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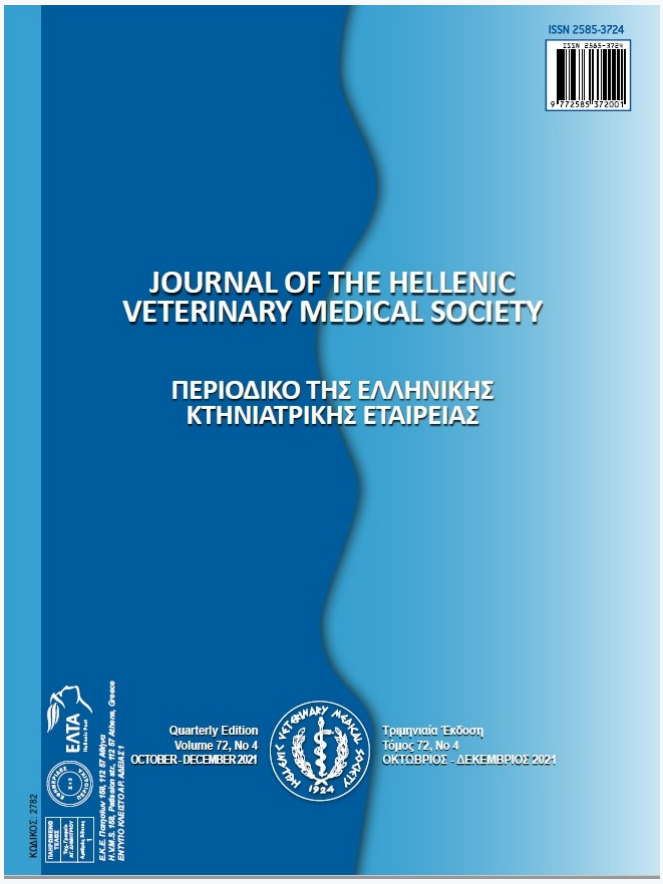
Journal of the Hellenic Veterinary Medical Society

Vol 72, No 4 (2021)



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doi: 10.12681/jhvms.29374

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To cite this article:

ČOBANOVIĆ, K., PAJIĆ, M., RADINOVIĆ, M., SIMONOVIĆ, M., CSORBA, C., KUČEVIĆ, D., & BECSKEI, Z. (2022). Environmental factors affecting milk composition in Holstein cattle breed. *Journal of the Hellenic Veterinary Medical Society*, *72*(4), 3355–3362. https://doi.org/10.12681/jhvms.29374

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J HELLENIC VET MED SOC 2021, 72(4): 3355-3362
ΠΕΚΕ 2021, 72(4): 3355-3362

Research article
Ερευνητικό άρθρο

Environmental factors affecting milk composition in Holstein cattle breed

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ABSTRACT: The incidence of mastitis in dairy herds is one of the main difficulties faced by dairy farmers, with a negative effect on the productivity of the herd and the welfare of the animals. Somatic cell count in milk is an indicator of udder health and frequency of clinical and subclinical mastitis incidence in dairy herds, and it is also often used to determine quality payments to dairy producers. Milk urea can be an indicator of the nutritional status of the dairy cows. The interpretation of these parameters assists in making important management decisions with regards to the health status and nutrition of dairy cattle. The objective of this study was to identify and evaluate environmental factors (farm, season, parity and stage of lactation) which affect the milk production and composition of Holstein breed, using field data. The presented research included 25,460 individual milk samples which were analysed as part of the Dairy Herd Improvement program, from 11 Holstein dairy farms in the region of Vojvodina, Northern Serbia. Analyses of raw milk samples were carried out on the FOSS instruments - CombiFoss™FT+, a combination instrument consisting of the MilcoScan™FT+ and the Fossomatic™FC. Statistical data processing was carried out by applying General Linear Model procedure, Statistics 13. Farm, season of milk control, parity and stage of lactation were included in the models as fixed effects. Significant differences in milk urea concentrations and somatic cell count were observed between farms ($P<0.01$), seasons ($P<0.01$), parity ($P<0.01$) and stage of lactation ($P<0.01$). Results showed that there were a highly significant ($P<0.01$) positive relationships between milk urea (MU) concentration and milk yield, MU and milk fat content, and between MU and solids non-fat (SNF), also between somatic cell count (SCC) and milk fat content, between SCC and protein content, as well as in between SCC and SNF. Highly significant negative relationships were found between milk urea and protein content and SCC, and between SCC and daily milk yield and lactose content. Proper analyses and interpretation of obtained results of milk samples obtained within the Dairy Herd Improvement program could contribute to better health management on the farms and it could have a positive impact on composition and nutritional value of milk, as well as on milk safety. It would be important to carry out further research in order to facilitate the detection of subclinical mastitis with MU as a potential indicator.

