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Milk urea concentration in Holstein and Simmental cows

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Summary

Determining the urea concentration in milk is a useful indicator of the nutritional protein status of the organism as well as of the ratio between the energy and the protein in ruminant rations, with increasing practical usage. In addition to nutrition, milk urea concentration is influenced by a whole range of factors, for example: breed, stage and number of lactations, body weight, daily production and chemical composition of milk, somatic cell count, season and milking. The objective of this research was to determine the impact of the cow breed (Holstein and Simmental), the number of lactation (1st, 2nd, 3rd, 4th, 5th), milking time (morning-evening) and season (spring-summer and autumn-winter) on milk urea concentration. The following was determined for each breed: daily milk yield, milk fat, protein and lactose content, urea concentration and somatic cell count in milk. Statistical data processing was carried out by applying General Linear Model procedure, SAS system (1999). The cow breed had a significant influence on daily milk yield and log somatic cell count ($P<0.001$), lactose content in milk ($P<0.01$), milk fat content and milk urea concentration ($P<0.05$). The number of lactations significantly influenced daily milk yield ($P<0.001$), protein content ($P<0.001$ and $P<0.01$) and milk urea concentration, but only for Holstein breed ($P<0.05$). Milking time significantly influenced the fat and protein content ($P<0.001$) in the milk of Holstein cows, that is, lactose content ($P<0.05$) and urea concentration ($P<0.05$) in the milk of Simmental cows. The season significantly influenced the fat and protein content of milk ($P<0.001$), that is, urea concentration and log somatic cell count ($P<0.01$). Determining of urea concentration in cow milk should also be systematically conducted in the Republic of Croatia, in order to determine standard physiological values characteristical for a particular cow breed, aiming to determine the balance of energy and protein in rations.

Key words: milk urea, breed, number of lactation, milking, season

Introduction

Urea is a water soluble molecule which can be found in blood, milk, urine and saliva. It is synthesized in the liver from ammonium obtained as a product of decomposition of food protein. Protein food consumed by an animal is divided in rumen on degradable protein and rumen undegradable protein. Rumen degradable protein is used by rumen microorganisms to sinthesize microbial protein which is a source of energy. Rumen microorganisms use soluble carbohydrates and proteins to synthesize microbial protein. Ammonia content in the rumen fluid depends on the protein content in the ration, the level of its digestability and available energy in rumen. Ammonia released in the process of protein degradation in rumen cannot be used by microorganisms,

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so it passes through the rumen wall into the blood circulation. Surplus of ammonia is absorbed through portal vein into the liver, where it is converted into urea. The purpose of urea synthesis is to eliminate excess of nitrogen and to achieve its balance in organism. Most of the produced urea is excreted through urine, and lesser part through milk.

There is a high and significant correlation between urea concentration in milk and blood, which was confirmed by a research conducted by Bed et al. (1997). The higher the urea concentration in milk and urine, the higher is also the urea concentration in blood. References in literature state different physiological values of urea concentration in milk, which vary from 10 to 30 mg/dL or from 1.7 to 5.0 mmol/L by Marenjak et al. (2004), while Young (2001) reports 12 to 16 mg/dL as a recommended urea concentration in cow milk. Urea concentration and protein content in milk are good indicators of the balanced diet in terms of energy and protein, so if the protein content is within normal ranges (3.2-3.8 %), and urea concentration is between 15-30 mg/100 mL, it is considered that the energy and crude protein supply are at optimum level. Rations have to be balanced and contain an adequate quantity and quality of protein and energy, because excess protein in rations otherwise leads to losses in production, causes metabolic and reproduction diseases and pollutes the environment. In addition to routine milk analyses and determination of the milk fat and protein content as well as somatic cell count and the total number of micro organisms, determination of milk urea concentration is used as a mean of nutrition control (Kampl and Stolla, 1995; Bed et al., 1997; Marenjak et al., 2004; Jilek et al., 2006). Determination of urea concentration in cow milk has found its practical usage in EU member states within health control and as a method of monitoring nutritional status of animals. However, this method is still not regularly conducted in the Republic of Croatia (Kuterovac and Dakić, 2004). For the purpose of this paper, urea concentration analysis was conducted on milk of Holstein and Simmental cows. Such analysis should be conducted in the future in order to determine the standard physiological values for a particular breed. To provide correct interpretation of results, not only that the influence of nutrition must be taken into account, but also all non-nutritional factors like: breed, stage and number of lactation, season, milking time, milk sampling, housing system, body weight, production and chemical composition of milk (Godden et al., 2001; Prpić et al., 2005). Namely, Jilek et al. (2006) reported that the influence of non-nutritional factors on milk urea concentration amounts to 13.3 %, and the influence of milk production and environment is 37 %. If urea concentration in milk exceeds the allowed standard values, then adequate corrections of nutrition should be made, because animal health and production might be affected.

