoluble ash (AIA) (Van Keulen and Young, 1977) was used as internal indicator to calculate digestibility. On the last day of each

Table 3. Daily intakes and body weight (BW) changes of cows fed total mixed rations varying in crude protein (CP) concentration

Item	CP level (%)				Effect (p<)		
						Contrast[d]	
	10.5	12.5	13.7	14.4	SEM	L	Q
Daily intake							
Dry matter (kg)	10.9	11.0	11.7	12.4	0.3	*	ns
Dry matter (%BW)	2.4	2.5	2.5	2.7	0.2	ns	ns
Organic matter (kg)	10.0	10.0	10.7	11.4	0.3	*	ns
Crude protein (kg)	1.2[a]	1.4[a]	1.6[b]	1.9[c]	0.1	**	ns
Neutral detergent fiber (kg)	5.4	5.0	5.7	5.9	0.2	ns	ns
Acid detergent fiber (kg)	3.7	3.4	3.5	3.4	0.1	ns	ns
Body weight (kg)							
Initial (kg)	463.2	440.6	439.4	439.4	9.5	ns	ns
Final (kg)	472.0	451.0	448.0	446.0	12.5	ns	ns
ADG[e] (kg/hd/d)	0.1	0.2	0.1	0.1	0.1	ns	ns

[a, b, c] Means with different superscripts within rows differ ($p<0.05$).

[d] L: Linear, Q: quadratic, ns: non-significant, * $p<0.05$, ** $p<0.01$.

[e] ADG: average daily gain.

month, 60-80 ml whole rumen fluid were collected by stomach tube from individual cows of each treatment at 0 and 4 h-post feeding. The pH of rumen contents was determined immediately after collection using a glass electrode pH meter. Ruminal fluid was centrifuged (3,000×g, 15 min) immediately and supernatant of centrifuged rumen fluid was preserved to be later analyzed for ruminal NH_3-N by the hypochlorite-phenol procedure (Beecher and Whitton, 1978).

Milk yields of each cow were recorded daily. Milk samples (in ratio of morning milk samples to afternoon milk samples at 60:40.) were collected twice daily during milking (05.00 and 17.00 h) on the last five days of each month and were analyzed for fat, protein, lactose and solids-not-fat using infared apparatus (Milko-scan 104, Foss Electric, Denmark). Sub-sample of the composite was analyzed for milk urea nitrogen according the method of Roseler et al. (1993) using a Sigma diagnostics kit #535 reading at 540 nm by using of spectrophotometer (Spectonic 20, Milton Roy company, USA).

Blood samples were collected from the coccygeal vein of each cow twice daily at 0 and 4 h after the morning feeding on the last day of each month. Samples were kept at room temperature for 2 h and then centrifuged at 3,500×g for 20 min. The serum was removed and analyzed instantly for blood urea nitrogen composition using automated clinical chemistry analyzers (Synchron CX7, Beckman).

Statistical analyses

Data were analyzed using Proc. GLM (SAS, 1989). The following models were used to determine treatment mean differences using Duncan's New Multiple Range Test. The model was:

$$Y_{ij} = \mu+\alpha_i+\beta_j+e_{ij}$$

where

Y_{ij} = the criteria under study, response of cow in treatment j of block i

μ = over all sample mean,

α_i = effect of block i,

β_j = effect of treatment j, and

e_{ij} = error

Multiple regression procedures of SAS (1989) were used to separate effects of dietary component (CP), NH_3-N, BUN and MUN when overall treatment effects were significant at $p<0.05$. Trend analysis for increasing dietary CP level was used using orthogonal polynomials analysis.

RESULTS

Nutrient intake and body weight

Daily DM intake did not differ significantly among treatments but there was a trend to a linear increase with increasing dietary CP concentrations ($p<0.05$) (Table 3). Neutral detergent fiber and ADF intakes were not different among treatments and increased linearly with increasing dietary CP concentration ($p>0.05$). The daily CP intake differed significantly ($p<0.01$) among treatments while CP intake increased from dietary Treatment 1 to Treatment 4 (Table 3). The body weights were not changed during the experimental period and did not differ significantly among treatments (Table 3).

