THE VARIATIONS IN BIOCHEMICAL PARAMETERS IN BLOOD AND MILK DUE TO MASTITIS INDICATION AND COWS PARITY*

Ivana Jožef[1], Marcela Šperanda[2], Mislav Đidara[2], Ranko Gantner[2], Muhamed Brka[3], Vesna Gantner[2]

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Summary

With the aim of determination of the variability of biochemical parameters in blood plasma and milk samples depending on mastitis incidence and parity, blood and milk samples were taken from 75 high-yielding Holstein cows. The mastitis incidence was defined accordingly to daily lactose content and daily somatic cell count (test-day records). The obtained results indicate that the differences between the analysed biochemical parameters in blood plasma, due to mastitis score classes (accordingly to daily somatic cell count, SCC) were present and statistically significant ($P < 0.05$) in some parameters (AST and GGT). Also, different patterns regarding mastitis scoring (DCL of SCC) were determined in some parameters. It was established that there was an increase in the levels of most of the analysed biochemical parameters in blood plasma and milk compared due to animal parity, except for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity. Therefore, when using test-day records as an indicator for the mastitis risk and health status of an animal both scoring ways should be used, but in the case of mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for explicit detection.

Key words: *biochemical parameters, test-day records, mastitis, Holstein*

INTRODUCTION

Inefficiency in milk production, such as the occurrence of animal disorders/diseases, can have great economic consequences. Although epidemic infectious diseases can result in large economic losses and generate much greater public attention, production disorders/diseases are economically more important for the overall efficiency of livestock production. Selection to increase milk yield imposes stress on the overall animal and especially on the udder, so high-yielding cows have an increased risk of mastitis. The disproportion between the genetically determined ability for milk

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[1] University in Novi Sad, Faculty of Agriculture, Osijek, Department on Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2] Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Department for animal production and biotechnology Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia
[3] University of Sarajevo, Faculty of Agriculture and Food Sciences, Department for Animal Breeding, Bosnia and Hercegovina
Correspondence: Vesna Gantner, Vladimira Preloga 1, Osijek, vgantner@fazos.hr

production and the limitations in improving the energy value of the ration may be the cause of metabolic disorders (Puppel & Kuczyńska, 2016). Metabolic burden in the liver function is often associated with abomasal displacement, ketosis, mastitis, parturient paresis, placenta retention and endometritis, which usually occur in postpartum cows. Therefore the priority for intensive milk production is the prevention of metabolic disorders/diseases. The identification of new and less expensive biomarkers can enable earlier detection of animals at risk and consequently improve treatment strategies and milk yield and reduce the use of antibiotics. Many studies conducted in various countries have represented the incidence rate of clinical mastitis (CM) and its estimated effects and risk factors. Jamali *et al.*, (2018) reported that parity was found to be a risk factor for new intramammary infection (IMI) in general, suggesting that the cow's intramammary and anatomical (e.g. teat sphincter patency) defense mechanisms may deteriorate with age. These non-standard defense mechanisms may explain the higher incidence of CM and CM recurrence in older cows. Furthermore, Gonçalves *et al*. (2018) noticed that second- and third-lactation cows had greater milk loss associated with somatic cell counts, than primiparous cows. Antanaitis *et al.* (2021) observed that the decrease in lactose concentration and the increase in the number of somatic cells in milk were directly related to the presence of the causative agent of subclinical mastitis in dairy cattle. Since the percentage of lactose in milk decreased with parity, while milk yield and number of somatic cells increased, it was hypothesized that changes in lactose percentage reflect the history of mammary gland infection and can be considered a mammary memory indicator (Costa *et al.*, 2020).
Metabolites can reflect the environment and the nutritional status of cells, the role of drugs and environmental pollutants, and the influence of other external factors (Hu *et al*., 2021). Furthermore, metabolites in the blood can completely reflect the physiological and biochemical state of dairy cows, so it is frequently used to detect the occurrence of various disorders/diseases in cows. Different metabolites in milk can also indirectly reflect whether cows have mastitis or not. The knowledge and analysis of blood and milk biochemical parameters could be useful to assess and prevent udder health disorders/diseases. The metabolic changes are closely related to clinical and subclinical disease after calving, lactation, and to reproductive performance - factors that significantly affect the profitability of dairy production (Kuczyńska *et al*., 2021).
The aim of this work was to determine the values of biochemical indicators in blood plasma and milk of Holstein cows regarding the classes of daily lactose content (which indicates the risk of mastitis) and the number of somatic cells (which indicates the animal's health condition) separately for each parity (second and third).

