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Comparison of hematological and biochemical profile between Podolian grey steppe and Holstein cows

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ABSTRACT. This research aimed to investigate the influence of different age of Podolian grey steppe and Holstein Friesian cows, as well as the effects of different breeds on their hematological and biochemical blood profile.A total of 128 adult cows aged from 3 to 12 years were used in the study. Gained results show statistically significant differences ($P < 0.05$) regarding all hematological parameters. The concentration of NE was recorded in the optimal range for Podolian grey steppe cows, while for Holstein Friesian breed was elevated.Values for LY was in the optimal range, while the values for MO, EO, and BA were slightly higher for both breeds of cow in all groups, with recorded significant differences ($P < 0.05$).Count of RBC in the Podolian grey steppe breed ranged between 5.83 and 11.21×10^{12}/L, with significant influence ($P < 0.05$) of cows age in obtained values. In contrast,Holstein Friesian cows breed recorded RBC values lover then optimal, without any significant difference ($P > 0.05$) related to the age of cows. Total protein has ranged between 7.10 and 8.50 g/dl (Podolian grey steppe), and 8.13 to 8.48 g/dl (Holstein Friesian), without recorded statistically significant differences ($P > 0.05$) between the age, breed as well as the interaction of age and breed. Asimilar tendency can be seen regarding the otherbiochemical values ($P > 0.05$), respectively.Gain results have mainly shown a significant influence of breed on the parameters above, while the significant influence of age of cows was not present as well the interaction between age and breed.

Keywords: cows, hematological profile, blood, enzymes, WBC, RBC

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INTRODUCTION

To evaluate animal health and welfare, regular monitoring of the health status of dairy herds is essential(Roland et al., 2014).Cows' health can be assessed and depends on the blood profile of hematology and biochemistry(Obućinski et al., 2019). Hematology, in addition to clinical examination or other diagnostic procedures, is exceptionally Informatique as a diagnostic instrument in bovine medicine(Roland et al., 2014). Dairy cow's nutrition could be the primary technological factor that can lead to significant modifications in the metabolic profile of animals.The mineral, protein,and vitamin deficiency in cows nutrition is a source of profound metabolism alterations(Coroian et al., 2017). Hematological parameters indicate the adaptability of cows and other stressors to adverse environmental circumstances. On the other hand, besides hematological parameters, biochemical blood status in a range of normal physiological limits reflects a good health status and is highly correlated with milk production(Obućinski, Soleša, et al., 2019). Over the years biochemical and hematological blood profile of cowshasinvestigated the age-related changes and comparison with blood composition in adults(Mohri et al., 2007), location-related changes (Hagawane et al., 2009), effects of parity, stage of lactation, and season of production(Cozzi et al., 2011), mineral dietary supplementation (Sobhanirad and Naserian, 2012), influence dependent on the management system (Obućinski, Soleša, et al., 2019; Radkowska & Herbut, 2014), changes during drying-off and the beginning of lactation (Bertulat et al., 2015), the prevalence of uterine infection(Sarkar et al, 2016), improvement of the biochemical and metabolic biomarkers in response to the therapeutic management in ketotic dairy cows(Biswal et al., 2017)milk and blood of ketotic dairy cows in and around Bhubaneswar, Odisha, India, before and after treatment. Thirty of 100 ketotic cows identified from a population of 1014 cows were equally divided into three groups of 10 animals each while group IV selected from the population under investigation was treated as control. Following treatment in group III, the ALT, AST, ALP and LDH levels observed in ketotic animals at pre-treatment were decreased maximum at post-treatment. It can be concluded that the treatment package comprising of Dextrose (25%, lactation stage on milk composition (El-Tarabany et al., 2018), even the prevalence of ticks in cattle (Ullah et al., 2019), respectively.

