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Responses of Milk Urea Nitrogen Content to Dietary Rumen Degradable Protein Level in Lactating Holstein Dairy Cows

Research Article

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ABSTRACT

Nine multiparous lactating cows averaging 171 days in milk were divided according to days in milk and milk production into three 3×3 Latin squares with three 3-week periods to investigate the effect of rumen degradable protein on milk urea nitrogen. Diets were formulated to provide 3 concentrations of dietary rumen degradable protein (9.8, 10.8, and 11.8% of dry matter), while rumen undegradable protein (4.6% of DM) remained constant. Each period was 3 weeks in length, with 2 weeks used for adjustment and one week used for sampling. Rumen degradable protein levels had a low effect on milk yield ($P>0.05$), but a significant effect on protein percentage and milk urea nitrogen content. There was linear increase in the milk urea nitrogen content of cows fed diets one to three ($P<0.01$). Milk urea nitrogen is a simple and non-invasive measurement that can be used to monitor nitrogen efficiency in dairy cows. These results indicated that milk urea nitrogen might be used as a parameter to monitor the change in dietary protein levels.

KEY WORDS milk urea nitrogen, rumen-degradable protein, rumen undegradable protein.

INTRODUCTION

Plasma urea nitrogen (PUN) is the major end product of N metabolism in ruminants, and high concentrations of it are indicative of inefficient utilization of dietary N (Nousiainen *et al*. 2004). However, PUN cannot be measured routinely due to difficulties in obtaining regular and reliable samples. It is well established that urea equilibrates rapidly with body fluids, including milk, and this can account for the close relationship between milk urea nitrogen (MUN) and PUN (Broderick and Clayton, 1997; Hof *et al*. 1997).

Since milk is easily collected and can be determined accurately for urea, it has often been suggested that MUN in bulk tank milk could be used as a diagnostic of on-farm efficiency of N utilization (Jonker *et al*. 1998; Kauffman and St-Pierre, 2001; Kohn *et al*. 2002). On the other hand, PUN varies throughout the day with levels highest 4-6 hours after feeding and lowest just before feeding. The level of MUN reflects PUN, but is less variable since milk is produced and stored in the mammary gland between milkings. MUN is a convenient way to estimate PUN levels and may be useful in monitoring protein nutrition in the dairy herd (Moharrery, 2004).

Because of these reasons, there is an increasing interest in using MUN as a biological indicator of the protein nutrition status and the efficiency of dietary protein utilization for dairy cows (Zhai *et al*. 2006). Variance in MUN has been shown to be related to the ratio of dietary CP to energy (Oltner and Wiktorsson, 1983; Kirchgessner *et al*. 1986), extent of CP degradation in the rumen and the amount of ammonia in excess of microbial N requirements (Roseler *et al*. 1993; Hof *et al*. 1997), and protein or energy intake in relation to feeding standards (Gustafsson and Carlsson, 1993; Carlsson and Pehrson, 1994). However, there is evi-

dence that MUN is more closely associated with changes in dietary CP content than the ratio of dietary CP to energy intake, efficiency of N utilization, or rumen ammonia concentration (Broderick and Clayton, 1997). Because of these reasons, in this research we used fixed level of nonfiber carbohydrates (NFC) as source of energy, and fixed level of RUP. The only changeable item that could affect the concentration of MUN was CP level (as changed from RDP) that increased from diet one to diet three. MUN gives a look at how cows utilize the CP they consumed. The range of MUN values was different in reports, which consist of 12 to 18 mg/dL (Kohn *et al*. 1997), and 12.7 to 15.5 mg/dL (Bucholtz and Johnson, 2007).

Dietary crude protein (CP) content has the greatest nutritional influence on MUN concentrations and has the potential to be used as a management tool for assessing dietary protein (Bucholtz and Johnson, 2007). However, the effects of dietary crude protein levels on MUN content were uncertain. In some trials, MUN content was significantly influenced by dietary protein levels (Reynal and Broderick, 2005; Kalscheur *et al*. 2006) but not in others (Haig *et al*. 2002; Wattiaux and Karg, 2004; Flis and Wattiaux, 2005). The objective of this study was to investigate the responses of MUN to changes in dietary crude protein and rumen degradable protein level.