Keywords: somatic cell count; milk urea; season; parity; stage of lactation

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Date of initial submission:
Date of revised submission:
Date of acceptance:

INTRODUCTION

The incidence of mastitis in dairy cattle herds is one of the main difficulties faced by dairy farmers, with a negative effect on the productivity of the herd and the welfare of the animals. Infection of the mammary gland is among the most important diseases of cows, causing high economic losses (Boboš et al., 2013). Somatic cell count (SCC) is often used to determine the milk price paid to dairy producers. The delivery control of milk with high SCC was established by the Regulation of the European Union 853 in 2004 for dairy cattle, which requires that bulk tank milk used for production of dairy products should have SCC levels below 400,000 cells/ml.

Milk urea (MU) and SCC are important parameters which can be used as indicators for formulating preventive and corrective measures for nutrition and health management in the herd. Somatic cell count in milk is an indicator of udder health and frequency of clinical and subclinical mastitis incidence in dairy herds. MU can be used as an indicator of the nutritional status of the herd. The interpretation of these milk parameters can assist in making important management decisions regarding the health status and nutrition of dairy cattle herds.

Urea as a part of the non-protein fraction of nitrogen in milk represents the final product of protein metabolism in the rumen of ruminants. Via the portal bloodstream, toxic ammonia is transported into the liver where it is transformed into urea, which later gets into milk through the bloodstream. This urea then can be measured in the bloodstream and milk (Rajala-Schultz et al., 2001). When milk samples are taken as a part of regular Dairy Herd Improvement (DHI) program, sampling involves no extra labor, and it is cheaper than sampling and analyzing blood. Nutrition and content of crude protein in the diet have the greatest influence on the milk urea content. Milk urea nitrogen (MUN) can be used as a tool to monitor protein feeding efficiency and dietary protein - energy ratio in dairy cows. Some other paragenetic factors, such as season, can have an influence on the milk urea content in addition to feeding, milk yield, stage of lactation, parity, breed, body weight, etc. (Godden et al., 2001; Hojman et al., 2004; Wattiaux et al., 2005; Fatehi et al., 2012).

The aim of this study was to identify and evaluate environmental factors (farm, season, parity and stage of lactation) that influence MU, SCC and milk production traits in Holstein dairy herds and to determine associations between SCC and MU (mg/dl) and milk production traits (milk yield - MY (kg), milk fat (%) and protein (%)).

MATERIALS AND METHODS

The study included 11 dairy farms (each with over 120 dairy cows) located in Vojvodina, Northern Serbia, with a total of 4,057 Holstein cows. Milk recording control was performed by AT4 method (ICAR, 2014). A total of 39,313 individual milk samples were collected at monthly DHI milk tests between February 2014 and January 2016. However, in accordance with the ICAR's Protocol (2020) for the Evaluation of Milk Analysers and in order to exclude additional factors that affect milk composition (as improper sampling and some health problems in cows), 13,853 samples were excluded from the study due to at least one of the following reasons: SCC < 50,000 or SCC ≥ 1,000,000; samples with milk fat < 2% or greater than 6% and milk protein < 2% and greater than 5.5%; thus, this research included a total of 25,460 individual milk samples of Holstein cows.

