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# The Variability of Biochemical and Hematological Parameters Depending on the Mastitis Occurrence in Dairy Cows

Varijabilnost biokemijskih i hematoloških parametara ovisno o pojavnosti mastitisa kod muznih krava

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# THE VARIABILITY OF BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS DEPENDING ON THE MASTITIS OCCURENCE IN DAIRY COWS

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## SUMMARY

***With the aim of determination of biochemical indicators variability in the plasma and milk and hematological indicators regarding the daily lactose content classes (indicating the mastitis risk) and somatic cell counts classes (indicating the animal's health status), blood and milk of 75 Holstein cows were sampled. The cows were reared on a commercial dairy farm. A statistical analysis demonstrated that the lactation stage, parity, and statistically significant monthly measurement  $P < 0.01$ ) affected both the biochemical and hematological parameters. The differences between the analyzed biochemical and hematological parameters due to the mastitis score classes (according to the daily lactose content, DLC, and the somatic cell count, SCC) were present and statistically significant ( $P < 0.05$ ) in some traits. Furthermore, different trends regarding the mastitis scoring (DCL of SCC) were determined in some traits. Therefore, when using the test-day records as an animal's mastitis risk and health status indicator, both scoring ways should be used. Finally, in the case of a mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for the sake of an unambiguous detection.***

***Keywords: mastitis, biochemical parameters, hematological parameters, dairy cows, animal recording***

## INTRODUCTION

Economic efficiency is one of the most important parameters of farm management and, if efficient, enables a dairy farm sustainability. Currently, there is a growing interest in the technologies that allow the measurement of physiological, behavioral, and production indicators of cows in order to optimize the dairy farm management. Precision dairy farming could be defined as a significant tool for farmers who know what they want from their herd well. One of the main goals of precision dairy farming is to maximize the animal productivity, enable early detection of possible diseases/disorders, and minimize production costs while applying the adequate preventive health measures and management optimization. Bewley (2010) emphasized that the benefits of the use of precision dairy farming technologies are as follows: an increased farm effi-

ciency, reduced production costs, improved product quality, minimized adverse environmental impacts and an improved animal health and welfare. Many studies identified mastitis as the most common (Seegers et al., 2003; Petrovski et al., 2006) and most expensive disease (Ibrahim, 2017; Gráff and Mikó, 2015) in dairy production worldwide. The occurrence of mastitis (i.e., the udder inflammation) on a dairy farm results in the significant business losses due to a reduced milk production and deterioration of individual udder quarters or that of the entire udder, which can lead to a premature culling, death, and high treatment costs. Mastitis in

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dairy cows could be manifested in the subclinical and clinical forms. In contrast to the clinical mastitis forms (CM), subclinical mastitis creates the costlier problems (Gráff and Mikó, 2015) because it is not easily observed, with dairy herd prevalence of 20% (Hasan et al., 2018), 26% (Kaki et al., 2019; Sumon et al., 2017), to as much as 60% (Mpatshwenumugabo et al., 2017). Even when mastitis does not affect the entire udder but only the individual quarters thereof, the impact extends to the udder's overall immune status and results in a change of milk composition and the neighboring quarters' health status (Paixão et al., 2017). Based on the milk recording data (daily lactose content and somatic cell count in milk), a possibility of early mastitis detection could facilitate the farmers to improve the farm management and prevent the development of clinical form and consequent milk production costs.