MATERIALS AND METHODS

Animals and diets

The experiment was conducted in the farm of Tehran University, Aboureihan campus, in the area around Varamin, tehran, Iran, over the period July to September 2007. Nine Holstein dairy cows on mid-lactation period, averaging 171 DIM (SD17), 24 kg (SD3) of daily milk yield and 635 kg (SD52) of body weight (BW) were classified according to DIM and milk production into three 3×3 Latin squares. Cows were housed in tie stalls, with free access to water. The cows within the squares were randomly assigned to 3 dietary treatment sequences. Each experimental period lasted 21 days and consisted of 14 days for adaptation and 7 days for sample collection. The individual dry matter intake (DMI) of cows was estimated during the adaptation period and was then offered their appropriate total mixed ration (TMR) at ad-libitum levels allowing them for 5 to 10% orts per day during the experimental period. The total amount of TMR for individual cows was offered 2 times a day at 1000 and 2000 h. During the experimental period the mean of relative humidity was 21.5% and Maximum, Minimum and mean temperatures were 36, 28 and 32, respectively.

Based on the NRC (2001) recommendations, 3 isocaloric TMR were prepared. Treatments were arranged with fixed level of nonfiber carbohydrates (40% of DM) and rumen undegradable protein (4.6% of DM), with three concentrations of rumen degradable protein (9.8, 10.8 and 11.8% of DM), to study the effects of different levels of RDP on MUN concentration of midlactation dairy cows. Dietary ingredients and chemical composition are shown in Table 1. The crude protein (CP) of diets was 14.3, 15.3 and 16.3% of DM for the 1, 2, and 3 diets, respectively. Urea added to increase the level of CP and RDP of diets. Content of CP for alfalfa hay, corn silage, barley straw, barley grain, cottonseed meal were 16.3, 7.5, 4, 10.8 and 21% (DM basis), respectively. Diets were balanced for cows that produced 24 kg of milk daily (4% fat and 3% CP), 20.4 kg of DMI daily, weighed 630 kg, and were 171 DIM. The concentrates were based on barley grain and cottonseed meal. The concentrates were top-dressed onto the alfalfa hay and corn silage mixture and completely mixed together before feeding.

Sample collection and analysis

During collection periods, feed intake and orts were measured and feeds were sampled daily for each cow. These samples were refrigerated until the end of the collection period, and then were composited by cow and the resulting samples were stored at –20 ºC for later analysis. The feed and orts samples were dried in a forced air oven at 60 ºC for 48 h and ground through a 2 mm screen in a Wiley mill. The samples were analyzed for CP, ash (AOAC, 1990) and for NDF and ADF (Goering and Van Soest, 1970) with sodium sulfite.

Cows were milked thrice daily at 400, 1200 and 2000 h. Milk weights were recorded in sampling period and averaged to determine mean milk yield production for the entire period.

Milk samples were collected for six consecutive milking at the days of 3 and 4 of sampling week and preserved with dichromate potassium ($K_2Cr_2O_7$) at 4 ºC until analysis. Milk samples were analyzed for fat, protein, solid not fat (SNF), lactose and MUN (Milk-O-Scan 134 A/B Foss Electric, HillerØd, Denmark).

Blood samples were collected from the coccygeal vein of cows on day 20 at approximately 2 h post feeding. The blood was collected in sodium heparin collection tubes and placed on ice for transport to the laboratory. These samples were centrifuged for 15 min at 2500×g, and plasma was collected and frozen at −20 ºC until analysis. The plasma urea concentration was determined using the method described by Chaney and Marbach (1962).

