Responses of milk urea nitrogen content to dietary crude protein level and degradability in lactating Holstein dairy cows

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ABSTRACT: Two experiments were conducted to investigate the effects of dietary crude protein level and degradability on milk urea nitrogen (MUN) content. In experiment 1, twelve multiparous lactating cows averaging 176 days in milk were divided according to DIM and milk production into three 4 × 4 Latin squares with four 2-week periods. Cows were fed four diets with different crude protein levels (13.0, 14.0, 15.0, and 16.0%, DM basis) with isocaloric, respectively. Crude protein levels had a low effect on milk yield and composition ($P > 0.05$), but a significant effect on MUN content. There were significant differences in the MUN content of cows fed either of the two diets ($P < 0.01$). In experiment 2, fifteen multiparous Holstein dairy cows averaging 91 days in milk were classified according to DIM and milk production into five 3 × 3 Latin squares with three 3-week periods. Cows were fed one of the three isocaloric and isonitrogenous diets with RUP being 30.8%, 36.2%, and 41.6% (CP basis), respectively. Milk yield, milk composition, and MUN content were not significantly affected by protein degradability, and there were no significant differences between any two dietary treatments ($P > 0.05$). These results indicated that MUN might be used as a parameter to monitor the change in dietary protein levels.

Keywords: protein level; protein degradability; milk urea N; lactating cows

There is an increasing interest in using milk urea nitrogen (MUN) as a biological indicator of the protein nutrition status and the efficiency of dietary protein utilization for dairy cows. MUN content is mainly affected by nutritional factors (Arunvipas et al., 2003). However, the effects of dietary crude protein levels or degradability on MUN content were uncertain. In some trials, MUN content was significantly influenced by dietary protein levels or degradability in some studies (Rodriguez et al., 1997; Promkot and Wanapat, 2005) but not in others (Davidson et al., 2003; Flis and Wattiaux, 2005). Furthermore, the MUN content may differ even in cows consuming the same diet, due to genetic differences in the ability to metabolize protein (Wood et al., 2003).

Little information is available about the effects of dietary nutritional factors on MUN content in Chinese Holstein dairy cows, which have more complicated genetic traits and dietary composition compared to other Holstein breeds. The objective of this study was to investigate the responses of MUN to changes in dietary crude protein level and degradability in Chinese feeding conditions.

MATERIAL AND METHODS

Animals, diets, and management

Experiment 1: 12 Holstein dairy cows, averaging 176 DIM (SD11), 26 kg (SD2) of milk yield per day and 570 kg (SD15) of body weight (BW) at the beginning of the study were classified according to DIM and milk production into three 4 × 4 Latin squares.

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The cows within the squares were randomly assigned to 4 dietary treatment sequences. Each experimental period lasted 14 days and consisted of 9 days for adaptation and 5 days for sample collection.

The experimental diets consisted of 22.1% maize silage, 14.8% ryegrass, 9.4% lucerne hay, 12.3% lucerne pellets, 4.0% sugar beet pulp pellets, and 37.5% concentrate (DM basis). By varying ground maize grain, rolled barley, wheat bran, soybean meal, whole cottonseed, cottonseed meal, and rapeseed meal in the concentrates, differences in protein levels and similar energy levels in dietary treatments were achieved. The protein levels for diets A, B, C, and D were 13.2, 14.1, 15.0, and 16.2% (DM basis), respectively. The net energy content in each diet was about 1.50 Mcal/kg (DM basis).

Experiment 2: 15 multiparous Holstein dairy cows, averaging 91 DIM (SD11), 35 kg (SD2) of milk yield per day and 590 kg (SD15) of body weight at the beginning of the study were classified according to DIM and milk production into five 3 × 3 Latin squares with 3-week periods. The 3rd week was used for sample collection. Cows were randomly assigned to one of the three isonitrogenous diets: low RUP (LRUP), medium RUP (MRUP), and high RUP (HRUP). The diets consisted of 17.9% maize silage, 3.1% ryegrass, 9.0% lucerne hay, 10.8% lucerne pellets, 4.1% cabbage, 5.5% apple pomace (dry), 5.7% distillers dried grain, and 43.9% concentrate (DM basis). By varying ground maize grain, wheat bran, soybean meal, whole cottonseed, cottonseed meal, and rapeseed meal in the concentrates, differences in protein degradability and similar protein and energy levels in dietary treatments were achieved. According to the National Station of Animal Production and Health in China (2000), the rumen undegradable protein levels for diets LRUP, MRUP, and HRUP were 30.8, 36.2, and 41.6% (CP basis), respectively. The protein and net energy content in diets was 14.9% and 1.67 Mcal/kg (DM basis), respectively.