The aim of this research was to determine how daily milk yield, changes in the chemical composition of milk, urea concentration and somatic cell count in milk are affected by breed (Holstein and Simmental), the number of lactation (1st, 2nd, 3rd, 4th, 5th), milking time (morning-evening) and season (spring-summer and autumn-winter). Correlation coefficients between particular parameters were calculated.

Material and methods

Data

Research of urea concentration in cow milk was conducted on the part of population of Holstein (H) and Simmental (S) cows in Koprivničko-križevačka, Bjelovarsko-bilogorska and Zagreb County. The total number of analyzed samples was 572 taken from 70 Holstein cows and 603 samples taken from 76 Simmental cows. Depending on the number of lactations, cows were grouped accordingly: 1st lactation 12 (H) and 13 (S); 2nd lactation 15 (H) and 16 (S), 3rd lactation 14 (H) and 16 (S), 4th lactation 14 (H) and 16 (S) and 5th lactation 16 (H) and 15 (S) cows. Cows were kept in a loose housing system, with similar conditions for milk production. Rations consisted of corn silage, haylage, and a concentrated part of the ration, which were fed to animals according to their production and vitamin and mineral supplements. Milk control of cows of both breeds during lactation was conducted once a month by means of AT method. Cows were milked twice a day, and milk samples were collected one month from the morning milking and in the following month from the evening milking. Cows were grouped according to the season: spring-summer (from March 21 to September 22, 2008) and autumn-winter (from September 23, 2008, to March 6, 2009). Extreme values of particular parameters were not analyzed in the following cases: daily milk yield <10 litres, milk fat content <2.0 %, and >6.0 %, and the somatic cell count $>10^6$ mL^{-1}.

Milk analyses

Daily milk yield (DMY) for each cow was determined by measuring the quantity of milk in one milking and projection of milk quantity in the second milking on the same day (HSC, 2004). Milk samples (50 mL) were taken during the morning (6.00 h) and the evening (18.00 h) milking, in the period from March 21, 2008, to March 6, 2009. Milk fat content (F), protein content (P), lactose content (L) and urea concentration (U) in milk were determined by means of the method of infrared spectroscopy (HRN EN ISO 9622:2001) on the instrument Milkoscan FT 6000, produced by Foss. Somatic cell count in milk was determined by means of the fluoro-optic-electronic method (HRN ISO 13366-2:2007) on the instrument Fossomatic FC 5000. Milk analyses were conducted in the Central laboratory for milk control in Križevci, accredited according to the norm HRN EN ISO/IEC 17025:2007 and included in interlaboratory tests.

Statistical analysis

All the variables were tested for normal distribution using the Shapiro and Wilk, (1965) test and milk SCC was converted into decimal logarithms to normalize their frequency distributions before performing statistical analysis. Data were analyzed using a General Linear Model procedure of SAS (1999) statistical software. The following were calculated: Least Square Means (LSM), minimal (Min) and maximal (Max) values, standard error (SE), standard deviation (SD) and coefficient of variation (CV) for individual parameters. The data were subjected to the analysis of variance using the following model:

$$Y_{ijklm} = \mu + B_i + P_j + M_k + S_l + e_{ijklm}$$

where:

Y_{ijklm} = U; F; P; L; DMY; $\log_{10}$SCC (dependent variable)

μ = overall mean

B_i = breed, i = 1 (Holstein), 2 (Simmental)

P_j = parity, j = 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}

M_k = milking, k = 1 (a.m.), 2 (p.m.)

