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Effect of Dietary Protein Levels on Rumen Metabolism and Milk Yield in Mid-Lactating Cows under Hot and Humid Conditions

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Abstract: An experiment was conducted to investigate the effects of 2 levels of dietary Crude Protein (CP) in concentrates with similar proportions of Rumen Undegradable Protein (RUP) on rumen metabolism, milk yield and composition in mid lactating cows in Thailand. Eight 87.5% Holstein×12.5% indigenous multiparous cows were used in a crossover design with two successive 25 days periods. Diets contained 30% paragrass and 70% concentrate on a Dry Matter (DM) basis. Concentrate feeds were formulated to provide low dietary CP (17.3%; LCP) or high dietary CP (19.04%; HCP). The proportion of Rumen Degradable Protein (RDP) and RUP was 61 and 39% in both diets. Diets were isocaloric in terms of net energy for lactation. Milk yield, milk lactose yield, Dry Matter Intake (DMI) and apparent digestibility of DM, CP and Neutral Detergent Fiber (NDF) were greater in cows fed HCP than in those fed LCP. Concentration of blood urea nitrogen was elevated in cows fed HCP diets. Rumen NH_3-N concentration and pH tended to increase in cows fed HCP diet. Rumen microorganism counts and volatile fatty acids levels in the rumen did not differ between treatments. The increasing CP content in mid-lactating cow was beneficial to increase DMI, apparent digestibility of DM, CP and NDF and therefore milk yield.

Key words: Cattle, milk production, protein, rumen metabolism, dry matter

INTRODUCTION

The majority of forage in the tropics is low in digestibility and nutrient contents and thus limits feed intake and milk output in ruminants (Camero and Franco, 2001; Leng, 1990). Consequently, lactating Thai dairy cows mostly ingest poor quality roughage. Therefore, it is common practice in Thailand to reduce the relative amount of roughage while increasing the proportion of concentrate in order to increase nutrient supply to the animals (Kanjanapruthipong *et al*., 2001). The protein level of the concentrates is important to ensure an adequate supply of dietary protein in supporting milk production in the tropics because of low Crude Protein (CP) content in typical tropical roughage (Korhonen *et al*., 2002).

Providing sufficient protein to dairy cows depends on the balance between the availability of nitrogen for microbial growth in the rumen and nitrogen for productive functions (Ferguson *et al*., 1988). At the same intake of dietary protein, a shortage of Rumen Degradable Protein (RDP) may reduce microbial growth in the rumen. Consequently, rumen digestibility (Chalupa, 1984), Dry Matter Intake (DMI) (Weigel *et al*., 1997) and microbial protein synthesis and thus protein flow from the rumen (Argyle and Baldwin, 1989) may be reduced. Now a days there is a tendency towards an increase of dietary protein supply above NRC requirements (NRC, 2001) to optimize the production of dairy cows under hot and humid climates. However, experimental data that corroborate this tendency are scarce. Therefore, the aim of the present study was to investigate the effect of 2 levels of dietary CP with equal proportions of rumen RDP and RUP on digestion, rumen metabolism, blood urea nitrogen, milk yield and milk composition in mid-lactating cows.

MATERIALS AND METHODS

Animals and feeding: Eight Holstein x Indigenous (87.5×12.5) multiparous dairy cows with 113±12 Days in Milk (DIM) and weighing 469±46 kg were used. The parity of the cows ranged from 2-5 and the mean was 3 (±1.3).