Ruminal fermentation, blood urea nitrogen and milk urea nitrogen

There was a linear trend of increased ruminal fluid ammonia-N and BUN with increasing dietary CP concentration. Cows consuming the 14.4% CP diet had higher ($p<0.05$) ruminal fluid ammonia-N and BUN concentration than cows eating the lower CP concentration diets both before and 4 hours after feeding. Four hours post-

Table 4. Effect of increasing crude protein (CP) level of total mixed rations on ruminal fermentation, blood urea nitrogen (BUN) and milk nitrogen (MUN) in dairy cows

Item	CP level (%)				Effect (p<)		
	10.5	12.5	13.7	14.4	SEM	Contrast^{d}	
						L	Q
Rumen ecology							
pH							
0 h, post-feeding	6.7	6.8	6.8	6.8	0.1	ns	ns
4	6.7	6.7	6.8	6.7	0.1	ns	ns
mean	6.7	6.7	6.8	6.8	0.1	ns	ns
H_3-N, mg %							
0 h, post-feeding	9.5^{a}	10.5^{a}	11.4^{a}	14.7^{b}	0.5	**	ns
4	12.5^{a}	14.3^{a}	17.6^{b}	20.0^{c}	0.4	**	ns
mean	11.0^{a}	12.4^{a}	14.5^{b}	17.4^{c}	0.8	**	ns
BUN, mg %							
0 h, post-feeding	8.9^{a}	9.5^{a}	10.9^{a}	14.3^{b}	0.5	**	ns
4	11.6^{a}	13.6^{a}	16.12^{b}	19.9^{c}	0.5	**	ns
mean	10.3	11.6	13.5	17.1	0.8	**	ns
MUN, mg/dl	10.63^{a}	12.8^{a}	15.0^{b}	18.5^{c}	0.4	**	ns

[a, b, c] Means with different superscripts within rows differ (p<0.05).

[d] L: Linear, Q: quadratic, ns: non-significant. * p<0.05, ** p<0.01.

Table 5. Effect of crude protein (CP) level of total mixed rations on nutrient digestion coefficients

Item	CP level (%)				Effect (p<)		
	10.5	12.5	13.7	14.4	SEM	Contrast^{d}	
						L	Q
Digestion coefficients,%							
Dry matter	55.2	55.9	56.6	58.1	0.7	ns	ns
Organic matter	57.9	58.5	59.6	60.0	0.8	ns	ns
Crude protein	56.1^{a}	59.8^{b}	62.6^{b}	63.2^{b}	0.7	**	*
Neutral detergent fiber	41.0	43.7	44.2	44.6	1.0	ns	ns
Acid detergent fiber	40.2	42.1	43.2	43.9	0.9	ns	ns
Digestible intake, kg/d							
Dry matter	6.0	6.2	6.6	7.2	0.3	ns	ns
Organic matter	5.8	5.9	6.4	6.8	0.2	*	ns
Crude protein	0.7^{a}	0.8^{a}	1.0^{b}	1.2^{c}	0.1	**	ns
Neutral detergent fiber	2.1	2.2	2.5	2.6	0.1	ns	ns
Acid detergent fiber	1.5	1.5	1.5	1.5	0.1	ns	ns

[a, b] Means with different superscripts within rows differ (p<0.05).

[d] L: Linear, Q: quadratic, ns: non-significant. * p<0.05, ** p<0.01.

Table 6. Effect of increasing crude protein (CP) level of total mixed rations on milk yield and composition in lactating dairy cow

Item	CP level (%)				Effect (p<)		
	10. 5	12.5	13.7	14.4	SEM	Contrast^{1}	
						L	Q
Milk yield (kg/d)	10.7	11.5	11.6	11.6	0.3	ns	ns
3.5% FCM^{2} (kg)	11.1	11.8	11.7	11.8	0.6	ns	ns
Milk composition (%)							
Fat	3.7	3.8	3.8	3.9	0.1	ns	ns
Protein	3.2	3.2	3.3	3.3	0.1	ns	ns
Lactose	4.6	4.9	4.7	4.6	0.1	ns	ns
SNF$^{3/}$	8.5	8.8	8.6	8.5	0.1	ns	ns
TS^{4}	12.4	12.8	12.2	12.4	0.1	ns	ns

[1] L: Linear, Q: quadratic, ns: non-significant. * p<0.05, ** p<0.01.

