

Age-associated and Breed-associated Variations in Haematological and Biochemical Variables in Mangalitsa, Mangalitsa×Durock and Large White Pig

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ABSTRACT

Background: Research of hematologic and biochemical parameters in pigs is of great importance considering the fact that pigs are used as a model in research of different health disorders in humans. There are many different breeds of pigs that have different health, productive and biologic characteristics that need to be studied. Hematologic and biochemical values can vary dependent on presence of inflammation and infection. The aim of this study was to determine the influence of breed, age and their interactions on hematologic and biochemical parameters on blood of mangalitsa, mangalitsa x durock and Large White pig.

Materials, Methods & Results: Experiment included 10 litters of mangalitsa, white variety, mated with mangalitsa boar, 10 mangalitsa litters, and white variety inseminated with durock boar and litters of great Large White inseminated with great Yorkshire boar. Six groups, each include 10 animals were formed and their blood was sampled (3 breeds and 2 age categories). Age groups were formed according to moment of blood sampling. First sample was taken in moment of 30 ± 5 kg of body weight. Second sample was taken when body weight was 100 kg for hematological analysis. Samples were taken with BD Vacutainer®. Complete classic blood analysis and leucocytes formulas were done by hematology analyzer ADVIA 120 Hematology Siemens, Germany. Biochemical analysis was done by biochemical analyzer A15 BioSystem with their standard colorimetric reagents. Concentration of total protein, albumin, urea, creatinin, cholesterol, total bilirubin, AST and ALT were determined. Globulin concentration was calculated. Results have showed that hematologic and biochemical parameters are influenced by breed, age and their interaction as: total leukocyte number (age, breed x age), neutrophils number (age, breed), number of monocytes and platelets (age, breed x age), eosinophils number and percentage (age), percentage of neutrophils, percentage of lymphocytes and cholesterol (breed). All of three factors (breed, age breed x age) have affected number of lymphocytes basophils number, % of monocytes, % of basophils, erythrocyte number, hemoglobin concentration, hematocrit, MCV, MCH, MCHC, RDW, protein, albumin, creatinin and ALT. Globulin value varied in function of interaction breed x age. RBC, Hgb, Hct, MCV, MCH, MCHC, total protein albumin, creatinin, and ALT variance can be explained by influence of breed, age and their interaction ($\eta^2 = 35-75\%$).

Discussion: Based on results of this study breed and age have significant influence on numerous blood parameters. Besides the differences in mean values of parameters it is proved presence of statistically significant difference in frequencies distributions in function of age and breed. Different age and breed categories have different frequencies distributions of many determined parameters. There is positive correlation between RBC, Hgb, Hct and albumin in all three breed ($R^2 = 67-84\%$). Negative correlation was found between WBC and RBC, Hgb, Hct that was statistically significant in mangalitsa breed ($R^2 = 58-69\%$) but not in other two breeds. In mangalitsa significant positive correlation was found between globulin and leukocyte number. Given values have showed that during interpretation of lab results breed, age and interactions of hematologic and biochemical parameters need to be considered. Mean values and frequencies distribution differences lead to redefinition of referent range in function of breed and age that requires further research.

Keywords: pig, hematologic parameters, Mangalitsa, Large White.

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INTRODUCTION

Mangalitsa is typically fat breed of pig. One half has 65-70% of fat and 30-35% of meat [11]. Mangalitsa is late pig because they become sexually mature at age 3 to 6 months but they are allowed to mate at the age of 13-15 months. There are many different breeds of pigs that have different health, productive and biologic characteristics that need to be studied. For example effectiveness of iron preparations in anemia prevention and treatment can be studied by determination of hematologic parameters [28]. Hematologic and biochemical values can vary dependent on presence of inflammation and infection [5,20,24,26,27,30]. Change of blood parameters is consequence of negative factors influence like health stress and transport [3,18]. Biochemical parameters in blood indicate metabolic status of pigs and they change under the influence of nutrition and breed [1]. It is very interesting that presence of metabolic adaptations in pigs fed with food that induce fat accumulation in pigs that show great fat growth [2,6,23] because they are used as a model for research in humane obesity. Newer research [16] has showed statistical importance of farm, breed and age influence on hematologic parameters in pigs.