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The trial had a 25×25 days cross over design with a 16 days wash out period (Cochran and Cox, 1957). Data were collected from days 17-25 of each experimental period. The animals were randomly assigned to the two treatments, i.e., a low protein (17%, LCP) and a high protein (19%, HCP) concentrate. The proportions of RDP and RUP were similar for both concentrates, i.e., 61 and 39%, respectively. The energy density was the same for both experimental concentrates (1.7 Mcal NE_l/kg DM). Each day, the cows were first fed with concentrates at a level of 2.1% of Body Weight (BW) in DM and then fed paragrass (*Brachiaria mutica*) at a level of 0.9% of BW in DM. Each feed was offered 3 times per day at 08:00, 14:00, and 21:00 h. Paragrass was harvested at a 45-50 days interval. Individual feed intakes and refusals were recorded daily. Concentrate ingredients and the chemical composition of the feedstuffs are given in Table 1 and 2, respectively. Cows were housed in tied-stalls with individual feed bins. Animals had free access to water. Cows were milked by a milking machine twice daily at 06:00 and 15:30 h. The experiment was conducted in the months of January and February with mean minimum and maximum temperatures at 21.3±2.4 and 33.1±2.51°C, respectively. Mean maximum and minimum relative humidity was 96.1±1.1 and 48.6±10.0%, respectively. The average maximum and minimum Temperature Humidity Index (THI) was 90.9±4.2 and 66.9±3.3, respectively. The climatic conditions during the study are shown in Fig. 1.

Table 1: Ingredient composition of the experimental concentrates

Ingredients (%)	LCP[A]	HCP
Full fat soybean	7.1	11.5
Cassava chip	30.9	23.4
Palm kernel cake	11.5	12.0
Soybean meal	1.0	6.0
Kapok seed meal	13.4	12.0
Whole cotton seed	10.0	10.0
Coconut meal	16.0	15.0
Molasses, sugarcane	5.5	5.5
Urea	0.6	0.6
Mono dicalcium phosphate	1.0	1.0
Sodium bicarbonate	1.5	1.5
Sulphur	0.3	0.3
Magnesium oxide	0.2	0.2
Trace mineral and vitamin mix[B]	0.5	0.5
NaCl	0.5	0.5

[A]LCP = Concentrate containing a Low CP content; HCP = Concentrate containing a High CP content; [B]Consists of (per kg) 2 million IU of vitamin A, 0.40 million IU of vitamin D3, 3,000 IU of vitamin E; 0.46 g vitamin K, 10.0 g of Fe, 4.0 g of Cu, 7.4 g of Mn, 0.20 g of Co, 15.0 g of Zn, 0.20 g of I and 0.08 g of Se

Table 2: Chemical composition of the experimental concentrates and grass

Chemical analysis (DM %)	LCP[A]	HCP	Grass
Crude protein	17.30	19.05	10.67
Rumen undegradable protein[B]	6.90	7.33	1.28
NDF	52.75	51.38	75.94
ADF	19.06	17.75	39.20
Ether extract	8.79	9.45	1.09
Ash	12.16	12.41	13.05
AIA	0.78	0.60	7.16
NE_L (Mcal kg^{-1} DM)	1.68[C]	1.71[C]	1.30[D]
TDN	73.47	74.67	57.84

[A]LCP = Concentrate containing a Low CP content; HCP = Concentrate containing a High CP content; [B]Calculated RUP and TDN based on nutrient compositions of Thai feed table (Angthong *et al*., 2004; Kanjanapruthipong, 2006; Moran, 2005); [C]NE_L (Mcal kg^{-1} DM) = 0.0245×TDN (%) -0.12 (NRC, 1989); [D]NE_L (Mcal kg^{-1} DM) = 2.3977 (0.0280×ADF%) (Ishler *et al*., 1996)

Sampling and chemical analyses: Cows were weighed on the first and again on the final day of both periods before morning feeding. Milk yields were recorded from days 16-24 in each experimental period. Milk samples (approximately 30 mL per milking) were collected during days 22, 23 and 24 at each of the two milkings. Milk samples were placed in bottles containing 0.02% of 2-bromo-2-nitro-1,3-propanediol and stored at 5°C until determination of milk composition (fat, protein, lactose and non fat solids) by infrared spectroscopy (Bentley, 2000, Agriyork Ltd. UK). Concentrates and grass were sampled once a week to determine DM content. The weekly samples were dried at 60°C and were pooled based on CP content per period. The pooled samples were analysed for crude protein, Ether Extract (EE) and ash according to the Association of Official Analytical Chemists (AOAC, 1990) procedure and Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) following the method by Van Soest *et al*. (1991).