S_l = season, l = 1 (spring-summer), 2 (autumn-winter)

e_{ijklm} = random residual

Finally, an analysis of correlation among all of the variables was performed using the CORR procedure (SAS, 1999). For all parameters, model effects were declared significant at P<0.05, unless otherwise noted.

Results and discussion

Since cow milk production in Croatia is based on Holstein and Simmental breed, a research was conducted to determine the influence of breed, number of lactation, milking time and season on milk urea concentration, daily milk yield, milk fat, protein and lactose content and somatic cell count. According to the literature, the breed is also one of the factors influencing milk urea concentration. Some authors very often state contradicting values for urea concentration in milk of some breeds (Rodriguez et al., 1997; Ferguson et al., 1997). The influence of the cow breed on daily milk yield, content of milk fat, protein and lactose, urea concentration and log somatic cell count is shown in the Table 1.

Cows of Holstein breed produced on average 29.10 kg, and those of Simmental breed produced 25.99 kg of milk (P<0.001). Minimum daily milk yield for both breeds was about 10 kg, and the maximum production was 58 kg. A lower milk production of Holstein-Friesian cows breed was reported by Pintić et al. (2007) and Abdouli et al. (2008), and in Simmental cows by Marenjak et al. (2004) and Pintić et al. (2007).

The content of milk fat in milk was also significantly (P<0.05) higher in the milk of Holsein cows (4.31 %) than in the milk of Simmental cows (4.19 %), while no significant differences were determined for the protein content of milk. Also, Pintić et al. (2007) did not determine any significant differences in the content of milk fat and protein in the milk of Holstein-Friesian and Simmental cows. Johnson and Young (2003) as well as Abdouli et al. (2008) reported a significantly lower content of milk fat and protein in Holstein cow's milk in relation to the values determined in this research.

The average urea concentration in Holstein cow's milk was 23.70 mg/100 mL, and in Simmental cow's milk it was 24.56 mg/100 mL, which is within usual physiological values reported for cow milk by most of the authors (Marenjak et al., 2004; Drudik et al., 2007). The lower milk urea concentration (15.5 mg/dL) in Holstein cow's milk was determined by Johnson and Young (2003). Urea concentration in Holstein cow's milk was for 40 % higher than in Jersey cow's milk (Rodriguez et al., 1997). Kauffman and St-Pierre (2001) reported a significantly lower urea concentration in Holstein

Table 1. Effect of breed on daily milk yield, milk fat, protein and lactose content, urea concentration and somatic cell count (log) in milk

	Holstein n= 572	Simmental n= 603	Significance of differences
	Daily milk yield (kg)		
LSM±SE	29.10±0.35	25.99±0.31	
CV	26.91	29.12	***
Min-Max	10.27-57.1	9.28-57.99	
	Milk fat (%)		
LSM±SE	4.31±0.01	4.19±0.03	
CV	19.28	20.63	*
Min-Max	2.09-6.0	2.17-6.0	
	Protein (%)		
LSM±SE	3.33±0.02	3.36±0.016	
CV	12.96	10.25	NS
Min-Max	2.16-4.72	2.58-4.96	
	Lactose (%)		
LSM±SE	4.51±0,01	4.55±0.01	
CV	3.84	3.44	**
Min-Max	3.85-5.07	3.84-4.97	
	Urea (mg 100 mL^{-1})		
LSM±SE	23.70±0.38	24.56±0.34	
CV	36.16	33.22	*
Min-Max	2.8-51.7	3.0-47.9	
	Log_{10}SCC (log_{10})		
LSM±SE	4.80±0.02	4.65±0.02	
CV	10.80	10.50	***
Min-Max	3.60-5.99	3.48-5.97	

NS - Non Significant; *P<0.05; **P<0.01; ***P<0.001

LSM = Least Square Means; SE = Standard Error; CV = Coefficient of variation; Min = Minimum; Max = Maximum

cow's milk (9.44 mg/dL), which is almost identical with the concentration in Jersey cow's milk, and they reported a significant influence of the breed on all milk ingredients except on urea concentration in milk and blood. Opposite to this, Abdouli et al. (2008) determined in Holstein cow's milk on average 30.39 mg/dL of urea.

