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Effect of some factors on relationships between milk urea levels and cow fertility

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Abstract

The effect of some factors on relationships between milk urea levels and fertility of 19 028 cows was analysed. The generalized linear model (GLM) of the SAS package (SAS 2004) was used in the statistical calculations. The increase in milk urea concentration was found to increase calving interval (r=0.05, $P \leq 0.01$), rest period (r=0.07, $P \leq 0.01$), service period (r=0.01, $P \leq 0.01$) and the number of services per conception (r=0.02, $P \leq 0.01$). Therefore, dairy breeders may find it advantageous to monitor urea concentrations, which could help to improve cow fertility. Herd milk production level, age of cow and lactation period were the factors that had the greatest effect on the relationship between milk urea levels and cow fertility parameters. The relationship between milk urea level and fertility was stronger in cows from herds with >6 000 kg milk yield, in first-calf heifers and in cows at 3 months of lactation.

Keywords: cow, Holstein Friesian, milk yield, fertility, urea

Zusammenfassung

Auswirkung von einigen Faktoren auf die Beziehungen zwischen Harnstoffspiegeln in der Milch und Fertilität von Kühen

Die Auswirkung einiger Faktoren auf die Beziehungen zwischen Harnstoffspiegeln in der Milch und der Fertilität von 19 028 Kühen wurde ausgewertet. Bei den statistischen Berechnungen wurde das generalisierte lineare Modell (GLM) des SAS-Pakets (SAS 2004) verwendet. Es wurde festgestellt, dass die Zunahme der Harnstoffkonzentration in der Milch die Zwischenkalbezeit (r=0,05, $P \leq 0,01$), Rastzeit (r=0,07, $P \leq 0,01$), Güstzeit (r=0,01, $P \leq 0,01$) und die Anzahl von Besamungen je Trächtigkeit (r=0,02, $P \leq 0,01$) vergrößert. Darum könnten es die Milchviehzüchter vielleicht als vorteilhaft betrachten, die Harnstoffkonzentrationen aufzuzeichnen, was dabei helfen könnte, die Fertilität von Kühen zu verbessern. Das Herdenmilchleistungsniveau, das Alter der Kuh, und der Laktationszeitraum waren die Faktoren, die die größte Auswirkung auf die Beziehung zwischen Harnstoffspiegeln in der Milch und Fertilitätsparametern in Kühen aufwiesen. Die Beziehung zwischen den Harnstoffspiegeln in der Milch und der Fertilität war in Kühen aus Herden mit >6 000 kg Milchleistung, in Erstkalbfärsen und in Kühen nach dreimonatiger Laktation stärker.

Schlüsselwörter: Kuh, Holstein Friesian, Milchleistung, Fertilität, Harnstoff

Introduction

Urea is the end product of nitrogen metabolism in cattle. Because of passive diffusion, the urea molecule regulates concentrations of body fluids, and serum and milk urea levels are positively and highly correlated (Butler *et al.* 1996, Castillo *et al.* 2003). In milk, urea nitrogen forms 2.5-3 % of total nitrogen (Melendez *et al.* 2000). Several experiments (Hamann & Kromker 1997, Skrzypek 1998, Castillo *et al.* 2003) showed that in terms of nutrition, the optimum milk urea concentration for large dairy breed cows is 15-30 mg/dl. Monitoring blood or milk urea content may help to identify reasons for reproductive failure or health disorders (Markiewicz 2003). It is assumed that both urea and ammonia can affect the reproductive system during follicular development, ovulation, oocyte fertilization, embryo development and implantation (Butler 1998). A negative effect of elevated urea content on cow fertility was confirmed by many studies with American, Canadian and Israeli populations of Holstein-Friesian cows (Melendez *et al.* 2000, Rajala-Schultz *et al.* 2001, Hojman *et al.* 2004). Melendez *et al.* (2000) demonstrated that cows with high urea levels (>16 mg/dl) during 0 to 30 days before first insemination and calving during summer months were characterized by a higher risk of infertility compared to cows with low milk urea levels that calved during winter months. Larson *et al.* (1997) suggested that high milk urea concentration might be associated with fertilization failure or very early embryo loss (still before detection of pregnancy).

Dairy cattle farms in Poland participate in the milk recording system, as part of which milk urea levels are determined at monthly intervals. The aim of this study was to analyse the effect of some factors on the relationships between milk urea levels and cow fertility.

