Relationships between milk urea and production and fertility traits in Holstein dairy herds in the Czech Republic

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ABSTRACT: The objective of this study was to determine how the concentration of milk urea (MU) and FPCM production affect reproduction in selected Holstein dairy herds in the Czech Republic. A retrospective, observational study comprising analyses of individual cow records from monthly dairy herd improvement (DHI) milk tests including milk urea (MU) concentration was conducted in six commercial Holstein dairy herds in cows that were bred from July 2000 to August 2003. A total of 1 333 cows with available breeding date, pregnancy status, and calving to first service interval from 31 to 150 days were selected. The data were evaluated using the mixed linear model and logistic analyses. The effect of MU concentration on the probability of conception at first service was not significant (P = 0.11). The results indicated a nonlinear relationship between MU concentration and the probability of conception. A significant effect of the length of calving to first service interval (P < 0.05) and FPCM (P < 0.05) was determined. The cows with the calving to first service interval longer than 2 months had higher probability of conception (10% higher). In the group with the highest FPCM a markedly lower level of successful pregnancy was determined (38.99%), which significantly differed from the group with average FPCM production (48.53%). The probability of conception at first service in the group with the lowest FPCM is at an average level (43.55%).

Keywords: dairy cow; reproduction; probability of conception

Urea is an end product of nitrogen conversion and there are two origins of it in ruminants. Firstly, the unused ammonia formed in the rumen is converted into urea in the liver; secondly, the urea originates during amino acid catabolism in the body (Ponter et al., 2001; Nousiainen et al., 2004). A small molecule of urea is able to diffuse through cell membranes including the mammary gland. Milk urea concentration (MU) is strongly correlated with blood plasma urea concentration (PU) (0.88–0.98) (Butler et al., 1996; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001), moreover the measurement of MU is convenient and noninvasive and may be useful as a management tool to improve the efficiency of production (Godden et al., 2001b).

Dairy cows are commonly fed diets containing high levels of crude protein to maximize milk production (Rhoads et al., 2006). Although the milk urea concentration may be affected by others factors (Jílek et al., 2006), it has been demonstrated that the excessive feeding of crude protein can lead to increased MU concentration (Broderick and Clayton, 1997; Hojman et al., 2004).

Because reproductive performance has a substantial impact on the economic profitability of dairy farms (Mourits et al., 1997), many studies

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have focused on the relationship between MU concentration and reproduction with the objective of obtaining a monitoring or diagnostic tool for reproductive performance.

Several studies have reported the negative effect of BU or MU on reproductive performance in dairy cows (Ferguson et al., 1993; Butler et al., 1996; Rajala-Schultz et al., 2001). Rhoads et al. (2006) tested the pregnancy rate after embryo transfer from donors with medium or high PU concentration to the heifers with medium or high PU concentration (2 × 2 factorial design). Their results indicated that a high PU concentration in lactating dairy cows decreases embryo viability through affecting the oocyte or embryo before recovery from the uterus 7 days after insemination.

However Guo et al. (2004) demonstrated only a slight negative association with the probability of conception at first service within herds. The MU may be related to conditions affecting the reproduction of individual cows within a herd. Ferguson et al. (1993) also determined that cows within herds with high MU were associated with reduced probability of conception at first service, but not at subsequent services. Others did not find any negative effects of high MU on the fertility of cows (Howard et al., 1987; Garcia-Bojali et al., 1998; Melendez et al., 2000; Godden et al., 2001b).

The possible association between MU and the pregnancy rate in an Israeli dairy herd was investigated by Hojman et al. (2004). Increasing levels of MU were negatively related to the reproductive performance of diary cows, but the risk of nonpregnancy caused by a high level of MU was lower than reported by other authors. Godden et al. (2001a) tested milk urea concentration as a tool to monitor reproductive performance in Ontario dairy herds and concluded that MU testing may have limited utility as a monitoring or diagnostic tool for reproductive performance.

The objective of this study was to determine the relationship among MU concentration, FPCM production and reproduction in selected Holstein dairy herds in the Czech Republic.

