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The effect of selected factors on urea concentration in the milk of Polish Holstein-Friesian cows*

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The objective of the study was to determine the relationships between milk urea concentration and factors such as lactation number, stage of lactation, month and season of the test day, age at calving, milk yield and protein percentage. Data for the calculations consisted of 7,731 test-day records from 1,078 Polish Holstein-Friesian cows. Test-day milking was performed for first, second and third lactations during the period from December 2010 to December 2011. Calculations were performed using the MIXED procedure in SAS/STAT. A mixed linear model using was applied in which parameters were estimated by the restricted maximum likelihood (REML) method. Least squares means for fixed effects in the model were compared by the Tukey-Kramer test. The first lactation differed significantly ($p<0.05$) from the second and third in terms of mean urea concentration, but there were no significant differences between the second and third lactations. For primiparous cows the milk urea concentration increased throughout lactation, but for older cows it increased only up to 7-8 months of lactation. Urea concentrations did not differ significantly in the same stages of consecutive lactations, i.e. the first and second or second and third. Statistically significant differences were noted between the first and third lactations only in months 9 and 10 of lactation. Seasonal changes in milk urea content varied depending on the lactation number. In the first lactation the milk urea concentration was lowest in spring and highest in autumn. This tendency was not observed in the second and third lactation. Milk urea concentration was positively associated with both milk yield and protein percentage.

KEY WORDS: dairy cattle / urea / non-genetic factors

Urea is formed in the liver from ammonia, which is toxic. The ammonia is produced in the rumen as a result of microbiological decomposition of crude protein. Excessive

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amounts of ammonia which are not used to synthesize bacterial protein enter the bloodstream and are transported to the liver, where they are transformed into urea, protecting the body against intoxication. Then a substantial portion of the urea is excreted with the urine, while some enters body fluids, including milk. Thus urea is one of the components of milk; it accounts for the largest proportion of non-protein nitrogen compounds in milk and is the end product of nitrogen metabolism in cows. Optimal urea concentration in milk is considered to be in a range from 150 to 250 mg/l, with protein content in milk ranging from 3.2% to 3.6%. Factors influencing urea content in milk include intake of crude protein and energy and their ratio in the feed ration [9]. Therefore the urea concentration in milk (together with protein content) is a good indicator of proper protein and energy balance in the feed ration [12]. High urea content is indicative of inefficient conversion of crude protein, which leads to higher costs associated with feeding, as well as contamination of the environment. Milk urea concentration depends on many factors, including the calendar month or season in which the sample is collected, the stage of lactation, the day in milk, the time of day, lactation number, and the value of other performance characteristics [1, 3, 4, 5, 6, 7, 10, 11, 16, 17, 20, 22, 23]. Differences in urea content in cow milk may also be due to individual variation, as heritability of milk urea concentration according to various authors ranges from 0.09 to 0.59 [2, 8, 13, 14, 15, 18, 21, 23].

Many authors have noted a significant effect of the month or season of sample collection on milk urea concentration [1, 4, 5, 7, 16, 17, 22]. Godden et al. [5] and Rajala--Schultz and Saville [16] found that milk urea content was highest in the summer, while Rajala-Schultz and Saville [16] emphasized that this relationship occurred only in low-yielding herds (with mean yield up to 7,000 kg milk/year), in which pasture feeding was used in the summer. According to these authors, in high-yielding herds (over 10,000 kg milk/year) a different tendency was observed, i.e. the milk urea content was lower in the summer than in the winter and spring. Fatehi et al. [4] and Hojman et al. [7] observed seasonal variability in milk urea concentration (with the highest values in the summer), despite the fact that the animals were housed indoors all year and received total mixed rations (TMR) with no fresh hay. Similar results were obtained by Rzewuska and Strabel [17].

The stage of lactation is another factor whose effect on milk urea concentration has been studied by many authors. Most of them have found that the curve illustrating changes in urea content on successive days of lactation was similar to the curve showing the relationship between milk yield and day in milk, with the lowest values at the start of lactation, the maximum value attained between the second and fifth months, and then a gradual decrease in urea concentration until the end of lactation [1, 4, 5, 10, 11, 17]. A different shape for the curve illustrating the relationship between urea content and the stage of lactation was presented by Hojman et al. [7], Rajala-Schultz and Saville [16] and Wood et al. [23], who observed the lowest urea concentration in the first or second month of lactation, after which the values rose until the end of lactation.

