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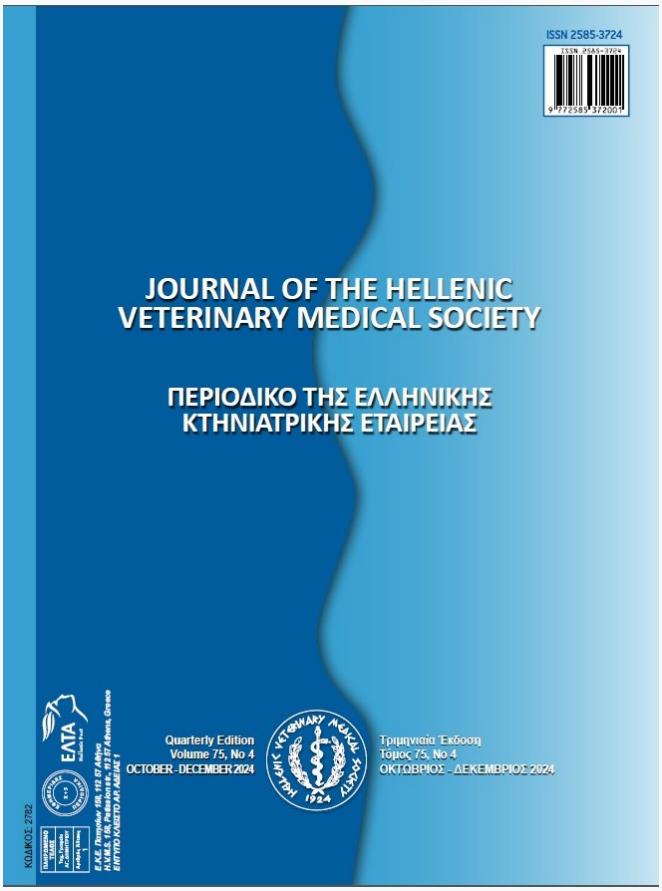

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Research article
Ερευνητικό άρθρο

Haematological and biochemical parameters of Podolian cattle breed cows

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ABSTRACT: The aim of this study is to set the reference blood work data for the Podolian cattle breed within conservation program. This particular breed (also known as Podolica, Podolsk, Hungarian grey or Grey steppe cattle) belongs to part of the group of primitive European wild cattle known as *Bos europeus primigenius* whom once roamed a vast region stretching from the Russian steppes to the northern Adriatic. In the mid-19th century, when Simmental breed made their way into Serbia, the Podolian cattle served as the foundation for breeding melioration, which led to a gradual but significant decline in population numbers. Consequently, the Podolsk cattle in the Republic of Serbia are now categorized as a "threatened and sustainable breed." This means that the breed is endangered, but there are ongoing conservation and preservation efforts in place. The use of adequate blood work analysis regarding physiological condition in order to monitor health, stress and welfare status is usual herd management practice. The published reference intervals of blood work results even for widespread cattle breeds in different climate conditions evoked the need for this study. To establish reference values for blood parameters, an observational cross-sectional study was conducted with a total of 107 cows, included in the study. Data were analysed on three age groups: 1) those aged 2 to 3.5 years (n=37), 2) 3.5 to 10 years (n=45), and 3) 10 to 15 years (n=25). This analysis included the examination of red and white blood cell parameters, haemoglobin, haematocrit, and various biochemical parameters, such as total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, urea, creatinine, calcium, phosphorus, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALT).

Attained results showed certain variation within some parameters, such as basophiles and neutrophils count both with coefficient of variation over 40%, but also revealed relatively stable parameters such as mean corpuscular haemoglobin concentration, red cell distribution width, haematocrit and haemoglobin. Biochemical blood parameters mostly varied were the triglycerides, HDL, LDL and total cholesterol, with coefficient of variation of over 20%. Relatively stable parameters were total protein, calcium, and albumin with coefficient of variation no larger than 10%. Concerning age group differences showed only in RDW and monocyte differential formula and only between youngest (group 1) and oldest (group 3) cattle while no other parameter got notable variation in all three groups. In comparison with parameters set for the highly productive dairy based Holstein Friesian breed, notable discrepancy was recorded at creatinine and albumin, which may lead to faulty interpretation in Podolians The constant scientific enthusiasm in Podolians conjoined with the fresh market demand for beef and dairy products of these cattle in later decades to justify preservation program and breed specific health management study.

Keywords: Podolica; haematology; biochemistry; indigenous breed; reference values.

