The Journal of Animal & Plant Sciences, 29(6): 2019, Page: 1558-1567
ISSN: 1018-7081

EFFECT OF DIETARY LEVELS OF PROTEIN ON MILK PRODUCTION, MILK-UREA AND NITROGEN USE EFFECIENCY IN PERI-URBAN MILKING BUFFALOES$^{\Phi}$

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$^{\Phi}$*The paper was presented in International Buffalo Congress 2019, February 18-20, Lahore, Pakistan*

ABSTRACT

In Pakistan peri-urban milking buffaloes are usually overfed protein in attempt to maximize milk production which adds to the cost of feeding with little or no positive response on performance of the buffaloes. The present study was performed to assess protein status and N use efficiency in peri-urban milking buffaloes maintained on conventional feeding system. One hundred and twentysix (126) buffaloes in early to mid-lactation were selected from 20 peri-urban dairy farms at three different locations in KP province, Pakistan. The farms were visited monthly and data on daily milk yield and feed intake were recorded from January to December 2008. Samples of feed offered and evening milk were collected for laboratory analysis. The data were analyzed using ANOVA according to RCB factorial design. The results were grouped on the basis of crude protein (CP) in total DM consumed as low CP (9-11%), medium CP (12-14%) and high CP (15-17%). CP levels did not influence DM intake (14.34±0.6 kg) or milk yield (9.13 ± 1.3 liter). Milk urea-N (MUN) linearly (r^2 =0.42) increased with increasing CP in the diet and ranged from 11.79 to 17.07 mg/100 ml. Nitrogen use efficiency for milk (MNE) ranged from 14.39% to 22.69% and negatively correlated (r^2=0.56) with CP intake. Majority of the buffalos (48%) had CP intake in excess of requirements associated with increase in protein-energy ratios and N loss in excreta. Feed conversion efficiency was not influenced by dietary CP and averaged 0.83±0.12. Excess CP intake resulted from berseem feeding which adversely affected performance of the buffalos. Lower CP intake with feeding of maize fodder supported higher milk yield, improved MNE and reduced MUN. The results concluded that CP intake was influenced by type of fodder and CP higher than 11% in DM did not increase daily milk yield while significantly reduced MNE. Nitrogen utilization for enhanced milk yield in peri-urban buffaloes can be improved by balancing protein-energy ratio of dairy rations.

Key words: Buffalo, milk production, Milk Urea-N, Nitrogen utilization.

INTRODUCTION

Peri-urban buffalo farms near big cities serve as major source of buffalo milk supply to urban population in Pakistan. In attempt to cater the increasing demand of milk in the cities, these farms keep milking buffaloes of high genetics and feed them luxuriously to maximize milk production. Protein is often overfed to these herds with a common belief that it leads to increasing milk yield. The nutrient composition of conventional dairy rations in peri-urban farms are mostly imbalanced (Habib *et al.* 2007) and never matched with the fluctuating supply of seasonal fodders, such practice not only constrain buffalo productivity (Habib *et al.*2007; Garg *et al.* 2013) but also at certain time may overburden the animal with nutrients like protein. However, biological potential of dairy animals is limited to transform nutrients inputs such as N into milk. Of the total feed-N consumed by dairy cow 65 to 85% is lost in excreta (Powell et al 2010) and is mostly caused by feeding protein in excess of animal's requirement. The excess protein is used with lower efficiency and may or may not be helpful in increasing feed consumption and milk yield in the animal (NRC, 2001). There is enough evidence available that elevated levels of circulatory urea associated with high protein feeding reduce reproductive performance of cows and buffaloes (NRC, 2001, Qureshi *et al.*2002). Concentrations of urea nitrogen in blood plasma and milk are highly related and quickly equilibrate. Therefore, milk urea-N values are representative of urea levels of blood and can be used to monitor protein status of milking animal (Broderick and Clayton, 1997)

A considered opinion is that while dairy farmers can do little to overcome the biological limitation of N utilization in buffaloes, they can improve N use efficiency through balancing dairy rations (Garg *et al.* 2013) and adapting recommended practices to avoid wasteful use of feed–N in the animals while still achieving high milk production at lower feed cost. As mentioned above, although productivity in commercial buffaloes is apparently constrained by imbalanced nutrition, there is no farms based investigation to this end in milking buffaloes in Pakistan. The short term controlled experiments using formulated rations are not representative of private buffalo farms where not only feeding practices are diverse and deviate from feeding standards but also confronted with inconsistent supply of seasonal fodders and market accessibility of feed ingredients. The present investigation was performed in peri-urban buffalo farms over a period of one year to determine the status of N consumption in relation to milk yield, milk urea concentrations and N use efficiency under conventional feeding system. The objective was to identify the impact of current feeding practices on N use efficiency and formulate recommendations for cost effective use of feed for enhanced productivity in buffaloes