Material and methods

Data used in the study were obtained from the SYMLEK system database. Analysis included milk urea levels from test-day records of 19 028 Black-and-White Polish Holstein-Friesian cows, milk recorded in the Pomerania and Kujavia regions. The cows had calved first in 2000 and 2001 and were used until 2008. Statistical calculations accounted for test-day yields obtained to 30 days before first insemination after calving.

- SYMLEK data were also used to calculate cow fertility indices:
- calving interval (CI) – number of days between calving and conception,
- rest period (RP) – number of days between calving and first insemination,
- service period (SP) – number of days between first and successful insemination,
- insemination index (II) – number of services per conception.

Numerical data were analysed statistically using the SAS package (SAS 2004). Two-way analysis of variance and the following linear model were used:

$$Y = \mu + a_i + b_j + (ab)_{ij} + e_{ijk} \quad (1)$$

where μ is the overall mean, a_i is the effect of *i*-th milk urea level (≤15, 15.1-30, >30 mg/dl), b_j is the effect of *j*-th herd milk production level (≤5 000, 5 001-6 000, >6 000 kg milk) or *j*-th age of cow (first-calf heifer, primipara) or *j*-th season of first insemination (II-IV, V-VII, VIII-X, XI-I) or *j*-th lactation period (≤60, 61-90, 91-120, 121-150 days), $(ab)_{ij}$ is the interaction between milk urea level and herd milk production level (or age of cow, or season of first insemination, or lactation period) and e_{jjk} is the random error of observations.

Significant differences were analysed using the Scheffe test.

In addition, the proportion of cows that conceived after first insemination (depending on milk urea level found in the first milking conducted up to 30 days before this insemination) was calculated, taking into account the effect of herd milk production level, age of cow, season of first insemination and lactation period.

Results and discussion

The level of urea in test-day milk obtained during 0 to 30 days before first insemination averaged 18.9 mg/dl and was within the range (15-30 mg/dl) considered optimal from the nutritional standpoint (Hamann & Kromker 1997, Skrzypek 1998, Panicke *et al.* 2000, Richardt *et al.* 2001a) (Table 1). In analysing reproductive parameter values, it should be pointed out that cows were characterized by low fertility (CI of 417 days, RP of 94 days, SP of 42 days, insemination index 1.92). The correlations between milk urea level and reproductive parameters were positive and statistically significant though very low (less than 0.07). Thus, the increase in milk urea concentration was due to longer RP, increased dose of semen needed for conception (II), and the resultant lengthening of CI. Butler *et al.* (1996), Panicke *et al.* (2000) and Melendez *et al.* (2000) showed that increased protein in cow diets and the resulting higher blood and milk urea concentrations cause changes in uterine pH during the luteal phase, which may lead to lower fertility.

Table 1
Mean values of traits ($\bar{x}$) and correlations (r) between milk urea levels and cow fertility indices

Trait	$\bar{x}$	r
Milk urea level, mg/dl	18.9	-
Calving interval, days	417	0.05034**
Rest period, days	94	0.06614**
Service period, days	42	0.01368**
Insemination index	1.92	0.02089**

**$P \leq 0.01$

Research carried out to date on the relationship between milk urea levels and cow fertility has been inconclusive. However, when a 30-day or shorter period before the insemination was applied as in the present study, poorest reproductive results were obtained for cows with high milk urea concentrations (Melendez *et al.* 2000, Castillo *et al.* 2003, Hojman *et al.* 2004, Skrzypek *et al.* 2005).

Milk urea concentration ranged from 17.5 mg/dl in medium-producing herds (5 001-6 000 kg milk) to 21.1 mg/dl in high-producing herds (over 6 000 kg milk) (Table 2). US studies also showed that herd milk production level has an effect on cow's milk urea concentration, with the mean production level of 8 883 kg and 10 916 kg for low- and high-producing herds and mean milk urea concentrations of 11.1 mg/dl and 13.6 mg/dl, respectively (Rajala-Schultz *et al.* 2001). The results shown in Table 2 suggest that cow fertility is significantly correlated with milk urea level and this effect increases with higher herd milk production levels. This concerns, in particular, parameters such as CI and RP. Coefficients of correlation (Table 3) between milk urea content and cow fertility fully confirm the results given in Table 2 that CI and RP steadily increase with increasing urea concentrations, which is evidence of declining

