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HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE IN EAST BALKAN PIGS AT DIFFERENT AGE AND SEASONS

Nadezhda PALOVA[1*], Yordan MARCHEV[2], Radka NEDEVA[2], Jivko NAKEV[2], Dimitrinka KRUSHEVA[1], Todor SLAVOV[3], Ivelina NEDEVA[3], Teodora POPOVA[4]

[1]Scientific Centre of Agriculture- Sredets, Bulgaria
[2]Agricultura Institute – Shumen, Bulgaria
[3]Trakia University – Stara Zagora, Bulgaria
[4]Institute of Animal Science- Kostinbrod, Bulgaria
*Corresponding author: nadejda_palova@abv.bg

ABSTRACT

Twelvehematological and fifteen serum biochemical parameters were determined in indigenous East Balkan pigs at different ages and two seasons. The experiment was carried out in the Scientific Centre of Agriculture- Sredets aiming to characterize the health status of the animals when reared organically. Blood samples were taken from pre-weaned piglets (n=10), growers (n=10) and sows (n=10) in spring and summer. The results were analysed through two way ANOVA to assess the influence of the age, season and their interaction on the hematological and serum biochemical profile of the animals. Both age and season interacted significantly in regard to the red blood cells count (RBC) ($P<0.001$), hemoglobin (HGB) ($P<0.001$) and hematocrit (HCT) ($P<0.01$), as well as in the most biochemical parameters including creatinine($P<0.001$), total protein (TP) ($P<0.001$), albumin (ALB) ($P<0.001$), alanine aminotransferase (ALT) ($P<0.001$), aspartate aminotransferase (AST) ($P<0.01$), uric acid (UA) ($P<0.01$), Mg ($P<0.001$), triglycerides (TG) ($P<0.05$) and cholesterol ($P<0.01$). Regardless of the season, the white blood cells (WBC) including lymphocytes and granulocytes, as well as platelets (PLT)decreased with age, while mean corpuscular volume (MCV) increased.The content of glucose, urea, gamma-glutamil transferase (GGT), alkaline phosphatase (ALP), Ca and P which displayed maximal values in pre-weaned pigs ($P<0.05$) compared to the other age groups ($P<0.05$). The season affected the number of lymphocytes ($P<0.01$), their percent ($P<0.001$), mean corpuscular hemoglobin (MCH) andmean corpuscular hemoglobin concentration (MCHC) ($P<0.001$), as well as the concentration of GGT ($P<0.001$) which increased during summer,and also granulocytes ($P<0.01$), their percentage ($P<0.001$), PLT ($P<0.05$), glucose, urea, ALP, Ca ($P<0.001$) and P ($P<0.01$) whichwere higher in spring.

Keywords: *Hematological and serum biochemical parameters, Pigs, East Balkan breed, Age, Seasons.*

INTRODUCTION

The study of the hematological and biochemical profile is an important method for monitoring of the health status in farm animals. It allows, on the one hand, to reveal the physiological-biochemical determination of some or other traits, and on the other, it is an internal indicator for prediction of the animal performance (Chemshirova, 2000). Although blood is known to be relatively persistent, it still remains one of the labile systems in the body. Comparing animals to haematological and serum biochemical parameters requires an accurate idea of the regularities in their variations. The main factors of affecting the variability of hematological and biochemical profile include animal age (Czech et al., 2017), level and type of nutrition (Fisher et al., 2013), environmental conditions (Mayengbam and Tolenkhomba, 2015) and hence these parameters can be a good indicator for determining the impact of different farming systems, food practices, seasonal and climatic changes on the health status of the animals. Indigenous pigs occupy an unique position in our animal genetic resource. East Balkan Pig is an indigenous domesticated pig breed in Bulgaria, distributed on the western coast of the Black Sea. The animals are well adapted to extreme climatic conditions of the area, robust constitution, longevity and resistance towards disease. So far, the studies on this breed refer to its genetics (Hirata et al., 2015), assessment of its performance influenced by feeding factors (Yordanov, 2017), reproduction (Palova and Marchev, 2009) and meat quality (Popova et al., 2015). For the development of the full performance potential of the breed, it is of crucial importance to investigate the influence of various factors affecting its health status. Some of the important parameters for its assessment are the hematological and serum biochemical profile of the animals. Palova et al. (2008) studied the hematological traits of fatteners from this breed when reared organically.The interpretation of these parameters comparing them to reference values might be difficult since most of the studies provide reference values for the modern breeds that are reared in intensive systems. Still very few report data about indigenous breeds that are reared in extensive conditions, affected by various environmental factors, such as seasonal climatic conditions. Therefore, it is necessary to complete and update the data for the profile of blood as affected by factors such as age and seasonal as well as the natural resistance in pigs from the East Balkan breed and their relationship to performance traits. Hence, this study was designed to assess the changes of the haematological and biochemical profile of East Balkan pigs depending on the age and season.

