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Biochemical and Hematological Parameters and Body Condition Score of Holstein Cows Under Different Welfare Conditions

Biokemijski i hematološki pokazatelji i indeks tjelesne kondicije krava holsteinske pasmine s obzirom na ocjenu dobrobiti

Đud, D., Gantner, V., Điđara, M., Pavlić, M., Šperanda, M.

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BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS AND BODY CONDITION SCORE OF HOLSTEIN COWS UNDER DIFFERENT WELFARE CONDITIONS

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SUMMARY

Animal welfare (AW) is a term denoting how the animals are coping with the conditions in which they live. A precise welfare assessment assumes a multidisciplinary approach, and modern evaluation protocol incorporates both the animal-based and non-animal-based measures. Due to the different welfare score classes, this study's objective was to determine the variability of biochemical and hematological parameters, as well as the body condition score. The study was conducted involving 145 Holstein cows, randomly chosen from the six commercial dairy cow farms. The dairy cows' welfare assessment checklist consisted of seventy items, pursuant to the CReNBA protocol. The biochemical parameters in blood and the milk plasma were determined using the **Beckman Coulter AU400** ***(Beckman Coulter, FRG) automatic clinical chemistry analyzer. The obtained results indicate that the lower albumin, triglyceride, iron, and calcium values were detected in the cows bred on the farms that were ranked lower concerning the farm management and farm infrastructure levels. In the milk plasma, the concentration of albumins was higher (P<0.05) on the farms that were ranked lower concerning the farm management, infrastructure level, and total score scale. Iron concentration was higher (P<0.05) in the cows from the farms ranked lower concerning farm infrastructure and on the farms that were ranked higher concerning the animal level. The erythrocyte sedimentation rate was higher on the farms with worse marks on the farm infrastructure level and total AW score.***

Keywords: animal welfare, dairy cows, biochemical parameters, hematological parameters, body condition score

INTRODUCTION

Animal welfare (AW) is an ethical approach to the animals that integrates all factors contributing to the health and an animal's optimal mental and physical-life conditions. The exploitation of animals is reduced, and unnecessary infliction of pain to the animals is avoided, following the animal species and age standards, general and veterinary care, and disease prevention and treatment (Loi et al., 2021).

Farm animal welfare should be viewed as a global condition, where the effects of infectious and non-infectious stressors cannot be easily discriminated against and can overlap, challenging the host's immune system (Razzuoli et al., 2016). The innate immune system can rapidly respond to both the infectious and non-infectious stressors, such as metabolic stress conditions, psychological stress, high/low temperatures, oxidative stress, and hypoxia (Trevisi et al., 2016).

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Thus, a resource-based assessment cannot answer the questions concerning animal welfare. For all these reasons, the attempts have been made worldwide to develop the animal-based measures (ABMs) to estimate the actual animal welfare (Webster, 2009; Peli et al., 2016). A precise welfare assessment assumes a multidisciplinary approach, and modern protocol for the evaluation incorporates both the animal-based and non-animal-based measures on dairy cattle farms (Ventura et al., 2021). Animal health is the objective and the key welfare and health-status component (e.g., of the presence/absence of a disease, organ function, metabolic processes, and internal body condition) that is primarily monitored using the hematological and biochemical tests (de Almeida et al., 2019). The need to measure the different animal health- and welfare-related outputs, as well as to control the effectiveness and efficiency of these measurements, is expanded globally (Loi et al., 2021).

A dairy cow's body condition score (BCS) is an assessment of the proportion of body fat it possesses, and it is recognized by the animal scientists and producers as being an important factor in the dairy cattle management. There are different methods for monitoring the dairy cows' body condition (body fat)—for example, an ultrasound scanner, manual observation, and digital imagery, as exemplified by Bell et al. in 2018. Most dairy-cattle BCS systems use the 5-point scoring method, on a scale of extremely thin (1) to very fat (5). Some evidence exists that the bovine BCS is associated with the risks to the animal health and that a higher BCS protects the animal during the winter season (Matthews et al., 2012). Unsurprisingly, the BCS measures are featured in the dairy cattle welfare assessment protocols. However, a degree to which the BCS predicts the welfare state, particularly concerning the level of experienced "hunger", is not professionally researched. For the dairy cows, the BCS is increasingly viewed as a welfare state measure.