MATERIALS AND METHODS

The research was performed on a commercial dairy cattle farm situated in East Croatia. During the research, blood and milk samples were taken from 75 cows of the Holstein breed with average daily milk production of 39.30±9.02 kg (Table 1). Blood samples of cows were taken from the coccygeal vein into tubes with lithium heparin anticoagulant

(Becton Dickinson, Plymouth, England, UK). Samples were centrifuged (1.500 g/10 min at 4°C) and plasma was separated and frozen at -80°C until analyses. Samples of milk were taken into clean tubes, centrifuged (12.000 g/30 min at 4°C) and milk plasma was separated and stored at -80°C until analyses. Biochemical parameters in blood and milk plasma were determined using the automatic clinical chemistry analyser Beckman Coulter AU400 (Beckman Coulter, Germany). The concentration of β-hydroxybutyrate (BHB) was determined using commercial kits (Randox Laboratories Ltd, Crumlin, UK) by the enzymatic colourimetric method.
Also, test-day records (from regular milk recording accordingly to the AT4 method) of selected cows were taken from the central database of HAPIH (Croatian Agency for Agriculture and Food). Test-day records were corrected accordingly to the ICAR guidelines (ICAR, 2017). In regard to daily lactose content (DLC), cows were divided into two classes: mastitis risk (DLC < 4.5%) and normal cows (DLC ≥ 4.5%). Furthermore, in accordance with the daily somatic cell count (SCC), animals were divided into three classes: normal healthy animals (SCC < 200,000/ml); cows in mastitis risk (SCC = 200,000 - 400,000/ml) and cows with mastitis (SCC > 400,000/ml). Tab. 1 presents the basic statistical parameters of daily production traits (daily milk yield, daily lactose content and somatic cell count).

Tab. 1. Variability of daily milk production traits of selected animals (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	2,205,156	155.22	31,818	10,844,296

The basic variability of analyzed biochemical parameters in blood plasma is shown in table 2, while table 3 shows the basic variability of analyzed biochemical parameters in milk.

Tab. 2. Variability of analyzed biochemical parameters in blood plasma of selected animals

Trait	**Mean**	**SD**	**CV**	**Min**	**Max**
Aspartate amino transferase (u/l)	139.319	68.090	48.873	56.290	396.100
γ-glutamil transferase (u/l)	33.561	16.391	48.840	8.700	106.600
Glucose (mmol/l)	3.036	0.466	15.334	1.600	3.940
Urea (mmol/l)	4.496	0.825	18.340	2.280	6.770
Protein (g/l)	84.374	5.562	6.592	73.200	103.400
Albumin (g/l)	32.043	2.547	7.948	23.100	36.000
Triglyceride (mmol/l)	0.115	0.022	19.422	0.070	0.190
β-hydroxybutyrate (mmol/l)	0.495	0.173	34.988	0.230	1.110
Fe (μmol/l)	23.722	7.018	29.587	6.000	42.600

Ca (mmol/l)	2.163	0.234	10,814	0.610	2.550

Tab. 3. Variability of analyzed biochemical parameters in milk of selected animals

Trait	Mean	SD	CV	Min	Max
Aspartate amino transferase (u/l)	15.095	11.379	75.387	3.755	53.955
γ-glutamil transferase (u/l)	335.803	83.286	24.801	110.125	632.900
Glucose (mmol/l)	0.519	0.174	33.450	0.163	0.945
Urea (mmol/l)	5.426	1.203	22.182	1.428	8.403
Protein (g/l)	35.689	5.186	14.530	14.825	47.350
Albumin (g/l)	22.373	2.390	10.685	9.900	26.625
Fe (μmol/l)	23.109	14.757	63.857	2.550	58.475
Ca (mmol/l)	3.179	0.551	17.343	1.303	4.523

The variability of biochemical parameters in blood plasma and milk due to different lactose content and somatic cell count classes were tested using least square means in GLM procedure in SAS (SAS Institute Inc., 2019) separately for each parity. Following statistical model was used:

$$y_{ijkl} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\, ln(305/d_i) + b_4\, ln^2(305/d_i) + M_j + D_k + e_{ijkl}$$