[2] 3.5% FCM = 0.4* (kg of milk)+15* (kg of fat)

[3] SNF: Solid not fat, [4] TS: Total solid.

feeding, cows on the 13.7% CP diet had higher ruminal fluid ammonia-N concentration than cows fed the two diets lowers in CP. Milk urea nitrogen results mirrored the ammonia-N and BUN results.

Nutrient digestibility

Digestibilities of DM, NDF, ADF were not affected by increasing dietary CP levels (Table 5). Digestibility of CP was found to be higher (p<0.01) in dietary treatments which contained cottonseed meal (12.5, 13.7 and 14.4% CP) than without cottonseed meal. Digestibility of CP increased with increasing CP content in the diet (p<0.01). Neutral detergent fiber and ADF digestibilities tended to increase (p=0.1) with increasing CP content of the diet. Digestible DM, NDF and ADF intake were not affected by increasing dietary CP levels. Digestible CP intake was increased with dietary CP content. Linear increase in digestible organic matter (OM) intake with increasing of dietary CP content was found (Table 5).

Table 7. Effect of increasing crude protein (CP) level of total mixed rations on feed cost and net profit in dairy cow

Item	CP level (%)				Effect (p<)		
	10.45	12.52	13.65	14.36	SEM	Contrast[a]	
						L	Q
Milk income (US$/hd/d)	2.77	2.9	3.03	3.22	6.3	ns	ns
Feed cost (US$/hd/d)	1.11	0.93	1.03	1.13	1.3	ns	*
Income over feed							
US$/hd/d	1.66	1.98	2.00	2.09	5.3	ns	ns
US$/kg of milk[b]	0.15	0.17	0.17	0.16	0.2	ns	ns

[a] L: Linear, Q: quadratic, ns: non-significant. * $p<0.05$, ** $p<0.001$.

[b] 1 kg of milk=0.26 US$.

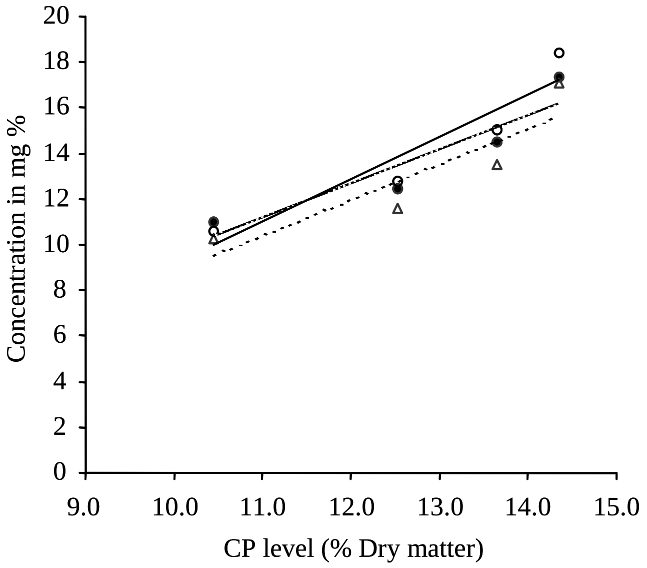

Figure 1. Relationship between crude protein (CP) level of total mixed rations and concentration of a ruminal ammonia N (o) (R^2=0.86), blood urea nitrogen (Δ) (R^2=0.81) and milk urea nitrogen (•) (R^2=0.89).

