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The effect of milk urea level on fertility parameters in Holstein–Friesian dairy cows

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Abstract: The objective of this study was to reveal the effect of milk urea levels on the fertility of dairy cows, taking into account their age, herd production level, number of cows, season of first insemination, which were analyzed using data from the SYMLEK national system. The study included data on milk urea levels in 88,745 test-day yields of 55,685 Holstein–Friesian cows, which were milked up to 30 days before first insemination, during the period preceding 180 days of first and second lactation. The GLM procedure of the SAS package was used in the statistical calculations. Considering the observed significant relationships and tendencies, a milk urea level higher than 300 mg/L was found to negatively affect reproductive efficiency in dairy cows, although the effects of the urea level could be masked by the more profound impact of environmental factors. Regardless of the factors, an increased milk urea level was paralleled by decreased cow fertility, with statistically significant differences occurring only for the interactions with herd production level and with herd size. The unfavorable effect of the increase in the level of urea in milk (from ≤150 to >300 mg/L) on cow fertility was particularly evident in the highest producing herds (>9000 kg milk).

Key words: Holstein–Friesian cows, fertility, milk urea level

1. Introduction

From a zootechnical and economic point of view, recording the urea content of test-day milk is a quick, inexpensive, convenient, noninvasive, and above all useful method, as it informs the farmer about the efficiency of protein utilization and provides a basis for proper ration formulation (1,2,3). Based on several studies (1,4,5,6), and keeping nutritional considerations in mind, the optimum milk urea concentration for large breeds of dairy cows (e.g., Holstein–Friesian) was determined as 150–300 mg/L (2.5–5.0 mmol/L). However, some authors (2,7,8) indicate that urea levels around 200 mg/L or lower may be responsible for poor reproductive performance in dairy cows.

Decreased or increased milk urea levels indicate that the diet is improperly balanced for protein and energy available for ruminal microbes (4,6,8). The inadequate amount of energy available for rumen microorganisms which accompanies the excessive amount of protein increases the urea level in the body, which has both indirect and direct negative effects on reproductive function. The negative effects apply to the development of ovarian follicles (number and size), ovulation, the oocyte fertilization process, and embryo development and implantation (6,8). It has been demonstrated in vitro that urea adversely affects sperm survival (6) and impairs oocyte development (9). Excess urea in the organism has the indirect effect of lowering uterine pH (6,9). The lower pH results in increased secretion of prostaglandins E_2 and $F_{2\alpha}$ through endometrial cells. In particular, it has been noted that increased $PGF_{2\alpha}$ content has a major role in linking high urea levels with reproductive abnormalities. This results from the fact that high $PGF_{2\alpha}$ levels effectively weaken peri- and postparturient secretion of estrogens and progesterone while disrupting the effect of these hormones on the uterus (6,8). Plasma urea concentrations of 190–200 mg/L have been set as a threshold beyond which the risk of the above effects increases significantly (6). These conjectures are supported by the results of research cited by Sawa et al. (5) and Roy et al. (8) concerning the negative effect of excess dietary protein on changes in uterine pH during the luteal phase. In turn, Jankowska et al. (7) suggest, based on the reports of other researchers, that excessive urea concentration in milk may be associated with early embryonic loss during the period when pregnancy still cannot be confirmed.

Skrzypek et al. (6) demonstrated the effect of urea concentration on reproductive parameters to be nonlinear, and also showed that urea concentrations of 201–250 mg/L (services per conception was 1.85) are most favorable in terms of reproduction, whereas exceeding 300 mg urea per liter of milk caused a significant decrease in conception rate (SPC was 2.52). Likewise, König et al. (10) reported

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poorer fertility in cows whose milk contained more than 300 mg urea/L. They showed that regardless of the milk protein level which accompanies such a high urea content, there was a decrease in percentage of cows with a 56-day nonreturn rate. Nourozi et al. (2) considered 12–160 mg/L as the optimal urea level for reproduction. In turn, research results from the Czech Republic (3) showed that milk urea level had no significant effect on SPC and SP, although the average values obtained were similar to our results (SPC: 2.12–2.36; SP: 51.7–60.8 days).

The objective of the study was to analyze the effect of milk urea concentrations on fertility of Polish Holstein–Friesian cows by considering the effect of other factors such as cow age, herd productive level, herd size, and season of first insemination.