The aim of this study is to determine influence of breed (mangalitsa-greasy breed, mangalitsa x durock and Large White as a meat breed) and aged (growth and fattening) on hematologic and biochemical parameters values, just like shape of frequencies distribution of parameters and correlations of dynamic changes of parameters values during research.

MATERIALS AND METHODS

Farm, nutrition and care

Research was conducted on a commercial pig farm in Serbia which produces high-selection noble breed of pigs and their hybrids, just as production of native breed of pig- mangalitsa. Farm has 400 fertile sows of landras breed and great Large White, 10 boars of landras, great Large White and durock breed, 140 sows and 20 boars mangalitsa breed. Experiment included 10 litters of mangalitsa, white variety, mated with mangalitsa boar, 10 mangalitsa litters, and white variety inseminated with durock boar and litters of great Large White inseminated with great Large White boar.

Formation of experimental groups was conducted by placing the animals into group boxes, marking them with ear marks, castration of male piglets (5 ± 2 days after dusting) and commercial fattening of pigs. When pigs reached 150 kg they were slaughtered.

Groups

Six groups, each include 10 animals were formed and their blood was sampled (3 breeds and 2 age categories). Age groups were formed according to moment of blood sampling. First sample was taken in moment of 30 ± 5 kg of body weight. Second sample was taken when body weight was 100 kg.

Sampling and laboratory analysis

Two samples from each animal were taken. One whole blood sample was taken in yellow test tube that contains separation gel which during centrifugation separates blood cells and serum. This sample was used for biochemical analysis. Second blood sample was taken in purple tube that contains EDTA and it was used for hematologic analyze. Samples were taken with BD Vacutainer[®]1.

Complete classic blood analysis and leucocytes formulas were done by hematology analyzer ADVIA 120 Hematology Siemens².

Biochemical analysis was done by biochemical analyzer A15 BioSystem³ with their standard colorimetric reagents. Concentration of total protein, albumin, urea, creatinine, cholesterol, total bilirubin, AST and ALT were determined. Globulin concentration was calculated.

Statistics analysis

Influence of breed, age and breed x age was determined by multivariate general linear model. As independent factors were used breed and age. Dependent factors were hematologic and biochemical parameters. Post-hoc LSD test was conducted in order to determine differences between different breeds and different age categories in the same breed. Effect of age, breed and their interactions and influence on variance of hematologic and biochemical parameters can be explained by noted factors and it is determined by partial eta-square value (η^2). Factor effect is significant if $\eta^2 \geq 0.3$ (30%). Influence of age and breed on shape of frequencies distribution of hematologic and biochemical parameters was determined by Colmogorov-Smirnow test of similarities of frequen-

cies distribution. For chosen parameter visualization of cumulative frequencies distribution with vertical lines that connect two distributions and represent K-S test value was done. Mutual relation of good health parameters (WBC, RBC, Hgb, Hct, Albumin, Globulin) were determined in all three breeds of pigs by determination of parameters of Pearsons linear correlation and regression with determination of coefficient of determination (R^2).

RESULTS

GLM analysis

Results of research have showed that on hematologic and biochemical parameters are influenced by breed, age and their interaction, just like: total leucocytes number (age, breed x age); neutrophil number

(breed, age); monocyte and platelets number (age, age x breed); eosinophil number and % (age), neutrophil %, lymphocyte and cholesterol % (breed); all three factors (breed, age, age x breed) affected lymphocyte and basophil number, monocytes %, basophil %, erythrocyte number, hemoglobin concentration, hematocrit, MCV, MCH, MCHC, RDW, protein, albumin, creatinin and ALT. none of factors had influence on MVP, bilirubin and AST (non-showed results). Globulin value varied in function of interaction breed x age. White cells count, platelets, cholesterol, bilirubin and AST variation can be explained by influence of breed age and their interaction. Their interaction was $\eta^2 < 0.3$. RBC, Hgb, Hct, MCV, MCH, MCHC, total protein, albumin, creatinine and ALT variation can be explained by influence of age, breed and their interaction ($\eta^2 = 0.35-0.76$; 35-75%). Results are shown in Table 1 and Figure 1.