MATERIALS AND METHODS

Selection of animals and farms: A total of 126 multiparous lactating buffaloes (Nili-Ravi breed; body weight 450 ± 35 kg) in early to mid-lactation were selected in 20 peri-urban dairy farms at three different districts in the KP province of Pakistan (Table 1). As part of the routine conventional practices, buffaloes in all selected farms were disposed towards the end of lactation and replaced by freshly purchased milking buffalo in early lactation. In such cases, the buffaloes selected for the data collection when culled were substituted by other buffaloes in early to mid-lactation at the same farm.

Table 1. Distribution of buffaloes selected for data collection at three different locations.

Location	Number of Farms selected	Herd size	Number of selected buffaloes
Peshawar	8	55 – 102	49
Nowshehra	8	50 – 100	51
Mardan	4	35 – 70	26
Total	**20**		**126**

Feeding practices: The buffaloes were housed tied in stall and offered forage and concentrate mixture separately twice a day. Seasonal fodders were fed *ad libitum*. Berseem (*Trifolium alanxdrium*) occasionally mixed with oats (*Avina sativa*) served as chopped from mid-November to June combined with 2-3 kg wheat straw to avoid bloat in the animals. Maize (*Zea mays*) was chopped and served during July to mid-November. In few cases sorghum (*Sorghum bicolor*) was also fed for short duration. Concentrate mixture was prepared at the farm by mixing equal parts of cottonseed cake (undecorticated), wheat bran and dried waste bread. The cake and bread parts were soaked in water for 2-3 hours and then mixed with wheat bran before feeding. The mixture was prepared on daily basis and offered at morning and evening milking time at flat rate 3 to 5 kg/buffalo/day mixed with about 2 kg wheat straw. The proportion of concentrate ingredients varied to some extent among different farms depending on availability and convenience of the farmers. Such information was recorded for calculating nutrient intake of the buffaloes. Average composition of feed ingredients is shown in Table 2.

Sampling and data collection: The selected farms were visited regularly once a month throughout the year 2008. Data on daily milk yield of individual buffalo was recorded. Representative samples of milk (30 ml) were collected immediately after evening milking in a centrifuge tube containing 20 µl of 1% Sodium azide solution, as a preservative. The samples were transported to laboratory in an icebox and further chilled in a refrigerator for 10-12 hours. These were then centrifuged at 2000 rpm for 5 minutes. After removing the top fat layer, the defatted sample was analyzed for urea concentration. Representative samples of forages and other feed ingredients were regularly collected for nutrients analysis.

Laboratory Analysis: All the laboratory analysis were performed in the animal nutrition department of Agricultural University, Peshawar, KP. Feed and forage samples were dried in hot air oven at 55 °C for 48 h. The dried samples were ground to 1 mm particle size using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). The ground feed samples were analyzed according to procedures of AOAC (2005) for dry matter (DM), ash, crude protein, ether extract and acid detergent fiber (ADF). Neutral Detergent Fiber (NDF) was analyzedwith the procedure of Van Soest *et al.* (1991). Urea concentration in milk samples was determined by modified colorimetric diacetylmonoxime (DAM) assay (Richter and Lapointe, 1962).

Data Calculation and Statistics: *Efficiency of Feed-N utilization for milk:* The feed-N use efficiency for milk production was calculated as the quantity of N in milk percent of feed-N consumed by the buffalo. The protein content in buffalo milk was estimated as3.84g/100g milk (Kanwal *et al.*, 2004) assuming that milk protein does not change with CP concentrations in the diet (NRC, 2001)

CP intake in relation to requirement: Total DM intake as calculated from the data recorded on feed offered and refused by the animals. The amount of CP consumed was calculated as DM intake multiplied by CP percent in the DM. The intake of CP was compared with the standard requirements calculated according to the following equation suggested by Campanile *et al.* (1998);

CP Balance g = g CP intake - (80 g CP x 100 kg live weight + 2.7 g CP x g milk protein yield)

N Excretion in feces and urine: N excretion in feces and urine was calculated based on the findings of Mahr-un-Nisa *et al.* (2008) and Neglia *et al.* (2014) in milking buffaloes. These workers estimated N excretion in feces and urine against the N intake of 285 to 381g/d and 119-332g /d, respectively. The data were combined for regression analysis and the regression equation was derived as follow;

N Excretion in feces plus urine (g/d)=0.617 N intake g+62.68; r^2=0.72

Feed conversion efficiency: It was determined as kg fat and protein corrected milk produced per kg feed DM consumed.