MATERIAL AND METHODS

Experimental animals, rearing and blood sampling

The trial was carried out in the Scientific Center of Agriculture – Sredets with threeage groups of East Balkan pigs – pre-weaned (n=10), growers (n=10) and sows (n=10). The growers and sows were reared according to the traditional scheme of feeding for this breed. The animals were taken daily to controlled pasture on the natural grassland (legumes 8.6%, cereals 44.7%, others 46.7%) in

Strandja mountain, as the duration of grazing depended on the climate and the pasture condition. In the evening, the animals were fed in groups with ground organic feed containing barley. The chemical composition of the grassland and barley are presented in Table 1. The pigs has *ad libitum* access to water.

Table. 1 Chemical composition of the grassland and barley

Item	Grassland	Barley
Water, %	27.31	9.05
Dry matter,%	79.69	90.95
Organic matter, %	66.93	88.59
% or Dry matter:		
Crude protein	13.48	8.09
Crude fat	2.17	0.93
Crude fiber	26.96	2.74
Minerals	7.92	2.36
Ca	1.76	0.11
P	0.62	0.45

Blood sampling was done by puncturing anterior *venacava*, the blood was then poured into test tube containing anticoagulant. Blood samples were taken in spring and summer. Cold chain was maintained during the transit of the samples from the farm to the laboratory.

Hematological and serum biochemical paramaters

Haematological analysis included the following parameters: WBC, number of Lymphocites and Granulocites, % of lymphocytes and granulocytes, RBC, HGB, HCT, mean corpuscular volume (MCV), MCH, MCHC, PLT and mean platelet volume (MPV). The paremeters were determined using automatic hematology analyser EXIGO in the Department of internal non contagious diseases in the Veterinary faculty of the Trakia University, Stara Zagora. Serum biochemical analysis was performed by automatic biochemical analyser SYNCHRON CX9 PRO and included determination of glucose, urea, TP, ALB, AST, ALT, GGT, ALP, UA, Ca, P, Mg, TG and cholesterol.

Statistical evaluation

Data were statistically evaluated by two-way ANOVA as the age, season and their interaction were included in the model. The Fit model procedure of JMP v.7 software package was used to perform the statistical analysis (JMP Version 7, SAS Institute Inc. Cary, NC). The effects were considered to be significant at $P<0.05$; $P<0.01$ and $P<0.001$. Significant differences among the means were determined using Tukey post hoc test ($P<0.05$). All data were expressed as mean values with pooled standard errors.

RESULTS AND DISCUSSION

Hematological parameters

As seen from Table 2, the age of the pigs had significant effect on the WBC as well as the number of granulocytes ($P<0.001$) and they decreased, reaching their minimum in the sows, when compared to the other two groups ($P<0.05$). This was also observed in regard of the the total platelet count ($P<0.05$). The last two parameters were also affected by the season, showing substantial decrease in their values in summer. On the other hand, the lymphocytes in the pigs remained unaffected by their age groups, however, their count and percentage increased in summer.

The increased number of leukocytes usually is associated with the presence of disease in the organism, however, it can also be attributed to strenuous exercises and feeding. Czech et al. (2017) stated that the higher WBC might occur in sows in the final stage of gestation, as well as sucking piglets. Jezek et al. (2018) reported higher WBC in younger pigs when compared to sows which is in agreement with our results. Contrary to our results, Mayengbam et al. (2014) found increase in the WBC with age, as the maximum values were observed in the adult pigs when compared to pre-weaned and growers, however, these authors detected no effect of the age on the lymphocytes which is in line with our observations.