Due to the different welfare score classes (assessed from the aspect of farm management, infrastructure, individual animal level, and a total farm score), this study's objective was to determine the variability of biochemical and hematological parameters, as well as a body condition score.

MATERIAL AND METHODS

The study was conducted on 145 randomly chosen cows from six commercial dairy cows farms located in East Croatia. The investigation was proved by the Ethical Committee Faculty of Agrobiotechnical Science Osijek.

The sake of a welfare assessment, the CReNBA dairy cow checklist (Bertocchi & Fusi, 2014) was applied by a trained veterinarian. The checklist consisted of seventy items, covering the following issues: animal-based measures, structure and equipment, and farm management. The animal-based measures section (eighteen items) included questions regarding mortality, mastitis, lameness, animal cleanliness, and skin lesions. The structure and equipment section (twenty-nine items) included the questions regarding floor and bedding space, feeding space, bedding material, microclimate conditions, calving, sick pen availability, and milking space and equipment. The farm management section (twenty-two items) included the questions regarding the training and the number of personnel, bedding material management, feed, and water management, milking management, and animal grouping. The AW was measured by the CReNBA protocol, demarcating the three different levels, as follows: a farm with inadequate welfare or biosecurity conditions, in cases in which the final score amounts to the lowest 33% concerning the obtainable score; a farm with the good welfare or biosecurity conditions, in cases in which the final score is between 33% and 66% concerning the obtainable score; and a farm with an excellent level of welfare or biosecurity, in cases in which the final score is between 66% and the maximally obtainable score.

During the study, the blood and milk samples were taken from 145 Holstein cows with an average daily milk yield of 36.24±10.72 kg (Table 1). Cow 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 minutes at 4°C), and the plasma was separated and frozen at -80°C till the 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 samples for erythrocyte sedimentation rate (ESR) were processed by the Westergren method (Vennapusa et al., 2011). The milk samples were taken into the sterile tubes and centrifuged (12.000 g/30 minutes. at 4°C), and the milk plasma was separated and stored at -80°C till the analyses. The biochemical parameters in blood and the milk plasma were determined using the *Beckman Coulter AU400* (Beckman Coulter (BC), Germany) and BC reagents. The β-hydroxybutyrate (BHB) concentration was determined using the commercial kits (Randox Laboratories Ltd., Crumlin, UK) by the enzymatic colorimetric method. The globulins in milk (GLOB) were calculated from the total protein (TP) and albumin (ALB) values (TP-ALB=GLOB).

Body condition scoring was performed pursuant to the AHDB (2020) for the Holstein cows (scale 1-5), including the assessment of an angle between the hooks and pins —that is, whether the hooks are rounded or angular—the visibility of sacral and tailhead ligaments and the short ribs, and the presence of a palpable fat pad on the pins, hooks, and short ribs.

Table 1. Variability of daily milk production traits and body condition score of selected animals (n = 145)
Tablica 1. Varijabilnost dnevnih svojstava proizvodnje mlijeka te ocjene tjelesne kondicije odabranih životinja (n = 145)

Trait / Pokazatelj	Mean	SD	*CV*	Min.	Max.
Daily milk yield (kg) / *Dnevna količina mlijeka (kg)*	36.24	10.72	29.59	10.70	68.70
Fat content (%) / *Udio masti (%)*	4.14	1.19	28.78	2.03	8.64
Protein content (%) / *Udio proteina (%)*	3.34	0.36	10.87	2.66	4.66
Days in milk / *Dani laktacije*	194.44	166.87	60.11	11.00	537.00
Body condition score / *Indeks tjelesne mase*	3.48	0.43	12.51	2.00	4.25

The variability of biochemical and hematological parameters, as well as the body condition score, were tested using the least square means in the GLM procedure in *SAS* (SAS Institute Inc., 2019) due to different welfare score classes. The following statistical model was applied:

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

where

y_{ijklm} = estimated biochemical or hematological parameters and body condition score;

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);

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

M_k = fixed effect of farm k (k = 1., …., 6.);

D_l = fixed effect of welfare score classes l (l = 1, 2); and

e_{ijklm} = residual.

Due to the different welfare score classes, the Tukey-Kramer's studentized range test in the GLM procedure in *SAS* (SAS Institute Inc., 2019) was applied to test the significance ($p < 0.05$) of differences in biochemical and hematological parameters, as well as in a body condition score.

