



## Influence of Varying Ruminally Degradable to Undegradable Protein Ratio on Nutrient Intake, Milk Yield, Nitrogen Balance, Conception Rate and Days Open in Early Lactating *Nili-Ravi* Buffaloes (*Bubalus bubalis*)

Mahr-un-Nisa, A. Javaid, M. Aasif Shahzad and M. Sarwar\*

Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad-38040 Pakistan

**ABSTRACT :** Twenty four early lactating *Nili-Ravi* buffaloes, eight in each group, were used in a Randomized Complete Block Design to evaluate the influence of varying ruminally degradable protein (RDP) to ruminally undegradable protein (RUP) ratio on feed intake, digestibility, N balance, milk yield and its composition, conception rate and days open. Three experimental diets were formulated to contain RDP:RUP of 50:50, 66:34 and 82:18 and were denoted as HRUP, MRUP and LRUP, respectively. Dry matter (DM) intake was higher ( $p < 0.05$ ) in buffaloes fed HRUP diet than in those fed MRUP and LRUP diets. Dry matter digestibility was higher ( $p < 0.05$ ) in buffaloes fed LRUP diet than in those fed HRUP and MRUP diets. Linear increase was observed in DM digestibility with increasing RDP:RUP while Neutral detergent fiber digestibility remained unaltered in buffaloes fed HRUP and MRUP diets, however, it was higher than in those fed LRUP diet. Crude protein digestibility remained unaltered across all treatments. Milk and 4 percent fat corrected milk (4% FCM) yield was higher ( $p < 0.05$ ) in buffaloes fed HRUP diet than those fed MRUP and LRUP diets. Linear decrease in milk yield was observed with increased RDP:RUP. Milk protein and fat yields were higher ( $p < 0.05$ ) in animals fed HRUP diet than those fed MRUP and LRUP diets. Milk protein percent in animals fed HRUP diet was higher than in those fed LRUP diet, whereas it did not differ with those fed MRUP diet. Percent of fat, total solids, solid not fat and lactose remained unaltered across all diets. Nitrogen balance was higher in buffaloes fed HRUP diet than in those fed other diets. Increasing the RDP:RUP resulted in a linear decrease in N balance. The blood urea nitrogen and milk urea nitrogen were lower ( $p < 0.05$ ) in buffaloes fed HRUP diet than those fed MRUP and LRUP diets. The blood pH remained unaltered across all treatments. Days open did not differ significantly. Conception rate was higher in buffaloes fed HRUP diet than those fed MRUP and LRUP diets. The findings of the present study indicate that feeding high (50% of the total crude protein) ruminally undegradable protein diet not only increased nutrient intake and milk yield but also improved conception rate in early lactating buffaloes. (**Key Words :** RUP:RDP, Milk Yield, Nitrogen Balance, Buffaloes)

### INTRODUCTION

Protein is one of the limiting nutrients in the diet of dairy animals in developing countries (Sarwar et al., 2002). Adequate protein supply becomes very important in early lactating buffaloes when dry matter intake (DMI) is relatively low and protein and energy requirements are high (Sarwar et al., 2004a; Shahzad et al., 2008). Poor efficiency of converting dietary protein into milk protein results partly from the extensive degradation of protein in the rumen with high rates of ammonia absorption and significant excretion of nitrogen (N) in the urine (Koenig and Rode, 2001; Oba et al., 2004; Sarwar et al., 2004b). Feeding a diet containing more protein is not a satisfactory solution because the

breakdown of dietary protein in the rumen is one of the most inefficient processes in ruminant nutrition (Sarwar et al., 2005).

During the last 25 years, the trend has been shifted from balancing rations for crude protein (CP) to balancing for ruminally degradable protein (RDP) and ruminally undegradable protein (RUP) fractions (Eastridge, 2006). Imbalance of RDP and RUP in a ruminant diet can compromise the microbial protein production, ruminal digestion and protein availability to dairy animals, if RDP is insufficient to meet microbial need (Santos et al., 1998; Reynal and Broderick, 2005). Lactating dairy animals require amino acids for milk production. Amino acids are supplied primarily by combinations of microbial protein and RUP. Therefore, CP should supply in an adequate amount of RDP for optimum microbial protein production that is of good quality for milk and protein production in

\* Corresponding Author: Muhammad Sarwar. Tel: +92-41-9201088, E-mail: drms01@gmail.com

Received October 1, 2007; Accepted January 30, 2008

**Table 1.** Ingredients and chemical composition of experimental diets (DM basis)

Ingredients	Diets <sup>1</sup>		
	HRUP	MRUP	LRUP
Wheat straw	35.00	35.00	35.00
Enzose (Dextrose)	13.60	28.00	45.66
Corn steep liquor	7.70	14.00	2.50
Wheat bran	28.50	9.38	0.62
Corn gluten meal 60 %	9.20	8.00	5.600
Urea	0.36	0.90	3.52
Oil	3.10	2.80	4.60
Dicalcium phosphate	2.00	2.00	2.00
Mineral mixture	0.50	0.50	0.50
Chemical composition			
NE <sub>L</sub> (Mcal/kg)	1.60	1.60	1.60
Organic matter (%)	91.20	91.80	94.00
Crude protein (%)	16.00	16.00	16.00
Neutral detergent fiber (%)	39.50	32.30	28.00
Ash (%)	8.80	8.20	6.00
RDP <sup>2</sup> % of DM	8.00	10.56	13.12
RUP <sup>3</sup> % of DM	8.00	5.44	2.88
RUP % of CP	50.00	34.00	18.00
RDP:RUP	50:50	66:34	82:18
NSC:RDP <sup>3</sup>	4	4	4

<sup>1</sup>HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup>Ruminally degradable protein.

