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Associations of days open with milk urea nitrogen and other herd traits in dairy cows

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ABSTRACT - We evaluated the association of monthly milk urea N (MUN) concentrations before conception and days open in high-producing dairy cows from commercial herds in Southern Brazil. Other herd traits were also investigated regarding their relationship with days open. A database containing 16,569 monthly test-day records (obtained from 3,926 lactations of 2,145 cows) for productive, reproductive, and milk quality data was assembled from three different herds. Maximum MUN before conception was divided into quartiles as follows: ≤15.5 mg/dL (1), from 15.6 mg/dL to 18.0 mg/dL (2), from 18.1 to 20.5 mg/dL (3), and >20.5 mg/dL (4). When cows calved in spring and winter, days open averaged (mean±SE) 171.2±2.71 and 155.4±2.31, corresponding to an increase in 12 and 2%, respectively, related to cows that calved in summer (152.7±2.44). When cows calved in fall, a 12% reduction in days open (135.5±1.79) compared with cows that calved in summer was observed. The estimated regression coefficients showed that multiparous cows are expected to have an increase of 12 and 6% in days open when compared with primiparous and secondiparous cows, respectively. Days open were positively associated with the highest MUN value obtained until conception. Cows in the highest quartile of maximum MUN (>20.5 mg/dL) had more days open (184.6±2.93) than cows in quartiles 1, 2, and 3 (123.7±2.12, 150.2±2.16, and 160.5±2.29, respectively), which represents reductions of 40, 21, and 14%, respectively. These results suggest that a maximum MUN concentration before conception higher than 15.5 mg/dL may negatively impact the fertility of high-producing dairy cows.

Keywords: livestock, protein, reproduction

1. Introduction

The cause of low fertility in dairy cows is multifactorial. It means that to improve the reproductive efficiency on dairy farms, we need to adopt adequate reproductive programs and techniques and also implement practices to mitigate heat stress effects. However, such strategies are better harnessed when coupled with good management and nutritional strategies. Poor feeding can affect nutrient availability and reduce fertility, while excessive feeding may also negatively influence reproduction. For example, excessive dietary protein may cause a slight decrease in uterine pH, changes in uterine secretions, reduce embryo survival, and impair embryonic development (Rajala-Schultz et al., 2001; Guo et al., 2004; Hojman et al., 2004).

Milk urea nitrogen (MUN) analysis is a useful tool to monitor protein nutrition and protein:energy synchronization in dairy cows. However, the recommendations for MUN concentration vary largely. For example, Butler et al. (1996) reported that a MUN concentration above 19 mg/dL is associated with a reduction of about 20% in conception rate in dairy cows. However, Rajala-Schultz et al. (2001) suggested that an even lower MUN concentration (>15.4 mg/dL) is negatively related to fertility in dairy cows. Another study (Hossein-Zadeh and Ardalan, 2011), performed with data obtained from 57,301 dairy cows, showed that MUN is correlated with fertility traits in early lactation. However, the MUN concentration at which substantial impairment of reproductive performance occurs still needs to be determined. Among the various fertility traits, days open, also known as calving to conception interval, can easily be determined and is useful in the monitoring of reproductive efficiency.

In Southern Brazil, dairy cows enrolled in a Dairy Herd Improvement (DHI) program have their milk analyzed monthly to provide farmers with data such as fat, protein, and lactose contents and somatic cell count (SCC) and MUN concentrations. As mentioned before, MUN is associated with fertility parameters, and these monthly milk analyses, which include MUN determination, could be an important tool for farmers as possible indicators of dietary adequacy, negative energy balance, and fertility. However, it is not clear how farmers should use this information or which MUN value is more associated with days open, i.e., if farmers are supposed to use the first MUN analysis after calving, the highest MUN value obtained from calving to conception, or the average MUN analyzed during days open.

Thus, this study was performed with the main objective of evaluating the association of monthly MUN concentrations (first, mean, or maximum MUN) before conception and days open in high-producing dairy cows from commercial herds in Southern Brazil. Given the data available, other herd traits (calving season, herd, and parity) were also investigated regarding their relationships with days open.