Where:
y_{ijkl} = estimated biochemical parameters;
μ = 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 day);
M_j = fixed effect of month of measurement j (j = V., VI., VII.),
D_k = fixed effect of lactose content classes k (k = normal / mastitis risk) or somatic cell count class k (k = normal / mastitis risk / mastitis),
e_{ijkl} = residual.
In order to test the significance ($p < 0.05$) of the differences in biochemical parameters due to different lactose content or somatic cell count classes the Tukey-Kramer's studentized range test in GLM procedure in SAS (SAS Institute Inc., 2019) were used.

RESULTS AND DISCUSSION

The statistical analysis showed that the effects of lactation stage, measurement month, and lactose content or somatic cell count class included in the used statistical model were statistically significant ($P < 0.01$). The values of the biochemical parameters in blood plasma regarding the mastitis score classes (accordingly to daily lactose content in milk and somatic cell count) in second and third lactation are presented in Table 4.
The value of aspartate aminotransferase (AST) in blood plasma showed a significant decrease in cows in the second lactation associated with an increase in the number of somatic cells, but also a slight decrease in relation to the increased lactose content. In

cows in the third lactation, lower values of AST were determined in animals at risk (DLC < 4.5%) and significantly ($P < 0.05$) lowest value was observed in cows with mastitis (scored by somatic cell count, SCC > 400,000). The highest value of γ-glutamyl transferase (GGT) in plasma was determined in animals at risk of mastitis (SCC = 200,000 to 400,000) and in normal animals (DLC > 4.5%) in the III. parity. Although LSMeans values of other analysed biochemical parameters were not statistically significantly different ($p > 0.05$), some patterns were observed. For biochemical parameters in blood plasma, depending on the mastitis classes from healthy to animals at risk and/or in mastitis, an increase, according to the daily lactose content (DLC), was observed in the levels of AST, glucose, albumin, BHB and Fe in cows of the II. parity; as well as protein and triglycerides in cows in the III. lactation, while Fe increased in all animals regardless of the parity. A consistent decrease in blood plasma concentration, according to an increased daily somatic cell count (SCC), was observed for urea, protein and Fe in cows in the II. parity and an increase in glucose concentration in cows in the III. lactation. The concentrations of other parameters varied, noting that the highest values of albumin and Ca, and the lowest values of TGC were found in animals at risk, in both parities.

Tab. 4. LSmeans of the biochemical parameters in blood plasma regarding lactose and SCC classes separately by each parity (II.; III.)

Trait	Parity	DLC, %			SCC	
		Mastitis risk	**Normal status**	**Mastitis**	**Mastitis risk**	**Normal status**
Aspartate amino transferase (u/l)	2	170.451^{A}	140.667^{A}	136.226^{A}	120.522^{A}	210.759^{B}
	3	120.160^{A}	148.158^{A}	107.912^{A}	196.589^{B}	162.151^{B}
γ-glutamil transferase (u/l)	2	30.987^{A}	33.561^{A}	35.310^{A}	27.065^{A}	31.109^{A}
	3	32.954^{A}	36.627^{A}	29.290^{A}	56.760^{B}	38.071^{A}
Glucose (mmol/l)	2	3.060^{A}	3.025^{A}	3.086^{A}	2.909^{A}	2.991^{A}
	3	2.989^{A}	3.157^{A}	3.118^{A}	3.069^{A}	2.856^{A}
Urea (mmol/l)	2	4.052^{A}	4.244^{A}	4.093^{A}	4.329^{A}	4.370^{A}
	3	4.590^{A}	4.788^{A}	4.732^{A}	4.485^{A}	4.550^{A}
Protein (g/l)	2	81.889^{A}	85.251^{A}	85.865^{A}	80.325^{A}	83.709^{A}
	3	85.210^{A}	83.471^{A}	83.807^{A}	84.014^{A}	86.905^{A}
Albumin (g/l)	2	32.277^{A}	31.456^{A}	31.624^{A}	32.010^{A}	31.487^{A}
	3	31.620^{A}	33.156^{A}	32.299^{A}	33.688^{A}	31.194^{A}
Triglyceride (mmol/l)	2	0.110^{A}	0.116^{A}	0.115^{A}	0.110^{A}	0.117^{A}
	3	0.119^{A}	0.105^{A}	0.115^{A}	0.104^{A}	0.116^{A}
β-hydroxybutyrate (mmol/l)	2	0.483^{A}	0.420^{A}	0.432^{A}	0.474^{A}	0.415^{A}
	3	0.503^{A}	0.556^{A}	0.555^{A}	0.453^{A}	0.467^{A}
Fe (µmol/l)	2	24.834^{A}	23.855^{A}	23.086^{A}	24.882^{A}	26.441^{A}
	3	23.004^{A}	23.781^{A}	21.705^{A}	30.402^{A}	24.481^{A}
Ca (mmol/l)	2	2.239^{A}	2.082^{A}	2.098^{A}	2.213^{A}	2.111^{A}
	3	2.208^{A}	2.131^{A}	2.162^{A}	2.291^{A}	2.181^{A}