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INTRODUCTION

The ancestral cattle breeds were used as a base of melioration programs worldwide. The Podolian cattle were already distributed across continental Europe and the Mediterranean basin during the Etruscan period in history. Thenceforth they expanded into the Balkans and Central Europe alongside human migration (Senczuk et al., 2021). This distribution was followed by several names for this breed such are: Podolica in Italy, Podolsk, Les gris des stteppes, Hungarian grey or Grey steppe cattle in Mediterranean and central European countries.

Even in contemporary animal science there is always active interest in Podolian cattle in the field of genetic resources especially in meat specifiers. Extensive rearing systems of farm animals are perceived by consumers as strongly linked to healthfulness, animal welfare, sustainability, and safety (Marino et al., 2011). Those qualities, backed by market demand were incorporated in the efforts for founding conservation efforts aligning with the convention on biodiversity and the FAO global strategy on the conservation and preservation of genetic resources of domestic animals.

The concept of reference values was firstly introduced at 1969 to replace concept of normal values. Today it's used to describe fluctuations observed in healthy populations or individuals, further implying interpretation of health or description of health status (Geffre et al., 2009). The future efforts in herd health management, animal welfare, and diagnosis are based on early identification and subsequent prevention of physiological imbalances. Serum parameters representing energy, protein, and minerals metabolic pathways reveal useful information for nutrition and animal health to optimize herds' potential *(Hussein et al., 2020). Blood analysis is not only indicator of reproduction and production performance but serves also as indicator of organism adaptability (Cerutti et al., 2018).*

Based on the available literature data, the biochemical and haematological parameter values of Podolian cattle in area of Republic of Serbia have not been established so far (Uzelac et al., 2019). Therefore, our research aims were to study the fundamental haematological and biochemical parameters of the Podolian cattle breed.

MATERIAL AND METHODS

Ethics approval and consent to participate

The study was performed in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09) and Ordinance on the conditions for registration for experimental animals and the keeping of such register, training programs on welfare on experimental animals, request forms for approval of conducting experiments on animals, standing, treatment and killing experimental animals and reproduction, circulation, or implementation experiments on animals (Official Gazette of the Republic of Serbia No 39/10). In order to prevent any additional stress for the animals, the blood samples were taken during the same puncture, as the yearly routine obligate sampling for health analysis for infection diseases required by before mentioned Law. All the procedures of the study were in compliance with the standard good clinical practices (EMA, 2021) and were performed by expert clinicians. Finally, the referring keepers were informed and in agreement with the purposes and methods assessed. They also gave oral consent for the study. The methods were reported following the STROBE guidelines (http://www.strobe-statement.org) for observational studies.

Animal Selection and Sampings

To perceive reference intervals of blood parameters, an observational cross-section study was designed, based on adequate sample size calculation with 5% of type 1 error and 95% confidence interval (Charan and Biswas, 2013). Standard deviation estimation is based on previous blood work investigations on various cattle breeds (Damptey et al., 2014; Hussein et al., 2020; Molefe and Mwanza, 2019; Vallejo-Timaran et al., 2020). The calculated sample size was minimum 96 samples. Animals were selected according to the following eligibility criteria: i) same housing system, ii) same management, iii) clinically healthy i.e.: health status was evaluated on the basis of rectal temperature, heart rate, respiratory rate, appetite, faecal consistency (Fadul et al., 2022; Herman et al., 2018). The eligible cows were a total population of 144 Podolian cattle registered in breeding farm in northern Serbia (Mladenovic et al., 2021). Further data study excluded top and low outliner samples values with more than three standard deviations from the mean (Cozzi et al., 2011), leaving the final 107 samples from total population of 144. To assess whether there is difference among parameters of animals of different age, the samples were divided into three groups: the first group (1) of animals aged 2 up to 3.5 years old (n = 37), the second group (2) aged 3.5 up to 10 years old (n = 45), while animals of the

third group (3) were 10 to 15 years old (n = 25). Blood samples of max 10 mL were taken from coccygeal vein in vacuum tubes (Vacutainer, Becton Dickinson, USA) coated with ethylene diaminetetraacetic acid for haematology and coated with sodium heparinize for plasma biochemical examination. Samples with visually detectable blood clots in the EDTA tubes were excluded. After sampling the test tubes were immediately transported to laboratory while kept in the refrigerator at a temperature of +4°C and analysed.