<sup>3</sup>Ruminally undegradable protein. Nonstructural carbohydrates.

terms of its amino acid content (NRC, 2001) and adequate amounts of RUP with an amino acid pattern that is complementary to that of microbial protein for maximal productivity at minimal N waste.

Ruminally undegradable protein is supplemented, when microbial protein synthesis alone is insufficient to meet the metabolizable protein requirements in dairy animals, especially during early lactation (Kalscheur et al., 2006). Supplementing ruminant diets with RUP can increase the efficiency of N utilization by increasing the flow of N and amino acids to the small intestine (Titgemeyer et al., 1989). Increasing the dietary RUP increased the milk yield (Chaturvedi and Walli, 2001; Flis and Wattiaux, 2005; Gulati et al., 2005) and milk protein and fat contents (Chaturvedi and Walli, 2001) in lactating cows during early lactation.

Higher level of RDP in a dairy ration causes excessive ruminal NH<sub>3</sub> production which ultimately results in an increased blood urea (Butler, 1998; Dhali et al., 2006). Increase in ruminal NH<sub>3</sub> and blood urea decreases DMI in cows (Huber and Cook, 1972; Fenderson and Bergen, 1974). Increase in blood urea increases the days open (Jordan et al., 1983), reduces conception rate (Butler, 1998; Dhali et al., 2006). Shorter days-open intervals were observed in cows supplemented with RUP (Wiley et al., 1991). Imbalance of RDP and RUP in a dairy ration also causes negative energy balance associated with metabolic disposal of excessive N

escaping from the rumen (Staples et al., 1993). Sufficient scientific literature is available regarding RUP and RDP effects on production in lactating exotic dairy cows. However, the scientific information regarding these aspects in early lactating buffaloes is very limited. Moreover, physiological status, environmental condition and feeding strategies of buffalo vary from that of exotic dairy cows in temperate regions and RDP:RUP responses of exotic cows may not be directly applicable to lactating buffalo. Therefore, the present study was designed to examine optimal RDP:RUP and its effect on feed intake, digestibility, N balance, milk yield and its composition, days open and conception rate in early lactating buffaloes.

## MATERIALS AND METHODS

Twenty four early lactating *Nili-Ravi* buffaloes (25±5 days post calving, liveweight 635±25 kg), eight animals in each group, were used in a Randomized Complete Block Design and were housed on a concrete floor in separate pens. Buffaloes which calved within a month were selected to avoid any variation because of days in lactation. Three experimental diets i.e. high ruminally undegradable protein (HRUP), medium ruminally undegradable protein (MRUP) and low ruminally undegradable protein (LRUP) were formulated (Table 1). The HRUP, MRUP and LRUP diets were balanced to contain a RDP to RUP ratio of 50:50, 66:34 and 82:18, respectively. All diets were formulated to be iso-nitrogenous and iso-caloric using NRC (2001). Diets were mixed daily and fed twice a day *ad libitum*. Feed offered andorts were sampled daily and composited by animal for analysis. Feed offered, orts and fecal samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ash (AOAC, 1990) and neutral detergent fiber (NDF; Van Soest et al., 1991).

Buffaloes were fed for 120 days. The first 30 days were adaptation while the last 10 days of each remaining month served as collection periods. Daily feed intake and milk production were averaged over 90 days. Milk samples (a.m. and p.m.) were taken during the collection period. Milk samples were analyzed for CP, fat, total solids and solid not fat using AOAC (1990) methods. Milk urea nitrogen (MUN) was determined as described by Dhali et al. (2005) using a colorimetric p-dimethylaminobenzaldehyde (DMAB) procedure. The milk (10 ml) samples were warmed at room temperature (30°C) and mixed well. Milk was deproteinised with 12% cold trichloroacetic acid solution (10 ml), allowed to stand for an hour, centrifuged at 3,000×g for 30 minutes and then filtered. Clear supernatant (2 ml) was mixed with 2 ml DMAB reagent (1.6 g DMAB+90 ml ethanol+10 ml concentrated HCl). Lactose content was determined with a Lactoscope<sup>TM</sup> Compact (Delta Instruments De Bolder 68, 9206AR

**Table 2.** Nutrient intake and digestibilities in early lactating *Nili-Ravi* buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
Intake (kg/d)						
Dry matter	14.85 <sup>a</sup>	12.30 <sup>b</sup>	11.12 <sup>c</sup>	0.91	0.001	0.070
Organic matter	13.55 <sup>a</sup>	11.29 <sup>b</sup>	10.46 <sup>c</sup>	0.35	0.001	0.044
Crude protein	2.38 <sup>a</sup>	1.97 <sup>b</sup>	1.78 <sup>c</sup>	0.06	0.001	0.070
Neutral detergent fiber	5.87 <sup>a</sup>	3.97 <sup>b</sup>	3.11 <sup>c</sup>	0.28	0.001	0.001
RUP	1.19 <sup>a</sup>	0.67 <sup>b</sup>	0.32 <sup>c</sup>	0.08	0.001	0.001
RDP	1.19 <sup>c</sup>	1.30 <sup>b</sup>	1.46 <sup>a</sup>	0.03	0.001	0.554
Digestibility (%)						
Dry matter	64.76 <sup>b</sup>	67.51 <sup>b</sup>	71.55 <sup>a</sup>	1.07	0.001	0.546
Crude protein	73.77	75.84	76.71	0.98	0.363	0.722
Neutral detergent fiber	62.70 <sup>a</sup>	60.81 <sup>a</sup>	51.26 <sup>b</sup>	1.85	0.001	0.026

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively. HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect.