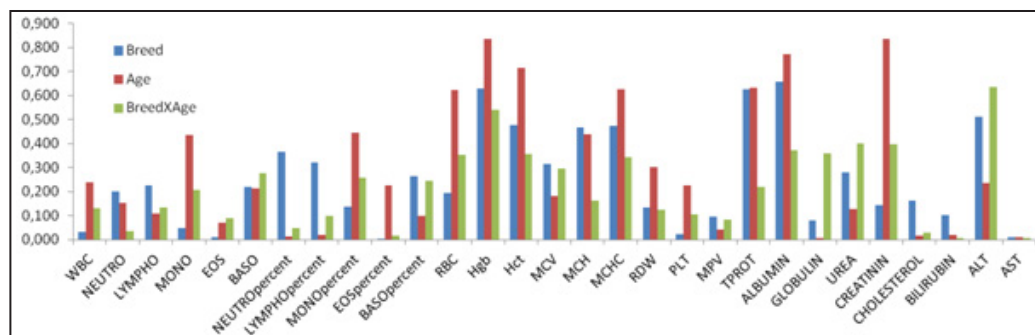


Figure 1. Age, breed and their interaction effects on values of hematology and biochemical parameters in swine blood.

Post-hock analysis

Results of post-hock analysis were determined by comparison of original values in Table 1 and significance of their differences in Table 2.

A) Breed influence in younger categories (30 kg) - Statistically significant difference ($P < 0.05$) was showed in hemoglobin concentration between groups WM (96.6 g/L) and DWM (108.4 g/L) in favor of DWM. That difference is highly significant ($P < 0.001$) between DWM and LW groups in favor of DWM. Hemoglobin 93.20 g/L concentration and hematocrit value in WM and LW groups were on lower referent border. Hematocrit value is statistically much higher in DWM (34.41%) compared to LW (29.53%). In other groups there was no significant difference. MCV and MCH values are statistically significant and higher ($P < 0.01$; $P < 0.001$) in WM (50.73 fL; 16.30 pg) and DWM (52.86 fL; 16.6 pg) compared to LW (45.67 fL; 14.44 pg). Between DWM and WM no

statistically significant differences were noted. MCHC was significantly higher ($P < 0.05$) in WM (321.7 g/L) compared to DWM (314.6 g/L) and LW (315.9 g/L). No significant statistical difference was noted between DWM and LW. RDW value was significantly higher ($P < 0.05$) in WM (21.87%) compared to DWM (19.08%). No significant differences were noted between other groups. Total leucocytes count showed no statistical significance in any of the group but there are differences between some leucocytes species. In WM group was noted statistically higher ($P < 0.05$) number of neutrophil granulocytes ($12.99 \times 10^9 / L$) compared to DWM ($8.49 \times 10^9 / L$). Other groups have not showed statistical differences. Statistically significant difference ($P < 0.01$; $P < 0.001$) in basophil number was noted between WM ($0.33 \times 10^9 / L$) and DWM ($0.43 \times 10^9 / L$) compared to LW ($0.18 \times 10^9 / L$). That difference is the same in given groups for monocytes number ($P < 0.05$). The greatest percentage of neutrophil granulocytes was determined in WM (45.63%)

Table 1. Hematology and metabolic parameters in White Mangalica (WM), Duroc × White Mangalica (DWM), and Large White (LW) in younger (30 kg) and older categories (100 kg) pigs.