RESULTS AND DISCUSSION

The animal welfare (AW) evaluation was performed following the relevant protocols and presented following the four different aspects: farm management, farm infrastructure, animal level, and total welfare score. It confirmed that the investigated farms belonged to the second and third score classes. The results indicate that more parameters have demonstrated variability with regard to the farm management: the activity of aspartate aminotransferase (AST) and γ- glutamyl transferase (GGT), as well as the concentration of albumin, triglyceride, iron (Fe), and calcium (Ca), whereby the values were higher under the better farm management conditions (Table 2). That signifies that the management incorporating animal care and mild procedures, with an amicable behavior, results in the more favorable biochemical parameters, but the activity of liver enzymes, which are elevated on the higher-ranked farms, is questionable. The AST and the GGT are commonly used as the liver damage markers (Moore, 1997). Nevertheless, in our investigation, the estimated means for both parameters were slightly above the referent range (Cozzi et al., 2011) and in the referent range, as discussed by Loi et al. (2021). Puppel & Kuczyńska (2016) stated that the changes in the AST activity in blood can be a consequence of its increased activity in cells, but it may also be the result of a structural cellular damage. A higher AST and the GGT activities are most frequently determined if there is a suspicion of an acute and chronic liver disease (Stojević et al., 2005) but also in connection with the fatty liver syndrome and hepatobiliary system diseases (Tenant, 1997). Opposite to these statements, Tsukano and Suzuki (2020) determined that there were no significant differences in the serum AST or GGT related to the liver function. According to Tainturier et al. (1984), the activity of AST and GGT enzymes manifested occasional irregular, small alterations during pregnancy and early lactation. El-Ghoul et al. (2000) found that the GGT activity in late pregnancy is much lower than in the first week subsequent to calving, and six weeks after delivery the activity increased. It could be assumed that the detected variabilities are not of major importance while assessing the impact of management on the activity of these enzymes, or that further investigations are necessary. The albumin, triglyceride, Fe, and Ca concentrations could be accepted as quality biomarkers for a farm management estimation because these parameters are in a direct correlation with a better bovine health. Similar results were confirmed by Loi et al. (2021), who approved a weak but significant correlation between the albumin concentration and farm management. It could be explained by a better liver status (i.e., by the albumin synthesis and a better energy supply) and consequently by the better health conditions (Fe, Ca). The correlation of GUK, AST, and GGT concerning the farm management and animal level is weak and non-significant (whereby the data are not shown). The animals in Group 3 had a higher production, glucose concentration was lower, and the activity of liver enzymes was also increased, yet without physiological relevance.

Similar trends in the analyzed parameters were also observed in the welfare scoring with regard to the farm infrastructure. The significantly higher LS means of glucose ($P<0.05$), albumin, triglyceride, β-hydroxybutyrate (BHB), Fe, and Ca concentrations were detected in the plasma of cows with the best welfare score class related to the farm infrastructure, and the LS means for the AST activity was lower (Table 2).

Table 2. The means of biochemical parameters in the plasma regarding the welfare score classes (farm management, farm infrastructure, animal level, and total score)

Tablica 2. Procijenjene srednje vrijednosti biokemijskih pokazatelja u plazmi u ovisnosti o razredu ocjene dobrobiti (ocjena menadžmenta te infrastrukture na farmi, ocjena vezana uz životinju te ukupna ocjena)

	Welfare score / *Ocjena dobrobiti*							
	Farm management / *Menadžment farme*		**Farm infrastructure** / *Infrastruktura*		**Animal level** / *Pokazatelji na životinjama*		**Total score** / *Ukupna Ocjena dobrobiti*	
Trait / *Pokazatelj*	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**
AST (U/L)	74.40^{A}	127.13^{B}	124.78^{A}	103.81^{B}	83.61^{A}	131.42^{B}	96.72^{A}	133.99^{B}
GGT (U/L)	21.06^{A}	32.97^{B}	30.92^{A}	31.66^{B}	26.40^{A}	32.76^{B}	30.27^{A}	31.63^{A}
GLU (mmol/l)	3.15^{A}	3.14^{A}	3.06^{A}	3.37^{B}	3.38^{A}	3.06^{B}	3.34^{A}	3.01^{B}
Urea (mmol/L)	3.28^{A}	4.44^{B}	4.22^{A}	4.37^{A}	3.71^{A}	4.46^{B}	3.97^{A}	4.46^{B}
PRO (g/L)	84.32^{A}	85.24^{A}	84.40^{A}	86.82^{A}	85.00^{A}	85.12^{A}	85.49^{A}	84.82^{B}
ALB (g/L)	31.50^{A}	32.76^{B}	31.88^{A}	34.22^{B}	32.39^{A}	32.62^{A}	32.13^{A}	32.85^{A}
TGC (mmol/L)	0.09^{A}	0.12^{B}	0.11^{A}	0.13^{B}	0.10^{A}	0.12^{B}	0.10^{A}	0.12^{B}
BHB (mmol/L)	0.58^{A}	0.53^{A}	0.51^{A}	0.60^{B}	0.56^{A}	0.53^{A}	0.52^{A}	0.55^{A}
Fe (μmol/L)	21.17^{A}	23.62^{B}	23.09^{A}	23.56^{A}	22.10^{A}	23.63^{A}	22.52^{A}	23.72^{A}
Ca (mmol/L)	2.09^{A}	2.27^{B}	2.15^{A}	2.48^{B}	2.24^{A}	2.25^{B}	2.22^{A}	2.26^{A}