Drachten, Netherlands).

Digestibility was determined by the total collection method. During collection periods, complete collections of urine and feces were made according to the procedure described by Nisa et al. (2006). The feces of each animal were collected daily, weighed, mixed thoroughly and 20% was sampled and dried at 55°C. At the end of each collection period, dried fecal samples were composited by animal and 10% of the composited sample was taken for analysis. For urine collection, small special metal buckets fitted with plastic pipe were made to surround the vulva. This plastic pipe ended in a large container (30 liters). The urine excreted by each animal was acidified with 50% H<sub>2</sub>SO<sub>4</sub> and 20% was sampled and preserved at -20°C awaiting analysis. At the end of each collection period, the preserved urine samples were composited by animal after thawing and 10% of the composited sample was used for analysis (Nisa et al., 2004).

Blood samples were collected from the jugular vein of each animal in heparinized syringes at 6 h post feeding to determine pH (AOAC, 1990). Blood samples were also collected from each buffalo without anticoagulant to harvest blood serum which was stored in aliquots at -20°C awaiting analysis for blood urea. The blood urea concentration was determined photometrically using an analytical kit (Cat # CS 612, Crescent Diagnostics, Saudi Arabia) following the Berthelot method. Days open and conception rate were calculated only for the animals that became pregnant during the experimental period.

Energy balance was calculated by the following equations (NRC, 1989).

$$\text{Energy balance (Mcal/d)} = \frac{(\text{NEL intake} - \text{NEL maint.} - \text{NEL lactation})}{\text{Efficiency factor}}$$

$$\text{NE}_L \text{ intake} = \text{DMI (kg)} \times \text{NE}_L$$

$$\text{NE}_L \text{ maint. (postpartum)} = \text{BW (kg)}^{0.75} \times 0.08 \text{ Mcal/kg}^{0.75}$$

$$\begin{aligned} \text{NE}_L \text{ lactation} \\ = \text{Milk (kg)} \times ((41.63 \times \text{fat (\%)} + 24.13 \times \text{protein (\%)} + 21.60 \\ \times \text{lactose (\%)} - 11.72) \times 2.2) / 1,000 \end{aligned}$$

An efficiency factor of 1 was used to calculate energy balance when it was positive and 0.82 when it was negative.

#### Statistical analysis

The data collected on DMI, digestibility, milk yield, milk composition, MUN, BUN, blood pH and N balance were subjected to ANOVA using the general linear model procedure of SPSS (SPSS 10.0.1., 1999). Linear and quadratic contrasts were determined using the same SPSS model.

## RESULTS

Dry matter intake (DMI) was higher (14.85 kg/d) in buffaloes fed HRUP diet than those fed MRUP (12.3 kg/d) and LRUP (11.12 kg/d) diets (Table 2). Similar trends were observed for OM, CP, NDF and RUP intakes. However, RDP intake by buffaloes fed HRUP diet was lower ( $p < 0.05$ ) than by those fed MRUP and LRUP diets. Linear decrease ( $p < 0.01$ ) in DM, OM, CP and NDF intake was noticed with decreasing dietary RUP level.

Dry matter digestibility by buffaloes fed LRUP diet was higher ( $p < 0.05$ ) than by those fed HRUP and MRUP diets. Linear increase ( $p < 0.01$ ) was observed in DM digestibility with decreasing dietary RUP level (Table 2). Neutral detergent fiber digestibility was higher ( $p < 0.05$ ) in buffaloes fed HRUP and MRUP diets than in those fed

**Table 3.** Milk yield and its composition in early lactating *Nili-Ravi* buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
Milk yield (kg/d)	9.55 <sup>a</sup>	8.29 <sup>b</sup>	8.02 <sup>b</sup>	0.19	0.001	0.028
4% FCM <sup>4</sup> (kg/d)	12.13 <sup>a</sup>	10.33 <sup>b</sup>	10.16 <sup>b</sup>	0.31	0.003	0.108
Protein yield (g/d)	412.56 <sup>a</sup>	353.98 <sup>b</sup>	334.43 <sup>b</sup>	10.56	0.001	0.195
Fat yield (g/d)	553.90 <sup>a</sup>	467.56 <sup>b</sup>	462.75 <sup>b</sup>	17.14	0.020	0.181
Protein (%)	4.32 <sup>a</sup>	4.27 <sup>ab</sup>	4.17 <sup>b</sup>	0.02	0.016	0.624
Fat (%)	5.80	5.64	5.77	0.15	0.941	0.660
Total solids (%)	15.41	15.23	15.35	0.21	0.918	0.753
Solid not fat (%)	9.54	9.59	9.57	0.09	0.876	0.867
Lactose (%)	5.63	5.57	5.42	0.06	0.190	0.722

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively. HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect. <sup>4</sup> Fat corrected milk.

