MONITORING OF PROTEIN NUTRITION BY MILK UREA NITROGEN AND MILK PROTEIN IN ANATOLIAN BUFFALOES

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ABSTRACT

There is an increasing trend in milk urea concentration has been used as an indicator for monitoring protein nutrition in ruminants because of linear relationship between milk urea concentration and dietary crude protein level. Milk urea analysis is easy to monitor and can be performed on buffaloes and has some advantageous under field conditions for estimating protein nutrition status of buffaloes. Thus, the objective of this study was to investigate the effect of nutrition status on milk urea concentration and milk protein content, and to monitor protein nutrition by determining milk urea nitrogen concentration and milk protein in Anatolian buffaloes. Ten lactating (in the 3rd lactation and 15±3 days in milk) Anatolian buffaloes having approximately accordant live body weights were used in this study.

Milk samples were collected biweekly to determine the milk urea concentration and milk nutrients. Individual samples of each feedstuffs consumed by lactating buffaloes were collected biweekly during the experiment. There was no change in milk fat, total solid, lactose and ash during the study. There was a positive correlation (P<0.001) not only between diet crude protein and milk urea concentration, but also between diet crude protein and milk protein values. There was a negative correlation (P<0.001) between energy value of diet and milk urea concentration, and also between energy value of diet and milk protein value. In conclusion, milk urea and protein analyses may provide an opportunity to get knowledge about dietary protein intake.

Keywords: Anatolian buffalo, milk urea concentration, milk composition, protein nutrition

INTRODUCTION

Bubalus bubalis is the name of domesticated buffalo and buffaloes are classified into two distinct classes: Swamp buffalo and River buffalo. While buffaloes are spread worldwide, the majority of the world's buffalo population is located in Asia with India, Pakistan and China. In Turkey, the buffaloes are mainly grown in Black sea, Central Anatolia and Aegean regions. The buffaloes in Samsun Province of Turkey are named as Anatolian buffaloes and are originated from Mediterranean buffaloes which are a subgroup of River buffaloes (Borghese and Mazi, 2005). Milked buffalo population and milk production of Turkey in 2013 are about 51.940 and 51.950 t, respectively. Although the most buffalo population is in Samsun province of Turkey (adult 11.349 and milked 6804), milk production in 2013 is only about 6.735 t (Tuik, 2013). As conventional, the majority of Anatolian Buffaloes in Doganca

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located in Bafra in Samsun Province are left to the Kizilirmak Delta from April to November for summer season (extensive). During this time, the buffaloes are fed on pasture of Delta and not milked, then, they are kept in barns for winter season (intensive). The buffaloes kept in barn for winter season are fed with very low quality roughages; some agricultural crop-residues such as rice and wheat straws and industrial by-products containing high fibrous materials which causes poor milk production. Therefore, buffalo milk production obtained from this location constitutes a very small part of total milk production.

There is an increasing trend in using milk urea concentration instead of blood urea concentration for monitoring of protein nutrition in dairy cows. Both blood and milk urea concentrations may be influenced by the amount of crude protein in the diet (Baker *et al*., 1995). Dietary protein level of lactating buffaloes may be equal to or below 12% (dry matter basis) according to Verna *et al*. (1992) wheras Rai and Aggarwal (1991) have suggested that the crude protein level of diet on dry matter basis must be between 11 and 14%.

The objective of this study was to investigate the effect of nutrition on milk urea concentration and milk protein content, and to monitor protein nutrition by determining milk urea nitrogen concentration and milk protein in Anatolian buffaloes.

MATERIALS AND METHODS

Experimental design

This study was conducted at a private farm in Doganca located in Bafra in Samsun Province of Turkey from April to November when Anatolian buffaloes were kept in barn for winter season. Ten lactating (in the 3rd lactation and 15±3 days in milk) Anatolian buffaloes whose have approximately accordant live body weights were used in the study. Composition of diets and feeding schedule of dairy farm were not changed during the study. Buffaloes were kept in individual pens and were fed individually daily at 8.00 am and 6.00 pm throughout the study. Fresh drinking water were provided as *ad libitum.*

Collection of milk and analytical techniques

Milk samples were collected to represent whole milk biweekly in the morning milking because of once a day milking schedule of dairy farm. The buffaloes were hand-milked. The daily milk production of the buffaloes was also recorded. Milk samples were collected separately in capped plastic bottle for individual sampling. After collection, milk samples were kept in a portable refrigerator at 4^{o}C and transported to laboratory. Fresh milk samples were analyzed for their chemical composition. Total solid and ash contents were determined by gravimetric method, milk protein was estimated according to the Kjeldahl method (FAO, 2011). For determination of milk fat content, 10 ml of milk was precipitated with 20% tricarboxylic acid (15 ml), filtered through a filter paper (Whatman, No.40) and the precipitate was subjected to ether extraction (Mech *et al*., 2007). Lactose was measured by polarimetric method. Milk urea concentration was determined by using the kit in minifoodlab portable analyzer.