Parameter	Body weight 30 kg			Body weight 100 kg			Influence of breed	Influence of age	Breed×Age
	WM	DWM	LW	WM	DWM	LW			
WBC (10 ⁹ /L)	28.63 ± 8.40	25.18 ± 4.86	26.76 ± 6.53	17.46 ± 3.25	22.65 ± 2.50	23.85 ± 3.35	NS	<i>P</i> < 0.001	<i>P</i> < 0.05
NEU (10 ⁹ /L)	12.99 ± 5.28	8.49 ± 1.28	11.74 ± 4.88	8.42 ± 3.28	6.03 ± 1.31	10.22 ± 3.12	<i>P</i> < 0.01	<i>P</i> < 0.01	NS
LYMPH (10 ⁹ /L)	12.65 ± 3.53	13.69 ± 4.71	12.95 ± 1.61	7.76 ± 1.60	14.31 ± 2.42	11.21 ± 3.07	<i>P</i> < 0.01	<i>P</i> < 0.05	<i>P</i> < 0.05
MONO (10 ⁹ /L)	1.51 ± 0.69	1.60 ± 0.55	0.94 ± 0.50	0.39 ± 0.15	0.60 ± 0.18	0.80 ± 0.38	NS	<i>P</i> < 0.001	<i>P</i> < 0.01
EOS (10 ⁹ /L)	1.05 ± 0.45	0.79 ± 0.17	0.83 ± 0.38	0.88 ± 0.30	1.29 ± 0.34	1.34 ± 1.06	NS	<i>P</i> < 0.05	NS
BASO (10 ⁹ /L)	0.33 ± 0.12	0.43 ± 0.19	0.18 ± 0.04	0.14 ± 0.05	0.24 ± 0.07	0.25 ± 0.07	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>P</i> < 0.001
NEU (%)	45.63 ± 8.75	34.63 ± 7.09	42.57 ± 6.91	46.54 ± 11.8	27.16 ± 6.39	42.9 ± 11.6	<i>P</i> < 0.001	NS	NS
LYMPH (%)	44.96 ± 8.25	53.38 ± 7.53	50.02 ± 7.18	44.93 ± 10.6	63.10 ± 6.13	47.0 ± 10.9	<i>P</i> < 0.001	NS	NS
MONO (%)	4.98 ± 1.16	6.26 ± 1.51	3.49 ± 1.40	2.31 ± 1.00	2.73 ± 0.92	3.28 ± 1.43	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.001
EOS (%)	3.75 ± 1.35	3.26 ± 1.00	3.01 ± 0.99	5.09 ± 2.15	5.55 ± 1.01	5.35 ± 3.61	NS	<i>P</i> < 0.001	NS
BASO (%)	1.18 ± 0.29	1.72 ± 0.81	0.66 ± 0.13	0.80 ± 0.21	1.02 ± 0.32	0.99 ± 0.21	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.01
RBC (10 ¹² /L)	5.95 ± 0.83	6.55 ± 0.74	6.46 ± 0.46	8.43 ± 0.65	8.09 ± 0.30	6.87 ± 0.50	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>P</i> < 0.001
HGB(g/L)	96.6 ± 11.17	108.4 ± 8.82	93.20 ± 7.13	157.6 ± 8.8	138.2 ± 2.82	112.5 ± 10.3	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
HTC (%)	31.04 ± 4.72	34.41 ± 2.98	29.53 ± 2.24	46.09 ± 2.82	40.83 ± 0.60	35.19 ± 3.18	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
MCV (fL)	50.73 ± 4.12	52.86 ± 2.08	45.67 ± 3.44	54.84 ± 2.13	50.57 ± 1.53	51.38 ± 2.71	<i>P</i> < 0.001	<i>P</i> < 0.01	<i>P</i> < 0.001
MCH (pg)	16.30 ± 1.33	16.62 ± 0.67	14.44 ± 1.18	18.77 ± 0.87	17.14 ± 0.70	16.43 ± 1.00	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.01
MCHC (g/L)	321.7 ± 7.3	314.6 ± 6.8	315.9 ± 3.2	342.0 ± 4.52	338.8 ± 4.98	319.5 ± 9.98	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
RDW (%)	21.87 ± 2.76	19.08 ± 1.70	20.02 ± 1.83	18.43 ± 0.55	18.43 ± 0.47	18.10 ± 1.06	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.05
PLT (10 ⁹ /L)	544 ± 255	446 ± 156	374 ± 151	268 ± 75	305 ± 67	334 ± 97	NS	<i>P</i> < 0.001	<i>P</i> < 0.05
T.protein (g/L)	59.64 ± 4.12	60.33 ± 6.77	43.28 ± 4.21	73.25 ± 4.23	66.09 ± 5.83	61.10 ± 3.95	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.01
Albumin (g/L)	24.24 ± 3.36	25.60 ± 3.62	19.25 ± 2.96	43.10 ± 3.63	35.07 ± 1.93	26.72 ± 4.39	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
Globulin (g/L)	35.10 ± 5.60	34.43 ± 8.15	23.73 ± 4.11	29.85 ± 3.62	30.72 ± 5.02	35.08 ± 3.76	NS	NS	<i>P</i> < 0.001
Urea (mmol/L)	4.71 ± 1.26	3.84 ± 0.54	2.07 ± 0.79	3.46 ± 0.78	4.95 ± 1.08	4.19 ± 0.84	<i>P</i> < 0.001	<i>P</i> < 0.01	<i>P</i> < 0.001
Creatinin (μmol/L)	57 ± 6.9	84.50 ± 13	75.31 ± 9.89	121.49 ± 10	112.93 ± 9.8	111.71 ± 9.24	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.001
Cholesterol (mmol/L)	2.49 ± 0.26	2.49 ± 0.17	2.69 ± 0.30	2.61 ± 0.35	2.42 ± 0.31	2.89 ± 0.52	<i>P</i> < 0.01	NS	NS
T.Bilirubin (μmol/L)	7.56 ± 5.41	6.51 ± 4.60	4.96 ± 1.10	9.67 ± 7.06	7.33 ± 3.47	5.44 ± 1.01	NS	NS	NS
ALT (IU/L)	52.99 ± 10.6	45 ± 10.3	146 ± 45.5	55.5 ± 10.4	70.97 ± 9.51	51.70 ± 10.08	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001