Statistical analysis

The experimental design was a replicated 3×3 Latin square. Data were analyzed using REPEATED MEASURMENT of SAS (SAS Institute, 2002). The original model included time×treatment interactions but this interaction was not significant for any variable and was pooled with the error

term in the final model. The final model included square, period, and treatment. Cow within square was the term of t-

Table 1 Composition of the three diets

	Diets[1]		
	1	2	3
Ingredient (% of DM)			
Alfalfa hay[2] (chopped)	22.65	23.74	23.51
Corn silage[2]	17.01	18.01	17.82
barley straw [2]	5.96	2.54	2.11
Barley grain[2](ground)	24.92	25.72	25.90
Cottonseed meal[2]	26.85	27.02	27.37
Urea	0.29	0.55	0.90
Calcium carbonate	0.48	0.49	0.49
Sodium bicarbonate	0.69	0.74	0.70
Salt	0.41	0.42	0.42
Mineral/vitamin premix [3]	0.75	0.77	0.77
Chemical composition (% of dry matter)			
Dry matter (%)	66.7	63.8	63.2
Organic matter	94.60	94.80	94.70
Net energy lactation (Mcal/kg)	1.47	1.48	1.48
Crude protein (CP)	14.3	15.3	16.3
rumen-undegraded protein (RUP)	4.6	4.6	4.6
rumen-degraded protein (RDP)	9.8	10.8	11.8
Nonfiber carbohydrates (NFC) [4]	39.9	40.1	39.5
Neutral detergent fiber (NDF)	38.5	37.4	37.0
Acid detergent fiber (ADF)	24.8	24.0	23.7
Calcium	0.6	0.6	0.6
Phosphorus	0.5	0.5	0.5

[1]Diets 1, 2 and 3 have 9.8, 10.8 and 11.8% rumen degradable protein, respectively. Diets formulated for 630 kg of BW, 24 kg/d of 4% FCM and 3.0% milk protein.
[2]Alfalfa hay, corn silage, barley straw, barley grain, cottonseed meal were 16.3, 7.5, 4,10.8 and 21% CP, respectively.
[3]Provided (per kilogram of DM): 196 g of Ca, 96 g of P, 20 g of Mg, 5500 mg of Na, 3000 mg of Zn, 2000 mg of Mn, 3000 mg of Fe, 300 mg of Cu, 100 mg of I, 100 mg of Co, 1 mg of Se, 500 KIU of vitamin A, 100 KIU of vitamin D, and 100 IU of vitamin E.
[4]NFC (nonfiber carbohydrates) calculated as 100-CP-NDF-ash-ether extract (NRC, 2001).

he RANDOM statement. Values reported as least squares means. The best fit covariance structure used was compound symmetry based on the lowest bayesian information criteria. Diet effect (i.e. different levels of RDP) was partitioned into linear and quadratic contrasts. Significance was declared at $P \leq 0.05$, and a trends was noted if $0.05 \leq P < 0.10$.

RESULTS AND DISCUSSION

Data for intake are presented in Table 2. DMI and OMI were decreased linearly from diet one to diet three ($P<0.01$) as RDP percentage decreased and diet one had significantly higher DMI and OMI than other treatments. Since this study conducted in hot ambient temperature (ranging from 28 to 36 ºC) and cows were in moderate to severe heat stress, reduction of DMI might be the result of higher amount of RDP (Higginbotham *et al*. 1989; Huber *et al*. 1994). Because of similar percentage of organic matter between treatments (Table 1), intake of OM followed the same pattern as for DMI and decreased linearly from diet one to three.

In agreement with treatment formulation, intake of CP increased from 2.64 to 3.05 kg/d for the lowest to the highest CP diets. Daily CP intake was significantly higher ($P<0.01$) for cows consuming diets two and three than diet one, with no significant difference between diets two and three.

These results agree with previous data indicating that decreasing the CP content of the diet of lactating dairy cows generally has minor effects on DMI unless dietary CP is reduced to extremely low percentages (≤12%; Ipharraguerre, 2004) Data for milk yield and composition are presented in Table 2. Rations were formulated assuming cows would produce an average of 24 kg/d.