Könyves et al. (2017)feed intake and feed efficiency of Holstein-Frisian cows in different seasons of the year. Five hundred and sixty three cows were monitored in spring, 557 cows in summer, 594 cows in autumn and 567 cows in winter, for a total period of two years. In contrast to the spring, autumn and winter periods, the summer period was characterized by heat stress conditions. Average T and THI exceeded the 25°C and 72 critical points, respectively, on all, 90% and 93% of test days for this period, indicating that the cows were exposed to heat stress during the summer trial. The heat stress reduced daily milk yield by 1.32 kg or 9.46%, by 0.92 kg or 9.62% and by 1.27 kg or 9.48% as the THI values went from 64 in the spring, from 66 in the autumn and from 42.34 in the winter periods to 79 in the summer period. Forage intake was decreased by 1.63 kg, by 1.42 kg and by 1.25 kg compared to those in spring, autumn and winter, respectively, and the efficiency of conversion of feed to milk was increased (from 1.6 to 1.59 kg milk/kg milkinvestigated the relationship of the temperature-humidity index with milk production and feed intake of Holstein-Frisian cows in different year seasons, while it has been confirmed that lactation phase the, as well as the duration of the production cycle,also influencethe biochemical profile of cows blood according to the research ofCoroian et al.(2017).Some investigations have shown that blood profile differs between different breeds of cows(Vordermeier et al., 2012; Widayati etal., 2018) indicating that breed as well the age of cows (Kumar et al., 2017) reflects on hematological and biochemical blood profile of cows.

Metabolic diseases are much more prevalent in cows with elevated milk yield than in indigenous breeds of the same species. In Podolian grey steppe cows, the diseases occur sporadically, while in the cows with high milk production, metabolic diseases are much more common(Di Lorenzo et al., 2018; Keros et al., 2013)BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824. Early detection of nutrient deficiency or metabolic disease can contribute to the successful diagnosis, faster recovery of animal and heart health approach to prevent deviations before they become significant. A significant number of factors such as dairy herd management, different cattle breed, various technological phases and productive life of cows, as many others, have a particular influence on cows blood parameters, each in their way(Coroian et al., 2017; Cozzi et al., 2011; Obućinski et al., 2019).

This study aimed to investigate differences in the hematological and biochemical profile between Podolian grey steppe and Holstein Friesian cows in different age groups.

MATERIALS AND METHODS

Animals and experimental design

All animals in this study were treated in complíancewith National legislation (Presidential Decree 56/2013 on harmonization of the Directive 2010/63/EU) on the protection of animals used for scientific purposes.

A total of 128 adult cows aged from 3 to 12 years were used in the study. Cows were divided into equal numbers of 64 Podolian grey steppe, and 64 Holstein Friesian cows breeds, respectively. Further, each breed was divided into three groups according to age. The first group included cows aged from 3 to 6 years, the second from 7 to 10 years, and the third group cow over ten years old.The cows both used breed were clinically healthy. All cows were in the early stage of lactationbetween 65 and 85 days.The cows were kept in stables with temporary access to pastures.

During the experiment, cows were fed with hay (94.7% dry matter, 9.7% crude protein and 1.4% crude fat, respectively) which was provided *ad libitum*, and with compound feedsupplementation (91.3% dry matter, 12.6% crude protein and 1.6% crude fat, respectively) of an average 6 kg/day/cow.Freshwater was available to cows all the time.

Sample collections

At the time of blood sample collection, all cows were clinically healthy.Ten cows from each breed group were randomly selected for blood samples collection (Total of 60 blood samples were collected; 10 blood samples from each group (10 samples × 3 groups of each breed × 2 breeds = 60 blood samples). Blood wasobtained from the jugular vein (*V. jugularis*) of each selected cowin the morning before feeding. Blood was collected in test tubes with and without EDTA as an anticoagulant and refrigerated at a temperature of 4 °C, and immediately delivered to the laboratory for further analyses. Haematological and biochemical blood parameters were analyzed with the devices ADVIA 120 (Bayer Diagnostics Siemens, Germany) and ANALYSER A15 (Biosystems S.A., Barcelona, Spain).

Hematological and biochemical blood analyses

All blood samples analyses were performed at accredited laboratory VetLab (Belgrade, Serbia).Analyzed hematological parameters include white blood cells (WBC), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV).All parameterswere determined on the hematological apparatus ADVIA 120 (Bayer Diagnostics Siemens, Germany).

Analyzed biochemical parameters included total protein (TP), albumin (AL), creatinine (Cr), urea, calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG). All mentioned parameters were determined on the biochemical auto ANALYSER A15 (Biosystems S.A., Barcelona, Spain).

Statistical analyses

For statistical analysis,statistical software Statistica 13 (TIBCO Software Inc., USA)was used. First, the data were checked for normal distribution and homogeneity of variances. Obtained values within each group used for comparisons were not normally distributed. Values transformations (x^2) were necessary to acquire normal distributionafter which one-way ANOVA analysis was performed. The mean values were compared by the Tukey HSD post hoc test. With the usage of factorial analysis of variance,the significance level of the factors has been estimated, as well as their interactions (age; breed; and age × breed) within the observed values.The results were presented as means ± SD, wherea significance level of $P<0.05$ was used.