Analyses of raw milk samples were carried out on FOSS instruments - CombiFoss™FT+. This device is a combination instrument consisting of the MilcoScan™FT+ and the Fossomatic™FC. To convert MU into MUN, the following conversion formula was used: MUN (mg/dl) = MU (mg/dl) × 0.4667 (Oudah EZM, 2009). The principle of analyzing of raw milk samples is based on the methodology by mid - infrared spectrometry method (ISO 9622 /2013). Milk and liquid milk products - Guidelines for the application of mid-infrared spectrometry) and flow cytometry (ISO 13366-2 /2006) Milk - Enumeration of somatic cells. Part 2: Guidance on the operation of fluoro-opto electronic counters).

For the statistical analysis of SCC data the absolute values were transformed into somatic cell linear scores (Log 2 SCC) by applying the following equation (Sant' Anna and Paranhos da Costa, 2011): Log 2 SCC = log2 (SCC/100.000) + 3.

Logarithmic transformations are the most appropriate for the SCC data because they yield normality and homogeneity of the variances, enabling the execution of statistical analysis taking into account the above assumptions (Ali and Shook, 1980).

Dataset included: farm code, date of test (season), days in milk (DIM - interval between date of calving and milk test day), daily milk yield, milk fat, milk

protein, lactose, and SNF content, MU concentration (mg/dl), somatic cell count (cells/ml) and parity. According to the season of sampling, milk samples were divided into four groups: 1 - winter (December - February), 2 - spring (March - May), 3 - summer (June - August) and 4 - autumn (September - November). For the analysis, cows were grouped in five categories regarding their parity status (first, second, third, fourth and fifth+). Lactation was divided into 4 DIM intervals (I - 30 to 100 days, II - 101 to 200 days, III - 201 to 300 days and IV - greater than 300 days). The average values and variability of examined traits (daily milk yield - DMY, milk fat - MF, protein - P, lactose - L, solid non fat - SNF, milk urea - MU and somatic cell count - SCC) as well as the effect of factors on mentioned traits were studied by means of the PROC UNIVARIATE and PROC GLM procedures within the Statistic software package (ver. 13 Stat Soft Company 2016). Post-hoc analysis (Duncan test) was used to determine the statistically significant differences between the mean values of different classes, with a significance level at P<0.05 and P<0.01. The model equation used for the evaluation was as follows:

$$\mathbf{Y_{ijkl} = \mu + Si + Fj + Pk + DIMm + eijkl}$$

Legend:
Yijkl - MU, MF, P, L, SNF, SCC and DMY (dependent variable) value of dependent variable;
μ - mean value of dependent variable;
Si - fixed effect the season of sampling i (i = 1, 2, 3, 4);
Fj - fixed effect the farm, j = 1(Farm 1), 2 (Farm 2), …, 11 (Farm 11);
P_k - fixed effect the parity, k = 1st, 2nd, 3rd, 4th, 5th+;
DIMm - fixed effect of the stage of lactation (days in milk), m = 1, 2, 3 and 4;
eijkl - other random effects.

Finally, the correlation between SCC and MU concentration and production variables was performed using the correlation procedure (Statistic. 13). For all parameters, model effects were declared significant at P<0.05 and P<0.01.

RESULTS

The average results for milk fat, total protein, lactose and SNF percentages, DMY, SCC and MU concentration are presented in Table 1.

The influence of the farm was included in the model as a fixed effect and as expected, management of the farm had a great influence on the content of milk urea, SCC and other examined parameters, Table 2.

According to the data in Table 3., season had a significant effect on SCC and MU concentration and other examined traits (the values of F-test in all cases are highly significant) in Holstein cows.

Table 4. shows that stage of lactation had a significant effect on SCC, MU concentration and other examined traits in Holstein cows (the values of F-test in all cases are highly significant).

The concentration of MU was significantly (P < 0.01) lower in the first 100 DIM (24.65 mg/dl) and after 300 days of lactation (24.64 mg/dl).

The content of SCC, MU, milk components and daily milk yield, were significantly influenced by the number of lactations (Table 5.). The high values of the F - ratios are the proof of the important influence of the parity on the examined variables.