Lactose is a carbohydrate synthesized by mammals. Its concentration in milk is influenced by metabolism, energy balance, and mammary gland's health status (Costa et al., 2019a). According to the numerous studies, the lactose content is correlated with the number of somatic cells in milk, where the lactose content below 4.5% represents an indicator of mammary gland's inflammation (mastitis) occurrence. Furthermore, a somatic cell count (SCC), or a somatic cell score (SCS), could be used as an udder health indicator (Ivanov et al., 2016). The healthy milk's SCC fluctuates from 20,000 to 100,000 cells/ml (Gráff and Mikó, 2015). Petzer et al. (2017) concluded that, due to a low specificity, the SCC level, being an indicator of different pathogen groups' presence, exerted a negative effect on the threshold accuracy of 150,000 cells/ml in the joint sample and 200,000 cells/ml in the individual quarter samples. Other studies have demonstrated a significant decline in the milk yield (Mikó et al., 2016), being directly related to an SCC increase: in the range from 50,000 to 100,000/ml, the decline amounts to 8%; from 100,000 to 250,000 ml, the decline amounts to 15%; and for the values higher than 250,000/ml, the decline amounts to up to 18% (Pfützner and Ózsvári, 2016). Some authors believe that the main cause of SCC variation is the presence of an intramammary infection pathogen type and the quarter location (posterior in relation to the anterior), and the animal's age and parity (Sumon et al., 2020). Phenotypic correlations between the milk lactose content and the SCS were recorded from -0,15 to -0,66. Therefore, the lactose content can be seriously taken as a highly informative indicator for mastitis diagnosis, along with the SCC and milk's electrical conductivity (Costa et al., 2019b).

A disproportion between a genetically determined nutrient intake requirements and a limited consummation potential leads to a negative energy balance (NEB) and can be a cause of different metabolic disorders. The NEB is a normal adaptive mechanism in the high-yielding dairy animals, and recent evidence indicates that the hepatic inflammatory responses during the

periparturient period are an important driving force for fat mobilization (Wankhade et al., 2017) too. The NEB stimulates the cows to mobilize the body fat in the form of non-esterified fatty acids (NEFA). The bovine liver has a limited capacity to metabolize the NEFA into triglycerides (TGCs). When the limit is reached, the TGC accumulates in the liver, and the acetyl CoA (resulting from the fatty acid oxidation) is not utilized in the tricarboxylic acid cycle, which is then converted into the ketone bodies such as acetone, acetoacetate, and  $\beta$ -hydroxybutyrate (BHB) whereafter the BHB is subsequently accumulation in the blood (Nelson and Cox, 2005). The cows with lipolysis are at a high risk to develop a fatty liver, because an excessive TGC content in the liver impairs its function and reduces the liver's gluconeogenic activity, which lowers the blood glucose (Goff and Horst, 1997). The activity of some liver enzymes, for example, that of the aspartate aminotransferase (AST) and  $\gamma$ -glutamyl transferase (GGT), can be used as a biochemical indicator for the evaluation of a metabolic balance (Liu et al., 2012; Jóźwik et al., 2012; Puppel and Kuczyńska, 2016).

The term *metabolic profile* refers to an analysis of sanguine biochemical parameters that are useful to assess and prevent the metabolic and nutritional disorders in dairy herds (Puppel and Kuczyńska, 2016). The cows with a low glucose level, ketosis, fatty liver, and the elevated NEFA are believed to have a poorer immune function (Burvenich et al., 2007). A metabolic adaptation to the inflammation and milk synthesis is interconnected, and therefore the high-producing dairy cows face an increased risk of glucose shortages in their immune cells, particularly during an early lactation (Habel and Sundrum, 2020). A research by Uyarlar et al. (2018) indicated that the hematologic parameters are significantly altered in the animals that have a subclinical or clinical ketosis form. The aforementioned authors also determined that a susceptibility to significant infectious diseases during the periparturient period, such as mastitis and metritis, increases, as is, resultantly, the rate of culling. Furthermore, the Fe serum concentrations represent a useful biomarker of an acute mastitis severity in cows and has a further advantage because it can be easily measured at a low cost (Tsukano and Suzuki, 2020).

Since the daily lactose content and the somatic cell count could be used as the mastitis occurrence indicators and as the animal's health indicators, being a part of precision dairy farming, this study's aim was to determine the values of biochemical indicators in the plasma and milk samples and hematological indicators in the blood samples, depending on the daily lactose content classes (indicating a mastitis risk) and the number of somatic cells (indicating the animal's health status).