Collection of feed and analytical techniques

Individual samples of each feedstuffs consumed by lactating buffaloes were collected during the experiment. Feed intake of the buffaloes were recorded daily.

The content of dry matter, crude ash,

crude protein, crude fiber and crude fat in the diets were analyzed according to FAO (2011). Acid detergent fiber (ADF) content were analyzed by using methods reported by Van Soest and Robertson (1981). The metabolizable energy level of feedstuffs were calculated by according to TSI (1991).

Statistical analysis

Data of milk composition were summarized with descriptive statistics for means, and the standard deviation of the means were analyzed with analysis of variance (ANOVA), using the Least Square Method of the GLM procedure of the SPSS. The differences between the groups were analyzed by tukey t test. Pearson's correlation coefficient was performed to determine the relationships among dietary protein, energy consumption, milk urea nitrogen, milk protein content.

RESULTS AND DISCUSSON

The chemical composition of the forage and concentrate consumed by the lactating buffaloes were given in Table 1 and 2, respectively. Dry matter, crude protein and metabolisable energy values of diets consumed by the lactating buffaloes were presented in Table 3. Total solid, lactose and ash contents of milk samples were presented in Table 4. It has been reported that buffalo milk contains higher protein, fat, lactose and total solid content than cow milk (Lidmark-Manson *et al*., 2003). However, Sekerden *et al*. (1999) have reported that nutrition, season, lactation period, milking frequency and method may affect on chemical composition of buffalo milk. Milk fat can be affected by many factors (particularly season and lactation period). During this study, the mean fat, total solid and protein contents in winter when buffaloes were kept in barn were similar to those reported by Sarfarz *et al*., 2008 and Gurler *et al*., 2013. During the experiment, the mean ash values of Anatolian buffaloes' milk were similar to the values reported by Sarfarz *et al*., 2008 and Gurler *et al*., 2013, but higher than the result of Sekerden and Avsar (2008). In the present study, the mean lactose level of buffalo milk was higher than that of Gurler *et al*. (2013) who has reported the same parameter as 4.19%. This result for lactose content was similar to that of Han *et al*., 2007. During the study, although there was no change in total solid, ash, fat and lactose contents of milk samples, there was a decrease in crude protein content of milk in relation to reducing crude protein level of diets.

Milk urea nitrogen, crude protein and crude fat values of milk samples were given in Table 5. Roy *et al*. (2003) have stated that milk urea concentration can be used to demonstrate the imbalance in protein nutrition and nutritional failures. In the present study, there was a positive correlation not only between dietary crude protein levels and milk urea concentration (correlation coefficient 0.548), but also between dietary crude protein levels and milk protein content (correlation coefficient 0.397). This result of the study was similar to that of Sporndly (1989) and Campanile *et al*., 1998.

In ruminants, dietary intake and the use of non protein nitrogen, protein metabolism and amino acid catabolism in mammary glands are sources for urea of milk. In other words, milk urea concentration is associated indirectly with the use of nitrogen in rumen (Kohn *et al*., 2007). Milk is more practical fluid than blood or urine for urea analysis (Roy *et al*., 2005). Drudik *et al*. (2014) have reported that a low milk urea nitrogen (less than 12 mg/dl) indicated low crude protein, improper

Table 1. The chemical composition (%) and metabolizable energy value (MJ) of forages on dry matter basis.

Forages	DM	Ash	OM	CP	ADF_{OM}	ME
CS	94.31	5.14	89.17	8.52	26.66	11.13
CS (nature)	26.96	1.46	25.50	2.43	7.62	11.13
RS	93.53	16.68	76.85	4.15	39.40	8.36

CS: corn silage, RS: rice straw, DM: dry matter, OM: organic matter, CP: crude protein, ADF: acid detergent fiber, ME: metabolizable energy.

Table 2. The chemical composition (%) and metabolizable energy value (MJ) of concentrates on dry matter basis.

Concentrates	DM	Ash	OM	CP	CF	CF	ME
C1	91.20	7.45	83.75	18.02	14.05	2.10	10.66
C2	90.64	8.70	81.94	15.40	9.40	1.85	10.28

C1: concentrate 1, C2: concentrate 2, DM: dry matter, OM: organic matter, CP: crude protein, CF: crude fiber, ME: metabolizable energy.

Table 3. Dry matter, crude protein and metabolisable energy values of diets consumed by the lactating buffaloes.