**Table 4.** Nitrogen balance in early lactating *Nili-Ravi* buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
N intake (g/d)	380.16 <sup>a</sup>	314.88 <sup>b</sup>	284.67 <sup>b</sup>	13.49	0.030	0.688
Fecal N (g/d)	99.72 <sup>a</sup>	73.05 <sup>b</sup>	66.3 <sup>b</sup>	6.41	0.115	0.695
Fecal N (% of intake)	26.23	23.20	23.28	0.98	0.043	0.722
Urinary N (g/d)	184.20	166.67	158.40	2.68	0.121	0.603
Urinary N (% of intake)	48.45 <sup>b</sup>	52.93 <sup>b</sup>	55.64 <sup>a</sup>	1.59	0.026	0.350
Milk N (g/d)	64.66 <sup>a</sup>	55.48 <sup>b</sup>	52.42 <sup>b</sup>	2.49	0.197	0.602
Milk N (% of intake)	17.01	17.62	18.41	0.30	0.181	0.349
N Balance (g/d)	31.58 <sup>a</sup>	19.68 <sup>b</sup>	7.55 <sup>b</sup>	2.81	0.011	0.407

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively. Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect.

**Table 5.** Blood pH, blood urea nitrogen and milk urea nitrogen in early lactating *Nili-Ravi* buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
Blood pH	7.82	7.83	7.87	0.01	0.050	0.785
BUN <sup>4</sup> (mg/dl)	25.25 <sup>c</sup>	29.20 <sup>b</sup>	34.18 <sup>a</sup>	1.11	0.001	0.667
MUN <sup>5</sup> (mg/dl)	13.99 <sup>c</sup>	16.5 <sup>b</sup>	21.03 <sup>a</sup>	0.79	0.001	0.235

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively. HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect. <sup>4</sup> Blood urea nitrogen. <sup>5</sup> Milk urea nitrogen.

LRUP diet, however, NDF digestibility remained unaltered between animals fed HRUP and MRUP diets. Linear ( $p < 0.01$ ) and quadratic ( $p < 0.05$ ) decrease in NDF digestibility was noticed with decreasing dietary RUP level.

Milk yield and four percent fat corrected milk (4% FCM) was higher ( $p < 0.05$ ) in buffaloes fed HRUP diet than those fed MRUP and LRUP diets (Table 3). Linear decrease ( $p < 0.01$ ) in milk yield was observed with decreasing dietary RUP level. Milk protein and fat yield were higher ( $p < 0.05$ ) in animals fed HRUP diet than in those fed MRUP and LRUP diets. Milk protein percent was higher ( $p < 0.05$ ) in

animals fed HRUP diet than in those fed LRUP diet, whereas it did not differ from those fed MRUP diet. However, fat percent, total solids, solid not fat and lactose remained unaltered across all diets. Linear decrease ( $p < 0.05$ ) in percent milk protein and its yield and fat yield was noticed with decreasing dietary RUP level.

Nitrogen balance, whether expressed as g/d or % of N intake was higher ( $p < 0.05$ ) in buffaloes fed HRUP diet than those fed MRUP and LRUP diets (Table 4). Nitrogen balance between buffaloes fed MRUP and LRUP diets was non-significant. Similar trends were noticed in N intake,

**Table 6.** Energy balance in early lactating Nili-Ravi buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
Energy intake (Mcal/d)	23.76 <sup>a</sup>	19.68 <sup>b</sup>	17.79 <sup>c</sup>	1.25	0.001	0.001
NE maint. (Mcal/d)	12.69	12.56	13.45	0.90	0.001	0.010
NE <sub>L</sub> lactation (Mcal/d)	9.57 <sup>a</sup>	8.14 <sup>b</sup>	7.87 <sup>b</sup>	1.05	0.001	0.001
Energy balance (Mcal/d)	+1.5	-1.25	-4.31	-	-	-

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively. HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect.

**Table 7.** Days open and conception rate in early lactating Nili-Ravi buffaloes fed diets containing varying RDP:RUP

Parameters	Diets <sup>1</sup>			SE	L <sup>2</sup>	Q <sup>3</sup>
	HRUP	MRUP	LRUP			
Days open	99	104	109	3.84	0.317	0.99
Conception rate (%)	75	50	50	-	-	-

Means within row with different superscripts differ ( $p < 0.05$ ).

<sup>1</sup> Ruminally degradable protein to ruminally undegradable protein ratio in HRUP, MRUP and LRUP diet is 50:50, 66:34 and 82:18, respectively. HRUP, MRUP and LRUP stand for high ruminally undegradable protein, medium ruminally undegradable protein and low ruminally undegradable protein, respectively.

<sup>2</sup> p value for linear effect. <sup>3</sup> p value for quadratic effect.

fecal N (g/d), milk N (g/d) and N utilization. Decreasing the dietary RUP level resulted in linear decrease ( $p < 0.05$ ) in N intake and its balance and utilization. Urinary N (% of intake) was higher ( $p < 0.05$ ) in buffaloes fed LRUP diet than in those fed HRUP and MRUP diets. Urinary N excretion (% of intake) increased linearly ( $p < 0.05$ ) with increasing degradability of protein. However, urinary N (g/d), fecal N (% of intake) and milk N (% of intake) remained unaltered across all diets.

