Milk Urea Nitrogen and Infertility in Florida Holstein Cows[1]

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ABSTRACT

The objective of this study was to evaluate the association between milk urea nitrogen (MUN) and risk of nonpregnancy after first breeding in a commercial dairy herd in Florida. A total of 515 and 558 cows were classified as having high (17 to 25 mg/dl) or low MUN (6 to 16 mg/dl) within 30 d before first breeding; a total of 158 (30.6%) and 189 (33.8%) cows were diagnosed as pregnant, respectively. Logistic regression was used to evaluate the association between MUN and risk of nonpregnancy controlling for other variables associated with fertility (parity, calving season, breeding season). An interaction was found showing that cows with high MUN that were bred during the summer were 18 times (OR = 17.9; 95% CI = 10.0 to 31.7) at higher risk of nonpregnancy compared to cows with low MUN that were bred during the winter.
(**Key words:** milk urea nitrogen, heat stress, dairy cattle, fertility)

Abbreviation key: BUN = blood urea nitrogen, **CaLCFA** = calcium salts of long chain fatty acids, **CI** = confidence intervals, **MUN** = milk urea nitrogen, **PUN** = plasma urea nitrogen, **OR** = odds ratio.

INTRODUCTION

Fertility is a major contributor to profitability of the dairy herd (25) and is a trait with a very low heritability value (17). Nutritional management plays one of the most important roles in achieving reproductive goals (12).

Milk urea nitrogen (**MUN**) corresponds to 2.5 to 3.0% of total milk N in dairy cattle (8). Milk urea nitrogen is highly correlated with blood urea nitrogen (**BUN**) and is closely related to dietary CP (2, 21,27). Therefore, MUN is a good estimator of BUN and can be used to monitor the protein nutritional status in dairy cows (18, 29).

The negative effect of BUN or MUN on fertility in dairy cattle has been widely reported (3, 5, 6, 9, 10, 11, 12, 13, 16, 24, 28). Some mechanisms that might explain this negative effect are related to the uterine environment and hormonal patterns during the estrous cycle and early pregnancy, thus impairing gametes and embryo survival. In most studies, values of MUN or BUN >19 mg/dl have been related to lower conception rates. Other studies have not found negative effects of MUN on fertility (7, 20).

The objective of this study was to evaluate the association between MUN concentrations and risk of nonpregnancy at first breeding in a commercial dairy herd in Florida.

MATERIAL AND METHODS

Dairy Cows and Farm Management

A commercial dairy farm in north central Florida with 3600 lactating cows and a yearly rolling herd milk production average of 10,500 kg per cow was used in this study. Cows were housed in a dry-lot system and fed a TMR. The same diet was fed to all milking herds, except cows in their first 20 d postpartum, which were fed a ration containing additional effective fiber. Cows were milked 3×/d and fed following each milking. The diet composition and chemical composition of the diet are shown in Tables 1 and 2, respectively.

Reproductive management consisted of a voluntary waiting period of 80 d. After that, cows were identified in estrus by visual observation or a computerized pedometer system (Afimilk, S.A.E. Afikim). Synchronization protocols were not used in a significant number of animals (11.3 and 12.7% of the cows for low and high MUN groups, respectively) ($P > 0.05$). Pregnancy diagnosis was performed by herd veterinarians by palpating per rectum the uterus and its contents approximately 42 to 49 d after insemination. Milk urea nitrogen values were determined on all cows every other month by the Dairy Herd Improvement Association (DHIA) (University Park, PA). Collection of milk samples was carried out during the first milking (0700 to 1400 h). All cows assigned to this study received the same TMR immedi-

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Table 1. Diet composition

Ingredient	Percent (DM)
Corn silage	20.02
Brewers grain	6.70
Hominy corn	26.12
Lactowhey[1]	6.75
Commercial protein/mineral concentrate	9.12
Cotton seed whole	8.96
Alfalfa hay	8.68
Citrus pulp	4.39
Coastal bermudagrass hay	4.30
Cotton seed hulls	3.78
Soybean meal 48%	1.19

[1]Lactowhey: Ammoniated whey (61.5% CP).

ately after each milking, thus, MUN concentrations were determined 6 to 8 h after the last feeding.