## MATERIAL AND METHODS

The research was conducted on a commercial dairy cattle farm situated in East Croatia. During the

research, the blood and milk samples were taken from 75 cows of the Holstein breed, with an average daily milk production of  $39.30 \pm 9.02$  kg (Table 1). The bovine blood samples were taken from the coccygeal vein into the tubes with the lithium heparin anticoagulant (Becton Dickinson, Plymouth, England, UK). The samples were centrifuged (1.500 g/10 mins. at 4°C), and the plasma was separated and frozen at -80°C until analyses. The samples for hematological analyses were taken into the Ca-EDTA tubes (Becton Dickinson, Plymouth, England, UK) and analyzed within two hours on the *Poch 100Veff* (Sysmex, Japan). The milk samples were taken into the clean tubes and centrifuged (12.000 g/30 min at 4°C), and the milk plasma was separated and stored at -80°C until analyses. The biochemical parameters in the blood and milk plasma were determined using the *Beckman Coulter AU400* automatic clinical chemistry analyzer (Beckman Coulter, Germany). The concentration of  $\beta$ -hydroxybutyrate (BHB) was determined using the

commercial kits (Randox Laboratories Ltd., Crumlin, UK) by the enzymatic colorimetric method.

Also, the test-day records of selected cows (from a regular milk recording) were taken from the central database of the Croatian Agency for Agriculture and Food (HAPIH). The test-day records were corrected according to the ICAR guidelines (2017). With regard to a daily lactose content (DLC), the records were divided into two classes: the records pertaining to the cows with a mastitis risk (DLC < 4.5%) and normal records (DLC  $\geq$  4.5%). Furthermore, in accordance with a daily somatic cell count (SCC), the records were divided into three classes: normal healthy animals (SCC < 200,000/ml), the cows with a mastitis risk (SCC = 200,000 - 400,000/ml), and the mastitis-affected cows (SCC > 400,000/ml). Table 1 presents the basic statistical parameters of daily production traits (daily milk yield, daily lactose content, and somatic cell count).

**Table 1: Variability of daily milk production traits of selected animals (n = 75)**

Tablica 1. Varijabilnost dnevnih svojstava mliječnosti odabranih životinja (n = 75)

Trait	Mean	SD	CV	Min	Max
Daily milk yield (kg)	39.30	9.02	22.95	18.60	59.80
Daily lactose content (%)	4.46	0.22	4.95	3.57	4.85
Somatic cell count	1,420,673.94	2,205,156.92	155.22	31,818.18	10,844,296.59

The variability of biochemical and hematological parameters due to a different lactose content and somatic cell count classes were tested in the SAS (SAS Institute, Inc., 2019) using the least square means in the GLM procedure. The following statistical model was used:

$$y_{ijklm} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + P_j + M_k + D_l + e_{ijklm},$$

where

$y_{ijklm}$  = estimated biochemical or hematological parameters

$\mu$  = intercept

$b_1, b_2, b_3, b_4$  = regression coefficients (lactation curve by Ali and Schaeffer, 1987)

$d_i$  = days in milk,  $i$  ( $i = 11$  to 537 days)

$P_j$  = fixed effect of parity,  $j$  ( $j = I, II, III, IV, V$ )

$M_k$  = fixed effect of the month of measurement,  $k$  ( $k = V, VI, VII$ .)

$D_l$  = fixed effect of lactose content classes,  $l$  ( $l =$  normal / mastitis risk) or somatic cell count class,  $l$  ( $l =$  normal / mastitis risk / mastitis),

$e_{ijklm}$  = residual

In order to test the significance ( $p < 0.05$ ) of differences in biochemical and hematological parameters due to a different lactose content or different somatic

cell count classes, the Tukey-Kramer's studentized range test in the SAS-based (SAS Institute Inc., 2019) GLM procedure was administered.