2. Material and Methods

A database containing 16,569 monthly test-day records (obtained from 3,926 lactations of 2,145 cows) for productive, reproductive, and milk quality data for lactating Holstein cows was assembled from three different herds in Southern Brazil. In all farms, cows were kept in similar conditions, confined in free-stall barns with wood shavings or sand for bedding. Diets were offered as a total mixed ration twice a day and based on corn silage. All diets contained similar ingredients and had a similar nutrient composition. Cows were milked three times a day with no additional feed provided at the milking parlor. Pregnancy status was weekly assessed in all three herds either manually (through rectal palpation) or via ultrasound.

The original dataset had 19,814 test-day records. Edits were made to exclude records with age at calving <18 or >150 months; days in milk >730 d, and days open >365 d. Records without yield data, milk yields <1 L/d, milk fat content <1.0% or >9.0%, and milk urea N concentration <1.0 or >50.0 mg/dL were also discarded. Such exclusions were made based on the understanding that they did not have any biological meaning. After all editions described above, 16,569 monthly test-day records remained in the dataset.

Milk samples were taken from three consecutive milkings at 30-d intervals, pooled on a yield basis, and stored at +4 °C with the preservative bronopol. Milk fat, protein, and lactose contents were analyzed via infrared spectrophotometry (Bentley model 2000, Bentley Instruments Inc., Chaska, MN, USA). Milk somatic cell counts were obtained using an electronic counter (Somacount 500, Bentley Instruments Inc., Chaska, MN, USA). Milk urea nitrogen concentration was analyzed via trans-reflectance spectrophotometry (ChemSpec150, Bentley Instruments Inc., Chaska, MN, USA).

Data were averaged on a per-lactation basis to improve the accuracy of the estimate of the true mean for each predictor and outcome variable. To adequately address MUN association with days open, three MUN values before conception were tested; first MUN post-calving, maximum MUN before conception, and average MUN from calving to conception. The MUN value with a higher correlation coefficient with days open, which was maximum MUN before conception, was chosen for model building and was divided into quartiles as follows: ≤15.5 mg/dL (1), from 15.6 mg/dL to 18.0 mg/dL (2), from 18.1 to 20.5 mg/dL (3), and >20.5 mg/dL (4).

The outcome variable analyzed in this study, days open, was determined as the interval between calving and conception. Pregnancy diagnosis was performed via transrectal ultrasonography or manually through rectal palpation, and days open calculation was done considering the effective conception date.

All predictor variables hypothesized to be related to the outcome of interest and the very outcome variable (days open) were initially screened for normality using the UNIVARIATE procedure of SAS (Statistical Analysis System, version 9.4). As the outcome variable was not normally distributed, all predictor variables that were considered for inclusion in the multivariable model were then screened for correlation with the outcome variable using Spearman's rank correlation.

Generalized linear models were built to assess whether days open and monthly report data were associated. Models were constructed using the GLIMMIX procedure of SAS. The model with gamma distribution was chosen to test the fixed effects of herd, parity (1, 2, and 3+ parities), calving season (spring, summer, fall, and winter), quartile of maximum MUN concentration before conception, and the covariate peak milk yield. Two-way interactions between fixed effects were tested and kept in the model whenever significant.

The following model was used:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + b(\varepsilon_{ijkl} - \varepsilon) + e_{ijkl}, \quad (1)$$

in which Y_{ijkl} = observation, μ = overall mean, α_i = the fixed effect of herd (i = 1-3), β_j = fixed effect of parity (j = 1-3), γ_k = fixed effect of calving season (k = 1-4), δ_l = fixed effect of quartile of maximum MUN concentration before conception (l = 1-4), b = linear regression coefficient, ε_{ijkl} = covariate peak milk yield, ε = average peak milk yield, and e_{ijkl} = error. Adjusted means were compared by Tukey's test, and results were considered statistically significant when $P<0.05$.

3. Results

Summary statistics for days open, milk yield, and milk composition are provided in Table 1. Spearman correlation coefficients among days open and the predictive variables were of moderate degree, such as for maximum MUN before conception (r = 0.33). A generalized linear model was built using herd, parity, calving season, and maximum MUN before conception as variables to predict days open. This model explained 81% of the variation in days open (Table 2), suggesting an acceptable fit.