The time of day when the cow is milked is another factor influencing the content of urea in the milk. Broderick and Clayton [3], Godden et al. [5] and Wattiaux et al. [22] found that urea concentration was lower in milk from morning milking than in milk from evening milking. A study by Gustafsson and Palmquist [6] showed that urea content decreased as the interval between feed intake and milking increased, which would explain the lower urea concentration in morning milk.

The relationship between urea content and lactation number has been studied as well. Fatehi et al. [4], Jílek et al. [10] and Johnson and Young [11] found that urea concentration in milk from the first two lactations was higher than in later lactations. In contrast, according to Arunvipas et al. [1], Wattiaux et al. [22] and Wood et al. [23], the first lactation is characterized by lower urea content than later lactations. Schepers and Meijer [20], on the other hand, found no significant correlation between lactation number and milk urea concentration.

In the available literature only Wood et al. [23] have studied the relationship between the age of cows at calving and urea concentration, but they showed no significant relationship between these characteristics.

Another subject of research by many authors has been the correlation between milk urea concentration and other production characteristics (milk yield or fat and protein yield and content). Wood et al. [23] observed no significant relationship between urea content and milk yield characteristics that repeated in successive lactations. Authors such as Arunvipas et al. [1], Fatehi et al. [4], Godden et al. [5], Hojman et al. [7], Jílek et al. [10], Johnson and Young [11] and Rajala-Schultz and Saville [16] investigated the relationship between urea content and other production characteristics for all lactations combined, in contrast to Wood et al. [23], who sought correlations within each of the first three lactations. Arunvipas et al. [1], Hojman et al. [7], Jílek et al. [10], Johnson and Young [11], Rajala-Schultz and Saville [16] and Rzewuska and Strabel [17] found that milk urea concentration increased with milk yield, although Rajala-Schultz and Saville [16] noted such a correlation only in high-yielding herds, but not in low-yielding herds.

Hojman et al. [7] and Rajala-Schultz and Saville [16] noted that urea content increased with fat content, although Rajala-Schultz and Saville [16] observed this relationship only in the case of high-yielding herds. On the other hand, Godden et al. [5], Jílek et al. [10] and Johnson and Young [11] found that milk urea concentration decreased as fat content increased.

Arunvipas et al. [1], Fatehi et al. [4], Godden et al. [5], Hojman et al. [7] and Johnson and Young [11] noted that urea content decreased as protein content increased in milk.

The aim of the study was to investigate the relationships between urea concentration in the milk of Holstein-Friesian cows and non-dietary factors: lactation number, the season or month when the sample is collected, stage of lactation, age at calving, milk yield, and milk protein content.

Material and methods

The material used for the calculations consisted of randomly selected data provided by the Osowa Sień breeding facility and the Cattle Breeding Department of the Agriculture University in Krakow. The data consisted of 7,731 test-day records of 1,078 Polish Black-and-White Holstein-Friesian cows, housed in three large barns (357, 470 and 251 cows) and fed total mixed rations (TMR). Test-day milking was performed during the first, second and third lactation from December 2010 to December 2011. Test-day records were obtained for 527 cows in the first lactation, 314 in the second lactation, and 268 in the third. Only cows with at least 5 test-day records per lactation were included in the study.

The effect of selected factors on urea content in the milk was tested using the MIXED procedure in the SAS/STAT software package [19]. In this procedure individual effects of the model are estimated by the restricted maximum likelihood method (REML). The following mixed linear model was used:

$$y_{ijklmnopr} = L_i + SW_j + F_k + MP_l + Ml_m + Bi_n + (Ml \times Bi)_{mn} + (L \times MP)_{il} + (L \times SW)_{ij} + (L \times F)_{ik} + (SW \times F)_{jk} + (L \times SW \times F)_{ijk} + KW_{io} + a_p + e_{ijklmnopr}$$

where:

$y_{ijklmnopr}$ – urea content in milk from the *r*-th test-day record from the *i*-th lactation of the *p*-th cow, having given birth in the *j*-th season, in the *o*-th age class at calving, for which test-day milking was performed in the *k*-th stage of lactation and in the *l*-th month of the calendar year; in addition, the *r*-th test-day record belonged to the *m*-th milk yield class and the *n*-th protein percentage class;

L_i – fixed effect of *i*-th lactation (*i*=1, 2, 3);

SW_j – fixed effect of *j*-th season of calving (*j*=1, 2);

F_k – fixed effect of *k*-th stage of lactation (*k*=1,…, 10);

MP_l – fixed effect of *l*-th calendar month of sample collection (*l*=1,…, 12);

Ml_m – fixed effect of *m*-th milk yield class (*m*=1,…, 4);

Bi_n – fixed effect of *n*-th protein percentage class (*n*=1, 2, 3);

$(Ml \times Bi)_{mn}$ – effect of interaction between *m*-th milk yield class and *n*-th protein percentage class;

$(L \times MP)_{il}$ – effect of interaction between *i*-th lactation and *l*-th calendar month of sample collection;

$(L \times SW)_{ij}$ – effect of interaction between *i*-th lactation and *j*-th season of calving;

$(L \times F)_{ik}$ – effect of interaction between *i*-th lactation and *k*-th stage of lactation;

$(SW \times F)_{jk}$ – effect of interaction between *j*-th season of calving and *k*-th stage of lactation;

$(L \times SW \times F)_{ijk}$ – effect of interaction between *i*-th lactation, *j*-th season of calving and *k*-th stage of lactation;

KW_{io} – fixed effect of *o*-th age class at calving within the *i*-th lactation;

a_p – random effect of *p*-th animal;

$e_{ijklmnopr}$ – random error.

The analysis was repeated with the month of sample collection (MP_l, *l*=1,…, 12) in the above model replaced by the season of sample collection (SP_l, *l*=1, …, 4). Least squares means for fixed effects in the model were compared by the Tukey-Kramer test.

In each lactation two calving seasons were considered: winter (October–March) and summer (April–September). Within each lactation 10 one-month stages were distinguished in which test-day milking was performed (5-35, 36-65, 66-95, 96-125, 126-155, 156-185, 186-215, 216-245, 246-275, and 276-305). Twelve calendar months and four seasons of test-day milking were taken into account: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). Each test-day record belonged to one of four milk yield classes – low (under 20 kg), intermediate (20 to 29 kg), high (30 to 39 kg), or very high (40 kg and more) – and to one of three milk protein percentage classes: low (under 3.2%), intermediate (3.2% to 3.6%), and high (over 3.6%). Classes for age at calving were as follows: first lactation – 5 classes (20-24, 25-26, 27-28, 29-30 and 31-45 months), second lactation – 4 classes (31-38, 39-41, 42-44 and 45-65 months), and third lactation – 3 classes (43-51, 52-55 and 56-74 months).

Results and discussion

Stage of lactation. Table 1 presents the mean milk urea concentration depending on the stage (month) of the first, second and third lactation.

Table 1

Least squares means (LSM) of milk urea concentration with standard errors (SE) by stage of the first, second and third lactations

Stage of lactation (DIM)	Lactation 1			Lactation 2			Lactation 3		
	N	LSM	SE	N	LSM	SE	N	LSM	SE
1 (5-35)	323	168	4.8	208	154	5.3	141	145	6.6
2 (36-65)	370	172	4.8	193	163	5.6	140	162	6.9
3 (66-95)	364	179	4.7	218	179	5.1	161	172	5.9
4 (96-125)	396	195	4.2	234	188	4.8	203	179	5.1
5 (126-155)	400	208	4.1	254	191	4.7	214	200	5.0
6 (156-185)	405	211	4.1	263	203	4.6	230	205	4.9
7 (186-215)	412	219	4.1	241	204	4.8	218	205	5.0
8 (216-245)	395	218	4.2	216	220	5.0	194	201	5.2
9 (246-275)	372	231[a]	4.4	184	210[ab]	5.4	198	201[b]	5.1
10 (276-305)	295	232[a]	4.9	134	217[ab]	6.3	155	204[b]	5.7
Total	3732	203[a]	2.9	2145	193[b]	2.9	1854	188[b]	2.9

N – number of test-day records
a, b – least squares means within the same row with different superscripts differ significantly ($p<0.05$)

In each of the first three lactations the lowest milk urea concentration was noted in the first month of lactation. In the first lactation an upward trend was observed until the 305th day of lactation, while in the second and third lactations the upward trend lasted until about the 7th or 8th month.