## RESULTS AND DISCUSSION

The statistical analysis demonstrated that the effects of lactation stage, parity, and measurement month included in the statistical model applied were statistically significant ( $P < 0.01$ ). The values of biochemical parameters in the plasma regarding the mastitis score classes (according to the daily lactose content in milk and the somatic cell count) are presented in Table 2. The highest aspartate amino transferase (AST) value in the plasma was determined in the normal healthy animals for the both mastitis scoring methods, with the significantly lowest value ( $P < 0.05$ ) observed in the mastitis-affected cows (scored in accordance to the somatic cell count, SCC > 400,000). Similarly, the significantly lowest value ( $P < 0.05$ ) of  $\gamma$ -glutamyl transferase (GGT) was observed in the same animals. Those animals also had the significantly highest value

( $P < 0.05$ ) of glucose in the plasma when compared to the cows manifesting a mastitis risk and the normal ones. On the contrary, when a mastitis score was defined according to the daily lactose content (DLC), an insignificantly higher value of glucose in the plasma was determined in the normal cows. The urea value in the plasma did not differ significantly ( $P > 0.05$ ) with regard to the mastitis score, but a higher value was determined in the normal animals according to the lactose content, while it was determined according to the SCC in the animals manifesting a mastitis risk.

The protein value varied regarding the mastitis score, but the difference was not significant ( $P > 0.05$ ). An insignificant variability ( $P > 0.05$ ) was also determined in the albumin, triglyceride,  $\beta$ -hydroxybutyrate and Fe concentrations in the plasma, while the Ca concentration was proven to be significantly higher ( $P < 0.05$ ) in the animals manifesting a mastitis risk according to the lactose content. According to the SCC classes, the highest Ca value in the plasma was determined in the cows with SCC within 200,00 – 400,000/ml, that is, in those manifesting a risk of mastitis occurrence.

**Table 2. The estimated biochemical parameter means (Ismeans) in the plasma with regard to the mastitis score classes (according to the daily lactose content and the somatic cell count)**

*Tablica 2. Procijenjene srednje vrijednosti biokemijskih parametara u plazmi u ovisnosti o razredu ocjene mastitisa (temeljem dnevnoga sadržaja laktoze te broja somatskih stanica)*

Trait	Mastitis score daily lactose content (%)		Mastitis score somatic cell count ( $\times 10^3$ /ml)		
	< 4.5 RISK	$\geq 4.5$ NORMAL	< 200 NORMAL	200 – 400 RISK	> 400 MASTITIS
Aspartate amino transferase (U/L, AST)	111.181 <sup>A</sup>	120.609 <sup>A</sup>	166.547 <sup>A</sup>	149.398 <sup>B</sup>	102.307 <sup>B</sup>
$\gamma$ - glutamyl transferase (U/L, GGT)	25.257 <sup>A</sup>	29.121 <sup>A</sup>	34.588 <sup>A</sup>	35.752 <sup>A</sup>	24.388 <sup>B</sup>
Glucose (mmol/l, GUK)	2.990 <sup>A</sup>	3.058 <sup>A</sup>	2.812 <sup>A</sup>	2.951 <sup>B</sup>	3.057 <sup>B</sup>
Urea (mmol/L, UREA)	4.421 <sup>A</sup>	4.584 <sup>A</sup>	4.543 <sup>A</sup>	4.657 <sup>A</sup>	4.455 <sup>A</sup>
Protein (g/L, PRO)	87.080 <sup>A</sup>	86.591 <sup>A</sup>	89.140 <sup>A</sup>	85.183 <sup>A</sup>	86.755 <sup>A</sup>
Albumin (g/L, ALB)	31.748 <sup>A</sup>	32.566 <sup>A</sup>	31.638 <sup>A</sup>	33.224 <sup>A</sup>	31.988 <sup>A</sup>
Triglyceride (mmol/L, TGC)	0.116 <sup>A</sup>	0.106 <sup>A</sup>	0.110 <sup>A</sup>	0.105 <sup>A</sup>	0.133 <sup>A</sup>
$\beta$ -hydroxybutyrate (mmol/L, BHB)	0.488 <sup>A</sup>	0.505 <sup>A</sup>	0.441 <sup>A</sup>	0.477 <sup>A</sup>	0.506 <sup>A</sup>
Fe ( $\mu$ mol/L)	21.499 <sup>A</sup>	22.070 <sup>A</sup>	23.896 <sup>A</sup>	25.455 <sup>A</sup>	20.900 <sup>A</sup>
Ca (mmol/L)	2.253 <sup>A</sup>	2.139 <sup>B</sup>	2.232 <sup>A</sup>	2.325 <sup>A</sup>	2.189 <sup>A</sup>