In the two experiments, the diets were offered three times each day at 06:00, 14:00 and 20:00 h, the rate being adjusted daily to yield orts of about 5 to 10% of intake. The cows were milked three times daily at 06:30, 14:30 and 20:30 hours. They were housed in a tie stall on rubber bedding with separate mangers and had free access to water.

Sampling, measurements, and laboratory 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°C for later analysis. Milk yields were recorded daily. Two samples were taken for each cow every day. One sample was preserved with 2-bromo--nitropropane-1,3-diol and refrigerated until analyzed for milk components. The other sample was stored immediately at –20°C for later analysis of MUN.

The feed and orts samples were dried in a forced-air oven at 60°C for 72 h and ground through a 2-mm screen in a Wiley mill. The above samples and urinary nitrogen were analyzed for CP by the Kjedahl method (AOAC, 1990) and for NDF and ADF (Van Soest et al., 1991). The milk samples were warmed to room temperature and mixed thoroughly. The preserved milk samples were examined for concentrations of fat, protein, lactose, and

Table 1. Effect of dietary protein concentration on milk production

	Diet				SE
	A	B	C	D	
DMI (kg/day)	17.94	17.85	17.96	17.91	0.02
Milk yield (kg/day)	23.10	23.50	23.50	23.70	0.40
4% FCM (kg/day)	22.00	22.40	22.60	22.70	0.60
Milk composition (%)					
Fat	3.69	3.70	3.74	3.72	0.04
Protein	3.31	3.28	3.30	3.29	0.03
Lactose	4.83	4.81	4.85	4.80	0.03
Total solids	12.53	12.47	12.54	12.53	0.05

total solids with an infrared analyzer (System 3000, Foss Electric, Denmark). The method to determine MUN content was the same as that described by Zhai et al. (2005).

Statistical analysis

Data were processed by the GLM procedure of SAS (1999) using a linear model with the effects of diet, square, period within the square and cow within the square. The interaction between the square and treatment was tested, found to be nonsignificant, and then removed from the model. The main effects (diet) were tested using Ducan's multiple range procedure with the GLM procedure of SAS (1999).

RESULTS AND DISCUSSION

The lactation response from **Experiment 1** is presented in Table 1. The dry matter intakes of cows fed different diets were similar, and the milk yield and FCM yield increased with the increasing dietary protein levels, but these were not significant ($P > 0.05$). Milk composition was not affected by increasing CP levels. The lack of responses in milk production and milk composition 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 16.4 to 20.4% (Mulligan et al., 2004), from 16.4 to 18.0% (Wattiaux and Karg, 2004), and from 14.6 to 18.3% (Castillo et al., 2001). While some researchers observed significant differences in milk yield or composition with dietary protein from 13.1 to 17.0% (Frank and Swesson, 2002), it has been reported that milk production benefits from > 15% protein, but increasing the protein above 17% has no further effect (Groff and Wu, 2005), and dietary protein has only a low effect on milk fat and protein concentration (Sutton, 1989).