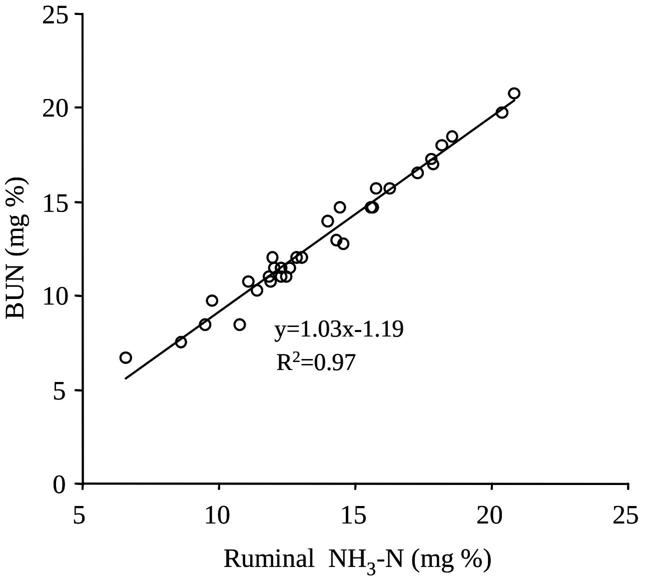

Figure 2. Relationship between concentration of ruminal ammonia N (NH_3-N) blood urea and nitrogen (BUN) in mid-lactating dairy cows fed with total mixed rations varying in crude protein concentration.

Milk yield and composition and cost and net profit

Milk yield and composition (Table 6) were not affected by level of CP in the diet. Milk yield tended to increase ($p=0.22$) with increasing CP content in the diet.

Feed cost (Table 7) was decreased when the CP level of the diet increased from 10.5 to 12.5% by completely replacing soybean meal with cottonseed meal at 12.1% of DM and was increased with the level of cottonseed meal in the diet (Quadratic effect, $p<0.05$) but there was no significant difference between treatments. Income over feed (US$/kg of milk) tended to be affected by level of CP (Q, $p=0.09$) and increased when CP level of diets increased from 10.5 to 13.7% and decreased at CP level above 13.7%, while there were no significant differences among treatments.

Correlations among dietary crude protein content, ammonia nitrogen (NH_3-N), blood urea nitrogen (BUN) and milk urea nitrogen (MUN)

Ruminal NH_3-N, BUN and MUN were linearly and positively correlated with dietary CP content ($p<0.05$). Coefficients of determination (R^2) were 0.86, 0.81 and 0.89 for NH_3-N, BUN and MUN, respectively (Figure 1). Correlations between BUN and ammonia-N, MUN and ammonia-N, and BUN and MUN were also positive and strong (Figures 2, 3 and 4).

DISCUSSION

Nutrient and dry matter intake (DMI)

There were no significant differences among treatments, these results being similar to a report of Christensen et al. (1993, 1994) who showed that intakes of DM were not altered by level (14.2 to 19.6%) of dietary CP. However, daily DM intakes in the present study were slightly lower than typical intake levels for lactating dairy cows of varying body weights as given by NRC (1989) due to the influence of NDF concentration. Many studies (NRC, 1988; Ruiz TM et al., 1995; Mertens, 1997) demonstrated that increased dietary NDF concentration linearly decreased DMI because of physical fill. It was thought that generally, cows would

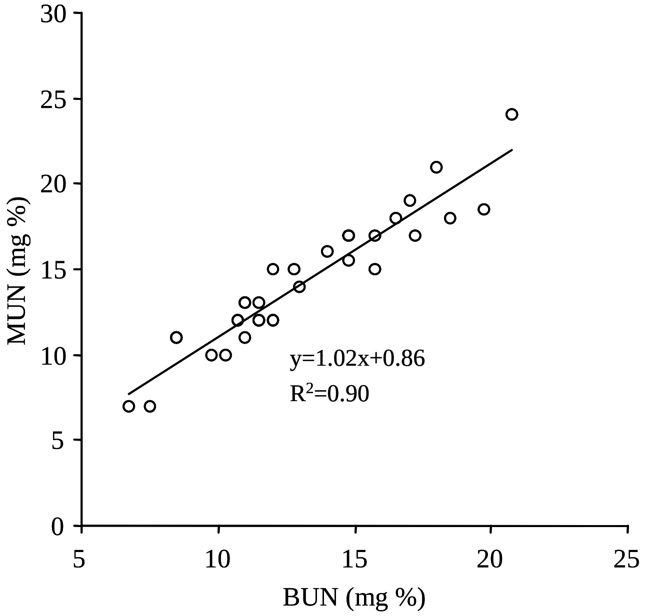

Figure 3. Relationship between concentration of blood urea nitrogen (BUN) and milk urea N (MUN) in mid-lactating dairy cows fed with total mixed rations varying in crude protein concentration.