Blood samples, 10 mL were collected from the jugular vein of each cow at days 23 and 24 of each period at 4 h after morning feeding. Then, blood samples were centrifuged for 5 min at 500 g. All serum samples were stored at -20°C until analysis for Blood Urea Nitrogen (BUN) concentration by spectrophotometry (Urease-Berthelot).

Faecal samples from each cow were collected after the morning feeding on the last day of each period. They

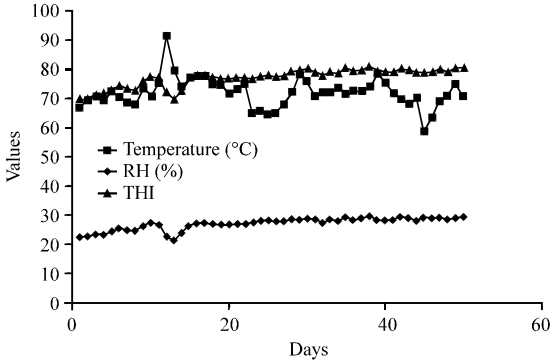

Fig. 1: The average temperature (°C), Relative Humidity (RH, %) and Temperature Humidity Index (THI) during the experimental periods

were dried in a 60°C forced-air oven and ground in a Wiley mill to pass through a 1 mm screen. Samples were subsequently analysed for NDF, CP and Acid Insoluble Ash (AIA). The AIA was used as an internal marker to determine apparent total-tract DM digestibility (Van Keulen and Young, 1977).

Rumen fluids were collected by means of a nasogastric tube on day 25 of each period. Whole ruminal contents (100-200 mL) were taken 4 h after the morning feeding. Rumen fluid pH was measured immediately by using pH meter (EC 20, HACH, USA). The rest of rumen fluid was strained through four layers of cheesecloth. Two sub-samples were taken from the strained fluid: one 10 mL sample was preserved by the addition of 1 mL of 50% (vol./vol.) H_2SO_4 for later analysis of ammonia (NH_3-N) and Volatile Fatty Acids (VFAs) and one 9 mL sample was preserved by the addition of 1 mL of 10% formalin solution for later analysis of total direct count of bacteria, protozoa and zoospores (Galyean, 1989). All ruminal samples were stored at -20°C. Just before analysis, samples were thawed, centrifuged (15000 g for 15 min) and analyzed for NH_3-N concentration (Bremner and Keeney, 1965) and for VFAs by gas chromatography following the method modified from Erwin *et al.* (1961).

Statistical analysis: Milk yield, milk composition, DM intake, digestibility, BUN, ruminal pH, NH_3-N, VFAs and microbial counts were subjected to ANOVA (SAS, 2001). Experimental period (fixed) and dietary treatment were factors (SAS, 2001). Effects were considered significant at $p \leq 0.05$.

RESULTS

Body weight, nutrient intake and digestibility: Body weight was not affected by dietary treatments. Mean BW at the end of the experiment was 468 kg (SE = 13.8; n = 8) which is similar to pre-experimental values ($p>0.1$). Nutrient intake and digestibilities of DM, CP and NDF are shown in Table 3. Total DM intake was 0.53 kg day^{-1} higher in cows fed HCP diet than in those fed the LCP diet which was mainly caused by the difference in concentrate intake. However, in contrast with the difference in grass intake between the two experimental diets, the difference in concentrate intake appeared to be non-significant. The intakes of CP, RUP, RDP, NDF and NE_L were significantly increased when the cows were fed the HCP diet. Digestibilities of DM, CP and NDF were influenced by dietary treatments. Cows that were fed the LCP diet had significantly lower digestibilities of DM, CP and NDF.

Milk production: Effects of dietary treatments on milk production are shown in Table 4. Cows fed the LCP diet had a significantly lower milk yield and lactose yield than those fed the HCP diet. The amount of 3.5% fat corrected milk and the contents of fat, protein, lactose and non-fat solids in milk were similar on both CP treatments.