fertility. The coefficients of correlation between milk urea content and length of CI increase from 0.02** in herds with <5 000 kg milk yield to 0.08** in herds with >6 000 kg milk yield, and for RP these range from 0.04** to 0.11**, respectively. In low-producing herds, SP length and II varied to a small extent according to milk urea concentration, whereas in high-producing herds SP was shortest (47 days) and II lowest (2.09) when urea content was in the 15.1-30 mg/dl range. With the urea content exceeding 30 mg/dl, SP increased to 54 days and II to 2.24. The effect of milk urea concentrations on cow fertility with regard to herd milk production levels was investigated by Rajala-Schultz *et al.* (2001). These authors showed that in the low-producing herds, cows in the lowest MUN (milk urea nitrogen) quartile were 2.5 times more likely to be confirmed pregnant than the cows with the highest MUN values, whereas the respective risk ratio in high-producing herds was 2.3. In low-producing herds, the hazard of being confirmed pregnant for cows in the other MUN categories did not significantly differ from that of cows with the highest MUN values.

Table 2
Effect of milk urea levels on fertility of cows in herds with different production levels

Fertility indices	Milk urea level, mg/dl		Production level of herd, milk/kg ≤5 000	5 001-6 000	>6 000	In general
Milk urea level, mg/dl			18.1	17.5	21.1	18.9
No. of cows	≤15		2 467	2 983	2 150	7 600
	15.1-30	-	2 333	2 736	3 549	8 618
	>30		771	744	1 295	2 810
Calving interval, days	≤15		411	412	417^{B}	414
	15.1-30	LSM	416	416	420	417
	>30		415^{A}	418^{a}	434^{ABa}	422
Rest period, days	≤15		92^{A}	89	85^{AB}	89
	15.1-30	LSM	95^{a}	92	90^{aC}	92
	>30		97	96	98^{BC}	97
Service period, days	≤15		35^{A}	41^{D}	50^{AD}	42
	15.1-30	LSM	36^{B}	41	47^{B}	42
	>30		34^{C}	37^{E}	54^{CE}	42
Insemination index	≤15		1.71^{AB}	1.90^{AE}	2.11^{BE}	1.91
	15.1-30	LSM	1.73^{Ca}	1.88^{Fa}	2.09^{CF}	1.90
	>30		1.74^{D}	1.75^{G}	2.24^{DG}	1.91
Effectiveness of	≤15		57.3	50.6	49.0	50.9
first insemination, %	15.1-30	-	56.8	51.7	45.2	50.4
	>30		58.2	56.5	43.7	51.1

^{A,B}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.01$, ^{a,b}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.05$

Table 3
Coefficients of correlation between milk urea levels and fertility of cows in herds with different production levels

Fertility indices	Production level of herd (milk, kg) ≤5 000	5 001-6 000	>6 000
Calving interval, days	0.02463**	0.02662**	0.07538**
Rest period, days	0.03926**	0.05744**	0.11300**
Service period, days	0.00337**	−0.01740	0.017010
Insemination index	0.00881**	−0.03344**	0.03073*

**$P \leq 0.01$, *$P \leq 0.05$

The decrease in first insemination success of the cows from high-producing herds (>6 000 kg milk) along with increased urea concentration (Table 2) is consistent with the results obtained for Israeli cows (Hojman *et al.* 2004).

The age of cows significantly differentiated milk urea concentration, with higher concentrations observed for first-calf heifers (21.8 mg/dl) than for older cows (18.8 mg/dl) (Table 4).

Table 4
Effect of age of cow on relationships between milk urea levels and fertility

Fertility indices	Milk urea level (mg/dl)		Age of cows	
			Primaparous	Multiparous
Milk urea level, mg/dl			21.8	18.8
No. of cows	≤15		294	7 306
	15.1-30	-	564	8 054
	>30		222	2 588
Calving interval, days	≤15		441^{A}	412^{Aa}
	15.1-30	LSM	455^{B}	415^{B}
	>30		460^{C}	421^{Ca}
Rest period (days)	≤15		111^{ABa}	88^{BE}
	15.1-30	LSM	123^{Cab}	90^{Cc}
	>30		136^{ADb}	94^{DEc}
Service period, days	≤15		45	41
	15.1-30	LSM	45	42
	>30		40	44
Insemination index	≤15		2.00	1.89
	15.1-30	LSM	1.96	1.93
	>30		1.87	1.98
Effectiveness of	≤15		50.0	50.9
first insemination, %	15.1-30	-	49.8	50.5
	>30		52.7	50.9