Cows in the first lactation had a higher milk fat content (3.78%), cows in the second lactation had higher protein content (3.33%) and in the third lactation had a higher daily milk yield (27.75 kg). Cows in the first lactation had the lowest SCC (255,300/ml) and SCC was increased with increased number of lactation, the highest SCC were cows in the fifth and greater lactation (308,430/ml).

Table 1. Means, minimum, maximum, standard deviation and coefficient of variation of analyzed variables

Trait	N	Mean	Minimum	Maximum	SD	CV
Fat (%)	25460	3.76	2.00	6.00	0.85	22.61
Protein (%)	25460	3.31	2.00	5.43	0.41	12.39
DMY (kg)	25460	26.75	2.00	67.20	9.88	36.93
SNF (%)	25460	8.74	5.59	10.98	0.47	5.38
Lactose (%)	25460	4.62	2.35	5.44	0.23	4.98
MU (mg/dl)	25460	25.49	10.00	92.00	8.11	31.82
SCC (10^3/ml)	25460	274.84	50.00	1000.00	227.25	82.68
Log 2 SCC	25460	3.99	2.00	9.62	1.17	29.32

Legend: DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; N - total number of individual cow milk samples; SD - standard deviation; CV - coefficient of variation

Table 2. Effect of farm on milk traits

Farm	N	Fat (%)	Protein (%)	DMY (kg)	SNF (%)	Lactose (%)	MU (mg/dl)	SCC (10^3/ml)	Log 2 SCC
1	5174	3.96^{a}	3.44^{a}	22.34^{a}	8.74^{a}	4.52^{a}	23.81^{a}	210.25^{a}	3.63^{a}
2	482	3.74^{b}	3.38^{b}	26.60^{b}	9.04^{b}	4.68^{bc}	22.52^{b}	256.02^{bc}	3.96^{b}
3	1014	3.85^{c}	3.20^{ce}	27.36^{c}	8.68^{c}	4.55^{d}	30.50^{c}	243.93^{bd}	3.82^{c}
4	4859	3.56^{d}	3.32^{d}	27.52^{c}	8.80^{d}	4.66^{be}	25.44^{d}	364.96^{e}	4.50^{d}
5	3495	4.09^{e}	3.23^{c}	27.26^{bc}	8.64^{e}	4.60^{f}	26.31^{ef}	312.84^{f}	4.18^{e}
6	2025	3.73^{b}	3.37^{b}	30.08^{d}	8.90^{f}	4.68^{bgh}	26.44^{e}	268.85^{c}	3.97^{b}
7	996	3.71^{b}	3.43^{a}	27.50^{c}	8.94^{f}	4.69^{cg}	23.32^{a}	233.64^{dg}	3.80^{c}
8	2997	3.45^{f}	3.23^{c}	27.84^{c}	8.75^{a}	4.66^{eh}	23.92^{a}	267.92^{c}	3.97^{b}
9	1956	3.25^{g}	3.22^{c}	23.59^{e}	8.74^{a}	4.65^{eh}	25.57^{df}	244.24^{bg}	3.81^{c}
10	1702	3.97^{a}	3.18^{e}	32.72^{f}	8.58^{g}	4.61^{f}	29.13^{g}	253.22^{bc}	3.91^{bc}
11	760	4.15^{h}	3.31^{d}	29.30^{g}	8.37^{h}	4.60^{f}	26.81^{e}	241.28^{bg}	3.82^{c}
F		267.7**	130**	242.5**	165**	188**	135.2**	148.8**	170.5**

Legend: N - total number of individual cow milk samples; DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; Means within the same column with different superscripts ($^{a,b,c...h}$) differ significantly ($P < 0.01$); significant differences: * $P < 0.05$; ** $P < 0.01$

Table 3. Effect of season of sampling on SCC, milk urea concentration, daily milk yield and milk components