Fat and protein corrected milk: The milk was converted to fat and protein corrected milk (FPCM) with 4.0 % fat and 3.3 % protein, using the following formula suggested by Opio *et al.* (2013);

FPCM (kg) = raw milk (kg) x[1+ 0.011 **x** {(10 x fat% - 40) + (10 x protein% -31)}]

Average fat and protein contents in buffalo milk were assumed 6.5% and 3.84%, respectively (Kanwal *et al.,* 2004).

Data analysis: The data were analyzed with ANOVA procedure according to RCB factorial design using SAS (Version 1.2, SAS Institute Inc. 1996). The main effects were months, locations, and farms and their interactive effects. The results were declared significant at $P<0.05$ and trends at $P\leq0.10$. The data were also grouped on the basis of CP % in DM consumed as low CP 9-11%; medium CP 12-14% and high CP 15-17%. Polynomial orthogonal contrasts were used to determine the effect of increasing levels of dietary CP and months. Regression analysis was performed to investigate the relationship among various parameters. Duncan's multiple range test and Turkey's test were applied for comparing means.

RESULTS

Buffalo performance in response to dietary CP levels: Average composition of feed used in the peri-urban farms for feeding of the buffaloes is shown in Table 2. Data on feed consumption, milk production and N utilization parameters in relation to dietary CP levels is summarized in Table 3. DM intake was not affected by CP levels. The difference in DMI was not pronounced and tended to show a quadratic response (P=0.08) to increasing CP concentrations. Daily intake of CP linearly ($P\leq0.0001$) increased with increasing dietary CP contents. Milk yield was not significantly affected by CP levels. Milk Urea-N (MUN) linearly ($P\leq0.0001$) increased from 11.79 mg/100 ml at low CP to 17.07 mg/100 ml with high CP levels. Conversely, N use efficiency for milk (MNE) sharply declined with increasing CP % in the diet ($P\leq0.0001$) and increasing CP consumption ($P\leq0.0001$). MNE was high (22.67%) with diet low in CP and was lowest (14.39%) on diets containing high CP showing an inverse correlation (r^2=0.56). The amount of dietary CP consumed on medium and high CP diets was 175 g/day and 675 g/day, respectively in excess of the buffalo requirements and negatively correlated with daily milk production (r^2 =0.21). Calculated N excretion in feces and urine progressively increased in response to rising levels of CP in the diet ($P\leq0.0001$). The efficiency of conversion of feed DM into milk (FCE) was not influenced by CP levels in the diet. Increasing CP in the DM increased protein-energy ratio (CP g/ME Mj) in the diets ($P\leq0.0001$) and ranged from 11.50 to 16.65.

Table2. Nutrient composition of main feed ingredients.

Nutrients	Berseem fodder	Maize fodder	Wheat straw	Concentrate mixture
Dry matter (DM)	23.67	28.49	91.75	92.03
Nutrient composition (% in DM)				
ME* Mcal/kg DM	2.27	2.50	1.51	2.27
Crude Protein	18.90	9.93	2.59	15.51
Ether Extract (EE)	2.24	2.30	1.12	5.00
Neutral Detergent Fiber (NDF)	44.34	57.66	75.45	24.28
Acid Detergent Fiber (ADF)	27.32	38.41	53.42	15.76
Non Fiber Carbohydrate** (NFC)	18.72	23.11	13.34	50.05
Ash	15.80	7.03	7.50	5.17

*Metabolizable energy calculated according to (NRC, 2001) using the values of analyzed feeds

**NFC= 100 - (%NDF+%CP+%EE+%Ash); (NRC, 2001).

Monthly variation in milk yield and N utilization related to seasonal fodder supply: Feeding practices over different months at the three locations were accompanied by large variation in CP intake, milk production and N utilization parameters in the buffaloes and the results are summarized in Table 4. Total DMI varied due to location ($P\leq0.0001$) and months ($P\leq0.01$) and was significantly lower during November-December (13.74 kg/d) compared to other months (average 14.5 kg/d). CP consumption linearly increased from November to June ($P\leq0.0001$) when berseem served as main forage and thereafter decreased with the onset of maize supply during July to October, showing a quadratic trend ($P\leq0.05$). CP intake significantly differed among the three locations ($P\leq0.05$). Daily milk production ranged from 8.6 to 10.7 liters and was influenced by both months (P≤0.0001) and location (P≤0.0001). Daily milk yield was higher from July to October and lower during Nov to June and showed linear (P≤0.0001) and quadratic (P≤0.001) trends. MUN results were same among the locations but inconsistently varied due to different months (P≤0.0001) and followed the monthly pattern of CP intake. MNE influenced by both location (P≤0.0001) and months (P≤0.0001) and was high from July to October (21.57-22.73%) when CP intake was low and progressively decreased during November to June (14.56-18.67%) in association with higher CP intake. Daily consumption of CP in the buffaloes above requirements ranged from 152g to 660g during January to June in association with high CP consumption caused by berseem feeding.

