Influence of Milk Yield, Parity, Stage of Lactation and Body Weight on Urea and Protein Concentration in Milk of Murrah Buffaloes

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ABSTRACT : The present study was carried out to investigate the effect of test day milk yield, test day evening milk yield, parity, stage of lactation and body weight on milk urea and milk protein concentration. A total of 319 milk samples was collected from buffaloes over four month's period and subjected to urea and protein analysis. Milk urea concentration (mg/dl) was significantly (p<0.01) increased with increasing test day milk yield. The lowest value (57.03±1.13) was observed in the milk yield group ≤4.5 kg/day and the highest value (64.15±1.13) in the group 7.7-10.7 kg/day. However, test day evening milk yield had no significant effect on milk urea concentration. Milk protein did not vary significantly with the test day milk yield as well as test day evening milk yield. A clear decreasing trend of milk urea concentration (mg/dl) was found with the increasing parity. The highest MU concentration (64.03±1.14) was found in the first parity and the lowest (55.67±1.22) was found in the sixth and above parity. Whereas, stage of lactation had no effect on milk urea concentration. Moreover, parity and stage of lactation did not have any significant effect on milk protein concentration. Body weight (kg) was also found negatively (p<0.05) related with urea content (mg/dl) in milk. The highest mean MU concentration (64.34±0.88) was found when body weight was between 532 and 598 kg and lower mean values (59.24±0.94 and 59.33±1.23) were observed in 599 to 665 kg and ≥666 kg group. Body weight also had significant (p<0.05) effect on milk protein content. The highest milk protein content (%) was found in ≥666 kg group and the lowest in <531 kg group. In conclusion, for proper interpretation of milk urea values to monitor protein nutrition status of the buffaloes parity, milk yield and body weight should be considered. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 9 : 1285-1290)***

Key Words : Milk Urea, Murrah Buffalo, Milk Protein, Parity, Body Weight, Stage of Lactation

INTRODUCTION

India has the privilege of having best breeds of buffaloes of which Murrah is the most popular because of its superiority on commercial ground. Buffalo milk contributes more than half of the total milk production of India (All India dairy business directory, 2001). Surprisingly, in India buffalo farming is practised on non-scientific lines and importance of animal health, reproduction or nutritional experts in identifying or addressing the relevant problems is yet to be recognised. Under conventional farming diets are not formulated according to the requirements of individual animal, resulting in adverse effect on overall production, health and reproduction.

Nutrients supply is a component of any management system that needs to be carefully evaluated. Protein is an expensive ingredient of dairy cattle feeds and thus overfeeding of protein could be costly to the producers. Several studies (Ferguson and Chalupa, 1989; Canfield et al., 1990; Dhali, 2001; Gooden et al., 2001) have shown an association between protein nutrition and reproductive performance of dairy cows, feeding excess amounts of protein may be detrimental to the fertility of cows and buffaloes (Qureshi et al., 2002). High levels of milk urea nitrogen (MUN) indicate inefficient protein use as a result of an imbalance between protein and energy, which may adversely affect fertility, feed costs, production efficiency and the environment. Any parameter (i.e. MUN) that provides information to fine tune dietary protein requirements will reduce the feed costs.

Excess dietary protein not needed by the ruminants is usually broken down into ammonia. Ammonia is toxic to animal tissues and therefore is rapidly converted into urea (an end product of protein metabolism) in the liver and subsequently comes into the blood and milk. Thus urea is then measurable in both the blood stream and milk. The concentration of urea in milk not only depends on the amount of protein ingested in relation to requirements but also on the protein and energy ratio in the ration, degradability of the protein and the availability of fermentable metabolisable energy in the diet (Oltner et al., 1985; Moore and Verga, 1996). So, milk urea concentration can be used as a tool to monitor protein feeding efficiency and dietary protein-energy ratio in dairy cows (Roseler et al., 1993; Moore and Verga, 1996; Hof et al., 1997).

The majority of the aforementioned studies were performed on individual animals under controlled experimental condition. However, in field condition, the interpretation of milk urea concentration may be hindered by some non-nutritional factors like season (Hwang et al.,

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2000, Dhali, 2001 and Gooden et al., 2001), parity (Kaim et al., 1983 and Canfield et al., 1990), stage of lactation (Kalchreuter, 1990 and Dhali, 2001) and body weight (Oltner, et al., 1985 and Broderick and Clayton, 1997) as reported by several workers from cows. The purpose of this study was to evaluate the effect of parity, stage of lactation and milk yield on MU and milk protein concentration. As buffaloes differ considerably in size, the possible effect of live weight on MU concentration also need to be studied.