In this research significantly higher (P<0.001) somatic cell count was determined in Holstein cow's milk (130.599 mL^{-1}), in relation to Simmental cow's milk (90.700 mL^{-1}). Hojman et al. (2005) determined in Holstein cow's milk on average 316.000 somatic cells per mL. Daily milk yield for both breeds was also significantly (P<0.001) influenced by the number of lactations. In the 1st and 2nd lactation, Holstein and Simmental cows produced significantly more milk than in the 4th and 5th lactation. Daily milk yield of Holstein cows was higher (P<0.001) than of Simmental cows in each particular lactation, which was reported by Pintić et al. (2007), although milk quantities were lower. What is interesting to mention is that for both breeds significantly higher (P<0.001) daily milk yield was determined from the 1st to 3rd lactation in relation to the 4th and 5th lactation. Yazgan et al. (2010) determined the peak milk production for Holstein cows in the 3rd lactation, while according to Hojman et al. (2004) an increase in milk production was evident until the 3rd lactation, after which there was a decrease in milk production.

Table 2. Effect of parity on daily milk yield, milk fat, protein and lactose content, urea concentration and somatic cell count (log) in milk

	Holstein						Simmental					
	Number of lactation					Significance of differences	Number of lactation					Significance of differences
	1st n=97	2nd n=125	3rd n=122	4th n=119	5th n=129		1st n=103	2nd n=126	3rd n=131	4th n=124	5th n=119	
Daily milk yield (kg)												
LSM±SE	31.99±0.87^{a}	30.67±0.82^{a}	29.34±0.79^{ab}	27.32±0.80^{b}	27.75±0.79^{b}	***	26.74±0.78^{a}	26.11±0.67^{a}	25.48±0.65^{a}	22.31±0.65^{b}	21.60±0.60^{b}	***
Min-Max	11.12-57.1	10.66-54.83	10.27-53.36	14.24-42.04	14.55-48.04		12.89-43.1	13.25-52.61	13.5-57.99	13.21-42.53	9.28-48.92	
Milk fat (%)												
LSM±SE	4.24±0.10	4.19±0.10	4.21±0.10	4.33±0.10	4.37±0.10	NS	4.10±0.10	3.89±0.09	3.91±0.08	3.89±0.08	3.93±0.08	NS
Min-Max	2.09-5.96	2.17-5.87	2.34-5.91	2.23-5.97	2.56-6.00		2.46-5.93	2.51-6.0	2.43-5.89	2.17-5.97	2.24-6.0	
Protein (%)												
LSM±SE	3.22±0.05^{ab}	3.06±0.05^{a}	3.25±0.05^{b}	3.36±0.05^{bc}	3.49±0.05^{c}	***	3.26±0.04^{ab}	3.13±0.03^{a}	3.23±0.03^{a}	3.37±0.03^{bc}	3.43±0.03^{c}	**
Min-Max	2.59-4.19	2.16-4.72	2.29-4.62	2.56-4.61	2.53-4.63		2.7-4.96	2.62-4.17	2.58-4.84	2.73-4.94	2.76-4.35	
Lactose (%)												
LSM±SE	4.49±0.02	4.52±0.02	4.54±0.02	4.53±0.02	4.47±0.02	NS	4.53±0.02	4.54±0.02	4.57±0.02	4.55±0.02	4.52±0.02	NS
Min-Max	4.12-5.07	4.03-4.93	4.07-5.03	4.17-4.96	3.85-4.88		4.02-4.85	4.54-4.97	4.57-4.86	4.55-4.87	4.52-4.94	
Urea (mg 100 mL^{-1})												
LSM±SE	21.43±0.80^{a}	21.56±0.75^{a}	23.61±0.72^{ab}	24.01±0.73^{ab}	24.46±0.72^{b}	*	23.32±0.94	23.62±0.81	23.91±0.78	25.71±0.79	25.74±0.72	NS
Min-Max	6.0-45.0	7.1-41.2	2.8-51.7	7.1-43.3	7.2-50.5		6.0-41.4	4.0-47.9	3.0-39.1	8.4-45.3	3.0-42.5	
Log_{10}SCC (log_{10})												
LSM±SE	4.81±0.06	4.79±0.06	4.73±0.06	4.74±0.06	4.89±0.06	NS	4.71±0.06	4.64±0.05	4.59±0.05	4.70±0.05	4.73±0.05	NS
Min-Max	3.60-5.97	3.78-5.81	3.90-5.88	3.60-5.99	4.04-5.96		3.70-5.95	3.60-5.91	3.48-5.97	3.60-5.96	3.70-5.97	