WBC: white blood cell; NEU: neutrophils; LYMPH: lymphocytes; MONO: monocytes; EOS: eosinophils; BASO: basophils; RBC: red blood cell; HGB: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; ALT: alanine aminotransferase; NS: non significant.

and it is statistically greater (*P* < 0.01) compared to DWM (34.63%). In LW (42.57%) is statistically greater (*P* < 0.05) compared to DWM. Statistically higher (*P* < 0.001) percentage of basophil was determined in DWM (%) and WM (1.18%) compared to LW (0.66%). Lymphocytes showed statistical differences (*P* < 0.05) between DWM (53.38%) and WM (44.96%). Percentage of monocytes was statistically different in all of three given groups. The

greatest difference (*P* < 0.001) was noted between DWM (6.26%) and LW (3.49%) while between WM (4.98%) and DWM and LW was less notable (*P* < 0.05). The greatest concentration of proteins, albumin and globulin was noted in DWM and WM. In these two groups and LW significant difference (*P* < 0.001) was noted. Higher urea concentration was noted in WM (4.71 mmol/L) and DWM (3.84 mmol/L). The statistical difference (*P*

< 0.001) was noted between these two groups and LW (2.07 mmol/L). Highest creatinin concentration was determined in DWM (84.50 μ mol/L), then in LW (75.31 μ mol/L). These two groups did not show statistical difference, but difference ($P < 0.001$) was noted between

them and WM (57.00 μ mol/L). There was no notable difference in concentration of cholesterol, total bilirubin and AST. IN LW extremely high concentration of ALT was noted (146.112 IU/L) in blood and it is over referent range in literature.

Table 2. Post-hock LSD test - influence of breed and age on hematological and biochemical parameters White Mangalica (WM), Duroc × White Mangalica (DWM), and Large White (LW) in younger (30 kg) and older categories (100 kg) pigs.