Exposure to High or Low MUN

During October 1997 and August 1998, from the herd computational system records a total of 515 and 558 cows with their DHIA MUN determination within 30 d before first breeding were identified as having high MUN (17 to 25 mg/dl) or low MUN (6 to 16 mg/dl). Cows were assigned to these two groups based on their frequency distribution (median). The outcome variable was conception rate at first breeding postpartum.

Data Collection

For each cow included in the study, the following data were collected: parity (1, 2, 3, 4+), calving season (winter months = November to April; summer months = May to October), dystocia (yes, no), breeding season (winter, summer), days to first service, and milk yield. From DHIA records, milk production data were collected on each cow at the end of the lactation or when it left the farm (ME-305-d milk yield and current test-day milk yield).

Table 2. Chemical composition of the diet

Nutrient	Value
DM, %	54.40
Net energy of lactation, Mcal/kg of DM	1.76
CP, % of DM	18.60
Soluble CP, % of CP	40.50
Undegradable intake protein, % of CP	29.72
Fat, % of DM	4.98
Acid detergent fiber, % of DM	22.34
Neutral detergent fiber, % of DM	36.34
Nonstructural carbohydrates, % of DM	33.06
Calcium, % of DM	1.04
Phosphorus, % of DM	0.49

Statistical Analysis

The null hypothesis was that cows with high MUN within 30 d before first breeding would have the same risk of nonpregnancy as cows with low MUN. Descriptive statistics and univariate OR were calculated to screen for statistically significant associations of the variables with nonpregnancy. Potential confounding variables were identified based on statistical significance and biological reasoning (parity, dystocia, calving season, breeding season, days to first service, ME-305-d milk yield and test-day milk yield). Continuous variables (MUN, days to first service, ME-305-d milk yield and test-day milk yield) were categorized into four groups based on their frequency distribution (quartiles). Adjacent categories were collapsed whenever it was biologically feasible and when they had similar stratum-specific odds of nonpregnancy. The data were modeled using logistic regression (19). A starting model was developed with variables that met the selection criteria and were considered to be biologically important (MUN, parity, dystocia, calving season, breeding season, days to first service, milk yield). The level of significance required for potential risk factors to enter the starting model was $P \leq 0.05$. Parity, calving season, and breeding season were forced into the model. A backward model-selection procedure was used in a sequential fashion starting with a full model parameter in the reduction process. Finally, the model's goodness-of-fit was explored with the Hosmer-Lemeshow goodness-of-fit X^2 statistic. In the final model, adjusted odds ratio (**OR**) and 95% confidence intervals (**CI**) were reported.

Differences in median number of days from MUN milk record to first breeding among pregnant and nonpregnant cows were tested by using the Mann-Whitney test. Proportion of cows with high or low MUN that were removed from the herd were compared among groups by use of a X^2 test. Statistical analyses were performed using Statistix for Windows (30).

RESULTS

A total of 1112 (35.4%) of 3146 records were considered for inclusion in the analysis (cows with MUN values within 30 d before first breeding). Fifteen records with data entry errors were not included. Twenty-four, 13 (2.5%), and 11 cows (2.0%) from high and low MUN groups, respectively, were removed for different health problems from the herd between first breeding and pregnancy diagnosis; the proportions of cows removed from the herd did not differ significantly among groups ($P > 0.05$). Total enrollment included 1073 cow records. One hundred and fifty eight (30.6%) and 189 (33.8%) cows with high and low MUN concentrations were diagnosed pregnant, respectively. Median (range) days from MUN milk record to breeding was 12 (0 to 30) and 13 (0 to 30) d among pregnant and nonpregnant cows, respectively; these were not significantly different ($P > 0.05$).