Many authors report that the urea concentration curve is similar in shape to the milk yield curve, with the lowest values at the start of lactation, a peak between months 2 and 5, and then a slow decline until the end of lactation [1, 4, 5, 10, 11, 16, 17]. Johnson and Young [11] state that the lower urea content at the start of lactation is explained by lower intake of dry matter during this period or different balancing of the feed ration in the first month of lactation as compared to later months. According to these authors, the decrease in urea concentration after the peak of lactation was associated with the decreasing need for crude protein during the period of declining milk production. Johnson and Young [11] found that the rate of the decline in urea content after it had attained its maximum value varied in different herds, and that a slower rate of decline was probably associated with excessive supply of crude protein after peak lactation. The results of our study may indicate a large supply of crude protein in the herds analysed, particularly in the first lactation, in which milk urea content was increasing even at the end of lactation. According to Godden et al. [5], changes in the composition of the feed ration or in feeding programmes for different lactations and different stages of lactation may contribute to the changes observed in milk urea content. Schepers and Meijer [20], who took into account the effect of factors directly related to diet, i.e. protein balance in the rumen, net energy balance and balance of protein digested in the small intestine, found no relationship between stage of lactation and milk urea concentration. Besides Godden et al. [5] and Johnson and Young [11], authors such as Arunvipas et al. [1], Fatehi et al. [4], Jílek et al. [10] and Rzewuska and Strabel [17] have also confirmed that the urea concentration curve is similar in shape to the milk yield curve, although the maximum urea content (4-5 month of lactation) occurred later than the lactation peak. According to Rajala-Schultz and Saville [16], the peak urea content overlapped with peak milk yield (about 2-3 months). Wood et al. [23] observed a different shape for the urea content curve, which was a mirror image (with respect to the horizontal axis) of a typical milk yield curve. According to the authors, at the start of lactation there was a gradual decrease in milk urea concentration until days 30-40 of lactation, when its minimum value was attained, after which the values increased up to the end of lactation.

Analysis of the effect of the stage of lactation on milk urea content revealed statistically significant differences in months 9 and 10 between the first and third lactations (Tab. 1). In the remaining months of lactation (1 to 8), no statistically significant differences were found between lactations, which indicates that the urea content remained at a similar level during these months, irrespective of lactation number.

The first lactation (203 mg/l) differed significantly in terms of urea concentration from the second (193 mg/l) and third (189 mg/l), but no significant differences were found in milk urea content between the second and third lactations. The literature contains studies confirming statistically significant differences in urea content between lactations, as well as studies that found no such relationships. Fatehi et al. [4] and Johnson and Young [11] came to similar conclusions as the authors of the present study, finding that the milk urea content in the first lactation was significantly higher than in the second and third. The lower milk urea content during the second and third lactations may be due to better balancing of the feed ration with respect to the production potential of the cows. The lower urea content in successive lactations may also be physiologically determined;

in older cows the liver becomes damaged and is unable to process ammonia. Different results, where milk urea content in the first lactation was significantly lower than in later lactations, were obtained by authors such as Arunvipas et al. [1], Wattiaux et al. [22] and Wood et al. [23]. On the other hand, Schepers and Meijer [20], who used a model taking into account the effect of dietary factors, found that lactation number had no significant effect on urea content.

Month and season of sample collection. Table 2 presents the least squares means of milk urea concentration in particular calendar months in the first three lactations.

No clear tendencies repeated in all lactations were noted for changes in urea content associated with the season in which the sample was collected. In the first lactation the lowest milk urea concentration was noted in the spring (182 mg/l) and the highest in the autumn (226 mg/l), while the summer and winter did not differ significantly in terms of urea content. During the second lactation no statistically significant differences were found in urea concentration between seasons. In the third lactation, milk urea content in the winter (178 mg/l) was significantly lower only in comparison with the value obtained in the summer (198 mg/l), whereas urea content in the summer was significantly higher than the values from both the winter and the spring (181 mg/l).