** DLC – daily lactose content, %; SCC – somatic cell count; Values within the same row marked with different letter differ statistically highly significant (P<0.05)*

The values of the biochemical parameters in cows' milk regarding the mastitis score classes (accordingly to daily lactose content and somatic cell count) in II. and III. lactation is presented in Table 5. Regarding the biochemical parameter in milk, depending on the mastitis classes from healthy to risky animals and/or in mastitis, a decrease was observed, according to the daily lactose content (DLC), in the levels of AST, GGT, urea, protein and Fe in cows of the II. parity; albumin in cows in the III. lactation. Furthermore, the concentration of glucose and Ca in milk were similar in cows in the II. lactation, but slightly decreased in the milk of cows in the next lactation. A consistent increase in the level of biochemical parameters in milk, according to the increased daily number of somatic cells (SCC), was observed for AST, GGT and urea, in both parities, and for the protein concentration in cows of the III. parity and a continuous decrease in the glucose concentration in cows in the III. lactation. The highest values of other biochemical parameters in milk were found in animals at risk regardless of the parity.

Tab. 5. LSmeans of the biochemical parameters in milk regarding lactose and SCC classes separately by each parity (II.; III.)

Trait	Parity	DLC, %			SCC	
		Mastitis risk	**Normal status**	**Mastitis**	**Mastitis risk**	**Normal status**
Aspartate amino transferase (u/l)	2	12.807^{A}	15.415^{A}	16.043^{A}	13.245^{A}	12.230^{A}
	3	17.129^{A}	15.402^{A}	18.621^{A}	14.802^{A}	10.557^{A}
γ-glutamil transferase (u/l)	2	316.493^{A}	337.885^{A}	349.199^{A}	344.042^{A}	271.057^{A}
	3	355.392^{A}	345.538^{A}	356.714^{A}	383.929^{A}	318.110^{A}
Glucose (mmol/l)	2	0.568^{A}	0.568^{A}	0.534^{A}	0.650^{A}	0.594^{A}
	3	0.464^{A}	0.538^{A}	0.437^{A}	0.594^{A}	0.622^{A}
Urea (mmol/l)	2	4.801^{A}	5.192^{A}	4.952^{A}	5.329^{A}	5.282^{A}
	3	5.563^{A}	5.480^{A}	5.601^{A}	5.386^{A}	5.373^{A}
Protein (g/l)	2	34.857^{A}	35.491^{A}	35.006^{A}	36.400^{A}	35.280^{A}
	3	36.760^{A}	36.005^{A}	37.366^{A}	37.014^{A}	33.234^{A}
Albumin (g/l)	2	22.791^{A}	22.198^{A}	21.761^{A}	23.319^{A}	21.935^{A}
	3	22.704^{A}	22.959^{A}	22.904^{A}	24.193^{A}	21.744^{A}
Fe (µmol/l)	2	16.685^{A}	23.865^{A}	20.146^{A}	26.779^{A}	23.060^{A}
	3	26.327^{A}	25.059^{A}	24.452^{A}	31.355^{A}	27.585^{A}
Ca (mmol/l)	2	3.190^{A}	3.190^{A}	3.192^{A}	3.491^{A}	2.903^{A}
	3	3.238^{A}	3.309^{A}	3.297^{A}	3.680^{A}	2.944^{A}

** DLC – daily lactose content, %; SCC – somatic cell count; Values within the same row marked with different letter differ statistically highly significant ($P<0.05$)*

Comparing the concentrations of biochemical parameters in blood plasma to milk, a considerable decrease in AST, glucose and protein, and a slighter decrease in the concentration of albumin in milk compared to blood were found in all cows. A large increase in concentration in milk compared to plasma was found for GGT, and a slightly lower increase in urea and Ca concentrations.