Table 3: Intake of DM and selected nutrients and digestibilities of DM, CP and NDF in cows fed either the Low (LCP) or the High CP (HCP) concentrates

	Diets			
Contents	LCP	HCP	SEM[A]	p-values
Intake				
Total DMI (kg day^{-1})	11.71	12.24	0.37	0.01
Concentrate DMI (kg day^{-1})	8.41	8.77	0.35	0.10
Paragrass DMI (kg day^{-1})	3.30	3.48	0.13	0.04
CP intake (kg day^{-1})	1.81	2.04	0.09	<0.01
CP intake (%)	15.43	16.63	0.18	<0.01
RUP intake (kg day^{-1})	0.63	0.69	0.03	0.01
RDP intake (kg day^{-1})	1.18	1.35	0.05	<0.01
NE_L intake (Mcal day^{-1})	18.41	19.42	0.61	<0.01
EE intake (kg day^{-1})	0.78	0.81	0.03	0.09
NDF intake (kg day^{-1})	6.94	7.16	0.20	0.03
Digestibility (%)				
Dry matter	54.88	63.06	2.70	<0.01
CP	63.80	72.22	1.84	<0.01
NDF	61.76	69.02	2.47	<0.01

Table 4: Lactational performance of cows fed either the Low (LCP) or the High CP (HCP) concentrates

	Diets			
Contents	LCP	HCP	SEM[A]	p-values
Milk production				
Milk yield (kg day^{-1})	12.28	13.31	0.65	<0.01
3.5% FCM yield (kg day^{-1})	12.90	13.65	0.79	0.24
Fat yield (kg day^{-1})	0.47	0.49	0.03	0.50
Protein yield (kg day^{-1})	0.36	0.38	0.02	0.21
Lactose yield (kg day^{-1})	0.57	0.62	0.04	0.01
Milk composition				
Milk fat (%)	3.81	3.66	0.19	0.37
Milk protein (%)	2.94	2.85	0.06	0.22
Milk lactose (%)	4.57	4.62	0.07	0.18
Solid not fat (%)	8.17	8.13	0.13	0.53

[A]Standard error of the mean

Rumen metabolism and blood urea nitrogen: Effects of dietary treatments on selected indices on rumen metabolism and BUN are presented in Table 5. Rumen NH_3-N concentrations and rumen pH were similar between experimental treatments. However, rumen NH_3-N concentrations and pH had a tendency to increase when the cows were fed the HCP diet. Mean BUN concentrations were significantly higher in cows fed the HCP diet. The profile of individual VFAs in the rumen and the acetate to propionate ratio did not show clear differences between the two experimental treatments. Furthermore, bacteria, protozoa and zoospore counts in the rumen content of the cows were similar for both dietary treatments.

Table 5: Selected indices of rumen fermentation, Blood Urea Nitrogen (BUN) and rumen microorganisms of cows fed either the Low (LCP) or the High CP (HCP) concentrates

	Diets			
Contents	LCP	HCP	SEM[A]	p-values
Ruminal metabolism				
pH	6.22	6.29	0.04	0.21
NH_3-N (mg %)	24.31	28.28	1.46	0.11
Acetate (%)	65.77	65.75	1.01	0.98
Propionate (%)	23.37	22.73	1.16	0.68
Butyrate (%)	10.23	10.85	0.43	0.24
Valerate (%)	0.63	0.66	0.06	0.67
Total VFA (mmol L^{-1})	138.47	141.81	5.02	0.82
A/P[B]	3.04	2.95	0.20	0.74
BUN (mg dL^{-1})	12.23	14.73	0.70	0.01
Rumen microorganisms				
Bacteria (10^{11} cell mL^{-1})	2.20	2.16	0.17	0.88
Protozoa (10^5 cell mL^{-1})	5.81	3.78	0.64	0.09
Zoospore (10^5 cell mL^{-1})	1.69	1.48	0.13	0.42