MATERIAL AND METHODS

Cows that were bred from July 2000 to August 2003 in six commercial Holstein dairy herds were included in the experiment. A total of 1 333 cows with individual records of monthly dairy herd improvement (DHI) milk tests, including MU, and with available breeding date, pregnancy status, and calving to first service interval from 31 to 150 days were selected. All the cows were milked twice a day, housed in free stalls, and fed a TMR twice a day, but no distinctions were made for management style or feeding scheme. Laboratory measurements were performed by the Czech-Moravian Breeders' Corporation, Inc. The MU concentration (mmol/l) was measured using a standardised procedure based on the rate of electrical conductivity change during urea hydrolysis with Ureakvant 2 (Agroslužby Olomouc, Czech Republic) (Hanuš et al., 2008).

Test day observations in the interval from 31 to 150 days in milk (DIM) and reproduction data were combined into a dataset that included herd code, date of test, milk yield, milk protein content, milk fat content, MU concentration (mmol/l), breeding date, parity, date of first service, reproduction status, and date of conception (available only for 1 319 cows). Each production variable was screened for normality and for the presence of the outliers. Records with milk protein content and milk fat content exceeding intervals of < 2%; 5.5% > and < 2%; 6.5% >, respectively, were excluded from the analysis to remove the outliers. Fat-protein corrected milk (FPCM) was calculated for each test day and averaged for each individual in the stated interval (31 to 150 DIM). Subsequently, these values were divided into three categories: low (FPCM < 29.5), medium (FPCM ∈ < 29.5; 37.2) and high (FPCM ≥ 37.2), using the mean value and standard deviation of data. The average of MU was used in the same manner.

The investigative analysis of the data showed heterogeneous variances in MU concentration among the particular farms. Consequently, the averages and standard deviations within the particular farms were used to divide MU concentrations into particular categories (low ($MU < x_i - s_i/2$), medium ($MU \in < x_i - s_i/2;\ x_i + s_i/2$)) and high ($MU \geq x_i + s_i/2$)), where: i = 1–6). During the dataset processing other effects were calculated into the model, including season, lactation number (1st, 2nd and further) and two- and multi-factorial interactions. None of these factors was significant ($P > 0.20$), so they were subsequently removed by backward elimination.

The selected reproductive variables (calving to first service interval, calving to conception interval, first service to conception interval and number of services per conception) were evaluated using the mixed linear model. Parameters were estimated by the REML method using a Mixed Procedure of SAS (SAS Institute, 2006) The following statistical model was used:

$$Y_{iklm} = \mu + h_i + F_k + U_l + FU_{kl} + e_{iklm}$$

The probability of conception at first service was analyzed using logistic analyses of the Glimmix Procedure of SAS (SAS Institute, 2006). The parameters were estimated by the maximum likelihood method. The final generalized linear model was as follows:

$$\ln\left(\frac{p_{ijklm}}{1-p_{ijklm}}\right) = \mu + h_i + I_j + F_k + U_l + FU_{kl} + e_{iklm}$$

where:

Y_{jklm} = dependent reproductive variable (calving to first service interval, calving to conception interval, first service to conception interval and number of services per conception) alternatively
p_{ijklm} = the probability of conception for a cow
μ = the intercept
h_i = the random effect of herd (i = 1 to 6)
I_j = calving to first service interval (j = 1 to 4), intervals of 30 days each, starting from 31 days after parturition
F_k = FPCM (k = 1 to 3)
U_l = MU concentration (l = 1 to 3)
e_{iklm} and e_{ijklm} = random errors

Least-squares means and standard errors of effects were estimated. The estimates of probabilities in the treatment groups were obtained by applying the inverse link function to the least squares means. The standard errors of probability predictions were computed on the basis of standard errors on the logit scale by the Delta method (Bishop et al., 1975). Multiple comparisons were made, with P-values adjusted using Tukey's procedure

RESULTS AND DISCUSSION

Relationship between MU concentration and selected reproductive variables

1 319 pregnant cows from 6 farms were included into the observation. Milk urea was determined for each test day and the average for each individual in the stated interval (31–150 DIM). On some farms the average MU in analysed samples ranged from 4.10 to 8.08 mmol/l (11.49–22.64 mg of MUN/dl). The cows were divided into 3 categories according to the MU concentration (low, medium and high – see Material and Methods). The results of the reproduction indicators are given in Table 1. A significant relationship ($P < 0.05$) was found only between MU and calving to first service interval. Surprisingly, the cows in the group with the lowest MU had the longest calving to first service interval. No relationships were found in the other indicators.