The milk yield and FCM yield increased with the increasing dietary protein levels, but differences were not significant ($P>0.05$). The lack of responses in milk production was consistent with others' observations that these did not change when dietary protein varied from 16.7 to 18.4% (Davidson *et al*. 2003), from 16.7 to 18.4% (Broderick, 2003), 17.2 to 19.0% (Sannes *et al*. 2002). While some researchers observed significant differences in milk yield with dietary protein from 13.1 to 17.0% (Frank and Swesson, 2002), it has been reported that milk production benefits from >15% protein (Broderick, 2003), but increasing the protein above 17% has no further effect (Groff and Wu, 2005).

Increasing dietary RDP resulted in a linearly increase in protein and solid non fat percentage, and also milk and plasma urea nitrogen ($P<0.01$). Overall, changes in milk fat and protein were small and inconsistent, agreeing with the literature (Leonardi *et al*. 2003).

Kung and Huber (1983) reported no change in milk fat or protein concentration with diets varying in protein from 11 to 17%, whereas, Cunningham *et al*. (1996) showed increase in milk fat and protein percentages and yields when dietary protein was increased from 14.5 to 16.5%, but no c-

Table 2 Effect of dietary RDP on intake, production and composition of milk*

Item	Diets[1]			SE	P-value[2]		
	1	2	3		RDP	Linear	Quadratic
DM intake (kg/d)	21.83[a]	21.29[b]	21.08[b]	0.59	<0.01	<0.10	0.74
OM intake (kg/d)	20.65[a]	20.18[b]	19.96[b]	0.55	<0.01	<0.10	0.82
CP intake (kg/d)	2.64[b]	2.91[a]	3.05[a]	0.10	<0.01	<0.05	0.62
Milk yield (kg/d)	22.9	23.0	23.4	0.92	0.36	0.48	0.83
4% FCM (kg/d)	20.8	21.2	21.8	1.11	0.39	0.38	0.92
Milk fat (%)	3.31	3.44	3.36	0.12	0.66	0.67	0.69
Milk fat yield (kg/d)	0.78	0.80	0.84	0.04	0.30	0.45	0.83
Milk CP (%)	3.00[b]	3.07[a]	3.08[a]	0.02	<0.01	<0.05	0.29
Milk CP yield (kg/d)	0.71	0.73	0.75	0.02	0.24	0.23	0.98
SNF (%)	8.12[b]	8.22[a]	8.26[a]	0.08	<0.01	<0.1	0.65
SNF yield (kg/d)	1.89	1.91	2.01	0.08	0.11	0.21	0.57
Milk lactose (%)	4.29	4.34	4.37	0.07	<0.1	0.30	0.93
Milk lactose yield (kg/d)	1.00	1.01	1.06	0.05	0.11	0.25	0.56
MUN (mg/dL)	11.20[b]	12.88[a]	13.90[a]	0.54	<0.01	<0.01	0.63
PUN (mg/dL)	15.20[b]	16.86[a]	17.76[a]	0.41	<0.01	<0.01	0.45
Feed efficiency [3] (kg/kg)	0.96	0.99	1.03	0.04	0.30	0.22	0.90

*The means within the same row that have at least one common letter, do not have significant difference (P>0.05).
[1] Diets 1, 2 and 3 have 9.8, 10.8 and 11.8% rumen degradable protein, respectively.
[2] Probability of a significant effect of RDP or of a linear or quadratic effect of RDP level in the diet.
[3] Feed efficiency (%)=4.0% FCM (kg/d)/DMI (kg/d) (Kalscheur et al. 2006).
[4] Rumen degradable protein (RDP).