RESULTS AND DISCUSSION

The novelty of this research is reflected in that way that in the field and available scientific literature exists luck in data on the specific parameters of the health status of this autochthonous cattle breed. Asimilar investigation has been conducted in other research(Bedenicki et al., 2014) on the Istrian cattle breed, which pointed constant genetic similarity to Podolian grey steppe cattle breed. Muchresearch has shown different values of hematological and biochemical factors that are understandable because of the many differences of genetic factors between different cattle breeds and many existing paragenetic factors(Keros et al., 2013) BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225, BM1824.Results in table 1 show hematological parameters related to white blood cells of both breeds Podolian grey steppe and Holstein Friesian for different age groups. Results of WBC for Podolian grey steppe ranged between 6.75 and 11.94×10^9/L without a statistically significant difference between the same breed and between different ages (P > 0.05). Statistically, a significant difference was observed within two investigated breed (P < 0.05) where WBC was significantly higher for Holstein Friesian breed compared to Podolian grey steppe, ranged from 11.68 to 14.90×10^9/L, but without present statically significant difference in age between same breed (P > 0.05). The overall effect of interaction between ages and breed shows a significant difference (P < 0.05) regarding the WBC parameters of cows.According to Merck's veterinary manual, values for Podolian grey steppe cows in our research wereoptimal, while WBC of Holstein Friesian breed was slightly elevated. Results of Coroian et al.(2017) study showed higher WBC values in the six lactations compared to those results obtained in the research of-Radkowska and Herbut(2014), who observed hematological changes which were related to the management system. Obtained results in our study and related studies are in agreement with results obtained in the research of Obućinski et al.(2019).

Table 1. Hematological white cell parameters (Mean ± SD) of two breed for different age groups

Breed	Age	White cell parameters, $\times10^9$/L					
		WBC	NE	LY	MO	EO	BA
Podolian grey steppe	3 - 6	$8.96^b\pm1.44$	$2.22^c\pm0.99$	$4.70^b\pm1.21$	$0.50^b\pm0.15$	$1.33^a\pm0.30$	$0.12^b\pm0.12$
	7 - 10	$6.75^b\pm0.21$	$0.81^d\pm1.17$	$2.60^c\pm0.75$	$0.29^c\pm0.11$	$0.96^b\pm0.05$	$0.04^c\pm0.01$
	> 10	$11.94^b\pm1.01$	$3.86^c\pm0.94$	$7.13^a\pm0.51$	$0.92^a\pm0.07$	$1.84^a\pm0.98$	$0.51^a\pm0.23$
Pooled SE		0.021	0.457	0.033	0.018	0.029	0.034
Holstein Friesian	3 - 6	$11.68^a\pm0.57$	$5.84^b\pm0.03$	$5.02^b\pm0.28$	$0.06^e\pm0.09$	$0.76^b\pm0.07$	$0.00^c\pm0.00$
	7 - 10	$13.80^a\pm0.31$	$5.30^b\pm0.27$	$7.30^a\pm0.03$	$0.14^d\pm0.05$	$0.99^b\pm0.07$	$0.00^c\pm0.00$
	> 10	$14.90^a\pm1.02$	$9.59^a\pm1.45$	$4.62^b\pm0.99$	$0.28^c\pm0.08$	$0.21^c\pm0.04$	$0.50^a\pm0.13$
Pooled SE		0.057	0.011	0.032	0.196	0.025	0.132
Statistical significance of effect	Age	NS	*	*	*	*	*
	Breed	*	*	*	*	*	*
	Age × Breed	*	*	*	*	*	*

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed (P < 0.05); * - in each column differ significantly (P < 0.05); NS – not significant;SD – standard deviation; SE – standard error

Results presented in Table 1 showed statistically significant differences (P < 0.05) regarding all other hematological parameters. High influence in our study was recorded regarding age, breed as the well significant influence of age and breed interaction (P < 0.05). The concentration of NE was recorded in the optimal range for Podolian grey steppe cows, while for Holstein Friesian breed was elevated.Differences tendency in NE concentration in our study is similarto thefindings of Aarif et al.(2011) with3 groups of Sahiwal cows in different physiological stages, while the difference in research ofKumar et al.(2017) was not present.According to Merck's veterinary manual values for LY werein the optimal range.