Table 3. Effect of increasing intake of CP on milk Urea-N (MUN), Milk production and N utilization efficiency (MNE) in commercial buffaloes at three different locations

	Treatments			Contrast (P value)		Treatment x location
	LCP (9-11%)	MCP (12-14%)	HCP (15-17%)	Linear	Quadratic	
Total DMI	14.31	14.33	14.41	0.68	0.08	0.85
Crude Protein Intake kg/d	1.50^{c}	1.82^{b}	2.28^{a}	0.0001	0.36	0.86
N Intake g/d	240^{c}	292^{b}	365^{a}	0.0001	0.36	0.86
Milk Production lit/d	9.14	9.22	8.86	0.56	0.33	0.10
MUN mg N/100 ml	11.79^{c}	14.23^{b}	17.07^{a}	0.0001	0.94	0.14
MNE %	22.67^{a}	18.70^{b}	14.39^{c}	0.0001	0.70	0.02
Crude Protein consumed relative to requirement g/d	$(-)141^{c}$	175^{b}	673^{a}	0.0001	0.02	0.20
Calculated N excretion in feces and urine g/d	211^{c}	242^{b}	287^{a}	0.00001	0.04	0.85
Feed Conversion Efficiency % (FPCM* kg /Feed DM kg)	0.84	0.84	0.79	0.20	0.26	0.05
Protein: Energy ratio (CP g/ME Mj)	11.50^{c}	13.78^{b}	16.65^{a}	0.0001	0.34	0.77

Means with different superscripts in the same row are significantly different (P≤0.05)
LCP: Low CP; MCP: Medium CP; HCP: High CP
* FPCM: Fat and protein corrected milk

DISCUSSION

Feed intake, milk production and N utilization in response to dietary CP levels: In the present study dietary CP ranged from 9.0 to 17% in DM and the increasing levels were not effective in stimulating feed intake in the buffaloes which averaged 14.34 kg/day or 3.2 kg per 100 kg body weight. Parallel to this, milk production was also not influenced by increasing dietary CP. The results agree with Haque *et al.* (2018) who did not find changes in DMI of buffaloes when dietary CP was increased from 11 to 14% in DM. Several other studies in buffaloes (Bovera *et al.* 2002; Olmos and Broderick 2003; Leonardi *et al.* 2003; Bartocci *et al.* 2006;) and in cows (Burgos *et al.* 2007) observed no effect of dietary CP varying from 10.0 to 21.0% on feed consumption. Generally, DMI increases when basal diet deficient in soluble N is supplemented with CP. In the present study buffalo requirements for CP supporting feed intake was apparently satisfied at low dietary CP levels of 9-11%. Neglia *et al.* (2014) reported that buffaloes are able to cope better than cattle to diets that are deficient in protein. Campanile *et al.* (1998) inferred that buffalo adapt to lack of dietary protein easier than dairy cows and their requirements can be satisfied at low levels such as 9% CP in DM. The other explanation could be that the present diets were low in non-fiber fermentable carbohydrate (NFC) which may have constrained the capacity of rumen bacteria to efficiently utilize the increasing N supply to rumen from high CP diets. Meta-analysis by Allen (2000) showed that feed intake in dairy cows is influenced by several animal and dietary factors and that positive response to increasing dietary CP was found in 17 out of 25 studies.Failure to find an increase in milk yield with increasing CP intake in the present study may also explain the absence of change in feed intake (NRC, 2001)

Daily milk production averaged 9.13 liter and was not influenced by CP in the diet. Milk production was numerically low (8.86 liter/day) with high dietary CP (15-17%) but did not qualify statistical significance. However, milk yield was found negatively correlated (r^2=0.21; Figure 1) with CP intake status above buffalo requirement. Haque *et al.* (2018) observed that milk yield in buffaloes marginally reduced when dietary CP was raised from 11 % to 14% in DM. In the present study protein-energy ratios (CP g/ME Mj) were unbalanced due to conventional feeding practices and