*AST – aspartate amino transferase (U/L), GGT – γ-glutamyl transferase (U/L), GLU – glucose (mmol/L), PRO – protein (g/L), ALB – albumin (g/L) TGC – triglyceride (mmol/L), BHB – β-hydroxybutyrate (mmol/L). The values within the same row regarding the same welfare score, marked by a different letter, are statistically significantly different ($P < 0.05$).

Regarding the level of animal welfare score, lower glucose, higher triglyceride, a higher concentration of Ca, as well as a higher AST and GGT activity, were determined in the plasma of cows evaluated by the better AW scores (Table 2). During the last decade, there is a growing interest in monitoring the biological animal markers for a fast and early diagnosis and animal health preservation. Therefore, numerous biomarkers are currently well-established (Zachut et al., 2018), and some of them are used routinely. Loi et al. (2021) have found a significantly strong-to-mild positive correlation with alfa-1 and beta globulin and the animal-based AW measurements but a mild negative correlation between the γ-globulin, basophils, MCHC, and the animal-based measurements. Having estimated the animal-based measures, the authors found that the most important symptoms to be detected are lameness, integument alterations, and body condition scoring, as well as a record of adult bovine and vituline mortality (Bertocchi et al., 2018). Regarding the total welfare score, a lower total protein content, glucose, and a higher triglyceride concentration were manifested by the cows bred on the farms achieving a better AW score.

The biochemical parameter values in milk concerning the welfare score classes (i.e., farm management, farm infrastructure, animal level, and total score, respectively) are presented in Table 3.

Table 3. The means of biochemical parameters in milk regarding the welfare score classes (farm management, farm infrastructure, animal level, and total score)

Tablica 3. Procijenjene srednje vrijednosti biokemijskih pokazatelja u mlijeku u ovisnosti o razredu ocjene dobrobiti (ocjena menadžmenta i infrastrukture na farmi, ocjena vezana uz životinje te ukupna ocjena)

Trait / *Pokazatelj*	**Welfare score** / *Ocjena dobrobiti*							
	Farm management / *Menadžment farme*		**Farm infrastructure** / *Infrastruktura*		**Animal level** / *Pokazatelji na životinjama*		**Total score** / *Ukupna ocjena dobrobiti*	
	2	**3**	**2**	**3**	**2**	**3**	**2**	**3**
AST (U/L)	11.75^{A}	14.02^{B}	14.61^{A}	11.68^{A}	10.53^{A}	14.68^{B}	13.34^{A}	14.03^{A}
GGT (U/L)	394.14^{A}	361.88^{A}	344.97^{A}	419.64^{A}	373.46^{A}	363.39^{A}	381.72^{A}	355.92^{A}
GLU (mmol/l)	0.53^{A}	0.48^{A}	0.52^{A}	0.39^{B}	0.47^{A}	0.49^{A}	0.50^{A}	0.48^{A}
Urea (mmol/L)	3.01^{A}	5.01^{B}	5.05^{A}	4.14^{B}	3.62^{A}	5.11^{B}	4.03^{A}	5.24^{B}
PRO (g/L)	33.47^{A}	33.47^{A}	35.29^{A}	28.88^{B}	32.80^{A}	33.67^{A}	34.15^{A}	33.06^{B}
ALB (g/L)	25.16^{A}	21.80^{B}	22.46^{A}	20.67^{B}	22.14^{A}	21.90^{A}	22.49^{A}	21.64^{B}
GLOB (g/L)	11.67^{A}	10.31^{B}	12.82^{A}	8.20^{B}	10.66^{A}	11.76^{B}	11.67^{A}	11.43^{A}
Fe (μmol/L)	15.65^{A}	19.97^{A}	21.72^{A}	13.78^{B}	15.36^{A}	20.66^{B}	20.96^{A}	18.57^{A}
Ca (mmol/L)	3.18^{A}	3.28^{A}	3.17^{A}	3.52^{A}	3.03^{A}	3.34^{A}	3.22^{A}	3.31^{A}