In contrast, the values for MO, EO, and BA were slightly elevated for both breeds of cow in all groups, with recorded significant differences (P < 0.05).Higher increase of MO number in the blood occurs in response to chronic infections, in autoimmune disorders, in blood disorders, and in certain cancers, while elevated levels of EO in the blood can be an indicator of an illness or infection, what was not the situation in our study, as well as the elevated levels of BA which cannot be related to basophilia.In the study of Coroian et al.(2017), lymphocytes number was under the influence of lactation within extensive limits, which was not following the results in our study, as well as the other studies (Obućinski et al., 2019).The results obtained byKumar et al.(2017) shown that the breed did not have a significant influence on cow's lymphocyte profile.

Table 2. Hematological red cell parameters (Mean ± SD) of two breed for different age groups

Breed	Age	Red cell parameters				
		RBC ($\times10^{12}$/L)	HGB (g/L)	HCT (%)	MCV (fL)	MCH (10^{-12})
Podolian grey steppe	3 - 6	$7.49^{b}\pm1.56$	$128.6^{a}\pm0.89$	$36.99^{a}\pm2.04$	$50.85^{a}\pm7.28$	$17.84^{a}\pm2.40$
	7 - 10	$5.83^{c}\pm0.25$	$112.0^{b}\pm0.93$	$32.50^{a}\pm1.92$	$33.60^{b}\pm3.48$	$12.30^{b}\pm1.27$
	> 10	$11.21^{a}\pm1.11$	$139.0^{a}\pm0.58$	$39.30^{a}\pm1.51$	$60.20^{a}\pm6.46$	$20.50^{a}\pm2.55$
Pooled SE		0.058	0.096	1.027	0.983	1.203
Holstein Friesian	3 - 6	$6.62^{c}\pm0.21$	$102.2^{b}\pm1.98$	$26.10^{b}\pm0.02$	$39.40^{b}\pm1.61$	$15.60^{b}\pm0.37$
	7 - 10	$6.69^{c}\pm0.69$	$105.8^{b}\pm0.96$	$27.60^{b}\pm0.97$	$39.90^{b}\pm1.12$	$15.80^{b}\pm0.88$
	> 10	$6.93^{c}\pm1.23$	$105.5^{b}\pm0.32$	$27.90^{b}\pm0.50$	$39.50^{b}\pm1.00$	$15.60^{b}\pm0.24$
Pooled SE		0.262	0.011	0.021	0.046	0.033
Statistical significance of effect	Age	*	NS	NS	NS	NS
	Breed	*	*	*	*	*
	Age × Breed	*	NS	NS	NS	NS

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed ($P < 0.05$); * - in each column differ significantly ($P < 0.05$); NS – not significant; SD – standard deviation; SE – standard error

Table 3. Hematological red cell parameters (Mean ± SD) of two breed for different age groups

Breed	Age	Red cell parameters			
		MCHC (g/L)	RDW (%)	PLT ($\times10^{9}$)	MVP (fL)
Podolian grey steppe	3 - 6	$351.1^{a}\pm0.90$	$20.09^{b}\pm0.93$	$231.50^{b}\pm65.67$	$10.51^{b}\pm1.30$
	7 - 10	$331.0^{a}\pm0.72$	$18.20^{b}\pm0.18$	$154.00^{b}\pm35.14$	$7.80^{b}\pm0.97$
	> 10	$370.0^{a}\pm0.66$	$21.80^{b}\pm0.56$	$386.00^{a}\pm69.22$	$12.00^{a}\pm1.28$
Pooled SE		0.299	0.029	3.470	0.150
Holstein Friesian	3 - 6	$394.0^{a}\pm0.31$	$24.11^{a}\pm0.27$	$401.00^{a}\pm82.11$	$13.48^{a}\pm0.15$
	7 - 10	$397.0^{a}\pm0.22$	$24.96^{a}\pm0.13$	$397.21^{a}\pm53.18$	$13.11^{a}\pm0.55$
	> 10	$394.0^{a}\pm0.54$	$23.18^{a}\pm0.85$	$342.39^{a}\pm51.10$	$13.79^{a}\pm0.63$
Pooled SE		0.874	0.013	2.997	0.183
Statistical significance of effect	Age	NS	NS	NS	NS
	Breed	NS	*	*	*
	Age × Breed	NS	NS	NS	NS