Table 1 - Descriptive statistics of herd information, milk production, and milk quality

Item	Mean	SEM	Minimum	Maximum
Days open (d)	154.4	77.57	35.0	365.0
Age (mo)	44.9	21.19	19.0	150.0
Days in milk (d)	101.3	73.11	2.0	335.0
Milk yield (kg/d)	43.5	10.09	11.2	72.3
Maximum milk yield (kg/d)	48.6	9.72	13.3	82.1
Fat (g/kg)	32.1	6.62	16.1	69.6
Protein (g/kg)	29.1	2.89	22.6	45.1
Lactose (g/kg)	46.3	2.32	34.8	52.9
Total solids (g/kg)	116.7	8.62	92.2	189.9
First MUN (mg/dL)	13.9	3.83	3.8	36.7
Mean MUN (mg/dL)	15.4	2.77	4.9	28.0
Maximum MUN (mg/dL)	18.1	3.57	4.9	40.0

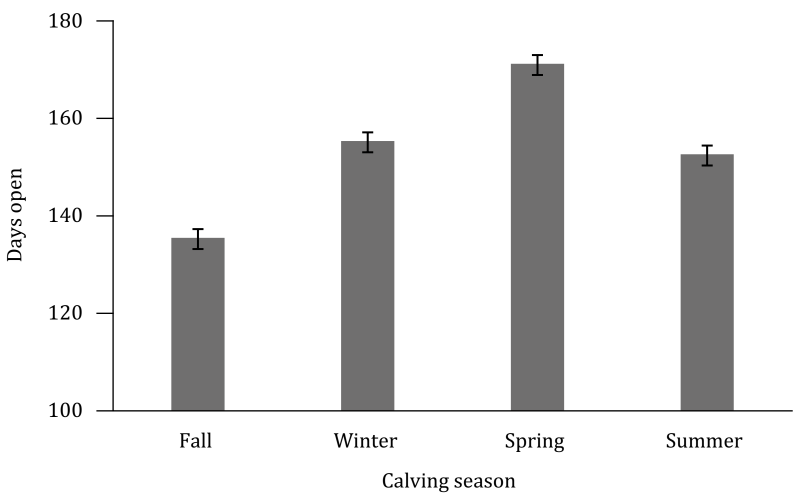
MUN - milk urea N; SEM - standard error of the mean.

The herd from which these data were obtained was significantly (P = 0.0002) associated with days open (Table 2). Days open were significantly (P<0.0001) associated with calving season and averaged 152.7±2.44 for cows that calved in summer (Figure 1). When cows calved in spring and winter, days open averaged 171.2±2.71 and 155.4±2.31, respectively, corresponding to an increase of 12 and 2% when compared with cows that calved in summer (Table 2). When cows calved in fall, an average of

Table 2 - Generalized linear model for associations of days open with explanatory variables

Item	R^2	β	SE	95% Confidence interval		P-value
				Lower	Upper	
Intercept	0.81	5.21	0.060	5.09	5.33	<0.0001
Season						<0.0001
Fall (n = 1249)		−0.12	0.021	−0.16	−0.08	-
Winter (n = 978)		0.02	0.018	−0.03	0.06	-
Spring (n = 861)		0.12	0.023	0.07	0.16	-
Summer (n = 838)		Ref	-	-	-	-
Herd						0.0002
1 (n = 1655)		−0.03	0.019	−0.07	0.01	-
2 (n = 1207)		0.04	0.020	0.01	0.08	-
3 (n = 1064)		Ref	-	-	-	-
Parity						<0.0001
1 (n = 1338)		−0.12	0.021	−0.16	−0.07	-
2 (n = 1068)		−0.06	0.019	−0.10	−0.03	-
>3 (n = 1520)		Ref	-	-	-	-
Milk urea N						<0.0001
≤15.5 (n = 857)		−0.40	0.024	−0.45	−0.35	-
15.6 to 18.0 (n = 1074)		−0.21	0.022	−0.25	−0.16	-
18.1 to 20.5 (n = 1066)		−0.14	0.021	−0.18	−0.10	-
>20.5 (n = 929)		Ref	-	-	-	-

β - estimated regression coefficients; Ref - reference value.