[A]Standard error of the mean; [B]A/P = Acetate/Propionate

DISCUSSION

Dry matter intake and digestibility: In this experiment, the mean Temperature Humidity Index (THI) was 77 which is above the upper point of 72 for optimal dairy cow productivity (Ravagnolo *et al*., 2000). The THI in the current study indicated that the cows experienced mild heat stress (THI 72-78) (Armstrong, 1994). Feed intake in cows with heat stress may be reduced by 8% or more, thereby negatively affecting milk production (Kabuga, 1990). In the current study, feed intake was lower than the NRC (2001) estimation for these cows, i.e., 14 and 11% lower for the LCP and HCP treatment, respectively. However, NRC (2001) estimations of feed intake are based on temperate climatological conditions, it may be suggested that the climatological condition during the current experiment was at least partly, responsible for the relative low level of feed intake. The lower DMI was likely to reflect the poor quality of roughage in tropical regions (Leng, 1990). During heat stress, cows reduce feed intake in particular with high fiber diets (Kanjanapruthipong *et al*., 2010). In contrast, the present study showed that roughage intake was greater in cows fed HCP diet than those fed LCP. This high DMI for cows fed HCP diet can be explained in part by differences in apparent DM, CP and NDF digestibility between diets. This is in agreement with other studies (Blauwiekel and Kincaid, 1986; Jones-Endsley *et al*., 1997).

In the current study, DM, CP and NDF digestibility were increased by 15, 13 and 12%, respectively when the HCP diet was fed. This observation is corroborated by Tyrrell *et al*. (1981), Weigel *et al*. (1997) and Kendall *et al*. (2009) whom observed an increased nutrient digestibility when the dietary CP content was raised from 11 to about 18% (DM basis). Concentration of ruman microbial yield did not differ between cows on HCP and LCP. This result on digestibility was in agreement with earlier reports in sheep that the increasing levels of concentrate in the diet resulted in increased total tract apparent digestibility of DM, organic matter and CP and similar microbial population (Ramos *et al*., 2009).

Milk production and milk composition: An increase in dietary CP intake may affect milk yield by increasing the availability of NH_3, peptides and amino acids for microbial growth in the rumen (Bequette *et al*., 1998). Providing adequate protein to dairy cows increases milk production under tropical conditions (Promma *et al*., 2002). Supplementing diets with CP increased DMI and as a consequence also milk yield (Reynal and Broderick, 2003). Similar results were observed in the present study, cows on the diet containing HCP produced more milk than those fed the LCP diet. The responses of milk yield agree with higher intakes of NE_L (+2.12 Mcal) and CP (+220 g) intake than recommended by NRC (2001). Promma *et al*. (2002) suggested that for dairy cows in tropical ambient conditions the energy and CP intake should be 20% higher than the NRC recommendation for dairy cows in moderate climatic condition. Similarly, Kalscheur *et al*. (2006) showed that cows fed a high RDP supplement (11% RDP, 17% CP) produced more milk yield than cows on a low RDP supplement diet (8.2% RDP, 14% CP). In contrast, supplemental CP at a higher level did not affect milk composition in the current study. This observation is in line with the results reported by Reynal and Broderick (2003) and Mulligan *et al*. (2004).

Rumen metabolism and blood urea nitrogen: When CP intake exceeds the requirements for microbial growth or when there is an insufficient supply of fermentable carbohydrates for microbial growth, there is a potential for Nitrogen (N) loss from N surplus in the form of ammonia (Kim *et al*., 2000; Agle *et al*., 2010). In this study, rumen NH_3-N concentration ($p = 0.1$) tended to increase in cows fed HCP diet. However, these rumen NH_3-N concentrations (24.31-28.28 mg dL^{-1}) were in line with results of earlier studies indicating that the optimum rumen NH_3-N should be between 10-30 mg dL^{-1} for rumen fermentation on low-quality roughage (Khampa and Wanapat, 2006; Chanjula *et al*., 2004). As a consequence of increased rumen NH_3-N, NH_3-N will be converted to urea. In general there is a positive correlation between rumen NH_3-N and BUN concentration (Odensten *et al*. 2005; Ropstad *et al*. 1989). The current study also showed that BUN in cows fed HCP diet was higher than those fed LCP diet. However, BUN concentration in cows fed HCP diet was still in the normal range. The optimal BUN concentration ranges from 12-17 mg dL^{-1} according by Baker *et al*. (1995).

