Effects of Milk Production, Season, Parity and Lactation Period on Variations of Milk Urea Nitrogen Concentration and Milk Components of Holstein Dairy Cows*

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ABSTRACT : The study was conducted to assess the effect of milk production, parity, stage of lactation, season and individual milk components themselves on milk urea nitrogen (MUN) concentration and other milk components of 3,219 Holstein dairy cows in Korean dairy farms. The MUN concentrations in Korean dairy cows were estimated to 16.68±5.87 mg/dl. Milk yield was negatively correlated with fat and protein contents and somatic cell counts (SCC) in milk ($p<0.01$). The increasing MUN concentration has positive correlation with yield and fat content. By increasing somatic cell, milk yield was reduced and MUN level was increased. Cows in spring and winter produced more milk over 1.43 and 0.93 kg/day, respectively, than cows in summer ($p<0.01$). Milk urea nitrogen concentrations of milk produced in summer and fall were significantly lower ($p<0.01$) than those in spring and winter. Both MUN concentration and somatic cell counts were highest in winter. Milk yield was lower ($p<0.01$) in the first calving than other calving time and was tended to increase until the fifth parity and then decrease. Milk urea nitrogen and SCC were not related to parity of cows in this study. Milk yield and SCC were positively related to lactation period while MUN concentrations and milk fat and protein contents were negatively influenced by stage of lactation. In the present study, the relationship between MUN and reproduction of dairy cows was also investigated. Cow produced milk in high MUN concentrations (greater than 18 mg/dl) had more open days than cows in MUN concentrations less than 18 mg/dl. However, no significant difference between MUN concentration levels and frequency of artificial insemination was found in this study. It is suggested that although MUN values for nutritional management and measures of production or reproduction are used, non-nutritional factors should be considered. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 4 : 479-484)***

Key Words : Milk Urea Nitrogen, Milk Production, Non-nutritional Factors, Dairy Cows

INTRODUCTION

Dietary CP, RDP, RUP, dietary energy and protein/energy ratios are known to sensitively change serum urea and milk urea (MU) concentrations in the ruminant (Folman et al., 1981; Oltner and Wiktorsson, 1983; Macleod et al., 1984; Carroll et al., 1988; DePeters and Ferguson, 1992; Roseler et al., 1993). Thus, it can be predicted theoretically that concentration of urea in milk should increase if there is a surplus of rumen-degradable protein in the diet and/or if there is a deficiency of energy in the diet, and that the concentration should decrease if there is a deficiency of protein in the diet. It has been shown that a surplus of crude protein in the diet gives rise to a high concentration of urea in the blood and milk (Refsdal et al., 1985; Ferguson et al., 1988) and Carlsson and Pehrson (1993) observed very low MU concentrations in samples taken from cows on farms where the rations were low in protein.

There is increasing interest in using MU as a biological indicator of the efficiency of dietary protein utilization including improved efficiency and reduced cost of production, reduced nitrogen excretion into the environment, and improved fertility (Kaim et al., 1983; Ferguson et al., 1988; Tamminga, 1992; Baker et al., 1995). However, the biological mechanisms explaining the possible relationship between urea concentrations and fertility are still not well defined. Several researchers reported that cows fed high concentration of dietary CP had higher blood urea concentrations and lower conception rates (CR) (Jordan and Swanson, 1979; Canfield et al., 1990; Ferguson et al., 1988, 1993). However, others reported that there was no association between urea and CR in spite of a positive relationship between dietary CP and urea (Holtz et al., 1986; Howard et al., 1987). Again some studies have reported reduced fertility when MU levels were either very low or very high (Miettinen and Juvonen, 1990; Pehrson et al., 1992; Gustafsson and Carlsson, 1993). Parity, health status, lactation stage and season have been reported to be important modifying factors in some studies. Folman et al. (1981) reported that feeding increased levels of dietary protein resulted in a decrease in fertility only in cows in their fourth or greater lactation, even though plasma urea concentrations were similar between older and younger animals. Others have reported similar age effects (Ferguson et al., 1988; Bruckental et al., 1989). Barton et al. (1996) observed a negative relationship between feeding high CP

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Table 1. Analysis of milk components

Milk component	No. of samples	Mean±SD
Milk yield (kg/day)	3,219	26.48±8.38
Milk fat (%)	3,219	3.80±0.58
Milk protein (%)	3,219	3.13±0.30
Milk urea nitrogen (mg/dl)	3,219	16.68±5.87
Somatic cell (10^4/ml)	3,219	39.20±7.70

diets and fertility only when cows had a major health problem such as metritis or cystic ovarian disease. Recently, Roy et al. (2003) suggested that for proper interpretation of MU values to monitor protein nutrition status of the buffaloes parity, milk yield and body weight be considered. Therefore, the present study was conducted to examine the effect of parity, stage of lactation and season on MUN concentrations and milk production, investigating the relationship between MUN concentration and reproduction in Holstein dairy cows.