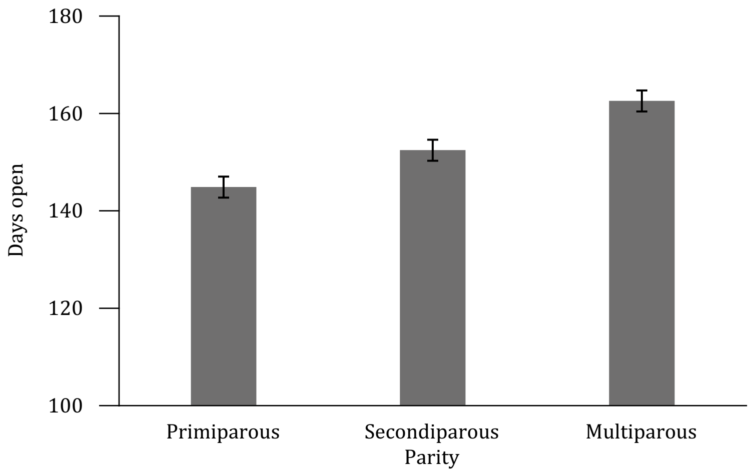
Values are estimated as least square means with their standard errors represented by vertical bars. Standard errors of the means were 1.79, 2.31, 2.71, and 2.44 days for fall, winter, spring, and summer, respectively. Cows calving in fall showed the lowest (P<0.0001) days open value. Cows calving in spring showed the highest (P<0.0001) days open value. There was no difference (P = 0.42) between cows that calved in winter and summer.

Figure 1 - Effect of calving season on days open.

135.5±1.79 days open was observed, which represents a reduction of 12% compared with cows that calved in summer.

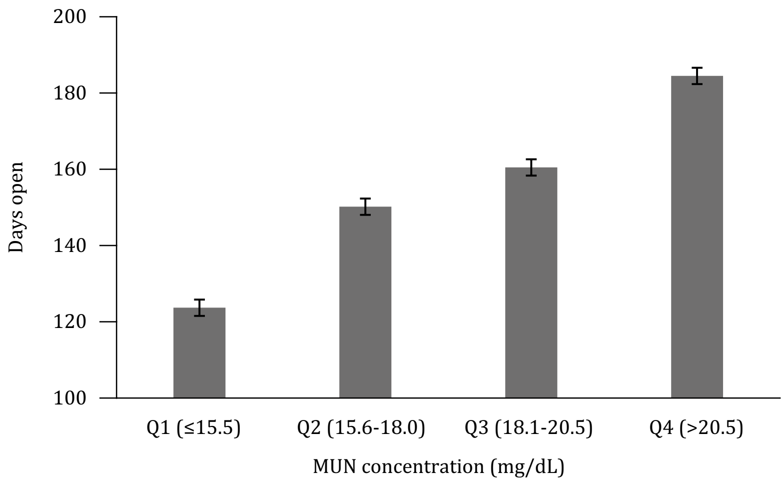
Days open were positively associated (P<0.0001) with parity. Multiparous cows with three or more calvings averaged 162.6±2.08 days open, while primiparous and secondiparous cows averaged 144.9±2.12 and 152.5±2.20, respectively (Figure 2). Estimated regression coefficients (Table 2) showed that multiparous cows, with three or more calvings, are expected to have an increase of 12 and 6% in days open when compared with primiparous and secondiparous cows, respectively.

Days open were positively associated (P<0.0001) with maximum MUN before conception. Cows in the highest quartile of maximum MUN (>20.5 mg/dL) had more days open, with a mean of 184.6±2.93 d, than cows in quartiles 1, 2, and 3, with mean days open of 123.7±2.12, 150.2±2.16, and 160.5±2.29, respectively (Figure 3). Estimated regression coefficients (Table 2) showed that cows in quartiles 1, 2, and 3 are expected to have reductions of 40, 21, and 14%, respectively, in days open than cows in the fourth quartile.