Table 4 Average protein intake, milk urea-N (MUN), Milk production and N utilization efficiency (MNE) in peri-urban buffaloes at different months of fodder supply

	Berseem Fodder (November-June)				Maize/Sorghum Fodder (July-October)		Contrast (P value)			
	November -December	January - February	March - April	May – June	July - August	September - October	Linear	Quadratic	Cubic	Treatment x location
Total DMI kg/d	13.74^{b}	14.43^{a}	14.51^{a}	14.34^{a}	14.63^{a}	14.65^{a}	0.0001	0.0294	0.01	0.007
Crude protein Intake kg/d	1.45^{d}	1.73^{c}	1.91^{b}	2.27^{a}	1.71^{c}	1.74^{c}	0.0001	0.0001	0.31	0.483
N intake g/d	232^{d}	277^{c}	306^{b}	363^{a}	273^{c}	279^{c}	0.0001	0.0001	0.31	0.483
Milk Production lit/d	8.59^{c}	8.67^{c}	8.51^{c}	8.90^{c}	9.92^{b}	10.70^{a}	0.0001	0.0007	0.70	0.011
MUN mg N/100 ml	10.98^{d}	13.71^{c}	15.39^{b}	17.17^{a}	13.12^{c}	13.51^{c}	0.005	0.0001	0.098	0.001
MNE %	22.17^{a}	18.67^{b}	16.51^{c}	14.56^{d}	21.57^{a}	22.73^{a}	0.178	0.0001	0.173	0.072
Crude protein intake relative to requirement g/d	-121^{d}	152^{c}	355^{b}	660^{a}	-28^{d}	-88^{d}	0.953	0.0001	0.47	0.281
N excretion in feces and urine g/d	206^{d}	233^{c}	252^{b}	286^{a}	231^{c}	235^{c}	0.0001	0.0001	0.293	0.483
Feed Conversion Efficiency % (FPCM[*]/Feed)	0.81^{c}	0.79^{c}	0.77^{c}	0.79^{c}	0.88^{b}	0.95^{a}	0.0001	0.0006	0.77	0.022
Protein: Energy ratio (CP g/ME Mj)	11.23^{d}	12.69^{c}	13.89^{b}	16.66^{a}	13.55bc	13.56^{bc}	0.0001	0.0001	0.41	0.422

Means with different superscripts in the same row are significantly different ($P \leq 0.05$)

[*] FPCM: Fat and protein corrected milk

the ratio increased from 11.5 on low CP to 16.65 on high CP diets suggesting energy was limiting with increasing dietary CP which may have influenced milk response to higher protein feeding. This is supported by the findings of Brun-Lafleur *et al.* (2010) and Bartocci and Terramoccia (2010) that milk yield in buffalo increased when CP in the diets was raised combined with high energy suggesting an interaction of protein and energy supply for enhancing milk yield. Burgos *et al.*, (2007) and Leonardi *et al.* (2003) observed no effect on milk yield of dairy cows when dietary CP was increased from 13.5 to 19.4% and from 16.1 to 18.9%, respectively. Olmos Colmenero and Broderick (2006) found that the negative effect of dietary CP on milk yield in dairy cows was apparent with CP above 16.5%. These observations indicate that excess protein in dairy ration may not be cost effective in supporting milk production. The efficiency of energy utilization decreases with high CP due to the energy cost of urea synthesis from excessive ammonia in the liver (NRC, 2001) which may have further constrained energy availability for milk synthesis on high CP and energy deficient diets. This is explained by negative correlation of milk yield with CP intake above requirement in the present buffaloes (Figure 1).