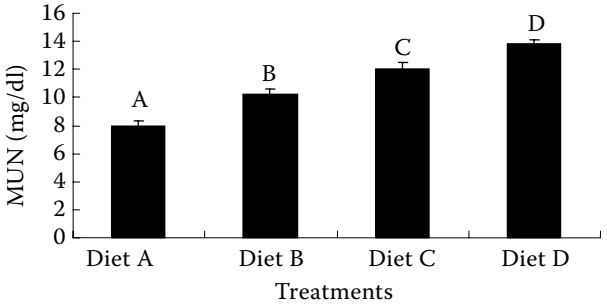

Figure 1. Effect of dietary protein levels on milk urea nitrogen content
A,B,C,D = the four means without a common superscript are different ($P < 0.01$)

The effect of dietary protein level on MUN content is shown in Figure 1. The MUN values were significantly affected by dietary treatments ($P < 0.01$), with the lowest values in cows fed diet A, followed by diets B and C, and the highest in cows fed diet D. The MUN values increased with CP levels. The observation was consistent with some studies (Davidson et al., 2003; Groff and Wu, 2005; Promkot and Wanapat, 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.

Lactation response from **Experiment 2** is presented in Table 2. No significant differences in DMI, milk yield and composition were found across dietary protein degradability ($P > 0.05$). Santos et al. (1998) summarized 108 studies on RUP supplement trials and concluded that possible reasons

Table 2. Effect of dietary protein degradability on milk production

	Diet			SE
	LRUP	MRUP	HRUP	
DMI (kg/day)	23.20	22.90	23.10	0.40
Milk yield (kg/day)	31.90	33.00	33.60	0.80
4% FCM (kg/day)	30.00	30.80	31.20	1.10
Milk composition (%)				
Fat	3.61	3.56	3.52	0.04
Protein	2.97	3.02	3.09	0.08
Lactose	4.93	4.94	4.96	0.02
Total solids	12.51	12.32	12.21	0.12

for the lack of response to increased RUP were as follows:
(1) microbial synthesis in the rumen decreased,
(2) the RUP source had a poor essential AA profile,
(3) RUP sources in the small intestine had low digestibility, and
(4) control diets were already sufficiently high in RUP.

They also stated that only fish meal and treated soybean meal could benefit lactation performance consistently. In the present experiment, the good RUP supplement sources (fish meal or treated soybean meal) were not utilized to alter dietary protein degradability, and the lactation performance was similar in the various treatments.

The effect of dietary protein degradability on MUN content is shown in Figure 2. The MUN values of cows fed LRUP, MRUP, and HRUP diets were not significantly different ($P > 0.05$). Based on theoretical considerations, both ammonia N absorbed from the rumen and absorbed AA not utilized for milk protein synthesis or retained in body tissues are metabolized to urea in the liver. Nousiainen et al. (2004) indicated that the absorbed AA from RUP not utilized for milk protein synthesis had similar effects on MUN as ruminal N losses. The result in **Experiment 2** confirmed this viewpoint. Some researchers also observed that dietary protein degradability did not affect the urea content in blood (Blouin et al., 2002; Reynal and Broderick, 2003) or milk (Rodriguez et al., 1997), whereas Davidson et al. (2003) reported that MUN was affected significantly by the varying dietary RUP level. It has been suggested that a restriction in energy supply increases MUN (Kirchgessner et al., 1986). It is possible that the energy supply did not meet the requirement for high-yielding dairy cows in the study of Davidson et al. (2003). For the studies which reported that protein degradability had no significant effect on MUN content, the energy supply might not have been deficient.

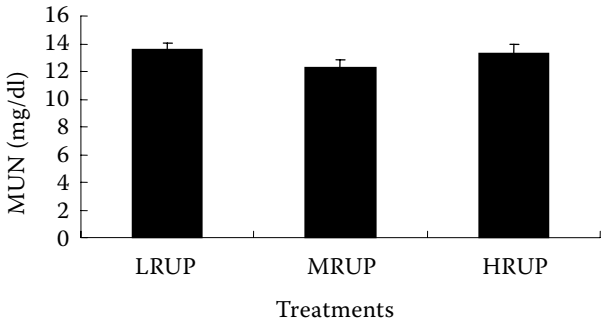

Figure 2. Effect of dietary protein degradability on milk urea nitrogen content

CONCLUSIONS

Milk production parameters were insensitive to the changes in dietary protein levels or degradability. Milk urea nitrogen did not reflect the change in dietary protein degradability, but it detected a difference in dietary protein levels of about 1%. It might be concluded that MUN can be used as a parameter to monitor the change in dietary crude protein levels.