Table 2

Least squares means (LSM) of milk urea concentration with standard errors (SE) by season and month of test day in the first three lactations

Season/Month of test-day	Lactation 1			Lactation 2			Lactation 3		
	N	LSM	SE	N	LSM	SE	N	LSM	SE
Spring	**1087**	**182**^{a}	**3.7**	**613**	**183**	**4.1**	**618**	**181**^{a}	**4.1**
March	308	187	4.8	187	188	5.6	199	187	5.4
April	407	191	4.3	211	190	5.2	217	189	5.2
May	372	138	4,4	215	151	5.2	202	151	5.4
Summer	**777**	**201**^{b}	**3.9**	**423**	**198**	**4.4**	**340**	**198**^{b}	**4.7**
June	363	158	4.5	197	173	5.4	176	176	5.6
July	175	194	5.7	69	202	8.2	47	186	9.7
August	239	267	5.1	157	228	5.9	117	229	6.6
Autumn	**1024**	**226**^{c}	**4.0**	**582**	**195**	**4.5**	**342**	**192**^{ab}	**5.1**
September	379	262	4.5	214	208	5.5	135	196	6.3
October	353	245	4.7	199	226	5.7	115	216	6.8
November	292	198	5.0	169	171	6.0	92	175	7.5
Winter	**844**	**200**^{b}	**3.9**	**527**	**192**	**4.2**	**554**	**178**^{a}	**4.3**
December	378	220	4.4	246	209	4.9	209	189	5.3
January	208	207	5.5	130	205	6.5	164	201	6.0
February	258	171	5.1	151	162	6.2	181	156	5.7

N – number of test-day records

a, b, c – least squares means within the same column with different superscripts differ significantly ($p<0.05$)

Similar results were obtained by Godden et al. [5], despite a slightly different division into seasons (three-month seasons beginning in January). These authors reported the highest urea content in the season from July to September, and the lowest from April to June. Rajala-Schultz and Saville [16] also found that urea concentration was highest in the summer, but only in low-yielding herds (less than 7,000 kg milk/year), in which the cows were pastured during this period. In the case of high-yielding herds (more than 10,000 kg milk/year), in which the cows were not pastured, the authors noted a reverse tendency, i.e. the urea content in the milk was lower in the summer than in the other seasons. Rajala-Schultz and Saville [16] attributed this reverse tendency to lower intake of dry matter due to the higher air temperature in the summer, and thus lower intake of crude protein, the final effect of which was lower urea content in the milk. This explanation was not confirmed in the present study, which involved high-yielding herds fed in a TMR system. Fatehi et al. [4] and Hojman et al. [7], who also studied TMR-fed herds, noted the highest urea content in the summer months (June and July) and the lowest in the autumn-winter months (November and December), which is consistent with the results of our study. Wattiaux et al. [22], who analysed the urea concentration in the milk of three breeds of cattle – Holstein, Brown Swiss and Jersey, showed high variation in this trait associated with the month of the test day, particularly in the case of Brown Swiss and Jersey. These authors observed no characteristic tendencies for changes in urea content depending on the calendar month that repeated in successive calendar years. The authors suggested that a model taking into account the season of the test day instead of the calendar month would better reflect the influence of such factors as temperature, humidity or daylight hours, as well as changes in the diet. After including the season of the test day in their linear model, they found that the lowest urea content in the case of the Holstein-Friesian breed occurred in the spring (as in our study for the first lactation), and the highest in the summer (as in most of the studies cited above). Rzewuska and Strabel [17], like the authors of the present study, noted high urea content in the milk of Polish Holstein-Friesians in the period from July to October, with the highest value attained in August (237 mg/l) and the lowest in January (192 mg/l).

Age of cows at calving. Table 3 presents the least squares means of milk urea content according to the age classes of the cows at the first, second and third calving. The urea content in the milk of cows giving birth for the first and third time was not dependent on their age, but in the second lactation a significant difference was noted between the youngest cows (31-38 months) and those a bit older (39-41 months). Similarly, Wood et al. [23] found no relationship between urea concentration and age at calving.