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed ($P < 0.05$); * - in each column differ significantly ($P < 0.05$); NS – not significant; SD – standard deviation; SE – standard error

Results in Tables 2 and 3 show the obtained values of red blood cell parameters between two different breed of cows used in our study. Statistically significant ($P < 0.05$) differences in RBC can be seen between these two breeds. Count of red blood cells in the Podolian grey steppe breed ranged between 5.83 and 11.21×10^{12}/L,whichisthe following values provided in Merck's veterinary manual, with significant influence ($P < 0.05$) of cows age in obtained values. On the other hand, Holstein Friesian cows breed recorded RBC values lover then optimal, without any significant difference ($P > 0.05$) related to the age of cows.Results obtained in our study regarding the RBC of Holstein Friesian cows agree with the results ofRadkowska and Herbut(2014). These differences in RBC cannot be attributed to acute hemorrhage or abnormal destruction of red blood cells, which wasn't the case in our study so that it could be related to cows breed. All other red blood cell parameters in our study are consist, and the overall statistical significance test has shown significant influence ($P < 0.05$) of breed on red blood cell values, while age and interaction of age and cows breed didn't show statistically significant differences ($P > 0.05$).

Some research has shown that red blood cell parameters and hemoglobin concentration do not show seasonal differences, while according toKoubková et al. (2002), seasonand oscillations in temperature could influence increase values of red blood cell count and hematocrit. Besides changes in blood parameters in dairy cows, similar changes have been recorded in blood parameters of meat cows(Miglior et al., 2017), respectively. The parameters of red blood cells are interrelated tightly, and rely, among other things, on hemoglobin levels, hematocrit count, and value.

Results of mean corpuscular hemoglobin concentration, red cell distribution width, platelets, and mean platelet volume (Table 3), gained in our study shown no statistically significant differences (P > 0.05) related to the age of cows, while significant differences (P < 0.05) was recorded between the breeds of cows, except for the MCHC values, which was not affected by the breed influence.Overall statistical significance of age × breed in our study has shown no significant influence (P > 0.05).Our results of MCHC values are in agreement with the results ofCoroian et al.(2017). The hematological profile of dairy cattle suffering from the reproductive problem was examined byRuginosu et al.(2011) compared with healthy cows with changes in hematocrit, hemoglobin and erythrocytes were reported. Some hematological parameters were influenced by the age of the cows(Botezatu et al., 2014).

Results of biochemical blood parameters of Podolian grey steppe and Holstein Friesian cows breeds gained in our study are shown in Tables 4, 5, and 6.

Total protein has ranged between 7.10 and 8.50 g/dl (Podolian grey steppe), and 8.13 to 8.48 g/dl (Holstein Friesian), without recorded statistically significant differences (P > 0.05) between the age, breed as well as the interaction of age and breed. The same tendency can be seen regarding the albumin values obtained in our study (P > 0.05). The indicators of protein metabolism are urea, total protein, and albumins, while creatinine is the primary parameter reflecting kidney function(Stojević et al., 2005). Having in mind that the optimal values for creatinine are between 53.0 to 159.0 (μmol/L), we can see that Podolian grey steppe cows breed over ten years old shows increased levels, which can be an indicator of renal problems in that age.

Statistically significant differences (P < 0.05) are present between thesetwo investigated breeds regarding the values of Cr in blood.

Table 4. Biochemical blood parameters (Mean ± SD) of two breed for different age groups