The haematological parameters were analysed using Advia 120 haematology System (Siemens AG, Erlangen, Germany) of the following data: i) white blood cells (WBC) (x10^6/μL), (neutrophils, lymphocytes, monocytes, eosinophils and basophils cells were counted with differential formulas calculated); ii) red cell line count (RBC; x 10^{12} /L); iii) haemoglobin (Hb; g/dL), iv) haematocrit; v) mean corpuscular volume (MCV); vi) mean corpuscular haemoglobin (MCH); vii) mean corpuscular haemoglobin concentration (MCHC; g/dL); viii) red cell distribution (RDW; %); ix) platelets count (PLT; x 10^3/μL); x) mean platelet volume (MPV).

Automatic analyser A15 (BioSystems, Barcelona, Spain) was used for the following biochemical parameters: i) total proteins (g/dL), ii) albumin (g/dL) iii) creatinine (μmol/L), iv) urea (mmol/L), v) total calcium (Ca, mmol/L), vi) phosphorus (P, mmol/L), vii) alkaline phosphatase (ALP, IU/L), viii) alanine aminotransferase (ALT, IU/L), ix) aspartate aminotransferase (AST, IU/L), x) high-density lipoprotein cholesterol (HDL, mg/dL), xi) low-density lipoprotein cholesterol (LDL, mg/dL), xii) triglycerides (mg/dL), xiii) total cholesterol (mg/dL).

Statistical Analysis

The Jasp 0.16.0.0 (JASP Team) software was used for statistical processing of the data. Data are presented descriptively as mean, minimum, maximum, standard deviation (SD). For evaluation of possible differences in age categories, a significance level of 0.05 ($p<0.05$) was set. After normality test was conducted, some parameters showed significant unconformity for parametric tests, so all comparisons were done by Kruskal-Wallis and Mann-Whitney U test.

RESULTS

The Podolian cattle blood parameters' analysis is presented as complete blood cell count (Table 1), biochemical parameters (Table 2) and p values of statistical differences between age groups (Table 3).

Table 1. Average values of haematological profiles of observed Podolian cows

Examined blood parameters		Middle value	Minimum	Maximum	Standard deviation (SD)	Reference values	reference
White bloodline	White blood cell (WBC) (x10^9 L^{-1})	8.96	6.75	11.94	1.44	4.9-12.0	Constable 2017
	Neutrophils	2.22	0.81	3.86	0.99	1.8-6.3	Constable 2017
	Lymphocytes	4.70	2.60	7.13	1.21	1.6-5.6	Constable 2017
	Monocytes	0.50	0.29	0.92	0.15	0-0.8	Constable 2017
	Eosinophils	1.33	0.96	1.84	0.30	0-0.9	Constable 2017
	Basophils	0.12	0.04	0.51	0.12		
Different forms of white blood cells	Neutrophils (%)	24.50	8.40	40.00	9.01	15.00-33.33	Kessell A (2015)
	Lymphocytes (%)	52.46	36.60	74.20	11.19	62.50	Kessell A (2015)
	Monocytes (%)	5.54	3.20	8.30	1.12	< 7.50-22.50	Kessell A (2015)
	Eosinophils (%)	15.13	10.20	22.00	3.78	< 6.00-20	Kessell A (2015)
	Basophils (%)	1.29	0.60	5.50	1.23	-	
Red bloodline	Erythrocytes (x10^{12} L^{-1})	7.49	5.83	11.21	1.56	5-10	Kessell A (2015)
	Haemoglobin *(g dL^{-1})*	12.86	11.20	13.90	0.89	8.5-12.2	Constable 2017
	Haematocrit	36.99	32.50	39.30	2.04	22-33	Constable 2017
	Mean corpuscular volume (MCV) (fL)	50.85	33.60	60.20	7.28	38-50	Constable 2017
	Mean corpuscular haemoglobin (MCH) (pg)	17.84	12.30	20.50	2.40	14-18	Constable 2017
	Mean corpuscular haemoglobin concentration (MCHC) *(g dL^{-1})*	35.11	33.10	37.00	0.90	36-39	Constable 2017
	Red cell distribution width (RDW) %	20.09	18.20	21.80	0.93	15.5-19.7	Constable 2017
Thrombocytes	Platelets (PLT) (x 10^3 μL^{-1})	231.5	154.00	386.00	65.67	200-650	Constable 2017
	Mean platelet volume (MPV) (fL)	10.51	7.80	12.00	1.30		