consume about 1.2% of their body weight/day as NDF (Merck, 1991). Average NDF intake as a percentage of BW in this study was 1.22%, which is similar to the NDF intake capacity suggested by Merck (1991).

However, DMI tended to linearly increase with increasing dietary CP and the mean increased DMI was 0.38 kg/d per percent unit increase in diet CP. This result agrees with the work of Allen (2000) who summarized that increasing CP content of the diets can increase DMI of lactating cows, particularly when the CP content of diets was low. When significant effects of CP content on DMI were detected, the range for increased DMI was 0.18 to 0.84 kg DMI/d with a mean of 0.63 kg DMI/d per percentage unit increase in diet CP content. Oldham (1984) and Roffler et al. (1986) showed that the CP content of diets was often related positively to DMI of lactating cows. These workers noted that the mechanism involved was presumably a reduction in distension as fiber and DM digestibility increase. For this study DM digestibility was highest in Treatment 4 with the highest DMI.

Intakes of ADF, and NDF were not different among treatments. Christensen (1993) stated that the amount of CP did not alter intakes of ADF, NDF and N when the TMR contained 25% alfalfa haylage, 25% corn silage and 50% concentrate and provided either 16.4 or 19.6% CP, when offered to Holstein cows.

There was a tendency (p=0.10 for NDF and p=0.15 for ADF) for intake of NDF and ADF to increase with intake of DM as CP level increased. The reason could be due to the higher digestibility in cows fed with high level of CP in the diet than low level group.

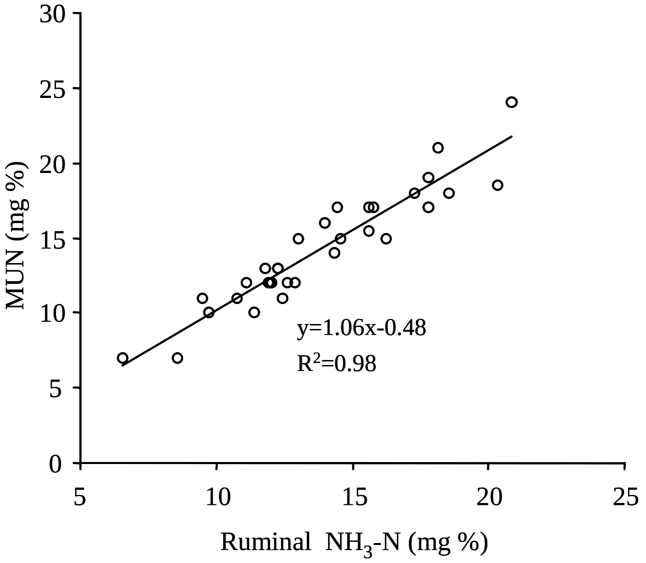

Figure 4. Relationship between concentration of ruminal ammonia-N (NH_3-N) and milk urea N (MUN) in mid-lactating dairy cows fed with total mixed rations varying in crude protein concentration.

Cottonseed meal intake

The result shows that supplementation of CSM at 2 kg/head/day did not depress feed intake of the ration containing rice straw and cassava chips and was without any adverse effects on health status of cows (assessed from clinical sign observations, data not shown). This is in agreement with results from previous work in which cottonseed meal was supplemented at 2 to 4 kg/day in diets based on rice straw and 5 kg/head/day of cassava chips in mid-lactating Holstein-Zebu crossbred cows with no effect on rice straw intake (Wanapat et al., 1996). On the other hand, intake of cottonseed meal plus whole cottonseed increased from 2.5 to 9.1 kg/head/day and resulted in increased DM intake of a ration containing alfalfa-based diets in early lactation (Grings et al., 1991). No adverse effects on health status was found in lactating Murrah buffaloes fed with cottonseed (Singh et al., 2004).