NS - Non Significant

$^{a, b, c}$ Means within the same row and not sharing the same superscript letter are significantly different (***P<0.001; **P<0.01; *P<0.05)

LSM = Least Square Means; SE = Standard Error; Min = Minimum; Max = Maximum

The content of milk fat and lactose in milk was not significantly influenced by the number of lactations. The content of milk fat in particular lactations varied. For example, the highest fat content in the milk of Holstein cows (4.37 %) was determined in the 5th lactation and in Simmental cow's milk it was in the 1st lactation (4.10 %). Hojman et al. (2004) reported that the content of milk fat decreases with the growing number of lactation. Opposite to this, Yazgan et al. (2010) emphasized that milk in higher lactations contains higher levels of milk fat. Lactose content for both breeds was the highest in the 3rd and 4th lactation, while it was the lowest in the 5th lactation. With small deviations, the average lactose content in each particular lactation exceeded 4.5 %.

Unlike milk fat, the protein content of milk was significantly influenced by the number of lactations. For Holstein and Simmental breed, higher protein content of milk was determined in the 5th lactation (3.49 % and 3.43 %), and in the 4th lactation (3.36 % and 3.37 %).

Prpić et al. (2005) reported that the increasing number of lactation resulted in a significantly increased urea concentration in milk. Opposite to this, Canfield et al. (1990) did not determine any significant connection between urea concentration in milk and the number of lactations.

In this research the highest daily milk yield was determined for both breeds in the 1st lactation, while urea concentration in milk was the lowest (21.43 and 23.32 mg/100 mL). In that period cows are still in the growth and development phase and they utilize amino-acids from rations more efficiently. This leads to the reduced deamination and synthesis of urea in the liver, which then results in lower concentration in milk of cows in the 1st lactation. As average

Table 3. Effect of milking on daily milk yield, milk fat, protein and lactose content, urea concentration and somatic cell count (log) in milk

	Holstein			Simmental		
	Milking		Significance of differences	Milking		Significance of differences
	a.m. n=298	p.m. n=274		a.m. n=299	p.m. n=304	
Daily milk yield (kg)						
LSM±SE	29.19±0.69	29.64±0.59	NS	24.18±0.43	24.71±0.46	NS
Min-Max	11.08-49.14	10.27-57.1		9.28-55.38	13.5-57.99	
Milk fat (%)						
LSM±SE	4.12±0.08	4.42±0.07	**	3.97±0.05	3.92±0.06	NS
Min-Max	2.34-5.97	2.09-6.0		2.17-6.0	2.18-6.0	
Protein (%)						
LSM±SE	3.19±0.04	3.36±0.03	**	3.28±0.02	3.29±0.02	NS
Min-Max	2.16-4.72	2.17-4.63		2.59-4.96	9.92-2.58	
Lactose (%)						
LSM±SE	4.50±0.02	4.52±0.01	NS	4.53±0.01	4.56±0.01	*
Min-Max	4.07-5.07	3.85-4.9		3.84-4.94	4.03-4.97	
Urea (mg 100 mL^{-1})						
LSM±SE	23.35±0.63	22.56±0.54	NS	25.20±0.52	23.71±0.55	*
Min-Max	2.8-51.7	7.2-43.3		3.0-42.5	3.0-47.9	
Log_{10}SCC (log_{10})						
LSM±SE	4.76±0.05	4.82±0.04	NS	4.66±0.03	4.68±0.04	NS
Min-Max	3.60-5.97	3.60-5.99		3.48-5.97	3.60-5.97	