* AST – aspartate aminotransferase (U/L), GGT – γ-glutamyl transferase (U/L), GLU – glucose (mmol/l), urea (mmol/L), PRO – protein (g/L), ALB – albumin (g/L), GLOB – globulin (g/L). The values within the same row regarding the same welfare score, marked by a different letter, are statistically significantly different ($P < 0.05$).

The biochemical parameters in milk demonstrated significant variability due to the AW score, as follows: a total protein content was lower in the better AW scores regarding the farm infrastructure and total score; albumin was lower on the better farms concerning the management, infrastructure, and the total score; and globulin was lower on the better farms concerning the management and infrastructure, but it was higher on the animal level. The LS means for the lacteal Fe was higher in the cows with the excellent AW scores (Table 3).

However, little is known about the levels of biochemical indicators in cow's milk in relation to the AW. Many authors compared the individual welfare issues with the actual results in animals (Tremetsberger & Winckler, 2015). In this research, according to all AW levels including a total mark, the significantly higher urea levels in milk cows having the higher AW scores refer to one of the good predictive factors and biomarkers in welfare prediction. The data indicate a need for greater nutrition care to balance the protein and energy in a total mixed ratio. While total lacteal protein was lower on the farms with a score of 3 (i.e., with a better AW), the albumin level was lower on all AW levels on the better farms regarding the farm infrastructure and total score. Globulin concentration was lower with regard to the farm infrastructure and management but higher with regard to the animal level on the better-scored farms. Iron concentration was lower in the cows achieving poor AW scores with regard to the farm management and animal level but higher with regard to the farm infrastructure. In the rumen, the proteins are decomposed and ammonia is formed, while the ruminants' microbiological flora is used for the amino acid production. Concerning the microflora growth rate in the rumen, the ratio of protein degradation and easily digestible carbohydrates is such that the microflora growth rate does not depend on the degradation rate of the consumed proteins but on the amount of energy consumed. The foods rich in protein will namely provide a large amount of ammonia in the rumen, while the foods rich in carbohydrates will condition its rapid consumption. It can be concluded that there is an unbalanced protein and carbohydrate ratio, so the energy in food will affect an increased or decreased concentration of ammonia in the rumen. The excess ammonia will be converted to urea in the liver. Thus, the level of urea in the blood serum serves as a nutritional imbalance indicator (Whitaker et al., 2004), which is, according to our results, an available predictive marker. The values exceeding 3.6 mmol/L refer, namely, to the protein excess in the feed and the impending reproductive disorders, even though the confidence interval amounts to 6.7 mmol/L (Cozi et al., 2011). It is supported by the fact that the estimated means for albumin concentration in our research was lower regarding the farm infrastructure, management, and total welfare score on the farms with a higher welfare score, also referring to a nutrition imbalance.

The values of hematological parameters due to the welfare score classes (i.e., farm management, farm infrastructure, animal level, and total score) are presented in Table 4.

Table 4. The means of hematological parameters regarding the welfare score classes (farm management, farm infrastructure, animal level, and total score)

Tablica 4. Procijenjene srednje vrijednosti hematoloških pokazatelja u ovisnosti o razredu ocjene dobrobiti (ocjena menadžmenta i infrastrukture na farmi, ocjena vezana uz životinje te ukupna ocjena)