Parameter	30 Influence of the group			100 Influence of the group			Influence of age in group		
	WM- DWM	WM-LW	DWM- LW	WM- DWM	WM-LW	DWM- LW	WM	DWM	LW
Leukocytes (10 ⁹ /L)	NS	NS	NS	$P < 0.01$	$P < 0.001$	NS	$P < 0.01$	NS	NS
Neutrophils (10 ⁹ /L)	$P < 0.05$	NS	NS	$P < 0.05$	NS	$P < 0.01$	$P < 0.05$	$P < 0.001$	NS
Lymphocytes (10 ⁹ /L)	NS	NS	NS	$P < 0.001$	$P < 0.01$	$P < 0.05$	$P < 0.001$	NS	NS
Monocytes (10 ⁹ /L)	NS	$P < 0.05$	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS	$P < 0.001$	$P < 0.001$	NS
Eosinophils (10 ⁹ /L)	NS	NS	NS	$P < 0.05$	NS	NS	NS	$P < 0.001$	NS
Basophile (10 ⁹ /L)	NS	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.001$	NS	$P < 0.001$	$P < 0.01$	$P < 0.05$
Neutrophils (%)	$P < 0.01$	NS	$P < 0.05$	$P < 0.001$	NS	$P < 0.01$	NS	$P < 0.05$	NS
Lymphocytes (%)	$P < 0.05$	NS	NS	$P < 0.001$	NS	$P < 0.001$	NS	$P < 0.01$	NS
Monocytes (%)	$P < 0.05$	$P < 0.05$	$P < 0.001$	NS	NS	NS	$P < 0.001$	$P < 0.001$	NS
Eosinophils (%)	NS	NS	NS	NS	NS	NS	NS	$P < 0.001$	NS
Basophils (%)	NS	$P < 0.001$	$P < 0.001$	NS	NS	NS	$P < 0.01$	$P < 0.05$	$P < 0.001$
Erythrocytes (10 ¹² /L)	NS	NS	NS	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
Hemoglobin (g/L)	$P < 0.05$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Hematocrit (%)	NS	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
MCV (fl)	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS	$P < 0.05$	$P < 0.05$	$P < 0.001$
MCH (pg)	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$	NS	$P < 0.001$
MCHC (g/L)	$P < 0.05$	$P < 0.05$	NS	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	NS
RDW (%)	$P < 0.05$	NS	NS	NS	NS	NS	$P < 0.01$	NS	$P < 0.05$
Thrombocytes (10 ⁹ /L)	NS	NS	NS	NS	NS	NS	$P < 0.01$	$P < 0.05$	NS
Protein (g/L)	NS	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.05$	$P < 0.001$	NS	$P < 0.001$
Albumin (g/L)	NS	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Globulin (g/L)	NS	$P < 0.001$	$P < 0.01$	NS	$P < 0.01$	$P < 0.05$	$P < 0.05$	NS	$P < 0.001$
Urea (mmol/L)	NS	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS	NS	$P < 0.05$	$P < 0.01$	$P < 0.001$
Creatinin (μ mol/L)	$P < 0.001$	$P < 0.001$	NS	NS	$P < 0.05$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
Cholesterol (mmol/L)	NS	NS	NS	NS	NS	$P < 0.05$	NS	NS	NS
ALT (IU/L)	NS	$P < 0.001$	$P < 0.001$	$P < 0.01$	NS	$P < 0.001$	NS	NS	$P < 0.05$

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; ALT: alanine aminotransferase; NS: non significant.

B) Influence of breed in older categories (100 kg) - total amount of erythrocytes was statistically higher in WM and DWM compared to LW (8.43 x 10¹²/L; 8.09 x 10¹²/L; 6.87 x 10¹²/L; $P < 0.001$). Significant statistical differences ($P < 0.001$) were determined between all of three groups when it comes to hemoglobin concentration and hematocrit. Both values were highest in WM (157.60 g/L; 49.09%), then DWM (138.20 g/L; 40.83%) and the least in LW (112.50 g/L; 35.19%). Highest values of

MCV and MCH ($P < 0.01$) were determined in WM (54.84 fl; 18.77 pg) compared to other two groups. MCHC value was significantly higher ($P < 0.001$) in WM (342.00 g/L) and DWM (338.80 g/L) compared to LW (319.5 g/L). Complete leucocytes count was significantly higher ($P < 0.01$; $P < 0.001$) in LW (23.85 x 10⁹ /L) and DWM (22.65 x 10⁹ /L) compared to WM (17.46 x 10⁹ /L). Statistically significant difference was determined in neutrophil granulocyte count in LW and DWM (10.22 x 10⁹ /L; 6.03 x 10⁹

/L). Lymphocytes were showed inverse situation and the highest level was determined in DWM ($14.31 \times 10^9/L$) but differences were significant in all of three groups. Statistically significant difference ($P < 0.05$) for eosinophils granulocytes was noted between WM ($0.88 \times 10^9/L$) and DWM ($1.29 \times 10^9/L$). Highest concentration of monocytes and basophil granulocytes was determined in LW ($0.80 \times 10^9/L$; $0.25 \times 10^9/L$), then in DWM ($0.60 \times 10^9/L$; $0.24 \times 10^9/L$). There was no statistical difference between these two groups, but there is between them and WM ($0.39 \times 10^9/L$; $0.14 \times 10^9/L$). Percentage of single leucocytes species showed statistical significance ($P < 0.001$) between WM (46.54%; 44.93%) and LW (42.96%; 47.00%) compared to DWM (27.16%; 63.10%) in neutrophil and lymphocyte count. DWM have showed the lowest count of neutrophil granulocytes below referent range but lymphocytes were above referent range.

