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DIURNAL VARIATIONS IN MILK UREA, PROTEIN AND LACTOSE CONCENTRATIONS IN HOLSTEIN DAIRY COWS

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Daily variations in milk urea, protein and lactose concentrations and their interrelationships were investigated in 36 and 40 moderate and high milk producer cows, respectively. Milk samples were collected from the morning and afternoon milking yield for up to one week from Holstein dairy cows in Urmia, Iran. The overall mean milk urea nitrogen (MUN), milk protein and lactose concentrations were 19.7 mg/dL, 3.6 g/dL and 4.13 g/dL, respectively. Daily mean concentrations for the milk parameters revealed the highest concentrations for MUN and milk protein in the afternoon and morning milking, respectively. Milk lactose was constant for both milking yields. Significant differences ($p<0.01$) were found for milk indicators for the moderate and high producers, morning and afternoon milking ($p<0.01$). The values for each cow within sampling times were also different ($p<0.01$). Positive correlations ($p<0.01$) were found between MUN/protein, MUN/lactose and protein/lactose in the morning, afternoon and daily milking except for protein/lactose in the afternoon milking. It was concluded that the concentrations in moderate producer cows were greater than in high producer cows. The values for MUN and milk protein were different between the afternoon and morning milking. MUN showed the highest relationships among milk parameters. Thus, MUN might be considered as a useful index in studies related to milk production and reproduction performance in cows.

Key words: cows, daily milking, lactose, MUN, protein

INTRODUCTION

Among ruminants' milk yield, cow's milk is known to be healthy, available, cheap and consumed widely by both animal and human offspring. Milk contains 4% protein. Milk proteins contain casein and other proteins which are easily digested and their nitrogen is converted to urea in the liver and excreted from the blood into urine, feces, milk and other body excretions (Burgos *et al*., 2007; Eicher, 1999; Park *et al*., 2007). Therefore, the amounts of urea nitrogen in the blood (BUN) or milk (MUN) could have an effect on the quality and quantity of milk

and their determination would be useful in understanding the health status of the animals. The normal value for MUN in cows has been reported to be up to 40 mg/dL (Trevaskis *et al*., 1999). Variations in MUN are based dietary energy and protein (Bed *et al*., 1997; Butler *et al*., 1996), animal breeding methods (Sekerden and Avsar, 2008), lactation period (Bed *et al*., 1997), pregnancy and parturition (Skrzypek *et al*., 2005).

Nowadays, the importance and determination of MUN as an indicator of blood urea nitrogen (BUN), which refers to the level of protein consumption, utilization and digestion has been discussed by many authors. In fact, milk testing and permanent recording programs, together with blood profile tests, are run constantly in cows for dairy herd improvement, and therefore, urea assessment could be included in these tests. Recently, the quantification of MUN has become practicable, rapid and also inexpensive by advanced spectrophotometric methods, which could be considered as an additional service for dairy herd management (Bashtani *et al*., 2009; Cao *et al*., 2010). These are the reasons that MUN in ruminants is probably more important than BUN measurements. The preliminary results on MUN in different species of animals (Arunvipas *et al*., 2007), its relationship with quality and quantity of the diet (Cannas *et al*., 1998), nutritional management (Jonker *et al*., 2002; KiTaeg *et al*., 2009), milk yield and composition (Park *et al.,* 2007) and reproductive performance revealed that MUN could be used as an index in the prediction of optimal production. Thus, the quality and quantity of the diet, capacity of rumen microflora fermentation, milk yield and reproduction performance are considered the main goals in that they are predictable and are modified by MUN determination in cattle or sheep industries (Roy *et al*., 2011).

Determination of MUN and its variations in cow's milk in different geographical locations (KiTaeg *et al*., 2009) is essentially necessary to access the mentioned objectives as reported for sheep and goats (Ramin *et al*., 2010) and buffaloes (Sekerden and Avsar, 2008; Sharma *et al*., 2009). Then, in the next step, determination of possible correlations between MUN and milk protein, lactose and other milk, blood and dietary parameters will lead to the prediction of suitable equations (Bet *et al*., 1997; Ramin *et al*., 2010). Thus, the aims of the present study were 1- Determination of the daily MUN, milk protein and lactose concentrations in Holstein dairy cows; 2 - Comparison of the diurnal variations of milk parameters in the morning, afternoon and daily milking; 3 - To present the relationships between milk parameters in dairy cows.

MATERIALS AND METHODS

Seventy six dairy Holstein milking cows including 36 cows with a moderate milk yield up to 20 kg/day and 40 cows with high milk yield up to 40 kg/day were selected in 2010 in the northwest of Iran. Fifty mL milk samples in the morning and afternoon milking were collected for up to seven days. Overall, 1064 milk samples were tested for undergoing milk parameters.