Weeks	CS,kg	RS,kg	C1,kg	C2, kg	DM, kg/d	CP g/d	ME MJ/d
0-2	1.61	8.41	2.28	-	12.30	968.30	112.51
2-4	1.61	8.41	2.28	-	12.30	968.30	112.51
4-6	1.61	9.35	1.82	-	12,78	919.09	115.47
6-8	1.61	9,35	-	2.26	13.22	943.85	119.30
8-10	1.61	10.28	-	1.35	13.24	830.20	117.72
10-12	3.23	12.15	-	-	15.38	829.90	137.51
12-14	3.23	12.15	-	-	15.38	829.90	137.51
14-16	3.23	12.15	-	-	15.38	829.90	137.51
16-18	2.69	12.15	-	-	14.84	781.25	131.50
18-20	2.69	12.15	-	-	14.84	781.25	131.50

CS: corn silage, RS: rice straw, C1: concentrate 1, C2: concentrate 2, DM: dry matter, CP: crude protein, ME: metabolizable energy.

Table 4. Total solid, lactose, ash and density values of milk samples.

Weeks	Total Solid x ± Sx	Lactose x ± Sx	Ash x ± Sx
2	17.60 ± 0.35	4.86 ± 0.03	0.79 ± 0.01
4	17.59 ± 0.34	4.88 ± 0.04	0.79 ± 0.01
6	17.56 ± 0.41	4.88 ± 0.05	0.78 ± 0.02
8	17.67 ± 0.47	4.86 ± 0.05	0.79 ± 0.01
10	17.79 ± 0.33	4.87 ± 0.04	0.78 ± 0.01
12	17.59 ± 0.50	4.87 ± 0.06	0.77 ± 0.01
14	17.66 ± 0.42	4.88 ± 0.04	0.78 ± 0.02
16	17.60 ± 0.44	4.88 ± 0.04	0.78 ± 0.01
18	17.63 ± 0.49	4.89 ± 0.04	0.78 ± 0.01
20	17.61 ± 0.52	4.89 ± 0.03	0.78 ± 0.01
P value	0.991	0.899	0.306

Table 5. Milk urea concentration, crude protein and crude fat values of milk samples.

Weeks	Milk urea concentration x ± Sd	Crude Protein x ± Sd	Crude Fat x ± Sd
2nd	12.39 ± 5.01[a]	4.55 ± 0.06[a]	7.05 ± 0.26
4th	12.92 ± 4.05[a]	4.55 ± 0.05[a]	7.05 ± 0.28
6 th	8.86 ± 2.51[ab]	4.52 ± 0.06[ab]	6.88 ± 0.33
8 th	9.02 ± 2.83[ab]	4.50 ± 0.07[ab]	7.03 ± 0.43
10 th	7.68 ± 2.98[b]	4.51 ± 0.07[ab]	7.22 ± 0.27
12 th	7.44 ± 2.54[b]	4.50 ± 0.07[ab]	7.02 ± 0.42
14 th	6.89 ± 2.38[b]	4.48 ± 0.06[ab]	7.02 ± 0.46
16 th	8.00 ± 2.00[b]	4.48 ± 0.05[ab]	7.03 ± 0.46
18 th	7.06 ± 1.77[b]	4.45 ± 0.04[b]	7.09 ± 0.51
20 th	6.51 ± 1.44[b]	4.46 ± 0.05[b]	7.12 ± 0.54
P value	0.000	0.004	0.915