NS - Non Significant
LSM within the same row are significantly different (**P<0.01; *P<0.05)
LSM = Least Square Means; SE = Standard Error; Min = Minimum; Max = Maximum

daily milk yield from the 1st to the 5th lactation was gradually growing, urea concentration in milk gradually decreased. Opposite to this, Jílek et al. (2006) reported a significantly higher urea concentration in milk in the 1st and 2nd lactation in relation to the 3rd and 4th. However, Pintić et al. (2007) did not determine any significant differences between the 2nd and the 3rd lactation of Holstein and Simmental cows. In every single lactation, urea concentration was higher in the milk of Simmental cows than in the milk of Holstein cows. Significant influence ($P<0.05$) of the number of lactations on urea concentration in milk was determined only in Holstein cows.

Although the most authors reported a significant influence of the number of lactations on somatic cell count, this was not confirmed in this research. Somatic cell count (log) was highest in the 5th lactation, and lowest in the 3rd lactation. Hojman et al. (2004) reported higher somatic cell count in higher number of lactations. Negative and significant correlation (-0.36 and -0.13 respectively) was determined between somatic cell count (log) and lactose content, that is, urea concentration in milk.

Daily milk yield was not significantly influenced by milking time, since daily milk yield for both breeds was similar for both morning and evening milking. Higher average daily milk yield was determined for Holstein breed compared to Simmental cows. Significantly higher ($P<0.01$) content of fat and protein in milk was determined during evening milking for Holstein breed. Although urea concentration in milk of both breeds was very similar, a significantly higher ($P<0.05$) concentration was determined only in Simmental cow's milk in evening milking. Some authors (Ferguson et al., 1997; Godden et al., 2001; Prpić et al., 2005) reported a lower urea concentration in the milk from morning milking, which they attribute to a different time interval between feeding and milking time. Bendelja (2009) reported higher urea concentration in Simmental cow's milk from morning milking, and in Holstein cow's milk from evening milking.

Table 4. Effect of season on daily milk yield, milk fat, protein and lactose content, urea concentration and somatic cell count (log) in milk

	Spring - Summer n = 311	Autumn - Winter n = 764	Significance of differences
	Daily milk yield (kg)		
LSM±SE	26.93±0.44	27.52±0.28	NS
Min-Max	10.27-50.94	10.66-57.99	
	Milk fat (%)		
LSM±SE	3.94±0.05	4.36±0.03	***
Min-Max	2.18-5.91	2.09-6.0	
	Protein (%)		
LSM±SE	3.25±0.02	3.38±0.01	***
Min-Max	2.29-4.59	2.16-4.96	
	Lactose (%)		
LSM±SE	4.54±0.01	4.53±0.01	NS
Min-Max	3.85-5.03	3.84-5.07	
	Urea (mg 100 mL^{-1})		
LSM±SE	25.39±0.47	23.83±0.30	**
Min-Max	4.4-47.9	2.8-51.7	
	$Log_{10}SCC$ (log_{10})		
LSM±SE	4.65±0.03	4.75±0.02	**
Min-Max	3.48-5.97	3.60-5.99	

NS - Non significant; **$P<0.01$; ***$P<0.001$
LSM = Least Square Means; SE = Standard Error; Min = Minimum; Max = Maximum

Generally, influence of the season and nutrition on milk production and composition should be taken into account when interpreting results (West, 2003). Daily milk yield was not significantly influenced by season, although a slightly higher quantity was determined in the autumn-winter season. Fat and protein content of milk was significantly higher in the autumn-winter season (4.36 %) than in the spring-summer (3.94 %), which was probably a result of cow feeding. Significant and positive correlation (r=0.11) was determined between the fat content and the urea concentration in milk, which can be explained by the fact that high content of crude protein in voluminous forage increases the fat content in milk as well as urea concentration at the same time, due to degradability of their proteins (Prpić et al., 2005). Correlation coefficient between fat content and protein content in milk was 0.29 (P<0.001), which resulted in higher protein content of milk in the autumn-winter season.