MATERIALS AND METHODS

Animals and their management

The experiment was conducted on Murrah buffaloes maintained at cattle yard, National Dairy Research Institute, Karnal, Haryana (India), during four months period from 15th January to 15th May. All the milking Murrah buffaloes available during the period of sampling except the diseased one, had sent to clinics for treatment were used for the experiment. Irrespective of the parity, milk yield, stage of lactation and body weight of the buffaloes, the milk samples were collected. The buffaloes were maintained in loose housing system, which covers large, open and brick paved paddock and were fed farm grown seasonal green fodders viz., berseem (*Trifolium alexandrium*, CP 15.80 to 17.48% and DM 12.30 to 16.64%) and Oats (*Avana sativa*, CP 8.02 to 8.20 and DM 20.51 to 27.60%) *ad libitum* and provided to the mangers in paddock twice daily, at morning (9.00 to 9.30 a.m.) and afternoon (3.00 to 3.30 p.m.). Concentrate mixture was fed in two parts to the buffaloes separately at the time of milking (morning 5.00 to 6.00 a.m. and evening 6.30 to 7.30 p.m.). Animals had free access to water throughout the day. Weaning is practised just after parturition. Machine and hand milking is practised in the farm.

Collection of milk samples

Samples were collected separately for all the buffaloes from the evening milking (6.30 to 7.30 p.m.), from the milk weighing buckets after complete milking and thorough mixing, in 100 ml capped plastic bottles. Samples were kept at 4°C until prepared for urea and protein analysis on the same day. Sampling of all the animals were completed within 5 days for a particular month and the gap between two successive sampling was 25 to 30 days.

Collection of data

Data regarding the following parameters were collected for individual buffaloes from the farm records.

Test day milk yield
Test day evening milk yield
Parity
Stage of lactation
Body weight

Estimation of milk urea

Samples were prepared on the same day of collection. Milk samples were warmed at room temperature (27° to 30°C) and mixed well. Milk samples were processed for urea estimation through 12% Trichloroacetitic acid (TCA) precipitation, filtration and centrifugation and kept in small plastic bottles (20 ml) at 4°C for 10 to 15 days, until analysed. Samples were subjected to urea estimation by a modified colorimetric DMAB (p-Dimethylaminobenzalde hyde) assay (Bector et al., 1998). Milk protein was determined from the same milk sample by Kjeldahl method as per AOAC (1995).

Statistical analysis

The data were subjected to least squares analysis of variance (Harvey, 1987) using fixed least squares model procedure. The mathematical model for the analysis was as under.

Mathematical model

$$Y_{ijklm}=\mu+E_i+D_j+P_k+L_l+e_{ijklm}$$

Where,

Y_{ijklm}=Observation under i^{th} test day evening milk yield, j^{th} test day milk yield, k^{th} parity and l^{th} stage of lactation

μ=Overall mean

E_i=Fixed effect of i^{th} test day evening milk yield (i=1, 2, 3 and 4)

D_j=Fixed effect of j^{th} test day milk yield (j=1, 2, 3 and 4)

P_k=Fixed effect of k^{th} parity (k=1, 2, 3, 4, 5 & 6 and above)

L_l=Fixed effect of l^{th} stage of lactation (l=1, 2 and 3)

e_{ijklm}=Random error, which is assumed to be normally and independently distributed with zero mean and constant variance σ^2_e.

To study the effect of body weight on MU and milk protein concentration the following model was used.