The values within the same row, with regard to the same mastitis score and marked by different letters, differ significantly ( $P < 0.05$ ).

The biochemical parameter values in milk with regard to the mastitis score classes (according to the daily lactose content and the somatic cell count) are presented in Table 4. The aspartate aminotransferase (AST) value did not differ significantly ( $P > 0.05$ ), but the highest values were determined in the animals manifesting a mastitis risk (DLC < 4.5%) and in the mastitis-affected ones (SCC > 400,000). The highest  $\gamma$ -glutamyl transferase (GGT) value was determined in the normal animals (DLC  $\geq 4.5$ ) and in the animals manifesting a mastitis risk (SCC = 200,000 to 400,000). The lowest

glucose, protein, and Fe concentrations in milk were determined in the animals manifesting a mastitis risk (DLC < 4.5%) and in the mastitis-affected animals (SCC > 400,000). Contrarily, the highest but still insignificant ( $P > 0.05$ ) milk globulin concentration was determined in the same animals. The Ca concentration in milk was insignificantly ( $P > 0.05$ ) higher in the normal animals (DLC  $\geq 4.5$ ), while the animals manifesting a mastitis risk according to the SCC had the significantly highest Ca value in milk ( $P < 0.05$ ).

**Table 3. The estimated biochemical parameter means (Ismeans) in milk with regard to the mastitis score classes (according to the daily lactose content and the somatic cell count)**

Tablica 3. Procijenjene srednje vrijednosti biokemijskih parametara u mlijeku u ovisnosti o razredu ocjene mastitisa (temeljem dnevnoga sadržaja laktoze te broja somatskih stanica)

Trait	Mastitis score daily lactose content (%)		Mastitis score somatic cell count ( $\times 10^3$ /ml)		
	< 4.5 RISK	$\geq 4.5$ NORMAL	< 200 NORMAL	200 – 400 RISK	> 400 MASTITIS
Aspartate amino transferase (U/L, AST)	16.340 <sup>A</sup>	15.816 <sup>A</sup>	10.355 <sup>A</sup>	13.228 <sup>A</sup>	17.321 <sup>A</sup>
$\gamma$ - glutamyl transferase (U/L, GGT)	352.847 <sup>A</sup>	365.560 <sup>A</sup>	316.587 <sup>A</sup>	381.214 <sup>A</sup>	360.630 <sup>A</sup>
Glucose (mmol/l, GUK)	0.424 <sup>A</sup>	0.478 <sup>A</sup>	0.539 <sup>A</sup>	0.548 <sup>B</sup>	0.419 <sup>A</sup>
Urea (mmol/L, UREA)	5.330 <sup>A</sup>	5.358 <sup>A</sup>	5.455 <sup>A</sup>	5.559 <sup>A</sup>	5.297 <sup>A</sup>
Protein (g/L, PRO)	35.369 <sup>A</sup>	35.591 <sup>A</sup>	33.886 <sup>A</sup>	35.840 <sup>A</sup>	35.627 <sup>A</sup>
Albumin (g/L, ALB)	21.512 <sup>A</sup>	22.167 <sup>A</sup>	21.211 <sup>A</sup>	22.756 <sup>A</sup>	21.723 <sup>A</sup>
Globulin (g/L, GLOB)	13.856 <sup>A</sup>	13.424 <sup>A</sup>	12.675 <sup>A</sup>	13.080 <sup>A</sup>	13.904 <sup>A</sup>
Fe ( $\mu$ mol/L)	17.581 <sup>A</sup>	19.960 <sup>A</sup>	21.362 <sup>A</sup>	21.950 <sup>A</sup>	17.685 <sup>A</sup>
Ca (mmol/L)	3.088 <sup>A</sup>	3.156 <sup>A</sup>	2.784 <sup>A</sup>	3.409 <sup>B</sup>	3.123 <sup>A</sup>