Season	N	Fat (%)	Protein (%)	DMY (kg)	SNF (%)	Lactose (%)	MU (mg/dl)	SCC (10^3/ml)	Log 2 SCC
1	5737	3.81^{a}	3.43^{a}	27.51^{a}	8.85^{a}	4.63^{a}	25.86^{a}	276.65^{a}	4.02^{a}
2	4932	3.74^{b}	3.25^{b}	27.87^{b}	8.79^{b}	4.65^{b}	27.34^{b}	283.71^{a}	4.04^{a}
3	6645	3.64^{b}	3.16^{c}	26.73^{c}	8.59^{c}	4.60^{c}	27.47^{b}	260.81^{b}	3.91^{b}
4	8146	3.83^{a}	3.38^{d}	25.54^{d}	8.75^{d}	4.59^{c}	22.49^{c}	279.61^{a}	4.02^{a}
F		69.3**	605**	73.8**	357**	93**	638.5**	12.30**	17.6**

Legend: Season: 1 - Winter; 2 - Spring; 3 - Summer; 4 - Autumn; N - total number of individual cow milk samples; DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; Means within the same column with different superscripts (^{a,b,c,d}) differ significantly ($P < 0.01$); significant differences: * $P < 0.05$; ** $P < 0.01$

Table 4. Effect of stage of lactation on SCC, milk urea concentration, daily milk yield and milk components

Lactation stage	N	Fat (%)	Protein (%)	DMY (kg)	SNF (%)	Lactose (%)	MU (mg/dl)	SCC (10^3/ml)	Log 2 SCC
1	5845	3.62^{a}	2.99^{a}	32.75^{a}	8.47^{a}	4.68^{a}	24.65^{a}	255.45^{a}	3.86^{a}
2	8257	3.63^{a}	3.22^{b}	29.51^{b}	8.67^{b}	4.65^{b}	26.68^{b}	265.82^{a}	3.93^{b}
3	7058	3.84^{b}	3.45^{c}	23.52^{c}	8.85^{c}	4.58^{c}	25.31^{c}	280.73^{b}	4.04^{c}
4	4300	4.06^{c}	3.68^{d}	18.56^{d}	9.05^{d}	4.51^{d}	24.64^{a}	308.84^{c}	4.22^{d}
F		328.1**	4267**	2915.2**	1747**	672**	98.0**	52.50**	91.2**

Legend: N - total number of individual cow milk samples; DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; F - values of F-test; Means within the same column with different superscripts ($^{a.b.c.d}$) differ significantly ($P < 0.01$); significant differences: * $P < 0.05$; ** $P < 0.01$

Table 5. Effect of parity on SCC, milk urea concentration, daily milk yield and milk components

Parity	N	Fat (%)	Protein (%)	DMY (kg)	SNF (%)	Lactose (%)	MU (mg/dl)	SCC (10^3/ml)	Log 2 SCC
1	10046	3.78^{a}	3.30^{ab}	25.61^{a}	8.80^{a}	4.68^{a}	25.58^{a}	255.30^{a}	3.88^{a}
2	7242	3.72^{b}	3.33^{a}	27.53^{b}	8.74^{b}	4.60^{b}	25.37^{a}	276.81^{b}	4.00^{b}
3	4462	3.76^{ab}	3.32^{ab}	27.75^{b}	8.69^{c}	4.56^{c}	25.25^{a}	296.38^{cd}	4.12^{c}
4	2107	3.75^{ab}	3.29^{b}	27.28^{bc}	8.64^{d}	4.53^{d}	25.32^{a}	290.05^{bc}	4.09^{c}
5	1603	3.74^{ab}	3.26^{c}	26.82^{c}	8.64^{d}	4.53^{d}	26.32^{b}	308.43^{d}	4.20^{d}
F		5.6**	11.0**	58.2**	91.0**	422.0**	6.0**	40.10**	53.4**

Legend: N - total number of individual cow milk samples; DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; F - values of F-test; Means within the same column with different superscripts (^{a,b,c,d}) differ significantly ($P < 0.01$); significant differences: * $P < 0.05$; ** $P < 0.01$

Table 6. Coefficient of correlation between milk composition components, DMY, SCC and MU