Breed	Age	TP (g/dl)	AL (g/dl)	Cr (μmol/L)	Urea (mmol/L)	Ca (mmol/L)	P (mmol/L)
Podolian grey steppe	3 - 6	$7.65^{a}\pm0.35$	$3.05^{a}\pm0.30$	$170.80^{b}\pm28.64$	$2.99^{b}\pm0.72$	$1.93^{b}\pm0.34$	$0.83^{b}\pm0.13$
	7 - 10	$7.10^{a}\pm0.18$	$2.20^{b}\pm0.94$	$145.10^{b}\pm29.14$	$1.70^{b}\pm0.66$	$1.30^{b}\pm0.47$	$0.70^{b}\pm0.22$
	> 10	$8.50^{a}\pm0.24$	$3.40^{a}\pm0.63$	$251.30^{a}\pm32.97$	$2.40^{b}\pm0.67$	$3.60^{a}\pm0.99$	$2.10^{a}\pm0.61$
Pooled SE		0.015	0.147	1.998	0.024	0.019	0.009
Holstein Friesian	3 - 6	$8.26^{a}\pm0.32$	$3.52^{a}\pm0.12$	$79.56^{c}\pm15.41$	$8.57^{a}\pm1.24$	$2.89^{a}\pm0.21$	$1.90^{a}\pm0.14$
	7 - 10	$8.13^{a}\pm0.41$	$3.14^{a}\pm0.19$	$83.98^{c}\pm19.20$	$7.99^{a}\pm0.86$	$3.00^{a}\pm0.55$	$1.96^{a}\pm0.05$
	> 10	$8.48^{a}\pm0.28$	$3.51^{a}\pm0.43$	$75.14^{c}\pm26.11$	$8.97^{a}\pm0.73$	$3.09^{a}\pm0.48$	$2.12^{a}\pm0.16$
Pooled SE		0.053	0.091	1.014	0.029	0.087	0.017
Statistical significance of effect	Age	NS	NS	NS	NS	NS	NS
	Breed	NS	NS	*	*	NS	NS
	Age × Breed	NS	NS	NS	NS	NS	NS

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed (P < 0.05); * - in each column differ significantly (P < 0.05); NS – not significant; SD – standard deviation; SE – standard error

Urea concentration in blood and milk is one of the indicators of nutritional status.The higher blood urea concentration in the Holstein Friesian cows breed compared to the Podolian grey steppe, maybe since Holstein Friesian breed had high rumen degradable protein content of the diet, which mainly are indicative of dietary nitrogen wastage, while thelow urea levels are characteristic of low dietary crude protein consumption.The urea content indicates that feed protein is efficiently used by rumen microflora(Calsamiglia et al., 2010)and while major improvements in our understanding of N requirements and metabolism have been achieved, the overall efficiency remains low. In general, maximal efficiency of N utilization will only occur at the expense of some losses in production performance. However, optimal production and N utilization may be achieved through the understanding of the key mechanisms involved in the control of N metabolism. Key factors in the rumen include the efficiency of N capture in the rumen (grams of bacterial N per grams of rumen available N.

Values of Ca and P obtained in our research was optimal with slightly lower and higher values, without any statistically significant differences (P > 0.05) between breeds, age, and their interaction. Our results show no significant differences in Ca levels between two breeds, while Mamun et al.(2013)founded a slight increase of calcium in females compared to the male, and alkaline phosphatase was higher in growing cattle. In research ofCoroian et al.(2017) Ca levels in the first three days of the postpartum period presented the lowest values, while the highest values were shown between 4 and 7 days, while phosphorus gave the most moderate average values on day one postpartum and the highest on day 7.

The alkaline phosphatase test is used to help detect liver disease or bone disorders. In conditions affecting the liver, damaged liver cells release increased amounts of ALP into the blood(Sato et al., 2005). The optimal range for ALP levels in cow's blood is 17.5 to 153.0 (U/L). The results of our study show no significant ($P > 0.05$) irregularities in ALP levels in Holstein Friesian cows breed (149.71 to 154.22 U/L). Significantly high ($P < 0.05$) levels of ALP in the blood of Podolian grey steppe cows breed over ten years old (314.40 U/L) indicates possible problems with liver disease or bone disorders.

Table 5. Biochemical blood parameters (Mean ± SD) of two breed for different age groups

Breed	Age	ALP (U/L)	ALT (U/L)	AST (U/L)
Podolian grey steppe	3 - 6	94.57^{c}±73.24	52.39^{a}±7.18	125.84^{a}±16.12
	7 - 10	14.50^{d}±2.84	38.40^{b}±4.12	93.70^{b}±17.31
	> 10	314.40^{a}±87.11	63.00^{a}±3.18	166.30^{a}±8.22
Pooled SE		2.144	0.991	1.034
Holstein Friesian	3 - 6	154.22^{b}±1.13	22.90^{b}±1.88	62.50^{b}±5.37
	7 - 10	149.71^{b}±2.36	23.30^{b}±2.67	63.80^{b}±8.22
	> 10	153.00^{b}±3.18	23.50^{b}±1.89	61.10^{b}±10.25
Pooled SE		1.065	0.084	1.001
Statistical significance of effect	Age	NS	NS	NS
	Breed	*	*	*
	Age × Breed	NS	NS	NS