Blood urea nitrogen (BUN) concentrations were significantly different, whereas, the blood pH remained unaltered across all treatments (Table 5). Milk urea nitrogen (MUN) concentrations were significantly different across all treatments (Table 5). Decrease in dietary RUP level resulted in linear increase ( $p < 0.01$ ) in MUN. Energy balance was positive in early lactating buffaloes fed HRUP diet, however, it was negative in buffaloes fed MRUP and LRUP diets (Table 6). Decreasing dietary RUP level resulted in decreased ( $p < 0.01$ ) energy intake. Days open did not differ significantly across treatments (Table 7). Conception rate was higher ( $p < 0.05$ ) in buffaloes fed HRUP diet than in those fed MRUP and LRUP diets (Table 7).

## DISCUSSION

Increased DMI by buffaloes fed HRUP diet might be attributed to sufficient supply of ruminal ammonia nitrogen for optimum rumen microbial proliferation which in turn might have increased feed intake by producing more enzymes per unit of time in ruminant animals (Chumpawadee et al., 2006). Synthesis or multiplication of rumen microbes is very much dependent on adequate

supply of nitrogen and carbon skeleton at rumen level (Ali et al., 1997). The HRUP diet might have favored optimum rumen microbial growth by providing corn steep liquor and enzose as precursors of nitrogen and carbon skeleton, respectively. This might have increased feed intake. Increased feed intake by increasing the rumen microbes has also been reported by Haddad et al. (2005). Decreased DMI by buffaloes fed LRUP diet was due to the high degradable protein portion of this diet. Rapid degradation of this protein portion might have overcome the capacity of rumen microbes to efficiently capture all the ruminal ammonia nitrogen for their growth and thus substantial ammonia nitrogen might have escaped from the rumen. This phenomenon was also supported by increased serum BUN concentration (Table 5). Reduced feed consumption by cows fed a diet containing high degradable N has also been reported by Wilson et al. (1975). Chaturvedi and Walli (2001) reported linear decrease in DMI in early lactating crossbred cows with increased RDP level. Similar findings have been reported by other researchers (Kridli et al., 2001; Lee et al., 2001; Haddad et al., 2005). Moreover, protein supplements, especially those high in RUP, stimulate DMI to a greater extent than diets containing highly soluble N in the form of urea (Kalbande and Chainpure, 2001). Fenderson and Bergen (1974) also reported decreased DMI with increased ruminal ammonia and plasma urea concentrations in steers fed excessive dietary N either as natural protein or nonprotein nitrogen. The plausible explanation of lowest DMI by buffaloes fed LRUP diet might be attributed to increased BUN concentration due to higher RDP (Table 5). Higher CP intake in buffaloes fed HRUP diet was due to increased DMI. Higher NDF and

RUP intakes in buffaloes fed HRUP diet were due to their higher dietary contents and increased DMI. Lower intake of RDP in buffaloes fed HRUP diet was due to its lower dietary RDP content.

Higher DM digestibility in buffaloes fed LRUP diet might be attributed to longer rumen retention time due to decreased DMI. Positive association between rumen retention time and digestibility has been reported by Sarwar et al. (2004b). Similar findings have also been reported by Griswold et al. (2003) who reported increased DM digestibility with increased dietary RDP in cows.

Higher milk yield in buffaloes fed HRUP diet was because of higher DMI. Increased milk production in lactating dairy cows with increased dietary RUP has also been reported by other researchers (Petit and Veira, 1991; Sklan and Tinsky, 1993; Shirley et al., 1998; Kalscheur et al., 1999; Westwood et al., 2000; Chaturvedi and Walli, 2001; Flis and Wattiaux, 2005). Similar findings were reported by Lee et al. (2001) in dairy goats. The increased milk yield with increased dietary RUP level might be due to increased feed intake (Westwood et al., 2000) as well as the increased supply of metabolizable protein and amino acids (Gulati et al., 2005). The RUP may increase the milk yield either directly or indirectly (Clark, 1975). The direct role may be through either increased supply of limiting amino acids to the mammary gland for protein synthesis or through enhanced gluconeogenesis in liver resulting in increased supply of glucose to the mammary gland for lactose synthesis. The indirect role may be mediated through altered hormonal status, especially increased concentration of plasma growth hormone which causes partitioning of nutrients in favor of growth and milk production and away from fat deposition (Sartin et al., 1985).

The increased milk protein content in buffaloes fed HRUP diet was due to increased intake of RUP that might have increased supply of limiting amino acids or total amino acids to the mammary gland for protein synthesis. Fat yield was higher in buffaloes fed HRUP diet than in those fed LRUP and MRUP diets. The increased fat yield in buffaloes fed HRUP diet was due to increase in milk yield.

Higher N intake in buffaloes fed HRUP diet than in those fed MRUP and LRUP diets was due to their higher DMI. A positive N balance was noticed across all diets. Higher N balance in buffaloes fed HRUP diet was due to higher level of RUP in their diet. Pattanaik et al. (2003) reported higher N retention in calves fed a low degradable protein diet than in those fed high degradable protein diet. Similar findings were reported by Paengkoum et al. (2004) who described that N retention decreased linearly as the dietary RUP level decreased. Kalscheur et al. (2006) reported linear increase in urinary N excretion with

increasing level of RDP in dairy cows. Similar results were reported by other workers (Castillo et al., 2001; Davidson et al., 2003; Pattanaik et al., 2003; Reynal and Broderick, 2005).