In the univariate analysis, calving season and breeding season were significantly associated with nonpregnancy after first service ($P \leq 0.05$). There was no association found between MUN and nonpregnancy (Table 3). In the multivariable analysis, terms for MUN, parity, calving season, and breeding season were retained (Table 4). Cows that calved during the summer months were at a higher risk of nonpregnancy (OR = 2.8; 95% CI = 1.9 to 4.0) compared with cows that calved during the winter months. Addition to the model of two-way interactions between independent variables already in the model showed that the interaction between MUN and breeding season was statistically significant. Cows with high MUN that were bred during the summer months were 18 times (OR = 17.9; 95% CI = 10.0 to 31.7) at a higher risk of nonpregnancy, compared to cows with low MUN that were bred during the winter months ($P \leq 0.01$). When compared to cows with low MUN bred in winter (OR = 1.0), the effect of MUN and breeding season acting together (OR = 17.9; high MUN in summer) was 3 times the combined effect of these variables acting separately (OR = 0.9; high MUN in winter and OR= 5.9; low MUN in summer).

Table 3. Univariate analysis (Pearson's statistics) for risk of nonpregnancy and associated variables in Holstein cows at first service

Factor	Cr1stS[1]	Unadjusted odds ratio	95% CI[2]	*P*
Milk urea nitrogen (MUN) (mg/dl)[3]				
<16	33.9	1.0		
≥16	30.7	1.2	0.9 to 1.5	0.26
Parity				
1	32.1	1.0		
2	31.4	1.0	0.7 to 1.4	0.86
3	36.9	0.8	0.6 to 1.6	0.23
≥4	29.7	1.1	0.8 to 1.6	0.55
Dystocia				
No	33.0	1.0		
Yes	28.8	1.3	0.9 to 1.7	0.07
Calving season				
Nov-Apr	29.1	1.0		
May-Oct	35.1	0.8	0.6 to 1.0	0.04
Breeding season				
Nov-Apr	53.8	1.0		
May-Oct	11.4	9.0	6.6 to 12.3	≤0.01
Days to first service				
135 to 245	31.7	1.0		
114 to 134	31.6	1.0	0.7 to 1.5	0.96
93 to 113	29.6	1.1	0.8 to 1.6	0.60
49 to 92	36.5	0.8	0.6 to 1.2	0.24
Mature equivalent 305-d milk yield				
5088 to 8875 kg	31.0	1.0		
8876 to 9896 kg	31.2	1.0	0.7 to 1.4	0.95
9897 to 10830 kg	33.2	0.9	0.6 to 1.3	0.58
10831 to 14322 kg	33.9	0.9	0.6 to 1.3	0.46
Test-day milk yield				
18.6 to 32.3 kg	31.6	1.0		
32.4 to 38.6 kg	32.4	0.97	0.7 to 1.4	0.85
38.7 to 45.0 kg	32.4	0.96	0.7 to 1.4	0.84
45.1 to 64.5 kg	33.7	0.91	0.6 to 1.3	0.60

[1]Conception rates at first service.

[2]Confidence interval (95%).

[3]MUN values within 30 d before first service.

Table 4. Logistic regression for risk of nonpregnancy in dairy cows

Factor	Adjusted odds ratio	95% CI[1]	*P*
MUN (mg/dl)			
<16	1.0		
≥16	1.0	0.7 to 1.5	0.90
Parity			
1	1.0		
2	1.1	0.7 to 1.6	0.71
3	0.7	0.5 to 1.0	0.07
≥4	1.0	0.7 to 1.5	0.91
Calving season			
Nov-Apr	1.0		
May-Oct	2.8	1.9 to 4.0	≤0.001
Breeding season			
Nov-Apr	1.0		
May-Oct	10.1	6.4 to 16.1	≤0.01
MUN × Breeding season	17.9	10.1 to 31.7	≤0.01

[1]Confidence interval (95%).

DISCUSSION

This study was designed to assess the association between MUN concentrations and risk of nonpregnancy in a commercial dairy herd in Florida. The unique finding of this study was the significant interaction between MUN and breeding season. We can speculate that high concentrations of MUN might be synergistic with the negative effect of heat stress or might have a direct negative effect on the reproductive physiological processes. Mishra et al. (26) found that rumen NH_3 and lactic acid levels increased significantly in cows under heat stress versus cows fed the same diet but in cool environment and low humidity. Furthermore, the energy cost of urea synthesis might be increased because of the effect of heat stress on other biological mechanisms such as less DMI, high metabolic rate, increased negative energy balance, and altered rumen patterns.