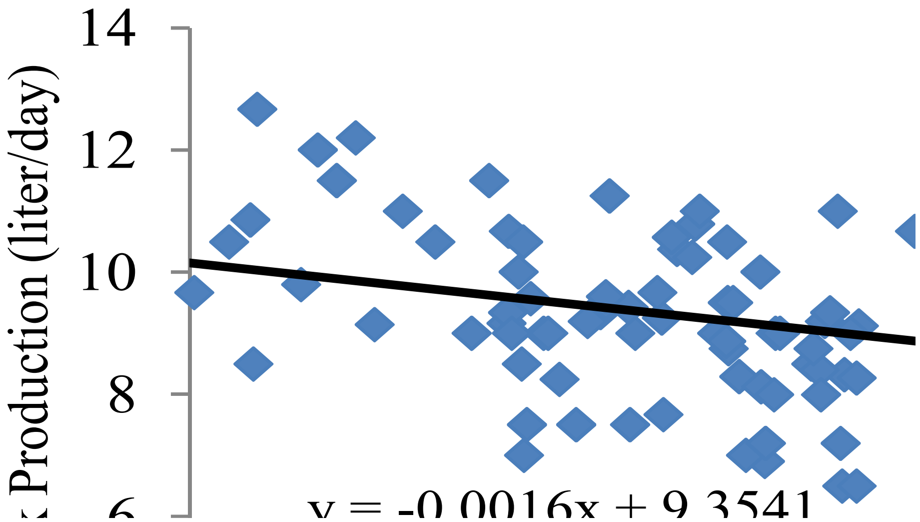

Figure 1. Relationship of protein intake balance with milk production in buffaloes

Milk urea-N (MUN) positively correlated to dietary CP (% in DM) in the buffaloes (r^2 =0.44; Figure 2). Similar linear relationship of MUN to increasing CP in diets was reported earlier in buffaloes (Campanile *et al.*1998; Dhali *et al.*2006; Rangel *et al.* 2013; Haque *et al.* 2018) and in cows (Hwang *et al.* 2000; Olmos Colmenero and Broderick 2006). MUN averaged 13.8 mg/100 ml and ranged from 11.79 on low CP (9-11%) diet to 17.07 on diet containing 15-17% CP. These values were within the MUN range reported in milking buffalo by Haque *et al.* (2018) and Roy *et al.* (2005). Several workers found larger variations in MUN in buffaloes ranging from 11.6 to 27.8 mg/100ml (Singhal *et al.* 1994); 12.0 to 25.4 mg/100 ml (Rangel *et al.*2013); 30.3 to 52.3 mg/100 ml (Sharma *et al.*2009). It is difficult to judge the variation in MUN concentrations among different studies because there could be more than one possible explanation for any particular MUN value. Baker*et al.* (1995) and Oltner and Wiktorsson (1983) inferred that protein intake, type of protein, degradability of protein and adequacy of fermentable energy influence MUN concentrations. In the present study majority of the buffaloes (48%) had MUN concentration above 14 mg/100 ml confirming high protein intake. MUN 7-10 mg/100 ml and 11-14 mg/100 ml was found in 16% and 36% buffaloes, respectively. The published data do not provide any clue for reference value of MUN in buffaloes due to high variation and limited observations.

There is strong evidence based close association of MUN with dietary CP that MUN can be used as tool for predicting CP intake in buffaloes (Dhali *et al.* 2006) and in dairy cows (Olmos Colmenero and Broderick 2006). In the present study 45% variation in MUN were attributable to CP intake. There was also an indication that the proportion of protein and energy in the diet influenced MUN results as evident from positive correlation of the two observations (r^2=0.40). Our observations are in line with the findings of Oltner and Wikstorson (1983) and Roy *et al.* (2005) who concluded that both protein intake and protein-energy ratio in combination influenced MUN in buffaloes and cows.

The efficiency of N use for milk (MNE) sharply declined as CP level in the buffalo diets increased (r^2 =0.56; Figure 3) and with each unit of CP intake (kg) the MNE declined by 10.7 percentage units. MNE averaged 22.7%, 18.7% and 14.39 % at low, medium and high dietary protein, respectively.

A similar response in MNE to increasing CP intake has been reported earlier in buffaloes (Haque *et al.* 2018) and in cows (Olmos Colmenero and Broderick