The increase in the concentrations of most of the examined biochemical parameters in blood plasma and milk compared due to parity was determined. The exception of the determined trend was observed for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity.
The increased levels of various enzymes in the milk occur mainly due to the increased permeability of microcirculatory vessels in inflamed areas along with the leakage from degenerated/necrotic parenchymal cells and leukocytes. Aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT) are important catabolic enzymes that play an important role in the function of animal liver. Also, variations in AST and GGT are frequently associated with abomasal displacement, ketosis, mastitis, parturient paresis; retain placenta and endometritis, which often occur in postpartum cows. Kuczyńska *et al.* (2021) stated that multiparous cows were characterised by a higher deterioration of the blood plasma parameters, particularly in the AST, GGT and BHB levels compared to primiparous. The same authors noticed the value of AST in the blood serum in the range from 67.00 U/L (the oldest cows at the end of lactation) to 100.39 U/l (cows in the second lactation in the first 100 days of lactation). Furthermore, the same authors emphasised that the influence of the parity on blood plasma GGT was significant with the lowest GGT activity found in the blood of primiparous cows at the early lactation. Djokovic *et al*. (2017) determined statistically significant ($P<0.01$) higher activity of AST in blood serum in early lactation cows compared to the mid-lactation cows, while there was no significant difference ($P>0.05$) in milk serum AST due to the stage of lactation. Liu et al. (2012) determined that AST activities in milk were significantly lower ($P<0.001$), and GGT activity was significantly higher ($P<0.001$) compared to blood plasma. Also, the same authors reported significant positive correlations ($P<0.001$) between the activities of these enzymes in milk and blood plasma. Batavani *et al.* (2003) found a similar distribution of AST in the milk of normal and subclinical ewes as well as a higher concentration of AST than in blood serum. Similarly, Babaei *et al*. (2007) reported no significant differences in milk AST in relation to subclinical mastitis, with a higher concentration in milk in relation to blood serum. However, a considerable amount of AST in homogenates from healthy mammary gland tissue suggests that a major source of AST in normal and mastitic milk is the mammary gland secretory cells (Batavani *et al*., 2003).
Increases in plasma glucose are primarily attributed to changes in feed intake and reduced demand for lactose synthesis in the mammary gland in response to intramammary infection (IMI) challenge, while lower plasma β-hydroxybutyrate (BHB) may be associated with the increased transfer into milk. Nyman *et al*., (2008) noticed that the greater serum concentrations of BHB and glucose before calving were associated with lesser SCC levels at first test milking. Primiparous cows have a lower incidence of mastitis than older cows, but in early lactation, they have as great or greater, the prevalence of mastitis than older cows, possibly indicating a lesser ability to cope with stressors occurring during the periparturient period. Guan *et al*., (2020) emphasised that although the results of BHB analysis showed an association with the risk of clinical or subclinical mastitis, the elevated BHB levels are mainly related to

ketosis in dairy cows and cause relatively minimal changes in immune function. Therefore, BHB could not be directly related to the inflammatory process.

Saleh *et al*. (2022) pointed out that the considerable rise in blood glucose in the subclinical mastitis cows could be attributed to higher cortisol levels. Furthermore, the biochemical examination of blood samples in some studies demonstrated a substantial decrease in serum total protein, albumin, and globulin (Saleh *et al*., 2022). Because vascular permeability is enhanced, this decrease may be linked to the leaking of albumin and other serum proteins into milk. Albumin is a negative acute-phase systemic protein that migrates to inflamed tissues via increased vascular permeability and performs a variety of physiological activities, including antioxidants, and is regarded as an immune-inflammatory biomarker (Kaneko *et al*., 2008). During the acute phase response, albumin production is suppressed and amino acids in the liver are diverted to the synthesis of acute phase proteins (Sadek *et al*., 2017). Furthermore, albumin breakdown is accelerated in the presence of elevated glucocorticoids; hence, illness stress and increased cortisol may result in a drop in blood albumin levels. Tripathy *et al*. (2018) indicated a significant ($p<0.05$) increase in various serum biochemical parameters such as protein and AST and a significant decrease in glucose and Ca concentration in cows with mastitis compared to apparently healthy ones (mastitis negative control cows). They also found a significant ($p<0.05$) decrease in protein value in mastitis cows' milk.