Platelets are fragments of the cells called megakayocytes in bone marrow. Wneh stimulated by thrombopoietin, the platelets break off the megakaryocytes and enter the blood stream. Generally the low number of platelets might be associated with disease or a genetic disorder, while a higher than normal count of platelets is known as thrombocytosis and can pose serious health risks (Campbell et al., 2008). The higher PLT in this study indicated that there might be less chance of disease in pre-weaned and grower pigs in comparison to adult sows, and also in spring when compared to summer. Chu and Song (2013) reported significant decrease in PLT in fatteners in summer when compared to spring, which is in line with our results.

Table 2. Effect of the age, season and their interaction on the hematological parameters in East Balkan pigs

Treatment	WBC 11.1-22. 0 $x10^9$l	Lymp $x10^9$l	Gran $x10^9$l	Lymp% %	Gran% %	RBC 5.0-9.50 $x10^{12}$l	HGB 99- 165 g/l	HCT, 32.0-50.0 %	MCV 51.0-68.0 fl	MHC 17.0-22.0 pg	MCHC 300-380 g/l	PLT 200- 700 $x10^9$l
Pre-weaned spring	25.35	8.47	13.42	33.03	53.23	8.76a	144.00a	46.51a	52.99	16.28	307.40	409.20
Growers spring	25.61	6.79	14.52	24.99	58.14	7.42b	127.10abc	41.13ab	55.55	17.09	308.50	484.20
Sows spring	17.97	5.64	10.23	31.22	56.97	5.97d	122.30bc	39.16abc	65.81	20.49	311.60	339.30
Pre-weaned summer	21.56	8.80	10.37	40.53	47.45	6.03cd	113.70c	33.11c	55.34	18.91	346.20	415.40
Growers summer	27.26	11.42	12.42	40.97	46.10	6.96bc	136.90ab	38.56bc	55.18	19.69	362.00	316.10
Sows summer	18.46	8.36	8.25	45.34	44.57	5.46d	127.80abc	35.11bc	64.34	23.60	373.80	222.00
Age (A)												
Pre-weaned	23.46a	8.64	11.89a	36.78	50.49	7.40a	128.90	39.81	54.17a	17.59a	326.80	412.30a
Growers	26.44a	9.11	13.47a	32.98	52.12	7.19a	132.00	39.84	55.37a	18.39a	335.25	400.15ab
Sows	18.22b	3.86	9.24b	38.28	50.77	5.72b	125.05	37.14	65.08b	22.04b	342.70	280.65b
Season (S)												
Spring	22.98	6.97	12.72	29.75	56.11	7.38	131.16	42.27	58.11	17.95	309.17	410.90
Summer	22.43	9.53	10.34	42.28	46.14	6.15	126.13	35.59	58.28	20.73	360.66	317.83
Sig.												
AxS	ns	ns	ns	ns	ns	***	***	**	ns	ns	ns	ns
A	***	ns	***	ns	ns	***	ns	ns	***	***	ns	*
S	ns	**	**	***	***	***	ns	***	ns	***	***	*
Pooled SEM	0.83	0.45	0.45	1.31	1.11	0.17	2.18	0.88	1.01	0.37	5.65	22.43

P<0.05; **P<0.01; ***P<0.001. Means connected with different letters are statistically different (P<0.05).

In regard to the RBC, both age and season as factors affecting this trait, interacted significantly ($P<0.001$). It was reflected by the different patterns that changes of the parameter followed with age, namely the gradual decrease in its values from the pre-weaned to growers and sows in spring, however in summer, the pre-weaned pigs showed lower RBC when compared to the growers ($P<0.05$). This was also observed in regard to the HGB and HCT. Seasonal variations were found only in RBC and HCT in pre-weaned pigs showing lower values in summer in comparison to spring season. Contrary to our results, Mayengbam et al. (2014) found increase in the RBC and HCT in the adult sows, when compared to pre-weaned and growers of and indigenous breed of pigs, however the same authors in another study with the same breed (Mayengbam et al., 2017) showed seasonal variations in these traits in growers and adult sows. MCV and MCH were significantly affected by the age of the animals ($P<0.001$), showing increase in the sows when compared to the pre-weaned and growers ($P<0.05$). Furthermore, MCH differed between seasons, showing higher content in summer, which coincided with the higher values of MCHC observed in this season. Mayengbam et al. (2017) reported effect of the age on the MCV and MHC showing decrease of these parameters between the pre-weaned and grower stage, and then increase in the adult sows. In our study we observed higher values of both MCV and MHC in the sows when compared to both pre-weaned and growers. Eze et al. (2010) did not report any difference in these parameters in piglets and adults. Furthermore, in the study of MCV decreased, while MHC and MCHC increased from winter to summer. This partly coincides with our results showing higher values of MHC and MHCH in summer, when compared to spring. The lower values of these parameters as well as the changes in the HB and HCT, especially in pre-weaned pigs might indicate inflammatory response (Odink et al., 1990).