Nutrient digestibility

The concentration of protein in rations for dairy cows had no effect on nutrient digestibility (p>0.05), except for CP (Table 4). This result agrees with the data of Klusmeyer et al. (1990) who stated that the amount (14.5 or 11.0%) of CP in the diet did not affect digestion in the rumen of ADF and NDF in Holstein cows. The results from Christensen et al. (1993) were also similar, where apparent total tract digestibilities for ADF and NDF of Holstein cows were similar among levels (16.4 or 19.6%) of CP.

Increasing ration CP content from 10.8 to 15% in DM had no effect on digestibility of energy of dry cows but there was an effect for lactating cows (digestibilty of energy increased by 0.04 to 0.08 digestibility units) (review by

Oldham, 1984). It seems that levels of CP in diet more than 4% unit of DM can affect DM digestibility. For this study the lowest and highest levels of CP were 10.5 and 14.4%. However, increasing trends of DM, NDF and ADF digestibilities were found with increasing dietary CP. OM digestibility was increased with increasing of dietary CP. Crude protein digestibility was higher ($p<0.05$) for cows fed diets containing higher levels of cottonseed meal (CSM) and linearly increased with level of CP. Christensen (1994) stated that apparent digestibilities of CP in the total tract were greater for cows fed a 17.5 % CP diet than a 14.2 % CP diet.

The suggested mechanism underlying this effect is that higher protein increased microbial fermentation in the rumen, which would improve digestion of DM and OM (Mehrez et al., 1977).

Milk yield and composition

The largest change in milk yield (0.82 kg/d) was due to increasing CP level from 10.5 to 12.5%, while increasing CP level above 12.5% of the ration has very small effects on milk yield (Table 6). However, there were no significant differences among the four levels of CP. Kalscheur et al. (1999) reported that milk yield in mid-lactation cows was not affected by increasing dietary CP from 13.3 to 15.3% of DM. Daily CP and TDN requirements of dairy cattle given by NRC (1989) for 450 kg of body weight with daily milk yield and fat composition at 12.5 kg and 3.5% were 1.4 and 7.2 kg/h/d, respectively. Crude protein intake of cow fed with 10.5% of CP content was 1.2 kg and it was lower than requirement. This might be the reason why the largest change in milk yield (0.82 kg/d) was found in increasing CP level from 10.5 to 12.5%.

The increase in milk yield was less than that observed in previous work in which milk yield increased by 1.4 kg/d as dietary CP increased from 13.8 to 17.5% by the addition of cottonseed meal (Grings et al., 1991). However, in terms of increasing milk yield per percent unit increasing of dietary CP by the addition of cottonseed meal, in previous work was similar to this experiment.

The increased dietary CP could have altered other chemical components (nonstructural carbohydrates) of the diet due to the decrease in chemical proportion of ingredient to diet (Grings et al., 1991). The proportion of grain (rice bran, broken rice) was decreased when CP increased in order to make the TDN of the different dietary treatments equal. This change could result in an increase of ratio CP to total non-structural carbohydrates that may affect rate and extent of digestion and microbial protein production that could result in changes in milk yield (Oldham, 1984).

Percentages of fat, protein, lactose, solids- not- fat and total solids in milk were not affected by dietary CP concentration by addition of cottonseed meal. Grings et al. (1991) observed no differences in milk composition as dietary CP was increased from 13.8 to 17.8%. Meanwhile Wanapat et al. (1996) found that supplementation of cottonseed meal up to 5 kg/head/day did not affect milk composition in crossbred dairy cows fed rice straw in the tropics. It was reported by NRC (2001) that dietary CP was not correlated with milk protein percent, and was correlated weakly ($r=0.14$) with milk protein yield. However, the cow generally uses protein supplements as a source of energy rather than a supply of protein to the udder (Mepham, 1982). Providing there is sufficient protein in the total diet, feeding protein supplements will result in a similar increase in protein percentage as feeding a similar amount of energy.