In this study, there was no association found between MUN alone and nonpregnancy. This result does not agree with many studies (3, 5, 6, 9, 10, 11, 13, 22, 23, 24), but does agree with several other investigations (1, 7, 14, 15, 20). These contradictions may be explained by one or more biological mechanisms. One of these mechanisms that might explain why excess dietary protein negatively impacts reproduction is that the addi-

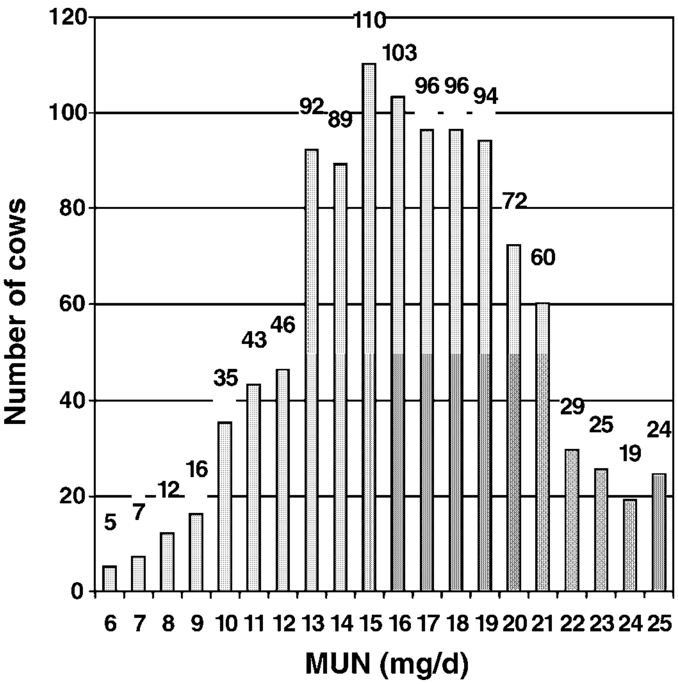

Figure 1. Distribution of the cow population by milk urea nitrogen (MUN) concentration.

tional energy the animal expends to detoxify NH3 in the liver (4, 15). If we analyze the diet composition for the cows 110 to 120 d in milk, where the average first breeding was conducted (Table 2), the excess of 1.91 Mcal/d of NE_L determined that cows were in a positive energy balance and the impact of the excess of dietary CP or urea synthesis excess could be minimal. Garcia-Bojalil et al. (15) did not find differences between nonlactating Holstein cows fed 12.3% of CP (9.8 mg/dl of PUN) and fed 27.4% of CP (21.3 mg/dl of PUN), in number or percentages of preovulatory, anovulatory, and ovulatory follicles induced by superovulation protocol. Furthermore, the numbers of recovered embryos, abnormal or retarded embryos, and unfertilized ova were similar between both groups. They concluded, using nonlactating cows, that the effect of high dietary CP on several measurements of reproductive efficiency were isolated from other possible interactions with factors such as milk yield, negative energy balance, changes in DMI and health problems. These findings do not eliminate the possibility that the concentration and rumen degradability of dietary CP may exacerbate one or more nutritional, metabolic, or management factors that could result in lowered reproductive performance of lactating cows for which changes in energy status are common. The same authors (14) in Florida also, found that diets containing two levels of both degradable protein (15.7 and 11.1% degradable intake protein) and calcium salts of long chain fatty acids (**CaLCFA**) affected reproductive performance of lactating Holstein cows during the first 120 d of latation. In that study, excess of ruminally degradable protein decreased the amount of luteal tissue present in the ovaries of early postpartum lactating cows. However, when they added CaLCFA to the highly degradable protein diet the development of total luteal tissue in the contralateral ovary was augmented. The supplementation of CaLCFA to the 15.7% degradable intake protein diet doubled the number of CL, reduced time to the first rise in P4 by 6 d, doubled the number of normal luteal phases, and restored the pattern of accumulated plasma P4 concentrations to a pattern that was similar to that induced by the other diets. They concluded that the supplementation of additional energy in the form of CaLCFA suggests that the negative effects of excess dietary degradable intake protein were at least partially of energy origin.

CONCLUSIONS

Our data indicated that cows exposed to high MUN concentrations (>16 mg/dl) 0 to 30 d before first service and were bred during the summer months were at higher risk of nonpregnancy, compared to cows with low MUN bred during the winter months.

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