CONCLUSION

It can be concluded that DMI and therefore milk production in mid-lactation cows under hot and humid conditions could be increased by increasing CP content higher than the CP level recommended by NRC (2001).

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REFERENCES

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Agle, M., A.N. Hristov, S. Zaman, C. Schneider, P. Ndegwa and V.K. Vaddella, 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. J. Dairy Sci., 93: 1625-1637.

Angthong, W., S. Pongpiajan and W. Panichpol, 2004. Table of nutritive values of animal feeds. The Agricultural Co-operative Federation of Thailand Research Report, pp: 3-37.

Argyle, J.L. and R.L. Baldwin, 1989. Effects of amino acids and peptides on rumen microbial growth yields. J. Dairy Sci., 72: 2017-2027.

Armstrong, D.V., 1994. Heat stress interaction with shade and cooling. J. Dairy Sci., 77: 2044-2050.

Baker, L.D., J.D. Ferguson and W. Chalupa, 1995. Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci., 78: 2424-2434.

Bequette, B.J., F.R.C. Backwell and L.A. Cromption, 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. J. Dairy Sci., 18: 2540-2559.

Blauwiekel, R. and R.L. Kincaid, 1986. Effect of crude protein and solubility on performance and blood constituents of dairy cows. J. Dairy Sci., 69: 2091-2098.

Bremner, J.M. and D.R. Keeney, 1965. Steam distillation methods of determination of ammonium, nitrate and nitrite. Anal. Chem. Acta, 32: 485-495.

Camero, A. and M. Franco, 2001. Improving rumen fermentation and milk production with legume-tree fodder in the tropics. Agroforestry Syst., 51: 157-166.

Chalupa, W., 1984. Discussion of protein symposium. J. Dairy Sci., 67: 1134-1146.

Chanjula, P., M. Wanapat, C. Wachirapakorn, S. Uriyapongson and P. Rowlinson, 2004. 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. Asian-Aust. J. Anim. Sci., 17: 1400-1410.

Cochran, W.G. and G.M. Cox, 1957. Experimental Designs. 2nd Edn., John Wiley and Sons, Oxford, pp: 131.

Erwin, E.S., G.J. Marco and E.M. Emery, 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44: 1768-1771.

Ferguson, J.D., T. Blanchard, D.T. Galligan, D. Hoshall, and W. Chalupa, 1988. Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. J. Am. Vet. Med. Assoc., 192: 659-662.

Galyean, M.L., 1989. Microscopy. In: Laboratory Procedure in Animal Nutrition Research, Laing, D. (Ed.). Department of Animal and Range Science, New Mexico State University, USA., pp: 163-168. New Mexico State University, USA., pp: 163-168.

Ishler, V., J. Heinrichs and G. Varga, 1996. Feed and Feed Nutrients for Dairy Cattle. In: From Feed to Milk: Understanding Rumen Function, Ishler, V.A., A.J. Heinrichs and G.B. Varga (Eds.). Pennsylvania State University, USA., pp: 422.

Jones-Endsley, J.M., M.J. Cecava and T.R. Johnson, 1997. Effects of dietary supplementation on nutrient digestion and the milk yield of intensively grazed lactating dairy cows. J. Dairy Sci., 80: 3283-3292.

Kabuga, J.D., 1990. The influence of thermal conditions on the conception rate of Holstein-Friesian cattle in the humid tropics. Agric. Forest Meteorol., 53: 33-43.

Kalscheur, K.F., R.L. VI Baldwin, B.P. Glenn and R.A. Kohn, 2006. Milk production of dairy cows fed differing concentrations of rumen-degraded protein. J. Dairy Sci., 89: 249-259.

Kanjanapruthipong, J., 2006. Dairy Herd Management. Kasetsart University Press, Thailand, pp: 230.

Kanjanapruthipong, J., N. Buatong and S. Buaphan, 2001. Effects of roughage neutral detergent fiber on dairy performance under tropical conditions. Asian-Aust. J. Anim. Sci., 14: 1400-1404.