Ruminal fermentation, blood urea nitrogen and milk urea nitrogen

Ruminal NH_3-N, BUN and MUN were linearly and positively correlated with dietary CP content ($p<0.001$), which is in agreement with previous studies (Gustafsson, 1993; Roseler et al., 1993; Baker et al., 1995). The results of the present study showed that simple regression for BUN on NH_3-N (Figure 2) and correlation between BUN and MUN were strongly correlated ($R^2=0.97$, $R^2=0.90$, respectively). This is supported by the study of Hammond (1983) and recent work, (Chanjula et al., 2004) where BUN was found to be highly correlated with ruminal ammonia. Preston et al. (1965) stated that the quantity of ammonia absorbed from the rumen was reflected in circulating BUN. Several studies (Roseler et al., 1993; Baker et al., 1995; Hong et al., 2003) had shown that MUN was highly correlated with BUN. In healthy ruminants, BUN and MUN concentrations are indicative of the protein to energy ratio in the diet. This situation was explained by Hammond (1983) who stated that when energy intake was held constant, increasing dietary protein would increase BUN concentrations. Also, in lactating dairy cows, an increase in BUN and MUN was caused by excess CP (Grings et al., 1991; Baker et al., 1995). Abeni et al. (2000) reported that BUN concentrations more relate to dietary CP than to energy ratio of diets. However, MUN may prove to be a better indicator of excess CP because concentrations are a result of equilibration over time. The reason was explained by Gustafsson and Palmquist (1993) who have observed diurnal variations in BUN, ruminal NH_3-N and MUN for the whole day and typically BUN concentrations peak about 4 to 6 h post feeding.

Expressed as digestible organic matter:crude protein (DOM:CP) the optimum ratio was about 1/7 (Moore et al., 1995). Balanced diets for lactating dairy cows were associated with average BUN concentrations of 15 mg % (Roseler et al., 1993) and average MUN concentrations of 15 to 16 mg % (Baker et al., 1995) or 11-17 mg % (Hwang et al., 2000). High levels of BUN and MUN indicated NH_3-

N losses from the rumen with loss of protein. Hwang (2000) summarized that cattle producing milk that contains a level of MUN and milk protein within the ranges of the standard reference values of 11-17 mg % and 3.0% milk protein was regarded as indicating a balanced protein and energy intake. BUN, MUN and CP:DOM ratio lower than this reference could be due to the insufficiency in CP per unit of energy, on the other hand, values higher than this reference could be due to excess in CP per unit of energy.

According to previous studies (Roseler et al., 1993; Moore et al., 1995; Hwang et al., 2000) the most balanced diets in the present study were dietary CP 12.5 and 13.7% which gave optimum levels of BUN, MUN and CP to organic matter ratio. The most imbalanced diet in terms of excess energy content was CP 10.5 diet %, with lower levels of MUN, BUN and CP to organic matter ratio. Income over feed, expressed as US$/kg of milk was high in the 12.5 and 13.7 diets CP %. The high level of dietary CP was reflected by high feed costs. Although high levels of dietary CP were offered to ruminants, the response of animals to protein may not occur if protein and energy imbalance from those resulting in loss of profit. Godden et al. (2000) noted that herds with low mean milk urea nitrogen had a positive relationship with feed costs per cow per day. Therefore, excessive feeding of dietary CP can be costly and economically, through increased feeding cost and the results of the present study showed loss of income with dietary CP level above 13.7%.

CONCLUSIONS

From the results of the current study, it can be concluded that increasing dietary CP levels from 10.5 to 13.8% by the addition of CSM was beneficial to cows fed cassava chips and rice straw-based diets during mid lactation due to increases in DMI, OM intake, total digestible nutrient intake and income over feed. Increasing levels of dietary CP increased ruminal NH_3-N, BUN, and MUN. The use of cottonseed meal should be highly recommended as a protein source in concentrate supplements, especially for small-holder dairy farmers. However, further research relating to the balance of energy to protein should also be considered in protein supplementation for dairy cows.

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