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed ($P < 0.05$); * - in each column differ significantly ($P < 0.05$); NS – not significant; SD – standard deviation; SE – standard error

Table 6. Bloodlipid profile (Mean ± SD) of two breeds for different age groups

Breed	Age	HDL (g/L)	LDL (g/L)	TC (g/L)	TG (g/L)
Podolian grey steppe	3 - 6	0.84^{b}±0.02	0.53^{b}±0.27	2.10^{a}±0.08	0.08^{a}±0.04
	7 - 10	0.55^{b}±0.01	1.11^{a}±0.16	1.60^{b}±0.01	0.08^{a}±0.02
	> 10	1.17^{a}±0.03	0.19^{c}±0.02	1.30^{b}±0.06	0.09^{a}±0.01
Pooled SE		0.143	0.074	0.033	1.225
Holstein Friesian	3 - 6	0.96^{a}±0.01	0.28^{b}±0.02	1.84^{b}±0.46	0.09^{a}±0.01
	7 - 10	0.94^{a}±0.07	0.31^{b}±0.02	1.89^{b}±0.34	0.09^{a}±0.09
	> 10	0.91^{a}±0.09	0.33^{b}±0.01	1.82^{b}±0.51	0.09^{a}±0.01
Pooled SE		0.022	0.554	0.021	0.086
Statistical significance of effect	Age	NS	NS	NS	NS
	Breed	*	*	NS	NS
	Age × Breed	NS	NS	NS	NS

The value (Mean ± SD) with a different superscript in each column differ significantly among the same age group of different cows breed ($P < 0.05$); * - in each column differ significantly ($P < 0.05$); NS – not significant; SD – standard deviation; SE – standard error

Determining AST activities in dairy cows is most often connected with fatty liver syndromelow appetite and the appearance of ketosis in dairy cows during early lactation. Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is subclinical. Ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis, is insignificant(Stojević et al., 2005). Results gained in our research (Table 5) show that the age of cows didn't influence significantly ($P > 0.05$) on ALT and AST enzyme activity. Statistically significant influence ($P > 0.05$) was recorded regarding the breed influence. Holstein Friesian cows have significantly lower levels of both ALT and AST enzyme activity compared to Podolian grey steppe cows breed, while the interaction of age and breed effects didn't show significant influence ($P > 0.05$). Our results have been in agreement with the results of other researchers(Cozzi et al., 2011; González et al., 2011; Krsmanovic et al., 2016; Sun et al., 2015), respectively.

Cholesterol, triglycerides, and different density lipoproteins are important constituents of the lipid fraction of the body. Cholesterol is unsaturated alcohol of the steroid family of compounds, and it is essential for the normal function of all animal cells and is a fundamental element of their cell membranes(Puvača et al., 2016; Puvača et al., 2015). Results presented in Table 6, show significant influence ($P < 0.05$) of breed on HDL and LDL levels in the blood of cows. Our results are in agreement with the investigation ofTajik and Tahvili(2011). In dairy cows, an important lipid-related metabolic disorder is the fatty liver, which has also been associated with the changes in TC and TG levels. Fatty liver develops when TG synthesis exceeds the export of TG as very low-density lipoproteins(Kessler et al., 2014).Levels of TC in Podolian grey steppe cows have ranged between 1.30 and 2.10 g/L, while in Holstein Friesian breed levels of TC ranged between 1.82 and 1.89 g/L, respectively. The significant difference in this parameter was recorded between thesetwo breeds ($P < 0.05$) only in cows 3 to 6 years old, while the interaction between age and breed did not show any statistical significance ($P > 0.05$). A similar tendency without statistically significant differences ($P > 0.05$) was recorded for levels of TG in the blood of both cows breed.

CONCLUSIONS

The results of our study have shown the influence of different age and breed of cows on hematological and biochemical parameters. Gain results have mainly demonstrated a significant influence of breed on the parameters mentioned above, while the significant influence of cows age was not present as well the interaction between age and breed.

It has been shown that the values of hematological and biochemical parameters were generally situated within the reference intervals. Due to the shortage of studies on the hematology and clinical biochemistry of the Podolian grey steppe ordinarily, the obtained results represent a novelty and contribution to achieving a better understanding of the metabolic profile and hematological indicators for estimating the physiological status of both Podolian grey steppe and Holstein Friesian breed, and for future diagnostic purposes, but more investigation in this field is certainly more than necessary.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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