Trait	Protein (%)	DMY (kg)	SNF (%)	Lactose (%)	MU (mg/dl)	SCC (10^3/ml)
Fat (%)	0.3362**	-0.2942**	0.1731**	-0.1477**	0.1106**	0.0571**
Protein (%)		-0.4663**	0.7551**	-0.2000**	-0.0270**	0.1260**
DMY (kg)			-0.2877**	0.2753**	0.0614**	-0.1231**
SNF (%)				0.2429**	0.0934**	0.0103^{NS}
Lactose (%)					0.0082^{NS}	-0.1983**
MU (mg/dl)						-0.0412**

Legend: DMY - daily milk yield; SNF - solid non fat; MU - milk urea; SCC - somatic cell count; Significant differences: * $P < 0.05$; ** $P < 0.01$; ^{NS} - Non Significant

Intensity of the correlation between analyzed parameters in milk and determined statistical significance is presented in Table 6. It can be noticed that MU concentration negatively correlated ($P < 0.01$) with protein (%), but positively correlated with milk fat (%), SNF (%), lactose (%) and DMY (kg).

SCC also negatively correlated ($P < 0.01$) with DMY (kg) and lactose (%), but positively correlated with milk fat and protein.

DISCUSSION

Mean values for milk fat (3.76%) and protein contents (3.31%) determined in this study were a higher than average values for total Holstein population in Vojvodina in the year 2015 (milk fat 3.71%, protein 3.25%, total milk yield 9,177 kg) given by Main breeding organization (2016).

In this research, the mean MU concentration (25.49 mg/dl) was within the optimal values of 15 to 30 mg/dl given by Carlsson and Pehrson (1993). Average MU concentration was higher than values reported in studies of Hof et al. (1997) and Kohn et al. (2004), but lower than values reported by Wattiaux and Karg (2004), Zadeh-Hossein and Ardalan (2011) and Fatehi et al. (2012) for Holstein dairy cows.

The average SCC (274,840 cells/ml) was higher than that reported by Konjačić et al. (2010), but was lower than that found by Yoon et al. (2004). Also, Sadeghi-Sefidmazgi and Rayatdoost-Baghal (2014) report an average SCC that is very similar to the result of this research.

The highest content of MU, 30.50 mg/dl, was found on Farm 3. On the contrary, the lowest MU content was estimated on Farm 2 (22.52 mg/dl). The effect of farm on MU and other examined parameters is related to the different ratio of energy and protein in feeding (Table 2.). Mean value of SCC on all examined farms was below 400,000/ml. The lowest SCC was on Farm 1 (210,250/ml) and the highest was on Farm 4 (364,960/ml).

Statistically significant differences in MU content between farms are reported by others (Wattiaux Met al., 2005; Konjačić et al., 2006). The effect of the farm is a very complex factor which reflects the action of numerous different systematic and non-systematic environmental influences, such as nutrition, type and quality of housing facilities, health status of cows, climatic conditions and farm management.

MU was lower in autumn (22.49 mg/dl) and highest during summer (27.47 mg/dl). Similar results have been reported by Hojman et al. (2004) and Fatehi et al. (2012). The highest values of SCC were evidenced during the spring (283,710/ml) and lowest values in the summer (260,810/ml). Ivanov et al. (2017) reported similar results, in which SCC showed significant elevation in the autumn-winter period compared to the spring and summer period. Some other authors found the lowest count of somatic cells in winter and highest during the summer (Wells and Ott, 1998; Memiši et al., 2011). Ferreira and De Vries (2015) evidenced higher SCC in warmer months (August, September and October) than the average SCC in colder months (February, March and April). According to Syridion et al. (2012), there was evidenced significantly higher SCC during the summer months compared to both autumn and winter seasons.

As presented in Table 4., the peak of lactation was in the first 100 days after calving. Some authors found that the peak of lactation was between 4 and 8 weeks after calving (Čobić and Antov, 1996; Park and Lindberg, 2004), but Piccardi et al. (2014) reported the peak of lactation around 122 days after calving. The highest MU level was evidenced between 101 and 200 DIM (26.68 mg/dl), this was a signal of the excess protein in diet of cows in the period after the

peak of lactation. Similar results were reported in other studies (Hojman et al., 2004; Fatehi et al., 2012).