2. Materials and methods

Study material was derived from the database of the SYMLEK Polish national system. Analysis was performed of milk urea levels in 88,745 test-day yields of 55,685 Holstein–Friesian cows during the period up to 180 days of first and second lactation. The test day preceded the insemination by 0–30 days. Data on length of calving interval (CI: number of days between consecutive calvings); length of service period (SP: number of days between first and successful insemination), services per conception (SPC: number of services required to conceive), were also collected for each cow.

Cow fertility (CI, SP, and SPC) was analyzed as the effect of interaction of urea level (mg/L) and selected factors (cow age, herd production level, season of first insemination, number of cows in herd), using the following linear model:

$$Y_{ijk....} = m + a_i + b_j + c_k + f_l + g_m + (ag)_{im} + (bg)_{jm} + (cg)_{km} + (fg)_{mn} + e_{ijklm},$$

where:

m – overall mean,

a_i – effect of i-th milk urea level (≤150, 150–300, and >300 mg/L);

b_j – effect of j-th age of cows (first, second lactation);

c_k – effect of k-th herd production level (<7000, 7000–9000, and >9000 kg milk);

f_m – effect of m-th season of first insemination (spring: March–May; summer: June–August; autumn: September–November; winter: December–February);

g_n – effect of n-th class of number of cows in the herd (≤20, 21–50, 51–200, and >200 cows);

$(ag)_{ij}$ – interaction between milk urea level and cow age;

$(bg)_{ik}$ – interaction between milk urea level and herd production level;

$(cg)_{im}$ – interaction between milk urea level and season of first insemination;

$(fg)_{in}$ – interaction between milk urea level and number of cows in herd;

e_{ijkmn} – random error of observation.

Significant differences were analyzed with the Scheffe test (SAS, 2014).

3. Results

Analysis of fertility parameters indicates that the cows were characterized by intermediate fertility (CI – 426 days, SP – 54.8 days, SPC – 2.20, success at first insemination – 44%) (Table 1).

Analysis of the results in Table 1 shows that milk urea levels from test-day milking preceding the insemination had an effect on the length of CI and SP (longest in cows producing milk with >300 mg urea/L). The obtained results suggest that 150–300 mg/L is the optimal urea level for high-yielding cows.

Table 1. Effect of milk urea level on fertility parameters of the cows.

Milk urea level (mg/L)	N	Fertitlity indicators of cows					
		CI (days)		SP (days)		SPC	
		LSM	SE	LSM	SE	LSM	SE
≤150	25611	453[a]	0.97	55.4[a]	0.94	2.20	0.01
150–300	45852	453[a]	0.59	55.2[a]	0.57	2.19	0.01
>300	14592	457[b]	1.06	58.6[b]	1.03	2.24	0.02

Statistically different values are marked with the letters a or b at $P \leq 0.05$.
CI – calving interval.
SP – service period.
SPC – services per conception.
LSM – linear square mean.
SE – standard error.
N – number of observations.

Analysis of the results in Table 2 allows for a more detailed evaluation of the relationships between milk urea and fertility of cows. Milk urea level was found to significantly influence fertility parameters (Table 1), but when additional factors were included at the same time, the intensity of its relationships with fertility was found to vary (Table 2). Regardless of the considered factors, an increased milk urea level was paralleled by poorer fertility of cows, with statistically significant differences occurring only for the interaction with herd production level and with herd size.

The shortest CI (438 days) was characteristic of cows whose milk contained ≤150 mg/L urea and which were used in herds producing >9000 kg milk; the longest CI (468 days) was found in cows with milk urea levels of >300 mg/L, in herds yielding <7000 kg milk. It was also found that regardless of milk urea level, the increase in herd productivity was accompanied by a significant decrease in CI, which suggests that fertility is influenced more by herd production level than by milk urea level. Similar trends occurred for SP and SPC values. In the case of cows from the highest-producing herds, CI and SP were longer (by 12 and 10 days, respectively), and SPC values increased (0.19) with increasing milk urea levels.

The results obtained show poorer fertility in primiparous cows compared to cows after second calving, as well as greater differences in the fertility parameters of the cows after second calving compared to primiparous cows, depending on the milk urea level. In 4 consecutive lactations, however, the differences were not statistically confirmed.

The season of first insemination did not create any significant differences in the effect of milk urea concentration on fertility in the cows. Regardless of the season, the increasing urea concentration in milk was paralleled by a tendency for slightly poorer fertility of the cows measured by indicators such as CI, SP, and SPC.