^{A,B}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.01$, ^{a,b}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.05$

The findings of other authors concerning milk urea content depending on age of cows are inconsistent (Panicke *et al.* 2000, Richardt *et al.* 2001b). In a study by Hojman *et al.* (2004) with 1 279 600 test-day measurements of Israeli cows, milk urea concentration ranged from 14.4 mg/dl in first-lactation cows to 14.9 mg/dl in second-lactation cows and decreased to 14.6 mg/l in fourth-lactation cows. Based on 61 928 test-day milk yields of cows from the Podlasie area, Guliński *et al.* (2008) found urea concentration to decrease from 20.2 mg/dl to 19.2 mg/dl with advancing age of the cows. Analysis of the results given in Table 4 indicates poorer fertility of first-calf heifers compared to older cows and larger differences in fertility parameters of first-calf heifers compared to older cows depending on milk urea concentration. It was found that with the increasing milk urea concentration, the RP of first-calf heifers increased from 111 to 136 days, so despite more favourable II values (a decrease from 2.00 to 1.87) their CI increased from 441 to 460 days. Also, the correlation coefficient values (Table 5) show closer relationships between milk urea concentrations and fertility of first-calf heifers compared to older cows.

Table 5
Coefficients of correlation between milk urea levels and fertility of cows depending on their age

Fertility indices	Age of cows	
	Primaparous	Multiparous
Calving interval, days	0.07698*	0.04158**
Rest period, days	0.15426**	0.04873**
Service period, days	−0.02563	0.01554**
Insemination index	−0.03357	0.0238*

**$P \leq 0.01$, *$P \leq 0.05$

Table 6
Effect of season of first insemination on relationships between milk urea levels and cow fertility

Fertility indices	Milk urea level, mg/dl		Season of first insemination			
			II-IV	V-VII	VIII-X	XI-I
Milk urea level, mg/dl			18.8	23.6	18.8	14.8
No of cows	≤15		2 330	1 093	1 686	2 491
	15.1-30	-	2 953	2 099	1 932	1 634
	>30		736	1 200	595	279
Calving interval, days	≤15		415	418	411	412
	15.1-30	LSM	419	418	416	416
	>30		426	428	419	420
Rest period, days	≤15		87ab	91	88	89
	15.1-30	LSM	94a	93	90	91
	>30		97b	99	95	94
Service period, days	≤15		45	43	40	39
	15.1-30	LSM	43	42	43	41
	>30		44	46	40	43
Insemination index	≤15		1.91	1.94	1.93	1.85
	15.1-30	LSM	1.91	1.92	1.96	1.93
	>30		1.95	2.04	1.88	1.97
Effectiveness of	≤15		51.7	50.1	49.1	51.7
first insemination, %	15.1-30	-	51.5	51.1	49.4	48.7
	>30		50.5	51.0	53.5	47.7

[a,b]Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.05$

The season of first insemination largely differentiated milk urea concentration, which was 14.8 mg/dl in winter, about 18.8 mg/dl in spring and autumn, and 23.6 mg/dl in summer (Table 6). Other authors (Guliński *et al.* 2008) also demonstrated the effect of season of the year on milk urea concentration, which was higher in summer than in winter. In the present study, the season of first insemination did not differentiate the effect of milk urea concentration on cow fertility. Regardless of the season, the increase in milk urea concentration was paralleled by a decline in cow fertility measured by parameters such as CI and RP. This is corroborated by positive values of correlation coefficients between urea concentration and CI and RP lengths (Table 7). The effect of urea concentration on the values of the other fertility parameters proved small and non-significant in each season (Table 6 and 7). Melendez *et al.* (2000) suggest that high concentration of MUN might be synergistic with the negative effect of heat stress or might have a direct negative effect on the reproductive physiological processes.