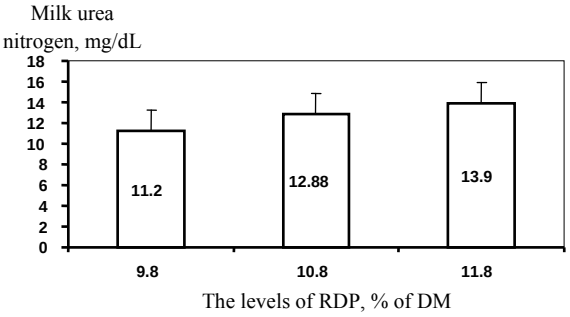

Figure 1 Effect of rumen degradable protein on milk urea nitrogen content

hanges when protein was further increased to 18.5%. Sutton (1989) indicated that dietary protein has only small effects on milk fat and protein concentrations. Analysis of data showed that MUN linearly increased from 11.20 to 13.90 mg/dL (SE 0.54) as dietary RDP was increased from the lowest to the highest levels (P<0.01). The effect of dietary RDP level on MUN content is shown in Figure 1.

The observation was consistent with some studies (Davidson *et al*. 2003; Broderick, 2003; Groff and Wu, 2005), while Flis and Wattiaux (2005) found that a 1% change in the dietary protein level did not cause a significant change in the MUN content. The values of MUN in this experiment for diet two and three were in the normal range (12 to 18 mg/dL reported by Kohn *et al*. 1997; 12.7 to 15.5 mg/dL reported by (Bucholtz and Johnson, 2007) but MUN value in diet one (11.2 mg/dL) was lower than normal range. This indicates that the amount of RDP or CP in diet one was less. The amount of PUN linearly increased from 15.20 to 17.76 mg/dL (SE 0.41) from diet one to three. Urea concentrations in plasma and milk were closely correlated (r=0.80). When MUN was regressed against PUN, a linear relationship was determined, with an intercept of 4.83 mg/dL (Figure 2). The relationship between PUN and MUN (in milligrams per deciliter) is described by the following equation:

MUN = 1.05 (±0.15) PUN – 4.83 (±2.60) (R^2 = 0.64)

Where, values in parentheses are standard errors. Treatment significantly affected concentrations of PUN and MUN. These results confirm that urea in the blood system is the major source of urea nitrogen in milk. Other researchers expressed different equations and their equations are expressed in Table 3.

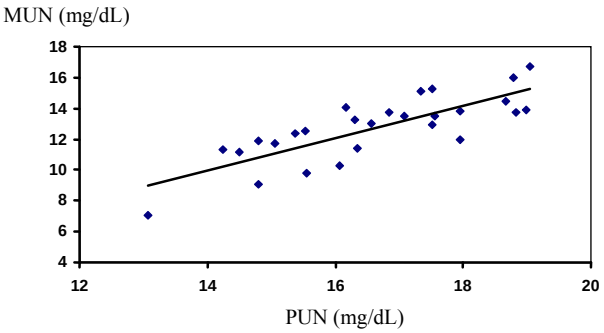

Figure 2 Relationship of plasma urea N (PUN) to milk urea N (MUN). The standard errors of coefficients are in parentheses. MUN (mg/dL)=1.05 (±0.15) PUN (mg/dL)–4.83 (±2.60); R^2=0.64

The concentration of dietary CP has a strong positive correlation with MUN (Broderick and Clayton, 1997) and can be affected by the proportion of RDP and RUP in the CP. In our experiment, diets were formulated to c ontain equal co concentrations of RUP, and the difference in CP was caused by addition of RDP.

Table 3 equations obtained by different researchers in the correlation of plasma and milk urea nitrogen

R-Square	Equations	Researchers
R^2=0.45	MUN=0.45 PUN+8.83	Moharrery (2004)
R^2=0.93	PUN=0.85 MUN+3.20	Baker et al (1995)
R^2=0.79	MUN=0.88 PUN−1.32	Roseler et al (1993)
R^2=0.84	MUN=0.62 PUN+4.75	Broderick et al (1997)
R^2=0.84	MUN=0.89 PUN−1.96	Kauffman et al (2001)

CONCLUSION

Results from this experiment indicate that milk production parameters were insensitive to the changes in dietary protein or rumen degradable protein levels. As dietary RDP content increased, MUN was increased. It might be concluded that MUN can be used as a parameter to monitor the change in dietary rumen degradable protein levels.

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