Mathematical model

$$Y_{ij}=\mu+B_i+e_{ij}$$

Y_{ij}=Observation under i^{th} body weight

μ=Overall mean

B_i=Fixed effect of i^{th} body weight (i=1, 2, 3 & 4)

e_{ij}=Random error, which is assumed to be normally and independently distributed with zero mean and constant variance σ^2_e

The least square means for different traits at different levels were compared by using Duncan's Multiple Range

Table 1. Effect of test day evening milk yield and test day milk yield on milk urea and milk protein concentration in Murrah buffaloes

Groups	Milk urea (mg/dl)	Milk protein (%)
Test day milk yield (kg/day)		
T_1 (≤4.5)	57.03±1.46[a]	4.15±0.08
T_2 (4.6-7.6)	60.40±0.99[b]	4.16±0.05
T_3 (7.7-10.7)	64.15±1.13[c]	4.11±0.06
T_4 (≥10.8)	63.13±1.59[bc]	4.18±0.09
Test day evening milk yield (kg/day)		
E_1 (≤ 2.7)	61.99±1.93	4.36±0.11
E_2 (2.8-4.8)	62.17±1.00	4.17±0.05
E_3 (4.9-6.9)	59.82±1.02	4.06±0.06
E_4 (≥7.0)	60.74±1.40	4.00±0.08

a, b, c Indicates values with different superscripts within a column differ significantly (p<0.01)

Table 2. Correlations of milk urea concentration with milk yield and milk protein content

Particulars	Correlation coefficient (r)
Milk urea: Test day milk yield	0.14*
Milk urea: Test day evening milk yield	0.06
Milk urea: Milk protein	0.11
Milk protein: Test day milk yield	-0.11
Milk protein: Test day evening milk yield	-0.09

* p<0.05

Test (DMRT) as modified by Kramer (1957).

For determining the association between the different traits the correlation analysis was performed.

RESULTS AND DISCUSSION

A total of 319 milk samples was collected over four months period and analysed during the study. For each month, sampling was completed within 5 days and the gap between the monthly sampling was 25 to 30 days. Test day milk yields were classified into four groups, T_1 (≤4.5 kg/day), T_2 (4.6-7.6 kg/day), T_3 (7.7-10.7 kg/day) and T_4 (≥10.8 kg/day) considering mean and standard deviation. Test day evening milk yields were classified into four groups, E_1 (≤2.7 kg/day), E_2 (2.8-4.8 kg/day), E_3 (4.9-6.9 kg/day) and E_4 (≥7.0 kg/day) on the basis of mean and standard deviation.

The average milk urea (MU) concentration (mg/dl) and milk protein content (%) in different groups of test day milk yield and test day evening milk yield have been presented in Table 1. It was evident from results that the MU concentration varied significantly (p<0.01) among the groups for test day milk yield and an increasing trend was observed with increasing test day milk yield. The highest value of MU concentration (mg/dl) was found in T_3 (7.7-10.7 kg/day) group and the lowest in T_1 (≤4.5 kg/day) group for the test day milk yield. However, test day evening milk yield had no significant effect on MU concentration. For the test day evening milk yield (kg/day), the highest concentration was found in group E_2 and the lowest in group E_3 and no definite pattern of change was observed (Table 1).

Milk protein (%) was also found to be decreasing non-significantly with the increase of test day evening milk yield. However, no such trend was found for test day milk yield. The highest value was found in T_4 group and the lowest in T_3 group (Table 1).

MU concentrations were significantly correlated (r=0.14; p<0.05) with the test day milk yield, but almost no correlation (r=0.06) found with test day evening milk yield (Table 2). Milk protein concentration showed non-significant correlation with MU (Table 2). Several workers have reported some relation of MU with milk yield and milk composition. But a definite pattern of relationship is yet to be established. Oltner et al. (1985) found that MU was positively correlated with milk yield. Broderick and Clayton (1997) reported a significant positive correlation between fat corrected milk yield and milk urea nitrogen (MUN). MU concentration was found positively correlated with test day milk yield and test day evening milk yield (Dhali, 2001). In contrast, a statistically significant negative correlation between the MU content and milk yield was found by Pestevsek et al. (1990), Diab Ismail and Hillers (1996) and Broderick and Clayton (1997). In the present study, MU content varied significantly (p<0.01) with test day milk yield, but not with test day evening milk yield. The findings follow the pattern as reported by Oltner et al. (1985), Gustafsson and Palmquist (1993) and Carlsson et al. (1995).