The SCC was lowest in the first 100 days of lactation (255,450/ml) and after it increased, reaching the highest value at the end of lactation (308,840/ml). The reports by Campos et al. (2006) showed that lactation curves of the content of somatic cells and milk yields usually show opposite patterns. Syridion et al. (2012) and Sitkowska (2008) also concluded that SCC increased with lactation progressing.

A lower MU concentration (25.58 mg/dl) was found in cows in the third lactation and mean MU concentration of cows in the fifth and greater lactations (26.32 mg/dl) was higher and differs significantly from other lactations (Table 5.). The overall differences between lactations are numerically small. Contrary to our results, Godden et al. (2001) recorded the highest MU concentration in cows in the first lactation. Hojman et al. (2004) found lower MUN content in the first lactation cows than the second or later lactation animals.

According to reports of Godden et al. (2001) and Hojman et al. (2004) negative correlation was found between the milk protein content and MU concentration. Contrary, Bendelja et al. (2011) found a positive correlation between milk protein and MU.

Milk fat content increased with the increasing MU level. Bendelja et al. (2011) reported a positive correlation between milk fat content and MUN. Hojman et al. (2004) explained that higher content of neutral detergent fibres in forage may increase milk fat content and at the same time caused an increased urea concentration, due to the high degradability of its proteins. A negative relation between milk fat and MUN was reported by Konjačić et al. (2010). A positive association between daily milk yield and MUN has also been reported by Godden et al. (2001) and Konjačić et al. (2010). Hojman et al. (2004) determined the correlation coefficient between the above stated parameters (r=0.17). A positive correlation between daily milk yield and MU was expected because cows with higher milk production were fed diets richer in protein component.

Some studies have examined urea transfer from milk to blood by measuring disappearance of injected labelled urea in the mammary gland of dairy cattle (Spek et al., 2016). Roy et al. (2001) claim that MU concentration decreased as intensity of infection increased from mild to moderate. Licata (1985) reported that udder quarters positive to California mastitis test was 0.45 mM lower in urea content than that from healthy quarters. It could be assumed that infections of the mammary gland, which cause increased permeability of the udder tissue, also increase MU transfer from milk into bloodstream.

A negative correlation has been evidenced between SCC and milk yield, as reported by Coffey et al. (1986). There was a negative correlation between SCC, fat/protein contents and milk yield in the report published by Yoon et al. (2014).

Very little research has been conducted concerning the relation between the SCC and the MU concentration. In the present research, a significant and negative correlation coefficient (r = -0.0412) between these parameters was determined. Increased somatic cell count was followed by reduced urea concentration in milk, also reported by Hojman et al. (2004) and Bendelja et al. (2011). Yoon et al. (2004) show that by increasing SCC, milk yield was reduced and MU level was increased.

CONCLUSION

Based on the present research results, the following conclusions can be drawn:

- The farm, season, parity and stage of lactation had significant effects (P<0.01) on SCC, MU concentration, milk fat and protein content and daily milk yield.
- MUN concentration and SCC should be eval-

uated considering the parity, season and stage of lactation.

- There are positive and statistically significant correlations between the MU concentration and milk fat and lactose content, as well as between MU concentration and milk yield; also between SCC and milk fat and protein content.
- Negative and statistically significant correlations were found between MU concentration and protein content and SCC, and between SCC and milk yield and lactose content.

Proper analyses and interpretation of obtained results could contribute to better health management on the farms and it could have a positive impact on composition and nutritional value of milk, as well as on milk safety. Moreover, individual milk samples can be taken easily, involving almost no extra labor and without causing stress to dairy cattle. Since there is no clear correlation between MU concentrations and occurrence of mastitis, it would be important to carry out further research on this topic in order to facilitate the detection of subclinical mastitis with MU as a potential indicator.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract 451-03-9/2021-14/200117).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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