Table 2. Average values of biochemical profiles of tested Podolian cows

Blood parameters examined	Mean	Minimum	Maximum	Standard deviation (SD)	Reference values	reference
Total proteins (g/dL)	7.7	7.1	8.5	0.352	5.7-8.1	Constable 2017
Albumin (g/dL)	3.0	2.2	3.4	0.300	2.1-3.6	Constable 2017
Creatinine (μmol/L)	170.8	145.1	251.3	28.642	88-175	Constable 2017
Urea (mmol/L)	3.0	1.7	4.2	0.724	2.142 - 9.639 mmol/L	Constable 2017
Ca (mmol/L)	1.8	1.7	2.1	0.133	2.425 - 3.1 mmol total 1 - 1.3 mmol/L Ionised	Constable 2017
P (mmol/L)	2.9	2.3	3.6	0.341	1.81 - 2.10 mmol/L	Constable 2017
ALP (IU/L)	94.6	14.5	314.4	73.242	0-200	Constable 2017
ALT (IU/L)	52.4	38.4	63.0	7.180	11-40	Constable 2017
AST (IU/L)	125.8	93.7	166.3	16.118	78-132	Constable 2017
HDL cholesterol (mg/dL)	83.7	55 .0	117.4	21.451		
LDS cholesterol (mg/dL)	53.3	19.5	111.3	27.497		
Triglycerides (mg/dL)	21.1	0.6	30.1	8.471	0-14	Constable 2017
Cholesterol (mg/dL)	141.2	87.0	195.8	33.952	65-220	Constable 2017

Table 3. Statistical differences among age group (p values)

	Group 1 vs. Group 2	Group 1 vs. Group 3	Group 2 vs. Group 3
White bloodline			
WBA	0.741	0.831	0.946
NE	0.636	0.434	0.701
LY	0.641	0.349	0.732
MO	0.246	0.273	0.685
EO	0.747	0.483	0.956
BA	0.304	0.602	0.433
Differential leukocyte formula			
NE neutrophils	0.458	0.092	0.700
LY lymphocytes	0.660	0.124	0.756
MO monocytes	0.098	**0.044***	0.695
EO eosinophils	0.602	0.462	0.997
BA basophils	0.258	0.564	0.396
Red bloodline			
Erythrocytes	0.343	0.165	0.336
Haemoglobin	0.682	0.947	0.514
Haematocrit	0.704	0.424	0.442
Mean corpuscular volume (MCV)	0.243	0.063	0.069
Mean corpuscular haemoglobin (MCH)	0.157	0.081	0.162
Mean corpuscular haemoglobin concentration (MCHC)	0.611	0.275	0.249
Red cell distribution width (RDW)	0.997	**0.02***	0.074
Thrombocytes			
Thrombocyte Normal values (PLT)	0.177	0.992	0.154
Mean platelet volume (MPV)	0.318	0.716	0.309
Biochemical parameters			
Total Cholesterol	0.154	0.419	**0.032***
HDL Cholesterol	0.066	0.803	0.168
LDL Cholesterol	0.324	0.675	0.123
Urea	0.623	0.810	0.446
Creatinine	0.278	0.336	0.475
AST	0.170	0.863	0.492
ALT	0.898	0.363	0.357
Ca	0.178	0.230	0.426
P	0.116	0.508	0.221
Triglycerides	0.780	0.828	0.773
Total proteins	0.770	0.535	0.350
(ALP	0.559	0.309	0.656
p<0.05 is considered significant			

In the term of variations between haematological values the largest variation was with basophiles counts (median 0.12 with range from 0.04 to 0.51), followed by neutrophils count (median 2.22 with range from 0.81 to 3.86). Other white blood cells had lower variations of individual values within studied population. Within biochemical parameters the largest variation was exhibited by ALP (mean 94.6 with range from 14.5 to 314.4 UI/L), followed by LDL cholesterol (mean 53.3 with range 19.5 to 111.3 IU/L) and triglycerides (mean 21.1 with range 0.6 to 30.1 mg/dL).

Other parameters had less fluctuation of measured values. Concerning different age groups noticeable difference recorded between groups 1 and 3 in the monocytes differential formula ($p=0.04$, $p<0.05$) and RDW ($p=0.02$, $p<0.05$), while blood biochemistry data revealed difference only in total cholesterol levels between animals 3.5-10 year old (group 2) and over 10 years ones (group 3) ($p=0.032$, $p<0.05$). Other results had no significant difference between age groups.