Highest protein concentration was determined in WM (73.25 g/L) and lowest in LW (61.10 g/L). Differences between all of three experimental are significant. They were the most significant between WM and DWM ($P < 0.01$), just like between WM and LW ($P < 0.001$). Differences in albumin concentration were significant between all three groups ($P < 0.001$) and the highest concentration was noted in WM (43.10 g/L) and the lowest in LW (26.72 g/L). Highest globulin concentration was determined in LW (35.08 g/L) and the lowest in WM (29.85 g/L). The difference between WM and LW was very significant ($P < 0.01$), between DWM and LW was significant ($P < 0.05$) and between WM and DWM significance was not noted. Urea concentrations showed statistically significant difference ($P < 0.01$) between WM (3.46 mmol/L) and DWM (4.95 mmol/L). Other groups were not showed significant difference. Between WM (121.49 μ mol/L) and LW (111.71 μ mol/L) showed statistical difference ($P < 0.05$) in creatinine concentration. This relation was not noted between other groups. Highest cholesterol concentration was determined in LW (2.89 mmol/L) while statistically significant difference ($P < 0.05$) was founded between LW and DWM (2.42 mmol/L). Other groups showed no statistical difference. No statistical difference between total bilirubin and AST activity was founded in any of the groups. ALT activity showed statistically significant difference

($P < 0.01$) between WM (55.56 IU/L) and DWM (70.97 IU/L) just like between ($P < 0.001$) DWM and LW (51.70 IU/L). WM and LW was not showed statistical differences.

C) Influence of age - there were statistically significant differences in hematologic parameters between age categories in same experimental group. So, WM pigs that weighted 20 kg and 100 kg weren't showed difference only in eosinophils count and relative percentage of neutrophils, eosinophils and lymphocytes. In DWM wasn't noted statistical difference between count of leucocytes, lymphocytes, MCH, RDW and MPV value. In LW statistically significant difference was showed in absolute and relative value of basophile granulocytes, hemoglobin, hematocrit, MCV, MCH and RDW value.

Statistically significant differences were found in biochemical parameters between different age categories in the same group. In WM group pigs that were 20 kg and 100 kg showed no significant difference in cholesterol, bilirubin, ALT and AST value, but there were differences between protein, albumin, globulin, urea and creatinin values. In DWM statistically significant differences were not noted between protein, globulin, cholesterol, bilirubin and ALT values, but difference between values of albumin, urea, creatinine and AST were significant. In LW group statistically significant differences were noted in every single biochemical parameter except cholesterol and bilirubin value.

Correlations of blood parameters

Positive correlation between RBC, Hgb, Hct and albumin was noted in all three breeds and regression model allows explanation of variance between parameters on level 67-84%. Negative correlation was noted between WBC and RBC, Hgb, Hct that was statistically significant in mangalitsa breed but not in other two breeds. Regression analyzes have showed a degree of explained variation between WBC and other parameters 58-69% in mangalitsa, while in other two breeds was 8-12%. It is interesting that in mangalitsa showed positive and significant correlation between globulin value and leucocytes count, while in other two correlation was negative and not significant. Graphic matrix with regression lines and disposition of original values for every breed are shown in Figure 2.

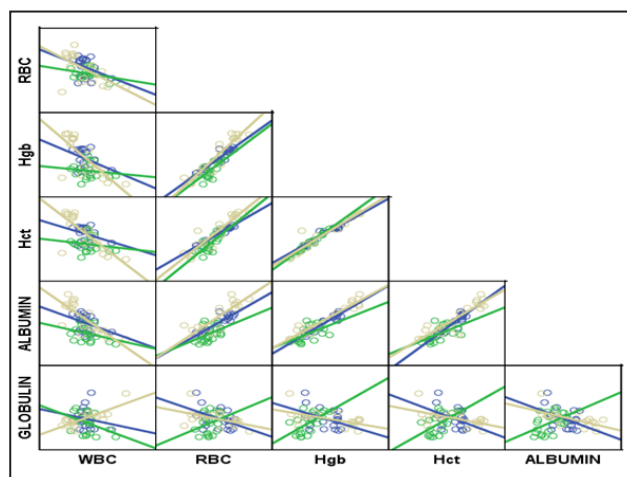


Figure 2. Correlation of health parameters and nutrition in three examined breeds of pigs.