MU concentration is influenced mainly by dietary factors, such as CP (crude protein), RDP (rumen

Table 1. Least squares means of calving to first service interval, calving to conception interval, first service to conception interval and number of services per conception by MU categories

Reproductive variable	MU category		
	low	medium	high
N	404	519	396
Calving to first service interval (days)	82.94[a]	81.07	78.20[b]
SE	4.042	4.000	4.045
Calving to conception interval (days)	134.81	137.01	139.18
SE	9.158	8.977	9.173
First service to conception interval (days)	51.69	55.72	60.81
SE	6.302	6.054	6.325
Number of services per conception	2.12	2.14	2.36
SE	0.087	0.080	0.088

different letters of the superscript mean a significant difference at the level of $P < 0.05$

Table 2. Partial *F* tests of fixed effects

Effect	Number DF	Dennum. DF	*F* value	Pr > F
Calving to first service interval	3	1 316	3.23	0.0217
FPCM	2	1 316	3.87	0.0210
MU	2	1 316	2.20	0.1109
FPCM × MU	4	1 316	1.66	0.1580

degradable protein), RUP (rumen undegradable protein), energy:protein ratio, and NSC (nonfibre carbohydrates) (Baker et al., 1995; Broderic and Clayton, 1997; Godden et al., 2001a).

Hypotheses suggest that a high urea concentration impairs reproduction through an indirect effect on the energy status (Jordan et al., 1983; Kaim et al., 1983; Holtz et al., 1986; Howard et al., 1987; Broderic and Clayton, 1997). An excessive intake of degradable protein and a relative shortage of energy to synthesize bacterial proteins will result in the accumulation of excessive ammonia in the rumen (Sinclair et al., 2000), which is absorbed through the ruminal wall and converted into urea in the liver. This detoxification process consumes energy and thus may exacerbate NEB early post partum (Leroy et al., 2008a).

Inconsistent results concerning the relationship between MU concentration and reproduction can be found in the literature. Thus, the effect of negative energy balance (NEB) on the reproductive performance is demonstrated (for review see Leroy et al., 2008a,b). NEB is associated with a high incidence of irregular cycles that can both increase the interval to first service and reduce conception rates (Wathes et al., 2007). Important was a hypothesis introduced by Oltner and Wiktorsson (1983), who reported that urea concentrations were not the results of absolute levels of either dietary protein or energy, but of the protein:energy (P:E) ratio. Three different P:E circumstances could occur: when the absolute levels of protein and energy fed are both underfed at low levels; when both are fed at recommended moderate levels or when both are overfed at high levels. While each of these circumstances could result in similar and moderate urea concentrations, we would not necessarily expect cows to reach the same fertility among these three circumstances.

In our study we can hypothesise that the NEB had a greater effect on the length of calving to first service interval than the MU concentration. In the feeding rate where the energy:protein ratio expressly benefits the crude protein content and the cow is able to ingest enough feedstuff to ensure enough energy for reproduction, and the start of ovarian cyclicity and the onset of full-value oestrus are not delayed, the crude protein intake is excessive and thus the MU concentration is high.

Factors affecting probability of conception at first service

Upon evaluating the effect affecting the probability of conception at first service together with the effect of herd, which was included in the model

Table 3. Means of the probability of conception by calving to first service interval categories

Calving to first service interval (months)	N	LSM	SE	Probability of conception	SE
2	312	–0.571[a]	0.145	0.3611	0.0335
3	563	–0.142[b]	0.118	0.4646	0.0294
4	302	–0.135	0.144	0.4664	0.0358
5	156	–0.174	0.187	0.4565	0.0463

different letters of the superscript mean a significant difference at the level of $P < 0.05$

Table 4. Means of the probability of conception by FPCM categories

FPCM (kg)	N	LSM	SE	Probability of conception	SE
< 29.5	425	–0.259	0.1332	0.4355	0.0328
< 29.5–37.2	492	–0.059[a]	0.1288	0.4853	0.0322
37.2 ≤	416	–0.448[b]	0.1362	0.3899	0.0324

different letters of the superscript mean a significant difference at the level of $P < 0.05$

as a random effect (not displayed in the table), a significant effect of the length of calving to first service interval ($P = 0.0217$) and FPCM ($P = 0.0210$) was found. The MU concentration ($P = 0.1109$) and interaction between FPCM and MU ($P = 0.1580$) had no significant effect (Table 2). During the dataset processing other effects were involved in the model, including season, lactation number (1st, 2nd and further) and two- and multi-factorial interaction. However, none of these factors was significant ($P > 0.20$).