During the sampling, the cow's ear identification number was recorded to determine the diurnal variations for each cow within a one week period. Age, pregnancy, number of parity and daily milk yield were recorded for further studies. Cows were fed *ad libitum*, a diet containing alfalfa, pomace, concentrate and silage, four times per day. During the study mastitis and other clinical diseases were not observed and the cows were all in good health.

Milk samples were first defatted by placing them in a cool area (4C^{o}) and/or centrifuged at 3000 g for 5 minutes. Milk casein was separated by 0.1 N HCl at pH 3.6. Milk serums were used to determine the MUN and milk protein concentrations. MUN and milk protein were measured using appropriate kits (Pars Azmon, IR) in an auto-analyzer (RA-1000, Pharmacia, LKB, Novaspec, USA). Whole milk was used to determine milk lactose and it was evaluated by polarimetry (B+S, UK). Data were analyzed by SPSS13 statistical program and Mean ± SEM were determined for milk parameters in the morning, afternoon, daily and overall milking yield. Students t-test and one way ANOVA were carried out to find out the differences in the parameters under study for each milk yield. Correlation tests were used to evaluate the relationships among parameters for different milking yields.

RESULTS

The overall means for MUN, milk protein and lactose concentrations in dairy Holstein cows in Urmia were 19.7mg/dL, 3.6 g/dL and 4.13 g/dL, respectively. The mean concentrations of milk parameters in the morning and afternoon milking between moderate and high producer cows are shown in Table 1. Milk protein in the morning milking yield and MUN and milk lactose in the afternoon milking yield were significantly different ($p<0.01$) between moderate and high producer cows (Table 2). The highest concentrations were observed in the afternoon milking in which MUN and milk protein were moderate, while lactose was in high producer cows. The variations between producer groups in the morning and afternoon milking for MUN, protein and lactose were 6.6%, 9% and 5% respectively (Table 1).

The daily mean concentrations for milk parameters (Table 1) revealed the highest concentrations for MUN and milk protein were in the afternoon and morning milking, respectively. Means comparison (ANOVA) of the milk values in moderate and high producers morning and afternoon milking yield were significantly different ($p<0.01$), while for the daily milking yield it was significant ($p<0.01$) just for the MUN concentration. The values in each cow within sampling times also showed significant differences ($p<0.01$).

The results of the correlation among milk parameters (Table 3) showed a significant positive relationship ($p<0.01$) between MUN/protein, MUN/lactose and milk protein/lactose in the morning, afternoon and daily milking yield, except for milk protein/lactose in the afternoon milking which was not significantly different.

Table 1. Comparison of mean ± SE of the milk parameters among morning (n=490), afternoon (n=510) and daily milk (n=1000) in moderate and high producer cows

Parameters	MUN (mg/dL)	Milk protein (g/dL)	Milk lactose (g/dL)
Moderate producer cows (morning milking)	19.4 ± 0.18^{a}	1.28 ± 0.02^{a}	4.12 ± 0.04^{ac}
High producer cows (morning milking)	19.2 ± 0.17^{a}	1.17 ± 0.03^{b}	4.14 ± 0.05^{ac}
Moderate producer cows (afternoon milking)	20.9 ± 0.16^{b}	1.19 ± 0.03^{b}	4.06 ± 0.05^{ac}
High producer cows (afternoon milking)	19.6 ± 0.42^{a}	1.14 ± 0.03^{bc}	4.18 ± 0.04^{ab}
Morning milking yield	19.2 ± 0.36^{a}	1.23 ± 0.03^{ab}	4.13 ± 0.03^{ac}
Afternoon milking yield	20.2 ± 0.33^{ab}	1.19 ± 0.03^{b}	4.13 ± 0.03^{ac}
Daily milking yield	19.7 ± 0.24	1.20 ± 0.02	4.13 ± 0.02

*Different letters in each column were significant different ($p<0.05$)

Table 2. Diurnal comparison of mean milk parameters in moderate and high producer cows (n=1000)

Parameters	Mean Square	df	Sum Square	F-Value
Moderate producer cows				
Urea	4357.3	13(461)	381.3	37.6**
Protein	7.2	13(461)	0.56	6.6**
Lactose	32.5	13(448)	2.5	6.3**
High producer cows				
Urea	4367.6	13(510)	336	34.6**
Protein	1.07	13(503)	0.83	10.6**
Urea	69.4	13(449)	5.34	15.1**
Morning milking				
Urea	4805.7	6(483)	801.1	69.1**
Protein	6.02	6(480)	1.5	18.2**
Lactose	63.5	6(481)	10.6	27.6**
Afternoon milking				
Urea	3302.2	6(503)	583.8	57.2**
Protein	6.81	6(498)	1.14	14**
Lactose	32.9	6(493)	5.49	14.8**
Daily milking				
Urea	90	1(998)	91	4.72*
Protein	0.28	1(990)	0.29	2.90
Lactose	0.02	1(974)	0.03	0.92
Overall milking				
Urea	7866.3	6(993)	1311.1	115.6**
Protein	14.46	6(985)	2.41	29.1**
Lactose	72.8	6(981)	12.13	30.5**