DISCUSSION

Mean values of erythrocyte parameters (MCV, MCH, MCHC, RDW), hemoglobin, hematocrit and platelets were determined in our experiment in piglets in nurture. They match referent range given by Cooper *et al.* [9]. They have been determining referential interval of hematologic and biochemical parameters in 6 weeks old hybrid piglet (Hampshire-Large White) that weighted 10-20 kg. Perri *et al.* [25] also have been determining the same parameters but in weaned piglets 22 days old. However, total leucocytes count was over upper referential limit [9] with significant deprivation of eosinophil and basophile granulocytes from maximal referential limit. As opposite to that, leucocytes value are in referential interval in research of Harvey *et al.* [14] who states experiment on piglet hybrid weighted from 30 to 50 kg. Biochemical parameters are in range of referential values in newer [17,25] and older [12] researches or from on-line sources that are available to many experts [21].

In pigs from farrow to end of reproduction period great influence of age on hematologic and biochemical blood parameters has been noted. Referent ranges [12] showed that upper and lower referential limit value are different in different age and productivity categories: referential range for hemoglobin, hematocrit, erythrocyte count, lower referential value for MCV, MCH and MCHC and mean value of total proteins and albumin was in increase during weaner pigs category, feeder pigs, gilts and sow. Results given by Czech *et al.* [10] showed linear increase of RBC, Hgb, Hct during age which is in accordance with our

results. Same author showed that WBC was lower in piglets than in weaners and both groups of fatteners, with linearly increasing LYM and a decreasing proportion of NEU in the leukocyte population. Chmielowiec-Korzeniowska *et al.* [8] studied influence of season and age (25 ± 2 ; 112 ± 4 kg) on hematologic and biochemical blood parameters. Results showed that with aging protein concentration, urea and creatinine increase while values of cholesterol, bilirubin, AST and ALT haven't showed significant variations.

Influence of breed on values on different parameters in pigs blood was showed in different experiments. Results showed that in Large White pigs 12-15 hematologic parameters have average or high level of heritability [22]. The following values for heritability of biochemical parameters were reported: two blood parameters (FFA, LDH) had $h^2 < 0.1$, six were in the range 0.1-0.2 (ALB, AP, Ca, CK, Mg, TG), five between 0.2-0.4 (ALT, CHOL, CREAT, GGT, LYA), while estimated values for the remaining seven were greater than 0.4 (AMYL, ASAT, C3, GLOB, HCA, IgG, TPROT) [12].

Research showed differences in hematologic parameters in mangalitsa and Large White [12]. It has been show that average values of WBC and Plt were higher in the Large White breed than in the Mangalitza breed, average values of other parameters RBC, Hg Band Hct were higher in the Mangalitza breed. Our results are in accordance with previous study [12] in fattening period (group of 100 kg body weight). Mangalitsa is expired from native pig, philogenetically from wild pig [13]. Study results [7] showed that in wild boars had higher HGB, PCV, MCH, MCHC, neutrophil count, and total protein, albumin, creatinin, and chloride concentrations than juveniles; in contrast, juveniles had higher values for lymphocyte count, cholesterol concentration, and ALP activity can be found.

Mangalitsa as a high percent fat pig and it is rich in fatty acids [29]. Differences in values of blood parameters between breeds that this experiment included can be interpreted as a influence of fat. So, low concentration of urea in mangalitsa compared to other two breeds can be interpreted by a fact that pigs with reduced growth have lower urea concentrations [19]. These differences are present in growth period and are lost in fattening period which is in accordance with results of other autors [1]. As a consequence of obesity low grade inflammation is present [2]. During

inflammation protein of acute phase are in increase (they are in globulin fraction), but albumin concentration decrease (negative protein of acute phase). Tendency of dropping concentration of hemoglobin and erythrocytes is also present [4]. Obesity can be biologic characteristic of mangalitsa that can explain bond between total leucocytes count and globulins with other parameters of red blood cells and albumins. This hasn't been found in other two breeds.

CONCLUSION

Based on results of this study breed and age have significant influence on numerous blood parameters. Besides the differences in mean values of parameters it is proved presence of statistically significant difference in frequencies distributions in function of age and breed. In mangalitsa was showed presence of negative correlation between parameters

of inflammation and nutrition that hasn't been significant in other breeds. Because of differences in mean values of metabolites and differences in frequencies distributions redefine of referent ranges in function of breed and age should be done which demand further research.

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