2006). The meta analysis by Huhtanen and Hristov (2009) estimated that concentration of dietary CP was negatively correlated and better predictor of MNE in dairy cows. In the present study a much stronger negative relationship of MNE was found with CP consumed in excess of requirement (r^2=0.97) suggesting that excess CP intake is detrimental to N use efficiency in buffaloes. The MNE across all CP levels averaged 19.46% which was close to the values reported by Bartocci *et al.* (2006); Mahr-un-Nisa *et al.* (2008); Garg *et al.* (2013); Neglia *et al.* (2014); Haque *et al.* (2018) in dairy buffaloes. High variation of MNE in buffaloes suggests large scope for improving N use efficiency impacted by a range of dairy practices. For example, Garg *et al.* (2013) observed that balancing dairy rations increased MNE from baseline level of 19% to 23% in smallholder buffaloes. The current values of MNE in buffalo (14.4 to 22.7%) were lower compared to Holstein cows (26.0 to 36.6%) with the similar range of CP concentrations (Olmos Colmenero and Borderick 2006) and the difference may be explained by higher milk yield in the cows. We found that improved MNE was associated with increase in milk yield (r^2=0.41) similar to that reported by Huhtanen and Hristov (2009). MNE in buffalo and cows can be logically compared based on the same level of milk production. Garg *et al.* (2013) calculated that MNE was not different between the two species and averaged 18% in both smallholder buffalo (n=480) and cows (n=280) in India producing same level of milk 8-12 lit/day. In our study the highest MNE was obtained with lowest level of dietary N intake across all the buffalo farms. This according to Powell *et al.* (2010) is because the rumen microbes are able to synthesize large proportion of N requirement from low level of dietary N consumed. In buffalo this could be more efficient and influenced by efficient urea recycling and microbial ecosystem in the rumen (Obitsu and Taniguchi, 2009). Thus feeding excess protein in buffaloes could be more damaging because their N requirements can be satisfied at lower CP in diet (Campanile *et al.* 1998). A number of studies have shown that reducing the CP content of the diet, above that needed to meet animal requirements, leads to better efficiency of N use for milk production and a lesser proportion excreted in urine and feaces (Krober *et al.* 2000; Kulling *et al.* 2001; Rotz 2004). In the present study MNE also significantly declined with increasing calculated N excretion in feaces and urine (r^2 =0.56) and was linked to excess CP intake in the buffaloes as discussed above.

In addition to N use efficiency the conversion of feed DM to milk (FCE) also determine the efficiency of dairy system and provide clue for calculating cost of milk production. In the current investigation FCE did not respond to increasing CP in the diet and was parallel to absence of changes in milk yield due to dietary CP. Numerically average FCE reduced with high CP levels but did not reach statistical significance due to interaction of dietary CP and farm locations ($P \leq 0.05$). This support the findings of Olmos Colmenero and Broderick, (2006) who reported lower feed efficiency at higher CP level in dairy cows. FCE in the buffaloes averaged 0.82 and was higher than (0.4 to 0.6) reported by Garg *et al.* (2013) in buffaloes but lower than 1.71 reported for high yielding Friesian cows by Olmos Colmenero and Boderick (2006). FCE in buffalo can be improved through ration balancing and the prospects are high in low producing buffaloes (Garg *et al.* 2013).

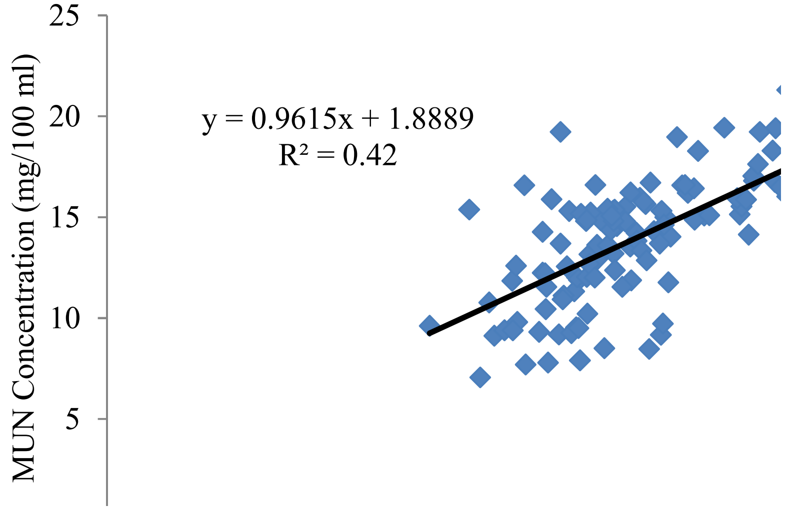

Figure 2. Relationship of Dietary CP level with Milk urea-N (MUN) concentrations in buffaloes

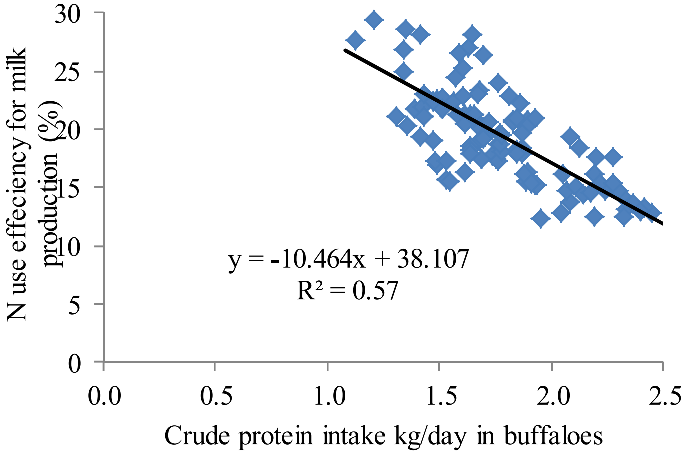

Figure 3. Effect of protein intake on N use efficiency (Milk N/Feed-N) for milk in buffaloes