The values within the same row, with regard to the same mastitis score and marked by different letters, differ significantly ( $P < 0.05$ ).

Similar to this study, Kuczynska and colleagues (2021) determined a significant lactation stage and parity effect on the AST and GGT activity in dairy cows. The aforementioned authors pointed out that the parity effect was characterized by a greater deterioration of changes in the blood plasma parameter dynamics, particularly in the AST, GGT, BHB and NEFA levels. Moyes and colleagues (2009) determined a significant positive relationship between the circulating AST and the development of clinical mastitis during an early lactation, with no differences observed between the healthy cows and the ones with subclinical mastitis, but a low AST specificity in the circulation concerning a particular disease may render it a less useful marker for the evaluation of the mastitis risk. Also, Moyes and colleagues indicated that the increased NEFA and BHBA concentrations are related to the development of mastitis. In a relationship analysis of an animal's production level and the AST activity, Jóźwik and colleagues (2012) determined that the AST activities in the blood serum were lower in the dairy cows with a lactation production of 7,000 kg when compared to the cows with a higher milk production (10,000 kg). The aforementioned authors also determined a GGT variation regarding the lactation stage, that is, they observed that the serum GGT is higher in the latter lactation days. Puppel and Kuczyńska (2016) stated that the changes in the blood-related AST activity can be a consequence of their increased cellular activity, as well as a result of cellular structural damage. The liver function effects on the inflammatory markers of serum's biochemical values in the cows with an acute mastitis were also studied by Tsukano and Suzuki (2020). They determined that there were no significant

differences in the serum AST or GGT related to the liver function. Furthermore, they found significant differences in the serum Fe concentrations and indicated the role of biomarkers for the acute mastitis severity in the cows. Some studies (Rathaur et al., 2020; Tripathy et al., 2018) indicated a significant increase in the serum AST and protein and a significant decrease in Ca and glucose in the mastitis-affected cows when compared with the healthy ones.

Comparable to this study's results, Liu and colleagues (2012) determined that the AST activities in milk, when analogized to those of the blood plasma, appeared to be significantly lower ( $P < 0.001$ ), while the GGT activities in milk were significantly higher ( $P < 0.001$ ). Therefore, they concluded that a detection of their milk levels may be an alternative to monitor the dairy cows' liver function.

Similar to our research findings, Silankova and others (2014) circumstantiated that the mammary gland inflammation results in a significant rise in the blood plasma glucose concentration, caused by a relatively higher glucose production rate. Furthermore, an increased immunosystem's energy demand can be satisfied by the maintenance of a high glucose production rate and a reduction in the mammary epithelial cells' extraction thereof from the blood plasma. Mastitis increases the mammary gland's epithelial barrier permeability, and this can consequently lead to a blood component transfer to the milk.

In this research, the highest BHB value was determined in mastitis-affected animals (SCC > 400.000) and in the normal ones according to the lactose content,

while the lowest value was determined in the cows with the SCC < 200.000 (normal status). Costa and colleagues (2019b) believe that the lactose percentage is strictly dependent on the circulating glucose, and therefore the milk of the (sub)ketotic cows usually demonstrates a lower lactose level and a higher BHB level than the one of the healthy animals, especially in early lactation.