Table 7
Coefficients of correlation between milk urea levels and fertility of cows depending on season of first insemination

Fertility indices	Season of first insemination			
	II-IV	V-VII	VIII-X	XI-I
Calving interval, days	0.05397**	0.04163**	0.04230**	0.03090*
Rest period, days	0.08134**	0.06169**	0.05996**	0.03196*
Service period, days	0.00048	0.01474	0.00687	0.02001
Insemination index	0.01629	0.01920	0.00187	0.02998

**$P \leq 0.01$, *$P \leq 0.05$

The milk of cows contained about 18 mg/dl urea to 60 days of lactation and about 19.7 mg/dl urea in the next months of lactation (up to 150 days) (Table 8). The changes found in urea concentration in the first five months of lactation are in agreement with those observed by other authors (Richardt *et al.* 2001c, Hojman *et al.* 2004, Guliński *et al.* 2008). The present study also analysed the effect of lactation period on the relationships between the analysed traits. Analysis of the results shown in Tables 8 and 9 shows that urea concentration in the 4th month of lactation would be most useful for possible prediction of cow fertility, although in this case the coefficients of correlation did not exceed 0.06. The CI of cows that were first inseminated within 60 days of calving increased with the increasing urea concentration from 386 to 392 days, and II increased from 2.03 to 2.17. For cows that were first inseminated within 91-120 days of calving, the respective values increased from 435 to 445 days and from 1.72 to 1.88.

Table 8
Effect of lactation period on relationships between milk urea levels and cow fertility

Fertility indices	Milk urea level, mg/dl		Lactation period, days			
			≤60	91-90	91-120	121-150
Milk urea level, mg/dl			18.0	19.7	19.7	19.7
No. of cows	≤15		3 587	2 032	1 029	481
	15.1-30	-	3 600	2 575	1 308	554
	>30		1 027	863	461	223
Calving interval, days	≤15		386^{ABC}	412^{AJK}	435^{BJP}	469^{CKP}
	15.1-30	LSM	388^{DEF}	412^{DLM}	440^{ELR}	462^{FMR}
	>30		392^{GHI}	417^{GNO}	445^{HNS}	461^{IOS}
Rest period, days	≤15		57^{ABCD}	88^{BKL}	116^{CKR}	147^{DLR}
	15.1-30	LSM	58^{EFG}	87^{EMN}	117^{FMS}	147^{GNS}
	>30		59^{HIJ}	88^{HOP}	116^{IOT}	147^{JPT}
Service period, days	≤15		45	42	35	39
	15.1-30	LSM	46	42	40	34
	>30		50	44	45	32
Insemination index	≤15		2.03^{A}	1.86	1.72^{A}	1.76
	15.1-30	LSM	2.05^{ab}	1.92	1.82^{a}	1.74^{b}
	>30		2.17	1.95	1.88	1.77
Effectiveness of	≤15		45.8	51.9	58.9	55.7
first insemination, %	15.1-30	-	46.2	50.9	54.4	56.1
	>30		45.9	49.9	53.8	61.3

^{A,B}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.01$, ^{a,b}Means in grounds of factors followed by the same letters differ significantly at $P \leq 0.05$

Table 9
Coefficients of correlation between milk urea levels and fertility of cows in herds with different production levels

Fertility indices	Lactation period (days)			
	≤60	91-90	91-120	121-150
Calving interval (days)	0.02768*	0.02447	0.05529**	−0.04145
Rest period (days)	0.04431**	−0.00013	0.00022	0.00627
Service period (days)	0.02078	0.01464	0.05856**	−0.05621*
Insemination index	0.03285*	0.02058	0.05391**	−0.00139

**$P \leq 0.01$, *$P \leq 0.05$

In conclusions, the present study is evidence of a statistical correlation (weak on Guilford's scale) between milk urea concentration and cow fertility. The increase in milk urea concentration increased CI (r=0.05**), RP (r=0.07**) and SP (r=0.01**) while increasing the number of inseminations per conception (r=0.02**). Therefore, dairy breeders may find it advantageous to monitor urea concentrations, which could help to improve cow fertility. Herd milk production level, age of cow and lactation period were the factors that had the greatest effect on the relationship between milk urea concentration and cow fertility parameters. The relationship between milk urea concentration and fertility was stronger in cows from herds with >6 000 kg milk yield, in first-calf heifers and in cows at 3 months of lactation. The differing values of correlation coefficients between milk urea concentrations and cow fertility indices within herd production levels, age of cows and lactation stage justify including these factors in measures aimed to improve cow fertility.

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