During the spring and summer, urea concentration in milk was significantly higher (P<0.01) than in the autumn-winter season. Also other authors reported high urea concentration in milk (Ng-Kwai - Hang et al., 1985; Carlsson et al., 1995; Ferguson et al., 1997; Rajala-Schultz and Saville, 2003; Hojman et al., 2004; Stoop et al., 2006). Godden et al. (2001) reported the highest urea concentration in milk in late summer months (July to September), stating that the total protein and crude protein (mostly casein) in milk are lower until NPN, which includes urea, increases them. Higher values for urea in milk during spring, that is lower values in the winter months were reported by Callberry (2003) and Abdouli et al. (2008), which is attributed to the influence of grazing.

Marenjak and Poljicak-Milas (2007) reported a significantly lower urea concentration in the milk of Simmental cows during the summer months, and the highest urea concentration during the autumn. This can be explained by reduced consumption of dry matter due to high temperatures. Oudah (2009) determined the lowest urea concentration in milk in February (29.1 mg/dL) and March (25.2 mg/dL), and the highest in May (36.1 mg/dL) and September (34.2 mg/dL). Miglior et al. (2006) reported lower urea concentration in Holstein cow's milk in winter months and early summer, and higher concentration in spring and autumn.

Coefficient of correlation between daily milk yield, chemical composition and somatic cell count are presented in Table 5.

Daily milk yield

A significant correlation coefficient (P<0.001) was determined between daily milk yield and urea concentration in milk (r=0.13). This can be explained by the fact that production animals have higher nutritional demands for crude protein, which leads to increased synthesis of urea in the liver. Accordingly, Arunvipas et al. (2003) and Hojman et al. (2004) determined the correlation coefficient between the above stated parameters (r=0.17). Konjačić et al. (2010) also reported that milking capacity increased along with the increased urea concentration in milk, but only up to 35 mg/dL, after which milking capacity started to fall.

Table 5. Coefficient of correlation

Trait	DMY	MF	P	L	U	$Log_{10}SCC$
DMY	-	0.05	-0.02	-0.04	0.13***	-0.06*
MF	-	-	0.29***	-0.13***	0.11**	0.05
P	-	-	-	-0.14***	0.14***	0.1**
L	-	-	-	-	0.01	-0.36***
U	-	-	-	-	-	-0.13***

*P<0.05; **P<0.01; ***P<0.001

DMY = Daily milk yield; MF = Milk fat; P = Protein; L = Lactose; U = Urea; $Log_{10}SCC$ = Somatic cell count (log)

Milk fat

A significant ($P<0.01$) and positive correlation coefficient ($r=0.11$) was determined between the fat content and the urea concentration in milk. The similar was reported by Hojman et al. (2004), explaining that the high content of neutral detergent fibres in forage can increase milk fat content and cause increased urea concentration at the same time due to high degradability of proteins. Johnson and Young (2003) determined a negative coefficient between fat content and urea concentration in Holstein and Jersey cow's milk.

Protein

Although a negative coefficient between protein content and urea concentration in milk was determined in most of the researches, in this research a positive coefficient was determined ($r=0.14$). Johnson and Young (2003) emphasized that lower urea concentration in milk can be related to better utilization of crude protein in a ration, resulting also in better nitrogen utilization. Godden et al. (2001); Hojman et al. (2004); Abdouli et al. (2008) reported a negative correlation coefficient between the protein content and the urea concentration in milk, while Rajala-Schultz and Saville (2003) did not report any significant relation.