Kanjanapruthipong, J., N. Homwong and N. Buatong, 2010. Effects of prepartum roughage neutral detergent fiber levels on periparturient dry matter intake, metabolism and lactation in heat-stressed dairy cows. J. Dairy Sci., 93: 2589-2597.

Kendall, C., C. Leonardi, P.C. Hoffman and D.K. Combs, 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. J. Dairy Sci., 92: 313-323.

Khampa, S. and M. Wanapat, 2006. Supplementation of urea level and malate in concentrate containing high cassava chip on rumen ecology and milk production in lactating cows. Pak. J. Nutr., 5: 530-535.

Kim, H.J., S.H. Park and W.J. Maeng, 2000. Effects of dietary carbohydrate sources on ruminal ammonia-N concentration and performance of dairy cows. Proceedings of the Announcement for the 3rd Joint Symposium of Japan and Korea on Rumen Metabolism and Physiology: Present and Future of Rumen Research, October 1-3, 2000, Miyazaki, Japan, pp: 31.

Korhonen, M., A. Vanhatalo and P. Huhtanen, 2002. Effect of protein source on amino acid supply, milk production and metabolism of plasma nutrients in dairy cows fed grass silage. J. Dairy Sci., 85: 3336-3351.

Leng, R.A., 1990. Factors affecting the utilization of poor quality forages by ruminant particularly under tropical condition. Nutr. Res. Rev., 3: 227-303.

Moran, J., 2005. Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmers in the Humid Tropics. Landlinks Press, Victoria, Australia.

Mulligan, F.J., P. Dillon, J.J. Callan, M. Rath and F.P. Mara, 2004. Supplementary concentrate type affects nitrogen excretion of grazing dairy cows. J. Dairy Sci., 87: 3451-3460.

NRC, 1989. Nutrient Requirements of Dairy Cattle. 6th Edn., National Academy Press, Washington, DC., USA., ISBN: 0309069971.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academy of Sciences, Washington, DC., USA.

Odensten, M.O., Y. Chilliard and K. Holtenius, 2005. Effects of two different feeding strategies during dry-off on metabolism in high-yielding dairy cows. J. Dairy Sci., 88: 2072-2082.

Promma, S., S. Puangdee, B. Cheva-Isarakul and B. Cheva-Isarakul, 2002. The proper levels of energy and protein for medium milk yield Holstein-Friesian crossbred cows fed ruzi silage as a major roughage source. Proceedings of 40th Kasetsart University Annual Conference (KUAC'02), Bangkok, Thailand, pp: 163-171.

Ramos, S., M.L.Tejido, M.E. Martinez, M.J. Ranilla and M.D. Carro, 2009. Microbial protein synthesis, rumenal digestion, microbial populations and nitrogen balance in sheep fed diets varying in forage-to-concentrate ratio and type of forage. J. Anim. Sci., 87: 2924-2934.

Ravagnolo, O., I. Misztal and G. Hoogenboom, 2000. Genetic component of heat stress in dairy cattle, development of heat index function. J. Dairy Sci., 83: 2120-2125.

Reynal, S.M. and G.A. Broderick, 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci., 86: 835-843.

Ropstad, E., L. Vik-Mo, and A.O. Refsdal, 1989. Levels of milk urea, plasma constituents and rumen liquid ammonia in relation to the feeding of dairy cows during early lactation. Acta Vet. Scand., 30: 199-208.

SAS, 2001. SAS/STAT User's Guide. Version 8.2, SAS Institute, Cary, NC.

Tyrrell, H.F., G.L. Haaland, P.W. Moe and A.C.G. Brown, 1981. Effect of level and solubility of protein on energy value of corn silage based rations fed to Holstein cows. J. Dairy Sci., 64 (Suppl.1): 123-123.

Van Keulen, J. and B.A. Young, 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci., 44: 282-287.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Weigel, D.J., J.P. Elliott and J.H. Clark, 1997. Effects of amount and ruminal degradability of protein on nutrient digestibility and production by cows fed tallow. J. Dairy Sci., 80: 1150-1159.