Values are estimated as least square means with their standard errors represented by vertical bars. Standard errors of the means were 2.12, 2.20, and 2.08 days for primiparous, secondiparous, and multiparous cows, respectively. Primiparous cows showed lower (P<0.0001) days open value than secondiparous and multiparous cows.

Figure 2 - Effect of parity on days open.



Values are estimated as least square means with their standard errors represented by vertical bars. Standard errors of the means were 2.12, 2.16, 2.29, and 2.93 days for Q1, Q2, Q3, and Q4, respectively. Days open were significantly increased (P<0.0001) from the bottom quartile to the top quartile.

Figure 3 - Effect of maximum milk urea N (MUN) quartile before conception on days open.

4. Discussion

In our study, maximum MUN levels before conception were associated with days open, and with increasing MUN concentrations, cows had a greater days open period. This agrees with the findings of Mitchell et al. (2005), who evaluated the association of MUN with performance and disease. As MUN is closely related to plasma urea nitrogen (PUN), the possible explanation for this is the effects that high PUN may have on the reproductive system. In a previous study, high PUN levels decreased embryo viability in lactating dairy cows (Rhoads et al., 2006). The physiological basis for this effect lays mainly in the fact that excessive PUN slightly changes uterine pH (Elrod and Butler, 1993; Elrod et al., 1993; Rhoads et al., 2004). As urea is a small molecule, it can diffuse through cell membranes, including those of the uterus, reducing the pH level (Rhoads et al., 2004), thus impairing embryo development (Ocon and Hansen, 2003). Other alterations, such as changes in urea concentration, Mg, K, P, and Zn in uterine fluid, have also been reported (Jordan et al., 1983). Another hypothesis is that excessive urea in the animal organism is a consequence of extensive liver metabolism to detoxify ammonia absorbed from the rumen, which could exacerbate the negative energy balance after calving. This may lead to irregular cycles and, consequently, impaired reproductive indices (Leroy et al., 2008). Altogether, these effects are possible explanations for the greater days open observed in the present study as maximum MUN levels before conception increased.

To better address the effects of maximum MUN levels on days open, lactations were categorized into four groups (quartiles) according to the maximum MUN before conception. Cows in the bottom quartile, with a maximum MUN lower than 15.5 mg/dL, showed a lower and more desirable days open value. However, it should be mentioned that only 21.8% of the cows in the study were in this quartile. This leads us to infer that the protein nutrition of the herds should be improved to obtain a better protein-fermentable carbohydrate balance. According to a previous study, the protein-fermentable carbohydrate ratio in the rumen is an important factor to maintain adequate blood and MUN levels (Rhoads et al., 2006).

Over the years, different MUN values affecting fertility have been reported in the literature. For example, Butler et al. (1996) studied the influence of dietary protein on dairy cows and concluded that a MUN concentration above 19 mg/dL adversely affects fertility. However, Rajala-Schultz et al. (2001) evaluated the effect of MUN on fertility in 1,249 dairy cows, using cows sorted into quartiles for milk urea, and concluded that MUN concentrations greater than 15.4 mg/dL resulted in a lower conception rate compared with lower MUN concentrations. These authors also reported that cows showing MUN levels lower than 10 mg/dL before conception had a 2.4 times greater possibility to become pregnant than cows with levels above 15.4 mg/dL. Hojman et al. (2004) also sorted cows into quartiles and observed that those with MUN concentrations lower than 11.8 mg/dL had a 1.4 times higher possibility to become pregnant than cows with MUN levels between 11.8 and 14.1 mg/dL. Ferguson et al. (1993) reported that MUN concentrations above 14.9 mg/dL decreased the probability of detecting pregnancy in cattle. Although these values vary from one study to another, the effect we observed in the present study was similar to those reported previously. However, it should be noted that we adopted the maximum MUN concentration analyzed from calving to conception, not the first MUN nor the mean MUN concentration after calving. This suggests that excessively high MUN values any time before conception may increase the days open period. Thus, efforts should be made by farmers and nutritionists to avoid excessively high MUN values after calving.