As regards the interaction of milk urea level and herd size on cow fertility, the latter exerted a stronger influence reflected in increasing CI and SP with the increasing number of animals in the herd at each urea level. Milk urea content caused no significant changes in length of CI and SP within herd size classes. On the other hand, there were differences of several and a dozen or so days in the length of CI and SP in cows from small and medium herds compared to cows from large and very large herds. Furthermore, higher milk urea level in herds of >200 cows was found to have a favorable but limited effect

Table 2. Change of fertility parameters by milk urea level and environmental factors.

Factor		Milk urea level (mg/L)									
		LSM	≤150			150–300			>300		
			LSM								
			CI (days)	SP (days)	SPC	CI (days)	SP (days)	SPC	CI (days)	SP (days)	SPC
Milk urea level (mg/L)		210	453	55.4	2.20	453	55.2	2.19	457	58.6	2.24
Lactation number	1	210	456	58.7	2.24	455	57.2	2.21	460	61.3	2.27
	2	209	449	52.0	2.16	451	53.1	2.17	455	55.9	2.20
Lactation milk yield (kg)	<7000	187	465^{a}	67.4^{a}	2.37^{a}	463^{a}	65.0^{a}	2.34^{a}	468^{a}	69.0^{a}	2.38^{a}
	7000–9000	213	455^{b}	58.2^{b}	2.26^{a}	454^{b}	56.4^{b}	2.20^{a}	455^{b}	56.2^{b}	2.17^{b}
	>9000	247	438^{c}	40.5^{c}	1.97^{b}	442^{c}	44.1^{c}	2.02^{b}	450^{b}	50.7^{b}	2.16^{b}
First insemination season	Winter	200	450	52.2	2.13	451	53.3	2.13	457	58.3	2.23
	Spring	202	456	57.5	2.21	455	56.7	2.19	457	58.6	2.20
	Summer	220	456	58.0	2.27	456	57.3	2.27	461	61.6	2.31
	Autumn	219	450	53.8	2.19	450	53.2	2.17	455	56.1	2.21
Size of the herd (number of cows)	≤20	185	447^{a}	47.7^{a}	1.99^{a}	450	50.6^{a}	2.06^{a}	452	51.8	2.07^{a}
	21–50	190	448^{a}	49.5^{a}	2.02^{a}	450	50.9^{a}	2.04^{a}	456	56.9	2.12^{a}
	51–200	214	455	58.9	2.29^{b}	457	60.3^{b}	2.32^{b}	463	64.0	2.37^{b}
	>200	254	462^{b}	65.3^{b}	2.50^{c}	456	58.7	2.34^{b}	459	61.9	2.39^{b}

Reproductive efficiency parameters that differ statistically within a factor are marked with the letters a, b, or c at $P < 0.05$.

on cow fertility, resulting in CI and SP being shorter by approximately 3 days.

4. Discussion

The fertility of cows in our research is similar to that described by Muller et al. (11). Using a study group of cows kept under South African conditions, Muller et al. (11) showed that during a period of less than 20 years (1986–2004), CI increased by 26 days (from 386 to 412 days). Albarráran-Portillo and Pollott (12) also highlighted the tendency for CI to increase in recent decades. The results of international studies show that in the years 1990–2000 CI increased by an average of 1.25 days/year, and the milk yield of cows was observed to increase in different countries (13).

Milk urea content is a trait of growing importance for dairy farming. Breeders and nutritional advisors use it as an important and objective indicator of a properly balanced ration for protein and energy in cow nutrition (10).