Iron, after adsorption from the digestive, passes into the bloodstream and is transported therein, attached to the glycoprotein transferrin, iron-binding protein. This protein is never fully saturated with iron and in that way ensures that no free iron remains in circulation (Ratledge and Dover, 2000). Ganz (2018) explained that the pathogenic microbes have evolved specialized mechanisms for obtaining iron from the host during infections, but the mammalian host has evolved multiple mechanisms of innate immunity that limit the availability of essential nutrient iron to infecting microbes. Antibacterial systems are helpless when iron becomes freely available and this result in rapid extracellular bacterial growth and greatly increased bacterial virulence. Furthermore, Tsukano and Suzuki, (2020) reported that the changes in the blood Fe concentration could be useful as biomarkers of inflammatory disease, including in cows with mastitis. Finally, El Zubeir *et al*. (2005) reported a significant negative effect of mastitis on the variation in Ca concentration in the blood serum that is they determined a significant decrease ($P < 0.01$) in blood serum Ca in samples from clinically infected cows.

CONCLUSIONS

This work aimed to determine the values of biochemical parameters in blood and milk samples, depending on the classes of daily lactose content (which indicates the risk of mastitis) and classes of the number of somatic cells (which indicates the animal's health condition) in cows of the II. and III. parity. The differences between the analysed biochemical parameters in blood plasma, due to mastitis score classes (accordingly to daily somatic cell count, SCC) were present and statistically significant (P < 0.05) in some parameters (AST and GGT). Also, different patterns regarding mastitis scoring (DCL of SCC) were determined in some parameters. It was established that there was an increase in the levels of most of the analysed biochemical parameters in blood plasma and milk compared due to animal parity, except for TGC and Fe in blood plasma, and milk glucose where the concentrations were mostly lower in the following parity. Therefore, when using test-day records as an indicator for the mastitis risk and health status of an animal both scoring ways should be used, but in the case of mastitis risk or mastitis occurrence, other diagnostic methods (such as various mastitis tests) should be used for explicit detection.

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VARIJACIJE BIOKEMIJSKIH PARAMETARA U KRVI I MLIJEKU USLIJED INCIDENCIJE MASTITISA I PARITETA KRAVE

Rezime

U cilju utvrđivanja varijabilnosti biokemijskih parametara u uzorcima krvne plazme i mlijeka ovisno o incidenciji mastitisa i paritetu krave, uzorkovani su uzorci krvi i mlijeka od 75 visokoproizvodnih krava Holstein pasmine. Incidencija mastitisa definirana je sukladno dnevnom sadržaju laktoze, DCL te dnevnom broju somatskih stanica, SCC (test-day record). Dobiveni rezultati pokazuju da su razlike između analiziranih biokemijskih parametara u krvnoj plazmi, ovisno o razredima mastitisa (prema dnevnom broju somatskih stanica), prisutne i statistički značajne ($P < 0,05$) u nekim parametrima (AST i GGT). Također, u nekih su parametara utvrđeni različiti obrasci varijablnosti ovisno o načinu incidencije mastitisa (DCL of SCC). Utvrđeno je povećanje razine većine analiziranih biokemijskih parametara u krvnoj plazmi i mlijeku u odnosu na viši paritet životinja, osim za TGC i Fe u krvnoj plazmi, te glukozu u mlijeku gdje su koncentracije uglavnom niže u sljedećim paritet. Stoga, kada se zapisi na kontrolni dan (test-day record) koriste kao pokazatelj rizika od mastitisa i zdravstvenog statusa životinje, trebaju se koristiti oba načina bodovanja, ali u slučaju rizika od mastitisa ili pojave mastitisa, druge dijagnostičke metode (kao što su različiti testovi za mastitis) trebaju biti korištene za nedvojbeno otkrivanje.

Ključne riječi: *biokemijski parametri, test-day records, mastitis, Holstein*