Somatic cells

Very little research has been conducted about the relation between the somatic cell count and the urea concentration in milk. A significant and negative correlation coefficient ($r=-0.13$) between these parameters was determined in this research. Increased somatic cell count was followed by reduced urea concentration in milk, which was confirmed by Rajala-Schultz and Saville (2003), but only in herds with high daily milk yield (>35 kg). A positive relation between somatic cell count and the non-protein nitrogen (NPN) content, which also includes urea, was determined by DePeters and Ferguson (1992), stating that the milk from cows with mastitis contains less casein, and more whey protein.

Conclusion

Based on the research results, the following conclusions can be made:

The breed of a cow significantly influenced daily milk yield and somatic cell count ($P<0.001$), urea concentration in milk and milk fat content ($P<0.05$).

As the number of lactations increased, in both cow breeds daily milk yield was decreasing and urea concentration in milk was increasing.

Milking time (morning-evening) significantly ($P<0.05$) influenced urea concentration, but only in the milk of Simmental cows, which was higher in the morning milking.

The season significantly influenced ($P<0.001$) the fat and protein content of milk as well as urea concentration in milk and log somatic cell count ($P<0.01$). In the summer and winter months a higher daily milk yield of milk with higher milk fat and protein content, but lower urea concentration in milk, was determined.

In addition to the routine analyses determining the milk fat and protein content, somatic cell count as well as the total number of micro organisms in milk, which are conducted on daily basis, determining the urea concentration in milk would be useful for milk producers with the aim of increasing their production profitability and correct milking cow's nutrition.

Koncentracija ureje u mlijeku holstein i simentalskih krava

Sažetak

Određivanje koncentracije ureje u mlijeku koristan je pokazatelj opskrbljenosti organizma proteinima, kao i odnosa energije i proteina u obroku preživača, te ima sve veću praktičnu primjenu. Na koncentraciju ureje u mlijeku, osim hranidbe utječe niz čimbenika kao što su: pasmina, stadij i redoslijed laktacije, tjelesna masa, dnevna proizvodnja i kemijski sastav mlijeka, broj somatskih stanica, sezona i mužnja. Cilj istraživanja bio je utvrditi utjecaj pasmine krava (holstein i simentalska), redoslijeda laktacije (1., 2., 3., 4. i 5.), mužnje (jutarnja - večernja) i sezone (proljeće-ljeto i jesen-zima) na koncentraciju ureje u mlijeku. Za svaku pasminu utvrđena je dnevna količina mlijeka, udio mliječne masti, proteina, laktoze, koncentracija ureje i broj somatskih stanica u mlijeku. Statistička obrada podataka izvršena je primjenom procedure General Linear Model, programskog sustava SAS (1999). Pasmina krava značajno je utjecala na dnevnu količinu mlijeka i log broj somatskih stanica (P<0,001), udio laktoze u mlijeku (P<0,01), udio mliječne masti i koncentraciju ureje u mlijeku (P<0,05). Redoslijed laktacije značajno je utjecao na dnevnu količinu mlijeka (P<0,001), udio proteina u mlijeku (P<0,001 i P<0,01) te na koncentraciju ureje u mlijeku ali samo kod holstein pasmine (P<0,05). Vrijeme mužnje značajno je utjecalo na udio mliječne masti i proteina (P<0,001) u mlijeku holstein krava odnosno na udio laktoze (P<0,05) i koncentraciju ureje (P<0,05) u mlijeku simentalskih krava. Sezona je imala značajan utjecaj na udio mliječne masti i proteina u mlijeku (P<0,001) odnosno na koncentraciju ureje i log broj somatskih stanica (P<0,01). Određivanje koncentracije ureje u kravljem mlijeku trebalo bi sustavno provoditi i u RH, kako bi se utvrdile standardne fiziološke vrijednosti karakteristične za pojedinu pasminu krava, a u cilju procjene izbalansiranosti obroka energijom i proteinima.

Ključne riječi: ureja, pasmina, redoslijed laktacije, mužnja, sezona

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