Nitrogen consumption and utilization in relation to supply of seasonal fodders: Annual fodder supply varied from January to December and two major categories of fodders; berseem (*Trifolium alexendrium*) and maize (*Zea may*) constituted bulk of fodder supply for feeding the peri-urban buffaloes. Berseem availability commenced in mid-November and continued until June followed by maize during next four months (July to mid-November). Results summarized in Table 4 demonstrate that N intake, milk yield, MUN, MNE and N excretion in the buffaloes were significantly influenced by difference in location and months. Monthly variations in the results were associatedwith availability of the two major divergent forages.

With the start of supply of CP rich berseem (containing 18 to 23% CP in DM), the N consumption gradually increased from 232g/d in November-December until it peaked to 363 g/d during May-June when berseem supply was in abundance. Onward feeding of maize fodder (CP 8-10% in DM) during July to October was associated with lower N intake of 273-279 g/d. Compared to maize fodder, berseem feeding was associated with high CP intake ($P \leq 0.05$), high MUN ($P \leq 0.05$), lower milk yield ($P \leq 0.05$) and lower MNE ($P \leq 0.05$). In line with the present findings large variation in CP intake and MUN concentrations at different parts of the year was also reported in dairy cows by Arunvipas *et al.* (2002) and Yoon *et al.* (2004). In consensus to our observation, Roy *et al.* (2005) also recorded higher MUN with berseem than oats feeding in buffaloes and was attributed to higher CP contents in berseem.

Nitrogen utilization parameters indicate that high N supply from berseem was not efficiently utilized by the buffaloes under the existing conventional feeding system in peri-urban dairy farms. The main reason was that not only the quantity and composition of the conventional concentrate supplement offered to the buffaloes were almost constant throughout the year but also these were unbalanced and deficient in energy feed. Thus high N supply from berseem was not combined with increase in dietary energy which constrained the N utilization for milk (NRC, 2001) and is supported by low MNE results (Table 4). MUN concentrations were consistently high with berseem feeding which together with low MNE indicate that excess N could not be efficiently utilized for microbial protein synthesis in the rumen and was largely lost through urinary N excretion. Calculated N excretion in feaces and urine was 83% of the total N intake representing substantial N losses with the current feeding practices of peri-urban buffaloes. Olsmo Colmenero and Broderick (2006) calculated that increase in N intake with high CP diets was associated with 81% N lost as urinary urea. The high N losses through feces and urine calculated in the present investigation would adversely impact the environment. With the existing improper manure management system in peri-urban farms in the country, the high N excretion in buffaloes resulting from protein overfeeding can cause leaching and soil nutrient imbalance and will be converted to nitrous oxide which has much higher GHG potential than carbon dioxide. On the basis of the findings of Buttler *et al.* (1995) and Qureshi *et al.* (2002) it can be postulated that high MUN levels with berseem that peaked to 17.7 mg/100 ml would adversely affect fertility of the peri-urban buffaloes. Throughout the duration of berseem feeding the feed conversion efficiency remained significantly lower than noted when maize fodder constituted major supply and was apparently linked with increasing protein-energy ratios caused by higher CP in berseem based diets explaining the difference in milk yield and MNE in the buffaloes.

Conclusions: Findings of the present study concluded that current conventional feeding practices in peri-urban farms were not tailored to the need of milking buffaloes. Under the conditions of the present study CP requirements of buffaloes were satisfied at low dietary CP of 9-11% in DM. For large part of the year most buffaloes were overfed with protein when berseem constituted fodder supply. Feeding excessive protein above the buffalo requirements did not increase feed intake, reduced milk yield and adversely affected the efficiency of N utilization for milk and contributed to increasing protein-energy ratio in the diet. Positive correlation of MUN with CP % in DM and CP intake and protein-energy ratios suggested that MUN can be used as a valued parameter to assess on-farm protein status of buffaloes and highlight the need of adjusting the protein-energy ratio of the diet. It is proposed that protein-energy ratio of conventional dairy rations need to be balanced by including energy source to improve N use efficiency and enhance productive performance of milking buffaloes. Matching feed supplements with quality of seasonal fodders would avoid wasteful use of feed–N in the buffaloes while still achieving high milk production at lower feed cost.

Acknowledgments: The research was conducted with the financial assistance of Pakistan Science Foundation, Islamabad**.**

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