In this study, the plasmatic protein levels were highest in the animals in a normal status (SCC < 200,000), while albumin was lowest in the same animals. This coincides with a research conducted by Turk and colleagues (2021), who found that most milk proteins were represented by some type of a higher quantity in subclinical and clinical groups, if compared to that of the healthy cows. The aforementioned authors reported a reverse trend in the serum, with most of the proteins being represented by a lower quantity in the subclinical and clinical groups, if compared to the healthy group. The increased milk protein quantity could be explained by a mix of local synthesis in the mammary gland and by the extravasation of blood proteins to the mammary gland through the blood–mammary gland barrier. The overall decrease in the quantity of serum proteins could be caused by consumption during a systematic inflammatory response in a negative acute-phase protein (APP) response and/or leakage and in the protein transfer to the mammary gland.

Erskine and Bartlett (1993) conducted an experiment with the induced mastitis that resulted in the decreases in serum Fe and Zn concentrations in approximately 4 to 8 hrs. subsequent to the peak bacterial concentrations in milk. Furthermore, similar to this research, Baydar and Dabak (2014) observed a reduced serum Fe concentration in the mastitis-affected cows and those affected by an acute traumatic reticuloperitonitis, if compared to the control group. Tsukano and Suzuki (2020) emphasized that the effects of liver function on the inflammatory markers alter the blood Fe concentrations in cows.

The hematological parameter values regarding the mastitis score classes (according to the daily lactose content and somatic cell count) are presented in Table 3. After eight hours, sedimentation was higher in the animals manifesting a mastitis risk (DLC < 4.5%), and sedimentation was at its highest in the animals with mastitis (SCC > 400,000). On the other hand, after 24 and 48 hrs., sedimentation was higher in the normal healthy cows (DLC ≥ 4.5%; SCC < 200.000). White blood cell count (WBC) was higher in the animals manifesting a mastitis risk (DLC < 4.5%), while the red blood cell count (RBC) was higher in the normal animals (DLC ≥ 4.5%). The hemoglobin (HGB) and hematocrit (HTC) values were higher in the normal animals (DLC ≥ 4.5%) and were at their highest in the animals manifesting a mastitis risk (SCC between 200.000 and 400.000/ml).

**Table 4. The estimated hematological parameter means (Ismeans) with regard to the mastitis score classes (according to the daily lactose content and somatic cell count)**

Tablica 4. Procijenjene srednje vrijednosti hematoloških parametara u ovisnosti o razredu ocjene mastitisa (temeljem dnevnoga sadržaja laktoze te broja somatskih stanica)

Trait	Mastitis score daily lactose content (%)		Mastitis score somatic cell count ( $\times 10^3$ /ml)		
	< 4.5 RISK	≥ 4.5 NORMAL	< 200 NORMAL	200 – 400 RISK	> 400 MASTITIS
Sedimentation after 8 hours (SED-8)	8.395 <sup>A</sup>	8.311 <sup>A</sup>	7.792 <sup>A</sup>	7.513 <sup>A</sup>	8.571 <sup>A</sup>
Sedimentation after 24 hours (SED-24)	31.544 <sup>A</sup>	32.246 <sup>A</sup>	32.645 <sup>A</sup>	30.976 <sup>A</sup>	31.800 <sup>A</sup>
Sedimentation after 48 hours (SED-48)	43.013 <sup>A</sup>	43.455 <sup>A</sup>	45.232 <sup>A</sup>	43.672 <sup>A</sup>	42.803 <sup>A</sup>
White blood cells ( $\times 10^9$ /L, WBC)	8.564 <sup>A</sup>	7.962 <sup>A</sup>	8.467 <sup>A</sup>	8.177 <sup>A</sup>	8.329 <sup>A</sup>
Red blood cells ( $\times 10^9$ /L, RBC)	6.267 <sup>A</sup>	6.425 <sup>A</sup>	6.089 <sup>A</sup>	6.818 <sup>A</sup>	6.304 <sup>B</sup>
Hemoglobin (g/L, HGB)	108.865 <sup>A</sup>	113.751 <sup>B</sup>	105.326 <sup>AB</sup>	116.211 <sup>A</sup>	110.941 <sup>B</sup>
Hematocrit (HTC)	0.284 <sup>A</sup>	0.294 <sup>A</sup>	0.270 <sup>A</sup>	0.301 <sup>B</sup>	0.289 <sup>A</sup>

The values within the same row, with regard to the same mastitis score and marked by different letters, differ significantly ( $P < 0.05$ ).