A linear increase in serum BUN was due to gradual reduction in dietary RUP. This higher BUN was due to higher renal resorption of urea in buffalo. Moreover, higher BUN values in buffaloes fed MRUP and LRUP diets were because of a higher level of RDP in their diets. The normal value of BUN in cows is 15 mg/dl (Roseler et al., 1993). Norton et al. (1979) reported higher blood urea concentrations in buffalo than cattle. Increased plasma urea nitrogen with increased dietary RDP level has also been reported by Roseler et al. (1993). The increased BUN with increased dietary RDP level can probably be explained by increased absorption of ruminal  $\text{NH}_3\text{-N}$ , resulting in greater quantities of  $\text{NH}_3\text{-N}$  being detoxified in the liver to form urea N. Wanapat and Pimpa (1999) reported a linear increase in BUN with increased ruminal  $\text{NH}_3\text{-N}$  concentration in swamp buffaloes. Similar findings were reported by Chumpawadee et al. (2006) and Vongsumphan and Wandapat (2004). Higginbotham et al. (1989) found an increase of 2.6 mg/dl in BUN in cows when RDP level was increased from 58 to 65% of dietary CP.

MUN was increased linearly with increasing RDP (Dhiman and Satter, 1997). Milk urea nitrogen arises primarily from passive transfer of urea from blood. Similarly, high correlation between MUN and BUN was also reported by Jonker et al. (1998) and Broderick and Clayton (1997). The BUN or MUN may vary under the influences of many different factors. They include CP intake (Jonker et al., 1998; Nousiainen et al., 2004), CP: Energy (Broderick and Clayton, 1997), imbalance of RDP: RUP (Roseler et al., 1993), RDP:NSC (Hoover and Miller, 1990) and diseases or medicines (Vestweber et al., 1989). Cows under negative energy balance tend to have slightly higher urea concentration in milk, which could be associated with the increase of body protein mobilization (Schepers and Meijer, 1998). In the present study, total CP, CP: energy and RDP:NSC were constant across all diets (Table 1) and protein degradability was the main controlling factor affecting BUN and MUN concentrations.

Positive energy balance was attributed to increased DMI due to higher dietary RUP level. Positive energy balance in buffaloes fed HRUP diet was due to higher energy intake (23.76 Mcal/d) than those fed MRUP (19.68 Mcal/d) and LRUP (17.79 Mcal/d) diets.

Although days open did not differ significantly across treatments, shorter days open were observed in buffaloes fed HRUP diet than those fed MRUP or LRUP diets. Sklan and Tinsky (1993) and Westwood et al. (2000) observed shorter days open for dairy cows fed high RUP than for

those fed low RUP diet. Kridli et al. (2001) also noticed similar findings in Awassi ewes. Dhali et al. (2006) reported a positive relationship between MUN and interval from parturition to first service. In the present study, MUN was increased with decreasing RUP level. Dhali et al. (2005) also reported increased service period with increasing MUN concentration in crossbred Karan-fries cows. Similar findings were reported by Gustafassan and Carlsson (1993).

Sklan and Tinsky (1993) examined the effect of protein degradability on reproduction in Israeli-Friesian cows. They reported that conception rate was higher (70.9%) in cows fed a diet containing 39 than in those (58.5%) fed a diet containing 34% RUP. Guo et al. (2004) reported negative effects of elevated levels of MUN concentration on conception rate. In the present study, there was a negative association between MUN concentration and conception rate. Change in MUN concentration from 13.99 to 21.03 mg/dl resulted in a 25% decrease in conception rate. Lower conception rates in buffaloes fed MRUP and LRUP diets were due to lower DMI because of higher RDP intake (Table 2). Higher dietary RDP level can adversely influence conception rate by increasing the BUN (Butler, 1998).

## CONCLUSION

The findings of the present study indicate that feeding high ruminally undegradable protein (50% of the dietary crude protein) diet not only increased nutrient intake and milk yield but also improved conception rate in early lactating *Nili Ravi* buffaloes.