Milk yield level and protein percentage. In the case of two milk yield classes, intermediate (20 to 29 kg) and high (30 to 39 kg), the milk urea concentration increased with the protein percentage, and the differences were statistically significant (Tab. 4). When milk yield was very high (over 40 kg), urea content remained constant irrespective of protein content (no statistically significant differences between protein percentage classes). In the case of low milk yield (less than 20 kg), statistically significant

Table 3

Least squares means (LSM) of milk urea concentration with standard errors (SE) by age at calving (Age) classes within the first, second and third lactations

Lactation 1				Lactation 2				Lactation 3			
Age (mo.)	N	LSM	SE	Age (mo.)	N	LSM	SE	Age (mo.)	N	LSM	SE
20-24	1695	203	3.0	31-38	948	201^{a}	3.6	43-51	603	190	4.1
25-26	1041	202	3.5	39-41	546	185^{b}	4.3	52-55	406	185	4.8
27-28	534	202	4.4	42-44	355	183^{ab}	4.9	56-74	845	187	3.7
29-30	286	214	5.7	45-65	296	202^{ab}	5.5				
31-45	176	196	7.2								

N – number of test-day records
a, b – least squares means within the same column with different superscripts differ significantly (p<0.05)

Table 4

Least squares means (LSM) of milk urea concentration with standard errors (SE) by classes of milk yield and protein percentage

Milk (kg)	Protein (%)								
	<3.2			3.2-3.6			>3.6		
	N	LSM	SE	N	LSM	SE	N	LSM	SE
<20	69	188^{ab}	7.9	289	169^{a}	4.2	436	191^{b}	3.6
20-29	391	185^{a}	3.6	1131	194^{b}	2.3	841	206^{c}	2.6
30-39	1053	197^{a}	2.5	1665	207^{b}	2.0	561	218^{c}	3.0
≥40	721	219	3.0	516	213	3.2	58	218	8.6
Total	2234	197^{a}	2.6	3601	196^{a}	1.7	1896	208^{b}	2.7

N – number of test-day records
a, b, c – least squares means within the same row with different superscripts differ significantly (p<0.05)

differences in urea concentration were found only between classes with intermediate (3.2% to 3.6%) and high (over 3.6%) protein content.

The results obtained in the present study are not confirmed by studies by Fatehi et al. [4] and Johnson and Young [11], who noted decreasing urea content in milk as the protein percentage increased, and observed this relationship in nearly all milk yield classes. It should be added that different protein percentage classes were adopted in the works cited (under 3.01%, from 3.01% to 3.20% and over 3.20%). A reverse relationship between urea concentration and protein percentage in milk was also obtained by Arunvipas et al. [1], Godden et al. [5], Hojman et al. [7] and Rajala-Schultz and Saville [16], with Godden et al. [5] reporting a non-linear relationship.

Within individual protein percentage classes the protein content increased with milk yield. In the case of protein under 3.2% the urea concentration in the very high milk yield class (over 40 kg) was significantly different from the urea content in the remaining milk yield classes, but no statistically significant differences were found between the remaining milk yield classes. In the case of intermediate (from 3.2% to 3.6%) and high (over 3.6%) protein content significant differences in urea concentration were noted between nearly all milk yield classes. Only the high (30 to 40 kg) and very high (over 40 kg) milk yield classes did not differ. Many authors have confirmed that milk urea concentration increased with milk yield [1, 4, 5, 7, 10, 11].

In conclusion, the urea concentration in the milk of the cows varied depending on lactation number, stage of lactation, the month and season of the test day, milk yield, and the protein percentage in the milk. The milk urea concentration was significantly higher in the first lactation than in the second and third, which may be due to better balanced feed rations in later lactations and to physiological differences between primiparous cows and older ones. Moreover, changes in urea content in the milk of cows in the first lactation were different from the changes noted in later lactations; in the first lactation an upward trend was maintained until the end of lactation, whereas in the second and third lactations it lasted until the seventh or eighth month. This may be indicative of an excessive supply of crude protein after the peak in milk yield, particularly in the first lactation. No tendency repeated in successive lactations for changes in urea concentration depending on the season when the sample was collected. The milk urea content increased with protein percentage and milk yield.

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