The positive association between MU and test day milk yield may be attributed to increase milk production that resulted from increased levels of dietary protein fed (Chalupa, 1984). Supplemental protein may increase milk yield by providing more amino acid (AA) for milk protein synthesis, by increasing the available energy through deamination of AA or by altering the efficiency of utilization of absorbed nutrients (Chalupa, 1984). The efficiency of utilization for metabolisable protein for milk production (0.68) is less than that of maintenance (1.00) (McDonald et al., 1995). So, as the milk production increases the overall protein utilization efficiency decreases and subsequently that leads more drainage of nitrogen in terms of urea through urine and milk. Because of some letdown problems, most of the low yielding buffaloes and buffaloes were in late lactation, which were milked only once in a day, either in the morning or evening. So, evening milk yield did not properly reflect the actual milk yield by the individual buffaloes. This may be the cause of non-significant relation between MU and test day evening milk yield. Reports on the association between MU and milk

Table 3. Effect of parity and stage of lactation on milk urea and milk protein concentrations in Murrah buffaloes

Particulars	Milk urea (mg/dl)	Milk protein (%)
Parity		
1	64.03±1.14[a]	4.03±0.06
2	63.84±1.20[a]	4.18±0.07
3	62.57±1.69[ab]	4.31±0.09
4	62.98±1.46[a]	4.21±0.08
5	57.98±1.66[bc]	4.12±0.09
6 & above	55.67±1.22[c]	4.06±0.07
Stage of lactation (months)		
1-3 (early)	60.03±1.20	4.20±0.07
4-6 (mid)	62.07±0.98	4.13±0.05
7 & above (late)	61.44±0.94	4.13±0.05

[a, b, c] Indicates values with different superscripts within a column differ significantly ($p<0.05$).

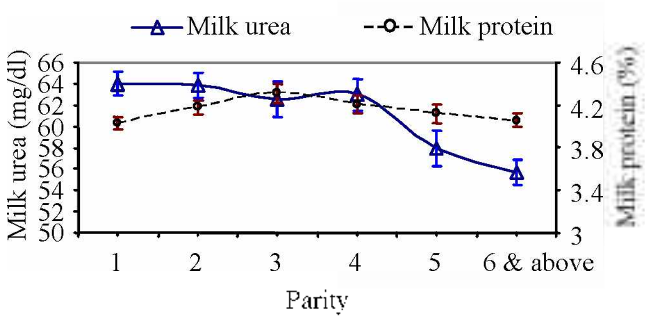

Figure 1. Effect of parity on milk urea and milk protein concentrations in Murrah buffaloes.

protein vary between positive (Pestevsek et al., 1990; Zadnik et al., 1993) and no association (Pecorari et al., 1993; Dhali, 2001). Whereas in dairy goats, an negative association was found between milk protein content, determined by the type of αs1-casein and urea content in blood and milk (Sauvant et al., 1992). In our study, a non-significant low positive correlation ($r=0.11$) was found between MU and milk protein content.

The effect of parity was studied at six different levels viz., 1, 2, 3, 4, 5 and 6 and above. The effect of lactation stage was studied at three different levels: 1-3 months (early), 4-6 months (mid) and 7 months and above (late). From Table 3, it is evident that the effect of parity was significant ($p<0.05$) on MU concentration. A clear decreasing trend of MU concentration (mg/dl) was found with the increasing parity (Figure 1). The highest MU concentration (64.03±1.14) was found in first parity, whereas, the lowest (55.67±1.22) in 6th and above parity. However, the effect of stage of lactation was not significant on MU concentration. But the lowest MU concentration (mg/dl) value (60.03±1.20) was found in early stage of lactation (1-3 months) and the highest (62.07±0.98) in mid lactation (4-6 months). Besides this, parity and stage of lactation did not have any significant effect on milk protein content. The milk protein content were lowest during first parity and mid and late stage of lactation (Table 3). There was a significant ($p<0.01$) negative correlation ($r=-0.31$,) between MU and parity and negligible positive correlation ($r=0.07$) between MU and stage of lactation.

Blood urea and MU concentration could be affected by parity and stage of lactation as indicated by few earlier reports. Kaim et al. (1983), Schepers and Meijer (1998) and Canfield et al., (1990) reported that the number of lactation did not influence the plasma urea or MU concentrations. But in case of first calver, it was found to be lower by 0.76 mmol per litre than multiparous cows (Oltner et al., 1985). Whitaker et al. (1995) also reported a similar trend. Gooden et al. (2001) found a statistically significant, but numerically small differences in MU due to parity. But contrast to this, in our study, MU concentration was significantly higher in the first calver than multiparous buffaloes. This result is supported by the finding by Broderick and Clayton (1997), who reported that parity was negatively related with MU nitrogen concentration. This may be due to the fact that animals' body weight increases with the parity and MU content decreases with the increasing live weight (Oltner et al., 1985).