DISCUSSION

The lack of data regarding Podolian cattle in the Republic of Serbia area, in both biochemical and haematological parameters' values (Uzelac et al., 2019) revealed the need for this study. General data for bovine breeds (Constable et al., 2016a; Kessell, 2015), or specific widely distributed breeds formed a basis for results interpretation due to availability of reports, but this practice is considered by some authors non diagnostic fundamental, since the literature data may be too general, derived from small population sample or reflecting a specific physiological phase (Cozzi et al., 2011). Comparing biochemical results to physiological ranges is complex because the values depend on many factors such are among others: sex, age, diet, pregnancy, animal health status, lactation and lactation phase (Giri et al., 2017; Kaneko, 1980; Sattar and Mirza, 2009; Vallejo-Timaran et al., 2020), therefore this study incorporated all suitable animals. Comparison with normal haematology of high productive dairy breeds is necessary to illustrate more clearly the characteristics of extensively reared Podolian breed.

In the red blood line, the average results of tested groups are elevated for haematocrit, and borderline higher for mean corpuscular volume and red cell distribution width. In some individual samples we came to phenomena of macrocytic anaemia and a phenomenon called anisocytosis where the average size of erythrocytes is bigger (data not shown). This macrocytic anaemia has also been described in puerperal haemoglobinemia of some high productive dairy cows (Rahmati et al., 2021). The haematology parameters in general don't deviate from the literature reference values range (Table 1).

Among blood biochemical values urea had average value of 3 mmol/L, which is low in comparison to reference values, but is known that indigenous cattle may have urea concentration below the reference range (Mapiye et al., 2009). In broader geographical view, results in terms of urea are similar to the results of grazing cattle Sanga from Ghana (Damptey et al., 2014) and Thai (Boonprong et al., 2007). Urea blood levels depict ruminal absorbed ammonia followed by neutralization in kidneys and liver to urea. Excessive protein contents in diet and suboptimal energy level of meal may be presented as blood urea levels of over 5 mmol/L, which is suggestive brake point in protein metabolism (Lebzien et al., 2006). Furthermore, blood urea level has an importance since it is correlated with subruminal acidosis in dairy cows (Coppa et al., 2023). Values of creatinine were slightly elevated in comparison to Holstein dairy cows (Mean ± 2SD), and despite that mean level is within physiological limits, it's still higher than average values in Holstein cows (Cozzi et al., 2011). Creatinine is a basic parameter in the assessment of renal function and its serum level is not significantly affected by nutrition or changes in liver function or the urea cycle (Kaneko, 1980; Sakkinen et al., 2001). Medical interpretation of creatinine levels is usually simultaneous with protein status, reflected by of total proteins and urea, associated with PCV and Hb, therefore in Podolian cattle focused attention on a single parameter, such as serum creatinine should be avoided.

Another parameter which might be specific is ALT. Function of liver is usually assessed based on AST, ALP and γ-GT (Stojevic et al., 2005), and despite ALT activity might be suggestive parameter it still remains with guarded use in cattle. The liver enzymes' activity in blood reflect leakage release from liver cells cytosol under functional disruption with physiological annual variation (Mazzullo et al., 2014). The measured values in ALT (52.4±7.18 IU/L) might be considered generally as high altogether with AST (125.8±16.12 IU/L), but the level of ALT (94.6±73.24 IU/L) altogether with total proteins (7.7±0.35g/dL) doesn't raise concern in liver health and function in Podolian

cattle. Low mean albumin level (3.0±0.30g/dL) in the blood and especially in animals with as low as 2.2 g/dL may raise concern in health status of skin, kidneys, liver or intestines if those values would be assessed from literature values (Constable et al., 2016b), but the co-evaluation of other blood parameters' values refute those problems.

Change of cattle blood values during ageing in a known phenomenon (Kessell, 2015; Shaffer et al., 1981). In order to see whether the Podolian cattle differ among age, the three age groups were set. Differences were found between groups 2.5-3 years old and 10-15 years old in the parameters of monocytes differential leukocyte formula ($p=0.044$) and red cell distribution width ($p=0.02$) and among groups of 3.5 to 10-year-old group and 10-15year old in total cholesterol ($p=0.032$). Although some significant differences were recorded, in general few differences among all tested parameters in all age groups in Podolian cattle are present.

CONCLUSION

The obtained results contribute to a better understanding of biochemical and haematological parameters of Podolian cattle breed, providing the basis for future clinical studies in order to deepen understand the physiology of this breed. All the obtained results can significantly contribute to perceive and meet the breed specific requirements for these cattle.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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