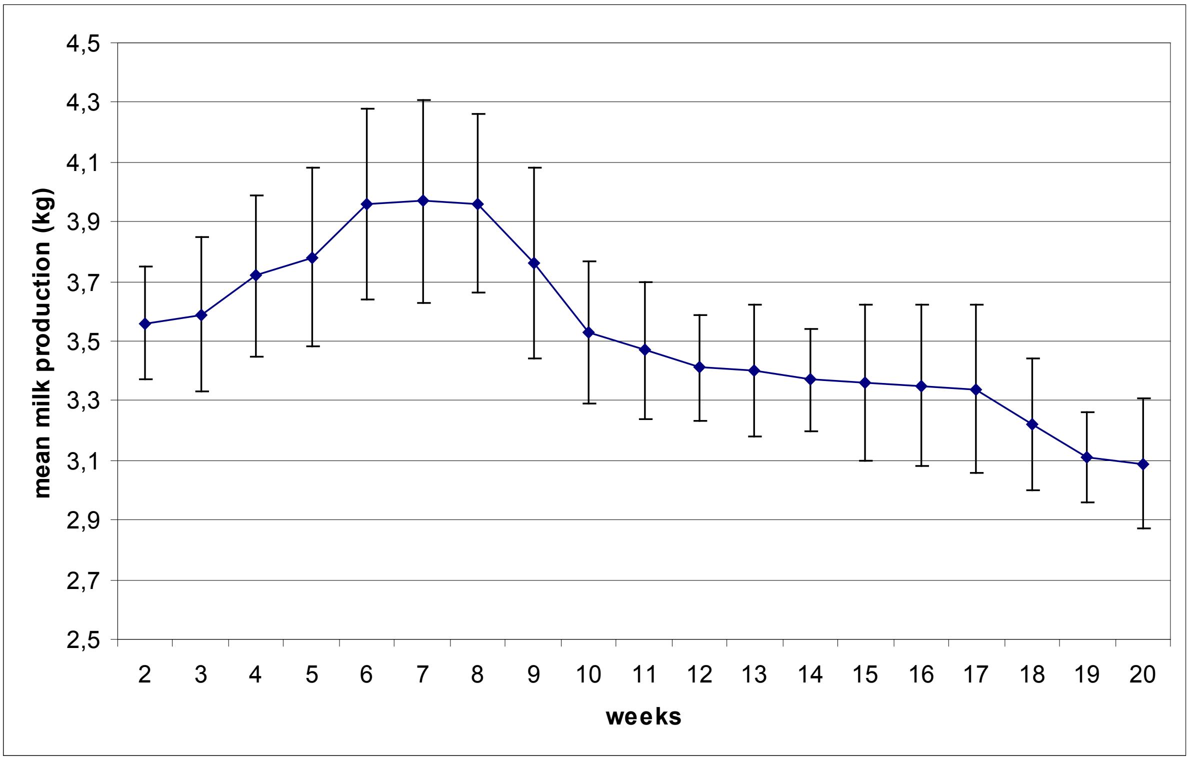

Figure1. Mean milk production of the lactating buffaloes during the experiment (n=10).

mix of rumen degradable and undegradable protein fractions and high rumen fermentable non-fiber carbohydrate in the diets. However, Zhai *et al.* (2006) have stated that insufficient dietary protein caused low milk urea nitrogen concentration. Except for the first and second sampling, milk urea nitrogen concentration was low (9.02 to 6.51 mg/dl) and this result was attributed to lower crude protein levels of diets than that of requirements. This finding was consistent with that of Promkot and Wanapat, 2005 and Zhai *et al*., 2006. Daily crude protein requirement of buffaloes in lactation varies depending on live weight and milk production. Although Terramoccia *et al*. (2005) have reported that crude protein requirement of lactating buffaloes was between 13% and 16%, Pahsa (2013) and Rai and Aggarwal (1991) have stated that dietary crude protein levels were 12% and between 11% and 14% on dry matter basis, respectively. However Rai and Aggarwal (1991) have mentioned that changes in dietary crude protein levels could affect on blood urea concentration and milk protein content. In the present study, the amounts of dietary crude protein were between 7.87% and 5.26% and this situation resulted in the failure in meeting optimal protein requirements of Anatolian buffaloes. However, there was a negative correlation ($P<0.001$) between energy value of diets and milk urea concentration (correlation coefficient -0.496) and also between energy value of diets and milk protein content (correlation coefficient -0.396). These results were compatible with the findings of Cannas *et al*. (1998) who stated that milk urea concentration increased when dietary gross energy was decreased.

Weekly milk production of the lactating buffaloes was given in figure 1 during the experiment. Generally, milk production increase towards peak after calving and then decrease by weeks and this is typical in dairy animals such as in buffaloes. In the present study, peak milk production was the highest between the sixth and eighth weeks and then it decreased by weeks. Peak productivity in the study was about in the second month post calving. This result was compatible with that of Peeva *et al*. (1988) who have reported that the peak milk production was in the second month after calving in Bulgarian Murrah and crossbreeds.

In the present study, daily milk production in Anatolian buffaloes was lower than that of reported by some researchers (Penchev *et al*., 2011; Sahin and Ulutas, 2014) and higher than the production reported by Sekerden *et al*. (1999). This result was attributed to the different genetic and environmental factors relating to nutrition, management and climate.

In conclusion, in Samsun Province, there were no intensive buffalo farms like dairy cow farms. Nutrition has significant effect on milk urea concentration. The result of this study, in can be said that the buffaloes are fed with diets containing insufficient protein level. Monitoring milk urea concentration may provide an opportunity to get knowledge about protein content of diets. Milk urea analysis is easy to monitor and can be performed on buffaloes and has some advantageous under field conditions for estimating protein nutrition status of buffaloes.

ACKNOWLEDGEMENT

This study was supported by Ondokuz Mayis University Scientific Research Foundation (Project no: PYO. VET. 1904.12.006)

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