MATERIALS AND METHODS

Data collection

A total numbers of 3,219 Holstein dairy cows from Korean commercial farms were used in the present study from May 1998 to April 2000. Test data included date, cow number, a.m., p.m. or pooled sample type, and individual cow information (calving date, parity, days in milk (DIM), insemination dates (pregnant and open days) and milk yield).

Milk samples were routinely analyzed for milk fat, milk protein, somatic cell counts (SCC) and MUN. Milk Urea nitrogen (mg/dl) was measured with an automated IR Fossmatic 4,000 Milk Analyzer (Foss North America, Brampton, Ontario, USA).

Statistical analysis

SAS (1996) was used as a tool of statistical analysis and multiple regression analysis, and statistical significance among treatment means was determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

Milk yield, milk fat and protein, MUN and SCC of 3,219 dairy cows in Korea are presented in Table 1. The mean of milk production in the data was 26.48 kg/day. The mean MUN was 16.68 mg/dl (with standard deviation of 5.87 mg/dl). The MUN concentrations in Korean dairy cows are very high compared with those data obtained from dairy herds in USA and Sweden, which are about 12 to 14 mg/dl (Godden et al., 2001abc; Rajala-Schultz et al., 2001; Jonker et al., 2002) and about 11 to 15 mg/dl (Gustafsson and Carlsson, 1993; Carlsson and Pehrson, 1994). This find suggests that Korean Holstein cows are fed diets in high protein, insufficient energy supply or influenced by some non-nutritional factors. It is possible that the high MUN may be caused by high concentrate ration. However, until more is known about the factors which affect MU concentration, the results must be interpreted with caution and only after taking into consideration the variations in feeding practices and management systems in different countries. Thus, it is necessary to consider non-nutritional factors such as days in milk, changes of season and parity affecting milk production and MUN concentration.

Table 2 shows the correlation of milk yield, milk fat and protein content, somatic cell, lactation period and MUN concentration. Days in milk had positive correlation with fat content and MUN ($p<0.01$). Milk yield was negatively correlated with fat and protein contents and SCC in milk ($p<0.01$). Milk urea nitrogen concentration tended to have a positive correlation with milk yield and milk fat component, while milk protein was negatively correlated with SCC ($p<0.01$). Oltner et al. (1985) reported that milk urea was positively correlated with milk yield and Broderick and Clayton (1997) reported a significant positive correlation between fat corrected milk yield and MUN. The increase in milk production would be expected to increase MUN due to higher protein requirements for milk production (Jonker et al., 1999). Supplemental protein may increase milk yield by providing more amino acids for milk protein synthesis, by increasing the available energy through deamination of amino acids or by altering the efficiency of utilization of absorbed nutrients (Chalupa, 1984). Consequently, the deamination of amino acids from supplemental protein increases drainage of nitrogen in terms of urea through urine and milk. Little research has been published that describes the relationship between SCC and MUN. Ng-Kwai-Hang et al. (1985) reported that a small but significant positive association between SCC and milk NPN levels (which includes urea). DePeters and Ferguson (1992), in a review of previous studies, reported that milk from mastitic glands was lower in casein and higher in noncasein

Table 2. Analysis of correlation in milk components

	Milk yield	Milk fat	Milk protein	Milk urea nitrogen	Somatic cell
DIM	-0.43**	0.19**	-0.26**	0.47**	0.025
Milk yield		-0.37**	-0.38**	0.01	- 0.07**
Milk fat			0.48**	0.03	0.02
Milk protein				-0.11**	0.01
Milk urea nitrogen					0.12**

** $p<0.01$.

Table 3. Analysis of relation between seasonal effect and milk components

Season	Milk yield (kg/day)	Milk fat (%)	Milk protein (%)	Milk urea nitrogen (mg/dl)	Somatic cell (10^4/ml)
Spring	28.41±0.37[a]	3.68±0.02[b]	3.06±0.01[b]	17.39±0.30[a]	44.13±4.00[b]
Summer	27.48±0.34[b]	3.74±0.02[ab]	3.12±0.01[a]	15.75±0.28[b]	40.92±3.69[b]
Fall	28.07±0.31[ab]	3.78±0.02[a]	3.14±0.01[a]	15.96±0.25[b]	42.57±3.41[b]
Winter	28.91±0.32[a]	3.74±0.02[ab]	3.06±0.01[b]	17.39±0.26[a]	55.38±3.50[a]

Different superscripts in the same column are significantly different at p<0.01.