	Welfare score / *Ocjena dobrobiti*							
	Farm management / *Menadžment farme*		**Farm infrastructure** / *Infrastruktura*		**Animal level**/ *Pokazatelji na životinjama*		**Total score** / *Ukupna ocjena dobrobiti*	
Trait / *Pokazatelj*	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**
ESR_8 (mm/H)	5.90^{A}	9.65^{B}	8.23^{A}	14.55^{A}	8.88^{A}	8.90^{B}	7.61^{A}	10.13^{B}
ESR_24 (mm/H)	17.86^{A}	28.10^{B}	28.35^{A}	20.40^{B}	24.88^{A}	26.90^{B}	28.73^{A}	24.76^{B}
ESR_48 (mm/H)	31.86^{A}	39.40^{B}	40.98^{A}	29.52^{B}	39.03^{A}	37.83^{A}	41.29^{A}	35.96^{B}
WBC ($*10^9$/L)	6.89^{A}	7.47^{A}	7.43^{A}	7.35^{A}	7.21^{A}	7.44^{A}	7.26^{A}	7.46^{A}
RBC ($*10^{12}$/L)	6.34^{A}	6.42^{A}	6.46^{A}	6.27^{A}	6.35^{A}	6.43^{A}	6.33^{A}	6.47^{A}
HGB (g/L)	95.99^{A}	107.12^{B}	107.20^{A}	100.76^{B}	98.05^{A}	107.98^{B}	103.94^{A}	106.32^{A}
HTC (L/L)	0.31^{A}	0.29^{B}	0.30^{A}	0.27^{B}	0.299^{A}	0.291^{A}	0.29^{A}	0.29^{A}

ESR_8 – erythrocyte sedimentation rate (mm/H), ESR_24 – erythrocyte sedimentation rate (mm/H), ESR_48 – erythrocyte sedimentation rate (mm/H), WBC – white blood cells ($*10^9$/L), RBC – red blood cells ($*10^{12}$/L), HGB – hemoglobin (g/L), HTC – hematocrit (L/L). The values within the same row regarding the same welfare score, marked by a different letter, are statistically significantly different ($P < 0.05$).

Hematological tests are aimed to monitor the health status and detect possible diseases (Brucka Jastrzębska et al., 2007). In clinical diagnostics, information about changes in the white blood cell count is of great importance. An erythrocyte sedimentation rate (ESR) is a type of blood test that measures how quickly the erythrocytes (or the red blood cells) sediment at the bottom of a test tube that contains a blood sample. Because of a very slow sedimentation in cattle, we had been waiting for eight, twenty-four, and forty-eight hours subsequent to the sampling, respectively, whereafter the citrated blood-filled standard hematocrit tube was placed in a vertical position. It is a nonspecific animal health evaluation method (Milinković Tur & Aladrović, 2012) and is connected with inflammation, which may be a sign of a chronic disease, an immune disorder, or other medical conditions. Therefore, we tested those parameters in comparison to the AW ones. In this research, a better farm management and a better animal-based measurement grade were responsible for a faster sedimentation rate after eight, twenty-four, and forty-eight hours, respectively, but an opposite situation was observed regarding the farm infrastructure: a higher sedimentation rate was recorded in the cows achieving the lower AW scores. Regarding the total score, the sedimentation rate was lower on the better-scored farm. We can therefore conclude that the ESR could be a good predictive marker for the estimation of total AW score. Concerning the farm management, farm infrastructure and animal level, and total score, the hemoglobin LSM was higher in the cows achieving a better AW score.

Farm animal welfare science has flourished during the last four or five decades, but it started with the animal-based measures as a direct measurement of different environmentally and behaviorally related factors. Thus, it commenced with the Welfare Quality Project (Welfare Quality, 2009), in which the animal measures were most important. There is a complaint concerning the WQ protocol, however, claiming that it cannot be applied frequently because of its time-consuming nature (Heath et al., 2014), as its multidisciplinary approach to animal welfare estimation justifiably has more supporters (Bertocchi et al., 2018). Pursuant to the obtained welfare scores, the results of biochemical and hematological parameter variability indicated a great significance of non-animal-based measurements, such as the farm management and farm infrastructure, according to Bertocchi et al. (2018) and Loi et al. (2021).