Serum biochemical parameters

Important interactions of age and season were found in regard to the most serum biochemical parameters that we use to describe the health status of the East Balkan pigs in this study (Table 3). Both factors interacted in creatinine, total protein and albumin ($P<0.001$). Although generally, creatinine increased with the age and lowered in summer, these changes followed different trend. In spring the concentration remained lower in pre-weaned pigs compared to growers and sows ($P<0.05$), however in summer the difference between the pre-weaned and growers were less pronounced. Seasonal changes existed only in pre-weaned and growers, while in sows the values remained relatively constant. Total protein increased gradually between all the age groups, while significant increase in the albumin existed only in the adult sows. These changes were observed only in summer, however significant difference between seasons were found. The increase in the total protein with age was observed in other species such as ruminants (Zarghan, 1994; Ahmadi et al., 2014; Habibu et al., 2017). This might be attributed to the higher albumin levels observed in the adult animals.

Table 3. Effect of the age, season and their interaction on the serum biochemical parameters in East Balkan pigs

Treatment	Glucose mmol/l	Urea mmol/l	Creatinine µmol/	TP g/l	ALB g/l	AST U/l	ALT U/l	GGT U/l	ALP U/l	UA µmol/	TG mmol/l	Cholesterol mmol/l
Pre-weaned spring	8.65	7.87	97.10b	74.90b	44.90a	129.80a	80.10a	50.10	472.90	115.70a	1.27ab	4.02a
Growers spring	9.57	6.09	135.20a	78.21ab	41.64ab	103.50a	54.40b	33.50	317.20	76.80ab	0.93b	3.38b
Sows spring	5.65	5.27	130.30a	78.64ab	43.31ab	92.80ab	51.40b	40.00	82.70	33.60b	0.54c	2.30c
Pre-weaned summer	5.39	5.23	73.40b	63.98c	31.10c	35.70c	51.90b	85.70	357.90	52.30b	1.27ab	3.13b
Growers summer	7.42	4.60	93.40b	71.63bc	33.52c	39.60bc	45.30b	60.80	217.30	46.60b	1.38a	2.83bc
Sows summer	4.69	4.31	139.70a	83.28a	40.57b	86.20abc	67.20ab	66.80	62.30	51.40b	0.93b	2.44c
Age (A)												
Pre-weaned	7.02a	6.55a	85.25a	69.44a	38.00a	82.75	66.00a	67.90a	415.40a	84.00a	1.27a	3.57a
Growers	8.50a	5.35b	114.30b	74.92b	37.58a	71.55	49.85b	47.15b	267.25b	61.70ab	1.16a	3.10b
Sows	5.17b	4.79b	135.00c	80.96c	41.94b	89.50	59.30ab	53.40ab	72.50c	42.50b	0.73b	2.37c
Season (S)												
Spring	7.96	6.41	120.87	77.25	43.28	108.70	61.97	41.20	290.93	75.35	0.91	3.23
Summer	5.84	4.71	102.17	72.96	35.06	53.83	54.80	71.00	212.50	50.10	1.20	2.80
Sig.												
AxS	ns	ns	***	***	***	**	***	ns	ns	**	*	**
A	***	***	***	***	***	ns	*	*	*	**	***	***
S	***	***	***	***	***	***	ns	***	***	**	***	***
Pooled SEM	0.37	0.23	4.05	1.11	0.75	6.68	2.73	3.75	27.64	5.78	0.05	0.09

*P<0.05; **P<0.01; ***P<0.001. Means connected with different letters are statistically different (P<0.05).