Serum urea concentration was found significantly influenced by the lactation stage (Reinartz and Hofmann, 1989). There was a trend of high MU values at the beginning of the lactation reported by Kalchreuter (1990). Since cows were on negative energy balance during the first 100 days of lactation and surplus energy intake in the last quarter of lactation. An increase in urea value was observed in the first 8 weeks of lactation (Ndibualonji and Godeau, 1993) and concentration of urea in milk and blood serum were to reach peak at 12 week postpartum and decreased slowly thereafter (Rajcevic et al., 1993). However, other workers found a different trend. During the first month of lactation lower MU concentration was found by Carlsson et al. (1995) and Whitaker et al. (1995) reported that cows in early lactation often have much lower MU level. Broderick and Clayton (1997) and Dhali (2001) reported significantly lower MU concentrations during the early stage of lactation and days in lactation positively related to MU nitrogen concentration. In contrast, no relation was reported between urea concentration in milk and stage of lactation by Erbersdobler et al. (1990) and values were relatively constant between 200 to 300 mg/l. Coustumier (1996) reported no correlation between lactation stage and urea levels except just after calving. Similar to our study, Schepers and Meijer (1998) also reported that stage of lactation had no influence on MU concentration.

Body weight should also be taken into account while interpreting the MU values, because buffaloes vary considerably in size. Few workers (Oltner et al., 1985, Broderick; Clayton, 1997; Dhali, 2001) reported that live

Table 4. Effect of body weight on milk urea and milk protein concentrations in Murrah buffaloes.

Body weight (kg)	Milk urea (mg/dl)	Milk protein (%)
B_1 (≤531)	62.42±1.26[a]	3.95±0.07[a]
B_2 (532-598)	64.34±0.88[a]	4.07±0.05[ab]
B_3 (599-665)	59.24±0.94[b]	4.16±0.05[b]
B_4 (≥ 666)	59.33±1.23[b]	4.19±0.06[b]

[a, b] Indicates values with different superscripts within a column differ significantly ($p<0.05$).

weight might have some effect on MU concentration. In present study, a total of 319 samples were analyzed to investigate the effect of body weight on MU concentration. The body weights of buffaloes were classified into four categories considering the mean and standard deviation of the data. These groups were B_1 (≤531 kg), B_2 (532 to 598 kg), B_3 (599 to 665 kg) and B_4 (≥666 kg). The average MU concentration (mg/dl) and milk protein content (%) for four different groups were presented in Table 4. The MU concentration was significantly ($p<0.05$) influenced by body weight. The highest MU concentration was found in B_2 category (64.34±0.88). Besides this, the body weight also had a significant ($p<0.05$) effect on protein content in milk and an increasing trend was observed with the increasing body weight (Table 4). The highest milk protein (%) value was found in the category B_4 and lowest in B_1 category. On the other hand, body weight had significant negative correlation ($p<0.05$; $r=-0.20$) with MU concentration and significant positive correlation ($p<0.05$; $r=0.15$) with milk protein.

MU concentrations were found to be related negatively with body weight. MU level decreases with increase in live weight at the rate of -0.06 mmol per litre per 100 kg (Oltner et al., 1985). Body weight and body weight changes were negatively related with plasma urea nitrogen concentration (Diab Ismail and Hillers, 1996). Broderick and Clayton (1997) also found a negative relationship between body weight and blood urea nitrogen. Dhali (2001) reported a weak negative correlation between body weight and MU concentration. In the present study also, the result follow the same pattern as reported by the earlier workers. The negative relationship between MU concentration and body weight might be explained partly on the basis of a simple dilution. In a big buffalo, the compartment for urea distribution is naturally larger than in a small buffalo, and for a given amount of urea is formed in the liver, concentration in blood and milk will obviously be lower. With simple dilution, the effect of body weight on MU concentration will also depend on the absolute amount of urea to be distributed in the body. Further, on diets producing higher urea and consequently higher MU concentrations, the effect of live weight on MU will be greater on a concentration basis (Oltner et al., 1985).

CONCLUSION

MU concentration varies significantly with milk yield, parity, and body weight. Hence, researchers should consider monitoring for these variables as potential cofounders when exploring the relationship between MU and nutritional management or measures of performance such as production or reproduction.

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