## REFERENCES

- Ali, C. S., T. Khaliq and M. Sarwar. 1997. Influence of various sources of non-protein nitrogenous sources on *in vitro* fermentation patterns of rumen microbes. *J. Anim. Feed Sci. Technol.* 10:353-363.
- Association of Official Analytical Chemists. 1990. Official Methods of Analysis (15th Ed.) Arlington, Virginia, USA.
- Broderick, G. A. and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964-2970.
- Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *J. Dairy Sci.* 81:2533-2539.
- Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby and F. France. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.* 79:247-253.
- Chaturvedi, O. H. and T. K. Walli. 2001. Effect of feeding graded levels of undegraded dietary protein on voluntary intake, milk production and economic return in early lactating crossbred cows. *Asian-Aust. J. Anim. Sci.* 14:1118-1124.
- Chumpawadee, S., K. Sommart, T. Vongpralub and V. Pattarajinda. 2006. Effects of synchronizing the rate of dietary energy and nitrogen release on ruminal fermentation, microbial protein synthesis, blood urea nitrogen and nutrient digestibility in beef cattle. *Asian-Aust. J. Anim. Sci.* 19:181-188.
- Clark, J. H. 1975. Lactational responses to postpartum administration of protein and amino acids. *J. Dairy Sci.* 58: 1178-1197.
- Davidson, S., B. A. Hopkins, D. E. Diaz, S. M. Bolt, C. Brownie, V. Fellner and L. W. Whitlow. 2003. Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. *J. Dairy Sci.* 86:1681-1689.
- Dhali, A., R. K. Mehla and S. K. Sirohi. 2005. Effect of urea supplemented and urea treated straw based diet on milk urea concentration in crossbred karan-fries cows. *Italian J. Anim. Sci.* 4:25-34.
- Dhali, A., D. P. Mishra, R. K. Mehla and S. K. Sirohi. 2006. Usefulness of milk urea concentration to monitor the herd reproductive performance in crossbred karan-fries cows. *Asian-Aust. J. Anim. Sci.* 19:26-30.
- Dhiman, T. R. and L. D. Satter. 1997. Effect of ruminally degraded protein on protein available at the intestine assessed using blood amino acid concentrations. *J. Anim. Sci.* 75:1674-1680.
- Flis, S. A. and M. A. Wattiaux. 2005. Effects of parity and supply of rumen-degraded and undegraded protein on production and nitrogen balance in Holsteins. *J. Dairy Sci.* 88:2096-2106.
- Griswold, K. E., G. A. Apgar, J. Bouton and J. L. Firkins. 2003. Effects of urea infusion and ruminally degraded protein concentration on microbial growth, digestibility, and fermentation in continuous culture. *J. Anim. Sci.* 81:329-336.
- Gulati, S.K., M. R. Garg and T. W. Scott. 2005. Rumen protected protein and fat produced from oil seeds and meals by formaldehyde treatment; their role in ruminant production and product quality: A review. *Aust. J. Exp. Agric.* 45: 1189-1203.
- Guo, K., E. R. Cohen, M. A. Vamer and R. A. Kohn. 2004. Effects of milk urea nitrogen and other factors on probability of conception of dairy cows. *J. Dairy Sci.* 87:1878-1885.
- Gustafassan, A.H. and J. Carlson. 1993. Effects of silage quality, protein evaluation systems and milk urea content on milk yield and reproduction in dairy cows. *Livest. Prod. Sci.* 37:91-105.
- Haddad, S. G., R. T. Kridli and D. M. Al-Wali. 2005. Influence of varying levels of dietary undegradable intake protein on nutrient intake, body weight change and reproductive parameters in postpartum Awassi Ewes. *Asian-Aust. J. Anim. Sci.* 18:637-642.
- Hoover, W. H. and T. K. Miller. 1990. Carbohydrate and protein considerations in ration formulation. Page 71 in Proc. Large Dairy Herd Manage. Conf., Cornell Coop. Ext., Cornell Univ., Ithaca, NY.
- Jonker, J. S., R. A. Kohn and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *J. Dairy Sci.* 81:2681-2692.
- Kalbande, V. H. and A. H. Chainpure. 2001. Effect of feeding bypass protein with urea treated jowar kadbi on performance of cross bred calves. *Asian-Aust. J. Anim. Sci.* 14:651-654.
- Kalscheur, K. F., R. L. 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.