Table 4. Analysis of relation between parity and milk components

Parity	Milk yield (kg/day)	Milk fat (%)	Milk protein (%)	Milk urea nitrogen (mg/dl)	Somatic cell (10^4/ml)
1	25.13±0.23[b]	3.83±0.01[a]	3.15±0.00[a]	16.52±0.18	45.64±2.48
2	27.36±0.25[a]	3.78±0.02[ab]	3.13±0.01[a]	16.70±0.21	40.13±2.77
3	28.10±0.25[a]	3.75±0.02[b]	3.09±0.01[b]	16.60±0.20	42.13±2.72
4	28.42±0.34[a]	3.77±0.02[ab]	3.05±0.01[c]	16.50±0.27	47.63±3.65
5	30.24±0.58[a]	3.53±0.04[c]	3.01±0.00[c]	17.51±0.47	47.93±6.28
6	29.49±1.04[a]	3.73±0.08[b]	3.16±0.04[a]	15.82±0.84	45.36±11.16
7	28.80±1.23[a]	3.77±0.09[ab]	3.08±0.04[b]	16.69±1.00	41.43±13.16

Different superscripts in the same column are significantly different at p<0.01.

protein. They suggested that casein breakdown products contributed to the whey protein fraction of mastitic milk. Whey protein is measured by the infrared test method as NPN and should not interfere with the urea estimate (D. McKenna, Foss North America, Eden Prairie, MN, USA; 1999, personal communication).

Table 3 shows the seasonal changes of milk yield and components. Cows in spring and winter produced more milk over 1.43 and 0.93 kg/day, respectively, than cows in summer (p<0.01). Milk fat content in milk produced in fall was 0.06% higher than that in spring (p<0.01). Summer and fall seasons increased (p<0.01) milk protein content over 0.06 and 0.08%, respectively, compared with spring and winter. Milk urea nitrogen concentrations of milk produced in summer and fall were significantly lower (p<0.01) up to 1.43 and 1.64 mg/dl, respectively, than those in spring and winter. In winter somatic cell counts in milk were highest (p<0.01). Dairy cattle across Korea are subject to high ambient temperatures and high relative humidity for summer season from June to August. Hot climates cause heat stress of animals and result in elevated body temperature, which in turn initiate compensatory and adaptive mechanisms to reestablish homeothermy and homeostasis. Stott (1981) stated that these readjustments to maintain homeostasis are referred to as adaptations and may be favorable or unfavorable to the economic interests of humans, and that, nevertheless, they are essential for survival of the animal. A reduced rate of metabolism, decreased DM and nutrient intake, and altered water metabolism all occur in response to heat stress. Unfortunately, responses to heat stress often have negative effects on the physiology of the cow and on milk yield. Feed DMI starts to decline and maintenance expenditures increase when environmental temperatures exceed 25°C (NRC, 1981). Milk yield declined when body temperature exceeded 38.9°C and for each 0.55°C increase in rectal temperature, milk yield and intake of TDN declined 1.8 and 1.4 kg, respectively (Johnson et al., 1963). Thus during summer and fall, hot weather influenced cows to produce less milk, but higher milk fat and protein contents. Lower MUN concentrations of milk produced in summer and fall may be caused by lower DMI of cows in hot climates. As DMI declines, the quantity of consumed nutrients, including CP, also declines and a negative protein balance may occur (Ibrahim et al., 1970; Kamal and Johnson, 1970; Hassan and Roussel, 1975). However, Hwang et al. (2000) reported that MU was significantly lower in the cool season than in the warm season in Taiwan because the lactating cows had a surplus of protein and/or inadequate energy intake in the warm season. Several reports (Dulin et al., 1983; Randy et al., 1991; Muggli, 1995; Wilson et al., 1995) indicating that the somatic cell counts are influenced by season, stage of lactation and productive stage are related to the highest SCC during winter in the present.

Milk yield was lower (p<0.01) in the first calving than other calving time and tended to increase until the fifth parity and then decrease (Table 4). Milk fat and protein contents tended to be reduced until the fifth parity and then recovered, in which milk fat content in the first calving was higher (p<0.01) than that in the third and fifth calvings and milk protein content in the first calving was higher (p<0.01) than that in the third, fourth, fifth and sixth calvings. Milk urea nitrogen and somatic cell were not related to parity of cows in the present study. While some studies reported that MU was lower in first-lactation heifers (Oltner et al., 1985), other studies reported only minor differences, with no significant association between parity group and MU (Canfield et al., 1990).