*– $p<0.05$; **– $p<0.01$

Table 3. Correlation among MUN, milk protein and lactose concentrations in the morning, afternoon and daily milking

Parameters	Milk Protein		Milk Lactose	
	df	r	df	r
Morning milking				
MUN	971	0.35**	930	0.19**
Milk Protein			923	0.14**
Afternoon milking				
MUN	486	0.38**	452	0.24**
Milk Protein			449	0.06
Daily milking				
MUN	489	0.34**	478	0.15**
Milk Protein			473	0.11**

**– $p<0.01$

DISCUSSION

In this study the mean MUN was 19.7 mg/dL with a range of 3 to 51 mg/dL. The reported MUN for cows was between 15 and 70 (Trevaskis and Fulkerson 1999; Wittwer *et al*., 1999), ewes from 10 to 30 (Ramin *et al*., 2010), goats 26.3 (Khaled *et al*., 1999) and buffaloes from 31.8 to 59.2 mg/dL (Sekerden and Avsar 2008; Yadav *et al*., 2007), therefore, the values were high for cows and low for goats. The reason for the differences in MUN could be related to animal species, nutrition, milk yield, reproduction and environmental conditions. The MUN in this study was partially lower than other references, meaning that this condition is appropriate for dairy herd production in Urmia, but it could be an indication of low protein consumption in the diet, as well. The cows were fed 14% protein in their diet, but it seems that it must be increased up to 18%. Basically, the MUN increases by the level of diet protein (Butler *et al*., 1996). MUN results of protein metabolism in the liver could be varied following pregnancy and lactation (Trevaskis and Fulkerson, 1999), season of year (KiTaeg *et al*., 2009), quality and quantity of milk yield (Arunvipas *et al*., 2008), and genotype (Hossein-Zadeh and Ardalan, 2010; 2011; Kauffman and Pierre, 2001).

Mean milk protein in Holstein dairy cows (3.6 g/dL) in Urmia was a little less than that recorded by other authors (4 g/dL). Milk protein was reported to be high in ewes (7.74 g/dL), and for cows it was up to 5.23 g/dL (Eicher, 1999; Rashida 2004) as observed in this study for a few cows. Milk protein is considered as one of the main ingredients in milk, thus it has a direct effect on milk quality. Low milk protein results from the imbalance in the protein diet. This information together with the result for MUN shows that the amount of protein should be increased up to 18% in the diet. Meanwhile, the diurnal variation in milk protein reported for the lactation period in cows (Fekadu *et al*., 2005) means that milk protein could affect the quality and quantity of milk. This variation in this study for the period of one

week was high (88%) and correction of the protein diet for high producer cows (18%) and low producer cows (12%) will be recommended.

The presence of significant differences in the concentrations of MUN, milk protein and lactose in the morning and afternoon milking yield between moderate and high producer cows (Table 2) indicates that milk yield has a significant effect on the amounts of milk parameters as concluded by Cao *et al*. (2010) and Arunvipas *et al*. (2008). According to the literature, the highest concentration of MUN in cows was recorded from 90 to 120 days (Arunvipas *et al*., 2008; Cao *et al*., 2010) and for ewes up to 135 days of lactation (Ramin *et al*., 2010). These results were based on fewer animals with constant milking up to 135 days of lactation, while in this experiment the moderate producer cows were in over 5 months of lactation with less than 20 kg milk per day and high producer cows from one day of lactation up to 15 months lactation with milk production up to 40 kg/day. The results of this study showed that the concentrations of milk parameters in moderate producer cows were higher than in high producer cows and the concentrations decline when milk production increases. These variations between producer groups in the morning and afternoon milking for MUN, protein and lactose were 6.6%, 9% and 5% respectively (Table 1).

MUN increased up to 5% in the afternoon milking, protein up to 3.4% in the morning milking and for lactose remained stable (Table 1). These results are supported by the findings of Carlsson and Bergstrom (1994) and Arunvipas *et al*. (2007), in which the highest and lowest concentrations of MUN were in the afternoon and morning milking, respectively. The literature shows that the variations in MUN during the lactation period were quite different. Eicher *et al*. (1999) mentioned that the lactation period had no effect on MUN and remained stable during lactation. Others reported that MUN was low in early lactation, increased up to 4 months and then declined up to the end of lactation (Arunvipas *et al*., 2008). These variations for ewes start from 2 months of lactation and then decrease up to the end of lactation (Khaled *et al*., 1999; Ramin *et al*., 2010). The main reason for diurnal variation in MUN could be related to the quality and quantity of the diet. In this study cows were fed concentrate feeding with 14% protein, leading to increased microfloral activity, ammonia production and finally low urea in the blood and milk.