As expected, herd had a significant effect on days open. It should be noted that these data were obtained from a self-selected group of farms; therefore, sampling was not random. The main criteria for choosing these three herds was that monthly DHI-MUN analysis was adopted early on these farms. Besides, these herds have been analyzed for MUN every single month since the beginning of lactation, which is important in a study aiming to investigate the influence of MUN on reproductive traits. Influences of herd on reproductive performance indices are commonly observed in field studies. Rajala-Schultz et al. (2001), Santos et al. (2009), and Aguilar et al. (2012) also reported that herd has a significant effect on reproductive parameters. Such effects are probably caused by different management practices and diverse reproductive and nutritional strategies adopted on each farm.

Similar to the present study, previous references have also shown calving season effects on fertility traits of high-producing cows (Rajala-Schultz et al., 2001; Santos et al., 2009). Such effects are generally attributed to seasonal changes in photoperiodic stimulation (Dahl et al., 2000) and feed quality (Rhodes et al., 2003). The herds included in this study were located in areas that facilitate the growth of high-quality forage in fall and winter, such as ryegrass and oat, which usually have higher nutritional value than summer or spring grasses. However, the total diet quality depends on the specific and individual nutritional strategies of each farm. Additionally, it is common among farmers in that area to adopt reproductive management strategies that contribute to calving in the cooler seasons of the year to obtain higher incomes, since milk prices are generally higher at this time. Our results suggest that calving in spring and winter increases the days open related to calving in summer and fall. Greater risks of anestrus (Walsh et al., 2007) or delayed postpartum ovulation (Opsomer et al., 2000) have been reported for cows calving in spring and winter. Altogether, these are potential explanations for the greatest period of days open observed in those cows that calved in spring.

In our study, multiparous cows showed the greatest days open period. As this period is related to postpartum initiation of estrous cycles, conception rate, and pregnancy maintenance, our results suggest that older cows could have more difficulties to conceive or to maintain a pregnancy than secondiparous and primiparous animals. Parity effects on reproductive performance, specifically on conception rate, are still the subject of debate. While some authors reported a higher conception rate for younger cows compared with multiparous ones (Rajala-Schultz et al., 2001; Tenhagen et al., 2001), others reported lower conception rates for primiparous cows (Guo et al., 2004). However, Santos et al. (2009) reported that although primiparous cows are less likely to initiate estrous cycles by 65 days postpartum, their conception rates at 30 and 58 days after artificial insemination were greater than those of multiparous cows. The authors attribute this to a possible lower pregnancy loss in primiparous cows. For the present study, we had no information on any potential disorders that cows might have shown during the data collection period. However, the greater susceptibility of multiparous cows to postpartum disorders is a potential explanation for their higher days open observed. Multiparous cows have been reported to be at greater risk of clinical endometritis than secondiparous and primiparous cows (LeBlanc et al., 2002), and it is well established that periparturient disorders are associated with reduced fertility in dairy cows (Eicker et al., 1996). Besides, multiparous cows are also more prone to metabolic problems than younger cows, including negative energy balance, which also may impair fertility (Erb and Grohn, 1988; De Vries et al., 1999).

5. Conclusions

Overall, our results suggest that monthly milk urea nitrogen records before conception, together with other factors such as parity and calving season, can be used to estimate days open in high-producing dairy cows. Maximum milk urea nitrogen concentration before pregnancy is more significantly associated with increasing days open rather than first postpartum milk urea nitrogen concentration or mean milk urea nitrogen concentration before conception. A maximum milk urea nitrogen concentration before conception above 15.5 mg/dL may negatively impact the fertility of high-producing dairy cows. Cows calving in fall have the shortest period of days open, while those calving in spring have the longest one. Primiparous cows have a shorter days open period than multiparous cows.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: R. Almeida, J.A. Horst and A.A. Valloto. Data curation: R. Almeida, J.A. Horst and A.A. Valloto. Formal analysis: R. Almeida. Funding acquisition: R. Almeida. Investigation: R. Almeida and M.C. Doska. Methodology: R. Almeida, M.C. Doska and G.T. Santos. Project administration: R. Almeida.

Resources: R. Almeida, J.A. Horst and A.A. Valloto. Writing-original draft: R. Almeida, M.C. Doska, G.T. Santos and L.S. Lima. Writing-review & editing: R. Almeida and L.S. Lima.

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