The cows with a calving to first service interval longer than 2 months had higher probability of conception (10% higher) (Table 3). The nadir of negative energy balance occurs in early lactation and it can be hypothesised that the increased probability of conception at first service in the 3rd and next months of lactation is related to NEB.

In the group of the highest FPCM a markedly lower level of successful pregnancy was estimated (38.99%) (Table 4), which significantly differed from the group with average FPCM production (48.53%). Surprisingly the probability of conception at first service in the group with the lowest FPCM is at an average level (43.55%). In the study by Westwood et al. (2002), higher production of FCM during early lactation was associated with a longer interval from calving to first ovulation and with reduced likelihood of oestrus expression and successful pregnancy by the 150th day of lactation. The same results were reported by Buckley et al. (2003) where cows with high cumulative corrected milk yield were less likely to become pregnant at first service. The cause of reproduction losses is NEB, which may restrict follicular development and reduce oocyte quality (Britt, 1992) and reduce the plasma progesterone concentration (Spicer et al., 1990). The severity and duration of NEB may be influenced by the genetic merit for milk yield (Buckley et al., 2000).

The lower probability of conception (43.55%) in the group with the lowest FPCM production cannot be explained from available data. It is possible that the FPCM production in this group is not necessarily in direct relationship to NEB, or, more precisely, lower production does not necessarily imply a better energy balance. Lower production may indicate e.g. other health disorders, or the correlation between NEB and production in this group is not necessarily as close, etc.

The probability of conception at first service was almost consistent in the cows with medium and low MU concentration (Table 5). The probability of conception in the cows with high MU tended to be the lowest, but the differences were not significant ($P = 0.11$).

Figure 1 shows the effects of FPCM × MU interactions on the probability of conception. In this case the differences are not significant ($P = 0.16$) either but they have an unusual tendency. The re-

Table 5. Means of the probability of conception by MU concentration categories

MU	N	LSM	SE	Probability of conception	SE
Low	411	–0.199	0.1326	0.4505	0.0328
Medium	523	–0.144	0.1238	0.4642	0.0308
High	399	–0.424	0.1356	0.3956	0.0324

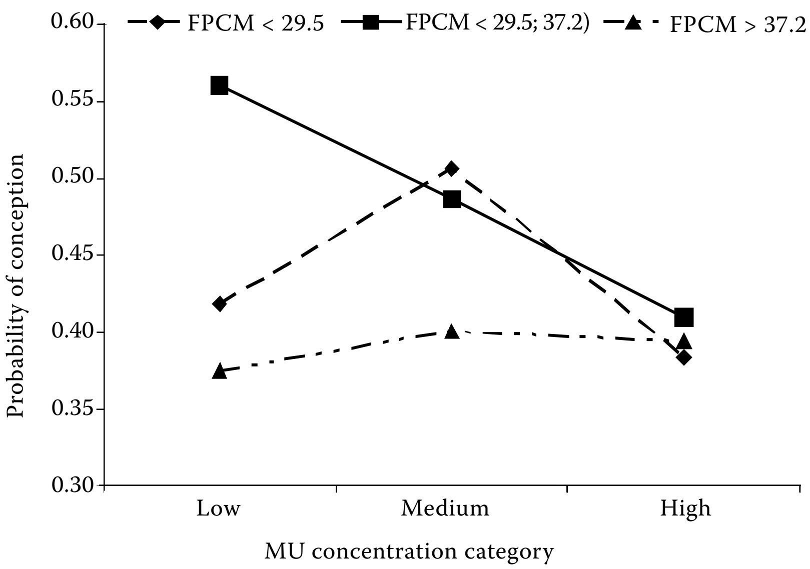

Figure 1. The effect of FPCM × MU interaction on the probability of conception

sults, with regard to the previous findings, imply a nonlinear relationship between MU and the probability of conception. Vallimont et al. (2003) also found that cows with very high and very low MUN within the 2-week period before insemination had reduced conception rates. In the study it was reported that cows with higher MUN had reduced conception rates. Many other authors also reported the lower probability of conception associated with high milk urea content (Rajala-Schultz et al., 2001; Guo et al., 2004).

CONCLUSION

The effect of MU concentration on the probability of conception at first service was not significant in this study. However, significant effects of the length of calving to first service interval and FPCM were found. These results imply a nonlinear relationship between MU concentration and the probability of conception. This study confirmed that further research would be required for the decision on the possible use of MU concentration as a selection or management tool to improve reproductive performance.

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