Similar results, comparing the healthy animals and the cows affected by subclinical mastitis (diagnosed by the California Mastitis Test), were reported by Zannatul and others (2015). They determined a higher erythrocyte sedimentation rate (ESR), RBC, HGB and HTC in the

normal cows than in the cows with subclinical mastitis, while the WBC in the cows affected by subclinical mastitis was lower than in normal ones. Many researchers have confirmed that the hematological parameters levels, such as those of the RBC, HGB, and the HTC



of the mastitis-affected cows, were lower than in the normal ones, while the WBC was increased (Tripathy et al., 2018; Rathaur et al., 2020; Ramesh et al., 2021). Furthermore, significantly higher average ESR values in the subclinical forms than those in healthy animals, with no significant change in values in clinical manifestations, were determined by Sarvesha and colleagues (2016) and by Das and colleagues (2018).

## CONCLUSION

The statistical analysis showed that lactation stage, parity, and measurement month statistically significantly ( $P < 0.01$ ) affected biochemical parameters in plasma and milk as well as hematological parameters. The differences between the analyzed biochemical and hematological parameters due to mastitis score classes (accordingly to daily lactose content, DLC and somatic cell count, SCC) were present and statistically significant ( $P < 0.05$ ) in some traits. Also, different trends regarding mastitis scoring (DCL of SCC) were determined in some traits. Therefore, when using test-day records as an indicator of the mastitis risk and health status of an animal both scoring ways should be used, and in a case of mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for unambiguous detection.

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## VARIJABILNOST BIOKEMIJSKIH I HEMATOLOŠKIH PARAMETARA OVISNO O POJAVNOSTI MASTITISA KOD MUZNIH KRAVA

### SAŽETAK

*U cilju utvrđivanja vrijednosti biokemijskih pokazatelja u uzorcima plazme i mlijeka te hematoloških pokazatelja u uzorcima krvi ovisno o razredima dnevnoga sadržaja laktoze (što ukazuje na rizik od mastitisa) i broju somatskih stanica (koji ukazuje na zdravstveno stanje životinje), uzorci krvi i mlijeka uzeti su od 75 krava holsteinske pasmine uzgajanih na komercijalnoj mliječnoj farmi tijekom tromjesečnoga razdoblja. Statistička analiza pokazala je da stadij i redosljed laktacije te mjesec uzorkovanja statistički značajno ( $P < 0,01$ ) utječu na biokemijske parametre u plazmi i mlijeku, kao i na hematološke parametre. Razlike između analiziranih biokemijskih i hematoloških parametara bile su zbog razreda mastitisa (prema dnevnome sadržaju laktoze, DLC, i broju somatskih stanica, SCC) prisutne i statistički značajne ( $P < 0,05$ ) kod nekih svojstava. Također, kod pojedinih su svojstava utvrđeni različiti trendovi ovisno o načinu ocjene mastitisa (DCL ili SCC). Stoga se pri korištenju rezultata kontrole mliječnosti kao pokazatelja rizika od mastitisa i zdravstvenoga stanja životinje treba koristiti obama načinima ocjene, a u slučaju procijenjenoga rizika od mastitisa ili pojave mastitisa treba se radi nedvosmislenoga otkrivanja koristiti drugim dijagnostičkim metodama (kao što su različiti testovi na mastitis).*

*Ključne riječi: mastitis, biokemijski parametri, hematološki parametri, mliječne krave, kontrola proizvodnosti*

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