Nowadays, MUN is widely applied as an index for monitoring protein nutrition status, milk yield and reproduction performance. MUN negatively affected oestrus, open days, milk composition and hygiene (Arunvipas *et al*., 2007; Park *et al*., 2007). Some reported MUN did not affect the number of parity, first service breeding and milk yield (Eicher, 1999). Similar studies were investigated by others (Ropsland *et al*., 1989; Wittwer *et al*., 1999). However, it is possible to decrease MUN by reducing diet crude protein (Jonker *et al*., 2002). Meanwhile, assessing MUN will allow the level of urea in the urine and ammonia in the rumen to be predicted (Burgos *et al*., 2007). Bed *et al*. (1997) and Ramin *et al*. (2010) applied MUN and milk lactose to determine the level of blood glucose, urea and protein and the energy diet necessary for ewe breeding.

The presence of significant positive correlations between milk parameters in the morning, afternoon and daily milk yield indicates their appropriate balances in

milk production among healthy animals. Ammonia is produced in the rumen following the catabolism of proteins and is converted to urea in the liver as the source of urea in the blood, urine and milk. Thus, the main source of MUN is blood urea and to a small extent the mammary glands metabolism. This is the reason for the strong relationships among blood protein, rumen ammonia, blood, urine and milk urea (Butler *et al*., 1996; Ropsland *et al*., 1989), as presented in this study too. MUN has been shown to correlate with milk protein and milk composition (Bed *et al*., 1997; Ramin *et al*., 2010), while MUN correlated with first service breeding, pregnancy and parturition (Butler *et al*., 1996; Nourozi *et al*., 2010; Roy *et al*., 2011). These results show that MUN would be a useful monitor index in ruminants, leading to suitable milk production and reproduction. It is concluded that the concentrations of MUN and milk protein in moderate producer cows were greater than in high producer cows. Milk lactose was quite stable. The highest values in MUN and milk protein were observed in the afternoon and morning milking, respectively. MUN revealed the highest relationships among milk protein and lactose. Thus, MUN might be a useful index in further studies related to milk production and reproduction performances in cows.

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DNEVNE PROMENE SADRŽAJA UREE U MLEKU, KONCENTRACIJE PROTEINA I LAKTOZE KOD MLEČNIH HOLŠTAJN KRAVA

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja dnevnih promena sadržaja uree u mleku, koncentracije proteina i laktoze i njihovi međusobni odnosi kod 36 i 40 krava sa srednjom i visokom proizvodnjom mleka, respektivno. Uzorci mleka su sakupljani tokom jedne nedelje od holštajn krava za vreme jutarnje i popodnevne muže u Urmiji, Iran. Srednja vrednost MUN (milk urea nitrogen), koncentracija proteina u mleku i koncentracija laktoze iznosile su 19,7 mg/dl, 3,6 g/dl i 4,13 g/dl respektivno. Srednja vrednost koncentracije MUN i proteina mleka je bila visoka u uzorcoma prikupljenim tokom popodnevne i jutarnje muže, dok je sadržaj laktoze bio nepromenljiv. Dokazane su značajne razlike ($p<0,01$) u koncentraciji ispitivanih parametara između uzoraka dobijenih od krava sa umerenom i visokom produkcijom mleka kao i između uzoraka prikupljenih prilikom jutarnje i popodnevne muže ($p<0,01$). Kod svih krava su takođe uočene statistički značajne razlike ($p<0,01$) u koncentraciji ispitvanih parametara u zavisnosti od vremena uzorkovanja. Pozitivna korelacija ($p<0,01$) je dokazana za koncentracije sledećih parametara: MUN/proteini, MUN/laktoza i proteini/laktoza prilikom dnevnih, jutarnjih i popodnevnih muža, ali ne i za odnos proteini/laktoza tokom popodnevne muže. Može se zaključiti da su koncentracije ispitivanih parametara bile veće kod krava sa umerenom produkcijom mleka nego kod onih sa visokom. Osim toga, vrednosti koncentracije MUN i proteina u mleku su bile različite u popodnevnim i jutarnjim uzorcima. Najveći stepen korelacije je utvrđen između koncentacije MUN i ostalih parametara i ovaj parametar se može smatrati korisnim u studijama o proizvodnji mleka.