Table 5 shows relation between days in milk and milk yield and composition. Milk yield was linearly decreased as days in milk increased while milk fat and protein contents were linearly increased (p<0.01). Milk urea nitrogen

Table 5. Analysis of relation between lactation period and milk components

Lactation period	Milk yield (kg/day)	Milk fat (%)	Milk protein (%)	Milk urea nitrogen (mg/dl)	Somatic cell (10^4/ml)
<100 days	32.93±0.33[a]	3.59±0.02[c]	2.99±0.01[c]	15.7±0.26[b]	41.49±3.52[b]
100 to 200 days	28.54±0.32[b]	3.71±0.02[b]	3.09±0.01[b]	16.9±0.26[ab]	45.08±3.43[b]
>200 days	23.19±0.30[c]	3.92±0.02[a]	3.21±0.01[a]	17.2±0.24[a]	50.68±3.21[a]

Different superscripts in the same column are significantly different at p<0.01.

Table 6. Comparison among milk urea nitrogen (MUN) concentration, open days and frequency of artificial insemination (AI)

MUN level (mg/dl)	Open days	Frequency of AI
<12	163.87±12.54[b]	2.08±0.22
12 to 18	154.46±11.32[b]	1.84±0.20
>18	201.72±14.87[a]	2.37±0.27

Different superscripts in the same column are significantly different at p<0.01.

concentration was affected by lactation period, which showed that MUN in the early lactation period (<100 days in milk) was lower (p<0.01) up to 1.5 mg/dl than that in the late lactation (>days in milk). Somatic cell counts in the early and middle lactation periods were lower (p<0.01) than those in the late lactation period. In two studies (Wolfschoon-Pombo et al., 1981; Buchberger, 1989), MUN increased during the first 1 to 2 months of lactation, remained at similar levels for 3 to 5 months and decreased until the end of the lactation, whearase two other studies reported a marked decrease during the first 1 to 2 months of lactation followed by a gradual increase towards the end of lactation (Ng-Kwai-Han et al., 1985; DePeter and Cant, 1992).

Cows produced milk in high MUN concentrations (greater than 18 mg/dl) had more open days than cows in MUN concentrations less than 18 mg/dl (Table 6). No significant difference between MUN concentration levels and frequency of artificial insemination (AI) was found in this study. The finding of this study was significant interaction between non-pregnant days and high MUN concentrations. Studies have reported conflicting results regarding the nature of the relationship between MU and fertility. Butler et al. (1996) reported that concentrations of MUN greater than 19 mg/dl were associated with decreased pregnancy rate. Several studies reported that cows fed high concentrations of dietary CP had higher blood urea concentration and lower CR (Carnfield et al., 1990; Ferguson et al., 1988, 1993; Jordan and Swanson, 1979). There are many theories as to why excess dietary CP decreases reproductive performance (Ferguson and Chalupa, 1989; Barton, 1996; Barton et al., 1996; Butler, 1998). The first theory relates to the energy costs associated with metabolic disposal of excess N. To the extent that additional energy may be required for this purpose, this energy may be taken from body reserves in early lactation to support milk production. Delayed ovulation (Beam and Butler, 1997; Staples et al., 1990) and reduced fertility (Buttler, 1998) have been associated with negative energy status. Another effect of negative energy status is decreased plasma progesterone concentrations (Buttler, 1998). Another theory is that excessive blood urea nitrogen (BUN) concentrations could have a toxic effect on sperm, ova or embryos, resulting in a decrease in fertility (Canfield et al., 1990). High BUN concentrations have also been shown to decrease uterine pH and prostaglandin production (Buttler, 1998). High BUN may also reduce the binding of leutinizing hormone to ovarian receptors, leading to decreases in serum progesterone concentration and fertility (Barton, 1996). Ferguson and Chalupa (1989) reported that by-products of N metabolism might alter the function of the hypophysealpituitary-ovarian axis, therefore decreasing reproductive performance. And last, high levels of circulating ammonia may depress the immune system and, therefore, may result in a decline in reproductive performance (Anderson and Barton, 1988).

The present study indicated that MUN concentrations are associated with fertility efficiency and milk production. However, there were some non-nutritional factors involved in variation of MUN concentration and milk production. Non-nutritional factors such as parity, health status, stage of lactation and season have been reported to be important modifying factors in some studies. Therefore, it is suggested that when MUN values for nutritional management and measures of production or reproduction are used, non-nutritional factors should be considered.

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