- Koenig, K. M. and L. M. Rode. 2001. Ruminant degradability, intestinal disappearance, and plasma methionine response of rumen-protected methionine in dairy cows. *J. Dairy Sci.* 84: 1480-1487.
- Kridli, R. T., S. G. Haddad and M. M. Muwalla. 2001. The effect of feeding ruminally undegradable protein on postpartum reproduction of Awassi Ewes. *Asian-Aust. J. Anim. Sci.* 14: 1125-1128.
- Lee, M., S. Hwang and P. W. Chiou. 2001. Application of rumen undegradable protein on early lactation dairy goats. *Asian-Aust. J. Anim. Sci.* 14:1549-1554.
- National Research Council. 1989. Nutrient requirements of dairy cattle (6th Rev. Ed.) National Academy Press, Washington, DC.
- National Research Council. 2001. Nutrient requirements of dairy cattle. Seventh revised edition. National Academy Press, Washington, DC.
- Nisa, M., M. A. Khan, M. Sarwar, W. S. Lee, S. B. Kim, B. S. Ahn and S. M. Kim. 2006. Influence of corn steep liquor on feeding value of urea treated wheat straw in buffaloes fed at restricted diets. *Asian-Aust. J. Anim. Sci.* 19:1610-1619.
- Nisa, M., M. Sarwar and M. A. Khan. 2004. Influence of *ad libitum* feeding of urea treated wheat straw with or without corn steep liquor on intake, *in situ* digestion kinetics, nitrogen metabolism, and nutrient digestion in *Nili-Ravi* buffalo bulls. *Aust. J. Agric. Res.* 55:235-342.
- Nousiainen, J., K. J. Shingfield and P. Huhtanen. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *J. Dairy Sci.* 87:386-398.
- Oba, M., R. L. Baldwin, S. L. Owens and B. J. Bequette. 2004. Urea synthesis by ruminal epithelial and duodenal mucosal cells from growing sheep. *J. Dairy Sci.* 87:1803-1805.
- Oltner, R., M. Emanuelson and H. Wiktorsson. 1983. Page 195 in Factors affecting the urea concentration in cows milk. Proc. 5th Int. Conf. Rod. Dis. Farm Anim. Swed. Univ. Agric. Sci., Uppsala, Sweden.
- Paengkoum, P., J. B. Liang, Z. A. Jalan and M. Basery. 2004. Effects of ruminally undegradable protein levels on nitrogen and phosphorus balance and their excretion in Saanen goats fed oil palm fronds. *Songklanakarin J. Sci. Technol.* 26:15-22.
- Pattanaik, A. K., V. R. B. Sastry, R. C. Katiyar and L. Murari. 2003. Influence of grain processing and dietary protein degradability on N metabolism, energy balance and methane production in young calves. *Asian-Aust. J. Anim. Sci.* 16: 1443-1450.
- Petit, H. V. and D. M. Veira. 1991. Effect of grain level and protein source on ruminal fermentation, degradability and digestion in milking cows fed silage. *Dairy Sci.* 74:2256-2267.
- Reynal, S. M. and G. A. Broderick. 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. *J. Dairy Sci.* 88:4045-4064.
- Roseler, D. K., J. D. Ferguson, C. J. Sniffen and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk non-protein nitrogen in Holstein cows. *J. Dairy Sci.* 76:525-534.
- Santos, F. A. P., J. E. P. Santos, C. B. Theurer and J. T. Hubber. 1998. Effects of rumen degradable protein on dairy cow performance: A 12-year literature review. *J. Dairy Sci.* 81: 3182-3213.
- Sartin, J. L., K. A. Cummins, R. J. Krempainen, D. N. Marple, C. H. Rahe and J. C. William. 1985. Glucagon, insulin and GH responses to glucose infusion in lactating cows. *Nutr. Abstr. Rev. (Series B) Abstract No.* 4371.
- Sarwar, M., M. A. Khan and Z. Iqbal. 2002. Feed Resources for Livestock in Pakistan. Status Paper. *Intl. J. Agric. Biol.* 4:186-192.
- Sarwar, M., M. A. Khan and M. Nisa. 2004a. Influence of ruminally protected fat and urea treated comcobs on nutrient intake, digestibility, milk yield and its composition in *Nili-Ravi* buffaloes. *Asian-Aust. J. Anim. Sci.* 17:171-178.
- Sarwar, M., M. A. Khan and M. Nisa. 2004b. Effect of organic acids or fermentable carbohydrates on digestibility and nitrogen utilization of urea treated wheat straw in buffalo bulls. *Aust. J. Agric. Res.* 55:229-235.
- Sarwar, M., M. A. Khan and M. Nisa. 2005. Chemical composition and feeding value of urea treated comcobs ensiled with additives for sheep. *Aust. J. Agric. Res.* 56:685-690.
- Shahzad, M. A., M. Sarwar and M. Nisa. 2008. Influence of altering dietary cation anion difference on milk yield and its composition of early lactating *Nili Ravi* buffaloes in summer. *Livest. Sci.* 113:133-143.
- Schepers, A. J. and R. G. M. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. *J. Dairy Sci.* 81:579-584.
- Sklan, D. and M. Tinsky. 1993. Production and reproduction responses by dairy cows fed varying undegradable protein coated with rumen bypass fat. *J. Dairy Sci.* 76:216-223.
- SPSS, 1999. SPSS user's guide: Release 10.0.1 edition. SPSS Inc.
- Staples, C. R., C. M. Garcia-Bojalil, B. S. Oldick, W. W. Thatcher and C. A. Risco. 1993. Protein intake and reproductive performance of dairy cows: a review, a suggested mechanism and blood and milk urea measurements. Pages 37-51 in Proc. 4th Annu. Florida Ruminant Nutr. Symp., Univ. Florida, Gainesville.
- Titgemeyer, E. C., N. R. Merchen and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. *J. Anim. Sci.* 67:262-273.
- Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.
- Vestweber, J. G. E., F. K. Al-Ani and D. E. Johnson. 1989. Udder edema in cattle: effects of diuretics on serum and urine electrolytes. *Am. J. Vet. Res.* 50:1323-1328.
- Vongsamphan, P. and M. Wanapat. 2004. Effect of levels of cassava hay (CH) supplementation in native beef cattle feed on rice straw. In: Proceeding of the Agricultural Seminar, Animal Science/animal husbandry. Held at Sofitel Rajaorcid hotel 27-28 January 2004, pp. 255-270.
- Wanapat, M. and O. Pimpa. 1999. Effect of ruminant NH<sub>3</sub>-N levels on ruminant fermentation, purine derivatives, digestibility and rice straw intake in swamp buffaloes. *Asian-Aust. J. Anim. Sci.* 12:904-907.
- Westwood, C. T., I. T. Lean, J. K. Garvin and P. C. Wynn. 2000. Effect of genetic merit and varying dietary protein degradability on lactating dairy cows. *J. Dairy Sci.* 83:2926-2940.



- Wiley, J. S., M. K. Peterson, R. P. Ansotegui and R. A. Bellows. 1991. Production from first-calf heifers fed a maintenance or low level of prepartum nutrition and ruminally undegradable or degradable protein postpartum. *J. Anim. Sci.* 69:4279-4293.
- Wilson, G., F. A. Martz, J. R. Campbell and B. A. Becher. 1975. Evaluation of factors responsible for reduced voluntary intake of urea diets for ruminants. *J. Anim. Sci.* 41:1431-1439.