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REFERENCES

AOAC (1990): Official Methods of Analysis. 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.

Arunvipas P., Dohoo I.R., Vanleeuwen J.A., Keefe G.P. (2003): Factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. Prev. Vet. Med., *59*, 83–93.

Blouin J.P., Bernier J.F., Reynolds C.K., Lobley G.E., Dubreuii P., Lapierre H. (2002): Effect of supply of metabolizable protein on splanchnic fluxes nutrients and hormones in lactating dairy cows. J. Dairy Sci., *85*, 2618–2630.

Castillo A.R., Kebreab E., Beever D.E., Barbi J.H., Sutton J.D., Kirby H.C., France J. (2001): The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci., *79*, 247–253.

Davidson S., Hopkins B.A., Diaz D.E., Bolt S.M., Brownie C., Fellner V., Whitlow L.W. (2003): Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early Holstein cows. J. Dairy Sci., *86*, 1681–1689.

Flis S.A., Wattiaux M.A. (2005): Effects of parity and supply of rumen-degraded and undegraded protein on production and nitrogen balance in Holsteins. J. Dairy Sci., *88*, 2096–2106.

Frank B., Swesson C. (2002): Relationship between content of crude protein in rations for dairy cows and milk

yield, concentration of urea in milk and ammonia emissions. J. Dairy Sci., *85*, 1829–1838.

Groff E.B., Wu Z. (2005): Milk production and nitrogen excretion of dairy cows fed different amounts of protein and varying of alfalfa and corn silage. J. Dairy Sci., *88*, 3619–3632.

Kirchgessner M., Kreuzer M.,. Roth-Maier D.A. (1986): Milk urea and protein content to diagnose energy and protein malnutrition of dairy cows. Arch. Anim. Nutr., *36*, 192–197.

Mulligan F.J., Dillon P., Callan J.J., Rath M., O'Mara F.P. (2004): Supplementary concentrate type affects nitrogen excretion of grazing dairy cows. J. Dairy Sci., *87*, 3451–3460.

National Station of Animal Production and Health (2000): Nutrient Requirement and Feeding Standard for Chinese Dairy Cows. 2nd ed. China Agricultural University Press, Beijing, China.

Nousiainen J., Shingfield K.J., Huhtanen P. (2004): Evaluation of milk nitrogen as a diagnostic of protein feeding. J. Dairy Sci., *87*, 386–398.

Promkot C., Wanapat M. (2005): Effect of level of crude protein and use of cottonseed meal in diets containing cassava chips and rice straw for lactating dairy cows. Asian-Aust. J. Anim. Sci., *18*, 502–511.

Reynal S.M., Broderick G.A. (2003): Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci., *86*, 835–843.

Rodriguez L.A., Stallings C.C., Herbein J.H., Mcgilliard M.L. (1997): Effect of degradability of dietary protein and fat on ruminal, blood, and milk components of Jersey and Holstein cows. J. Dairy Sci., *80*, 353–363.

Santos F.A.P., Santos J.P.E., Theurer B.C., Huber J.T. (1998): Effects of rumen undegradable protein on dairy cows performance: a 12-year review. J. Dairy Sci., *81*, 3182–3123.

SAS Institute Inc. (1999): What's new in SAS software for version 7 and the version 8 developer's release. SAS Inst. Inc., Cary, NC.

Sutton J.D. (1989): Altering milk composition by feeding. J. Dairy Sci., *72*, 2801–2814.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary, fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., *74*, 3583–3597.

Wattiaux M.A., Karg K.L. (2004): Protein level for alfalfa and corn silage-based diets: I. lactational response and milk urea Nitrogen. J. Dairy Sci., *81*, 3480–3491.

Wood G.M., Boettcher P.J., Jamrozik J., Jansen G.B., Kelton D.F. (2003): Estimation of genetic parameters for concentrations of milk urea nitrogen. J. Dairy Sci., *86*, 2462–2469.

Zhai S.W., Liu J.X., Ma Y. (2005): Relation between milk urea content and nitrogen excretion from lactating cows. Acta Agric. Scand., Sect. A-Animal Sci., *55*, 113–115.

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