Body condition score (BCS) values concerning the welfare score classes related to the animal and total score are presented in Table 5. The BCS parameter influence was of little significance regarding the welfare score. Concerning the infrastructure, a higher BCS was recorded in the cows with a lower AW score, and there was no evidence indicating that the BCS was an unimportant parameter for an individual AW class on the animal level. Dairy cattle's body condition scoring is an important managemental tool for maximizing the milk production and reproductive efficiency while reducing the incidence of metabolic and other peripartum diseases. An over-conditioning at the time of calving (BCS>4.0) frequently results in a reduced feed intake and an increased incidence of peripartum problems. An under-conditioning at calving (BCS<3.0), on the other hand, frequently results in a lower peak milk yield and less milk for the entire lactation. Also, the cows should not lose more than a 1.0 body score during early lactation. It has been proven, namely, that an excessive loss of body condition in early lactation may reduce reproductive efficiency. The target BCS for most high-producing Holstein cows is now in the range of 3.0 to 3.5, which has continued to decrease with a genetic selection of a high milk yield and the high yields of milk components (Garnsworthy, 2007). The animals falling into the optimal body condition ranges have been evidenced to have superior fertility in terms of conception rates and the days to the first estrus subsequent to the calving. Likewise, they were indicated to produce heavier calves when compared to the animals in the suboptimal body conditions. It has been proven, furthermore, that the maintenance of a herd in good body condition facilitates the producers to earn money due to the lower feeding costs and generates more income because of the larger calves and a better herd fertility. Poor environmental conditions, on the other hand, can affect several homeostatic functions and reduce the livestock's productive and reproductive performances. Stress factors and poor welfare can also compromise the host's immune system and lead to an increased susceptibility to diseases among the animals (Trevisi et al., 2016).

Table 5. The means of body condition score regarding the welfare score classes (farm management, farm infrastructure, animal level and total score)

Tablica 5. Procijenjene srednje vrijednosti ocjene tjelesne kondicije u ovisnosti o razredu ocjene dobrobiti (ocjena menadžmenta i infrastrukture na farmi, ocjena vezana uz životinje te ukupna ocjena)

Welfare score / *Ocjena dobrobiti*	**2**	**3**
Farm management / *Menadžment farme*	3.36^{A}	3.49^{A}
Farm infrastructure / *Infrastruktura*	3.52^{A}	3.36^{B}
Animal level / *Pokazatelji na* životinjama	3.39^{A}	3.50^{A}
Total score / *Ukupna ocjena dobrobiti*	3.44^{A}	3.49^{A}

CONCLUSION

The obtained results indicate that a few vital biochemical parameters (albumin, glucose, triglycerides, Ca, and Fe) and hematological parameters (ESR) could serve as a good predictor of farm management and infrastructure, as well as of a total AW score. In this statistical model, the BCS was not manifested as a crucial marker for the AW measurement, despite its quantification being a part of the AW protocol.

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BIOKEMIJSKI I HEMATOLOŠKI POKAZATELJI I INDEKS TJELESNE KONDICIJE KRAVA HOLSTEINSKE PASMINE S OBZIROM NA OCJENU DOBROBITI

SAŽETAK

Dobrobit životinja predstavlja procjenu stanja životinja kojima reagiraju na podražaje iz okoline. Precizna procjena dobrobiti podrazumijeva multidisciplinaran pristup, a suvremen evaluacijski protokol uključuje mjerenja na životinjama i mjerenja koja uključuju smještaj, hranidbu i upravljanje farmom. Svrha ovoga istraživanja bila je odrediti varijabilnost biokemijskih i hematoloških pokazatelja, kao i tjelesnu kondiciju, s obzirom na različite bodovne razrede dobrobiti. Istraživanje je provedeno na 145 krava holsteinske pasmine, nasumično odabranih na šest komercijalnih farma mliječnih krava. Kontrolni popis procjene dobrobiti mliječnih krava sastojao se od 70 pitanja prema protokolu CReNBA. Biokemijski pokazatelji u krvi i mliječnoj plazmi određeni su automatskim kemijskim analizatorom **Beckman Coulter AU400** ***(Beckman Coulter, SRNj). Dobiveni rezultati ukazuju da niže koncentracije albumina, triglicerida, željeza i kalcija u krvi imaju krave na lošije ocijenjenim farmama s obzirom na menadžment farme i infrastrukturu. S obzirom na pokazatelje infrastrukturnih karakteristika farme, menađmenta i ukupnu ocjenu farme, koncentracija albumina u mliječnom serumu bila je veća (P<0.05) u krava s lošijom ocjenom. Sedimentacija eritrocita bila je brža na lošije ocijenjenim farmama s obzirom na infrastrukturu farme i ukupnu ocjenu dobrobiti.***

Ključne riječi: dobrobit životinja, mliječna krava, biokemijski pokazatelji, hematološki pokazatelji, ocjena tjelesne kondicije

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