Higher uptake and utilization of dietary protein for growth and development in young animals as compared with adults may be responsible for the low blood total protein. Significant effect of the season ($P<0.001$) and of age ($P<0.05$) was found in regard of AST and ALT respectively, though these factors significantly interacted. ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis.Surprisingly, in summer we observed
dramatic decline in the AST, which was well defined in the pre-weaned and growers. Increase of the ambient temperature is associated with higher levels of increases which was showed in the studies of Nazifi et al. (2003) in goats and Chmielowiec-Korzeniowska et al. (2012), which we failed to observe.
Furthermore, important interactions were observed in regard to the UA content ($P<0.01$), Mg ($P<0.001$), serum TG ($P<0.05$) and cholesterol ($P<0.01$). All these parameters showed considerable decrease with age, and reaching minimal values in sows, however depending on the season.
Also differences between seasons were also detected but not in all age groups and not following the same patterns, which confirmed the significant interaction between both studied factors. The changes in the Mg content that we observed in this study does not agree with the findings of Mayengbam et al. (2017), while on the other hand it corresponds to the changes in the contents of Ca. The contents of TG and cholesterol decreased with age which corresponded with the reported effect of age in other studies (Yeom et al., 2012). Also we observed dependence of these parameters with the season showing decrease in the summer for the cholesterol, which could be associated with the changes in the diet of the animals.
Regardless of each other age and season affected the concentration of glucose and urea ($P<0.001$), showing decrease with age and lower values in summer. Contrary to us, Chmielowiec-Korzeniowska et al. (2012) found significant increase in the glucose levels in fatteners at higher temperatures (when comparing winter and summer). Also Chu and Song (2013), reported higher urea in pigs when comparing summer to spring which contradicts our results. On the other hand, Hooda and Upadhyay, 2014) reported decreased glucose with increasing temperature in kids and Yeom et al. (2012) showed lower urea with age in pigs which is in line with our findings. The age related changes in these two parameters might be considered normal, since their values do not deviate from the reference intervals determined by Friendship et al. (1984).
The same age variations were observed in regard to the GGT and ALP ($P<0.05$) which lowered their content with increasing the age of the groups, however, they did not showed the same affect of the season. Generally, GGT increased in summer, while ALP showed higher concentrations in spring. Age affected the content of Ca ($P<0.05$) and P ($P<0.001$) as it decreased in the older animals (Table 4). Significant decrease was also found in the summer in regard of these parameters. Our observations coincided with Chmielowiec-Korzeniowska et al. (2012), that showed higher GGT of fatteners in summer. Furthermore, our results are in line with those reported by Mayengbam et al. (2014), reporting decrease in ALP in adult sows and also lower values in the summer. The decrease in the ALP

might be associated with lower demands for this enzyme for skeletal growth that is observed in older animals (Rosol and Capens, 1999).

Table 4. Effect of the age, season and their interaction on the mineral contents of serum in East Balkan pigs

Treatment	Ca mmol/ml	P mmol/l	Mg mmol/l
Pre-weaned spring	4.19	3.29	1.50a
Growers spring	3.66	2.75	1.61a
Sows spring	3.76	2.06	0.96b
Pre-weaned summer	1.98	2.51	0.86b
Growers summer	1.50	2.15	0.84b
Sows summer	1.60	2.03	0.87b
Age (A)			
Pre-weaned	3.09a	2.90a	1.18a
Growers	2.58b	2.05b	1.22a
Sows	2.68ab	2.45b	0.91b
Season (S)			
Spring	3.87	2.70	1.36
Summer	1.69	2.23	0.85
Sig.			
AxS	ns	ns	***
A	*	***	***
S	***	**	***
Pooled SEM	0.16	0.09	0.05

*P<0.05; **P<0.01; ***P<0.001. Means connected with different letters are statistically different (P<0.05).\

Furthermore, as stated by Antonov and Malchevski (1983), up to 6 months, the alkaline phosphatase in pigs is of bone origin, while in adult sows it it from liver. This corresponded with the lower levels of P and Ca in the serum with increasing of the age of the pigs. On the other hand, the APL decreased in summer, which can be associated with the higher temperatures.Hooda and Singh (2010) and Sejan et al. (2010) found significant reduction in ALP in respectively in buffalo heifers and goats, which in the older animals can be attributed to disfunction of liver at high ambient temperature.

CONCLUSIONS

In conclusion, the age and season, as well as their interaction affected differently the hematological and serum biochemical parameters in East Balkan pigs. Their changes described the adaptive capabilities of the different age groups during two different season, however, more experiments are needed to fully clarify the

dependencies of the blood and serum parameters of the various factors which will allow to use them to improve the performance traits in East Balkan breed.

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