The results of the present study indicate that the fertility of high-yielding cows was the best when the level of the urea was in the 150–300 mg/L range, which is consistent with other reports (14,15,16). According to Jankowska et al. (7), in the case of high-yielding cows it is essential that the ration is properly balanced, especially for protein and energy content, which not only has an effect on the course of lactation, but is also crucial for normal reproductive function. The feeding of unbalanced rations has often been shown to reduce productivity and fertility in cows, especially when energy undernutrition is accompanied by excess protein, which increases blood urea concentration; however, it should be remembered that both protein and energy deficiency and excess will have negative consequences. Skrzypek et al. (6), who discussed the findings of other authors concerning the relationships between milk urea level and reproductive parameters, noted these contradictory results and the characteristic fact that cows with the highest milk urea concentration before insemination were characterized by the poorest reproductive parameters. Furthermore, very low urea concentrations were found to impede reproductive efficiency. In summing up the results, Skrzypek et al. (6) concluded that urea levels reported as optimal are inconclusive and ranged from 120–180 to 150–210 mg/L milk. Skrzypek et al. (6) demonstrated the effect of urea concentration on reproductive parameters to be nonlinear, and also showed that urea concentrations of 201–250 mg/L (services per conception was 1.85) were most favorable in terms of reproduction, whereas exceeding 300 mg urea per liter of milk caused a significant decrease in conception rate (SPC was 2.52). Likewise, König et al. (10) reported poorer fertility in cows whose milk contained more than 300 mg urea/L. They showed that there was a decrease in percentage of cows with 56-day nonreturn rate, regardless of the milk protein level which accompanied such a high urea content. Nourozi et al. (2) considered 120–160 mg/L to be the optimal urea level for reproduction. In turn, research results from the Czech Republic (3) showed that milk urea level had no significant effect on SPC and SP, although the average values were similar to our results (SPC: 2.12–2.36; SP: 51.7–60.8 days). This relationship appears to be due to the negative energy balance observed in high-yielding cows, and not to the urea level itself. Another possible explanation of this phenomenon is the cows' adaptability to a high protein content in their feed, which translates into reducing the negative effect of high milk urea content on their fertility (17). Sawa et al. (5) demonstrated that the urea level had a significant effect on the length of CI (417–434 days) in herds producing >6000 kg milk, with a particularly negative effect occurring at levels exceeding 300 mg/L.

The influence of the age of cows on the relationship of urea content in milk with their fertility has been described ambiguously in the literature. Jankowska et al. (7) observed that urea level had no significant effect on reproductive parameters regardless of the cows' age. They demonstrated that among primiparous and second-lactation cows, the best reproductive parameters were obtained by those producing milk with 150–300 mg urea, while in third- and fourth-lactation cows, by those yielding milk with <150 and 150–300 mg/L urea. In turn, Nourozi et al. (2) found a negative effect of elevated (>180 mg/L) urea concentration only for first-lactation cows, which they attributed to body size, feed intake capacity, and parturition-related stress, considering these factors to be important when calved primiparous cows were separated from the herd. Close to zero phenotypic correlation between milk urea level and fertility traits, regardless of the lactation number, was reported by Hossein-Zadeh and Ardalan (18): the correlation coefficients were 0.04 (SP) and 0.03 (SPC) for first-lactation cows, 0.06 (SP) and 0.02 (SPC) for second-lactation cows, and 0.07 (SP) and 0.02 (SPC) for third-lactation cows. For the reproductive rest period and days open, these indicators ranged from 0.04 to 0.09. Sawa et al. (5) found no significant effect of the urea level on first insemination success regardless of the cow's age, but showed CI to significantly increase for multiparous cows producing milk with >300 mg urea/L (421 days) compared to cows whose milk contained <150 mg urea/L (412 days).

In our own studies, regardless of the first insemination season, we found a tendency of the fertility of cows to deteriorate slightly (OMW, OU, and II) with an increase in the concentration of urea in milk. Melendez et al. (15) demonstrated that cows exposed to high milk urea nitrogen concentrations (16 mg/dl) 30 days before first service and

who were bred during the summer months were at higher risk of nonpregnancy compared to cows with low milk urea nitrogen bred during the winter months. The authors concluded that high concentration of milk urea nitrogen might be synergistic with the negative effect of heat stress, or might have a direct negative effect on the reproductive physiological processes.

Considering the observed significant relationships and tendencies, it is concluded that milk urea concentrations exceeding 300 mg/L negatively affect the reproductive efficiency of dairy cows, although the effect of urea content can be masked by a much stronger impact of environmental factors. This possibility is indicated by the studies cited by Rzewuska and Strabel (19), which suggest that a deterioration in reproductive parameters may be due to the compounded negative effect of high urea level and heat stress.

It is concluded that the increase in milk urea level from ≤150 mg/L to >300 mg/L had an adverse effect on cow fertility (SP and CI longer by approximately 4 days, SPC greater by approximately 0.04). The intensity of the relationships between milk urea level and cow fertility changed the most depending on the herd production level, which serves as a measure of rearing quality. The unfavorable effect of urea levels exceeding 300 mg/L on cow fertility was particularly noticeable in the highest producing herds (>9000 kg milk) (CI and SP longer by 12 and 10 days, respectively, services per conception greater by 0.19).

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