

Haematological indices, size of erythrocytes and haemoglobin saturation in broiler chickens kept in commercial conditions

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The study aimed at analysing and comparing haematological indices, blood saturation and dimensions of red blood cells of Ross 308 broiler chickens of both sexes. On day 44 of rearing, blood was withdrawn once from 60 randomly selected chickens (1:1 sex ratio). Prior to blood withdrawal the chickens were weighed and their heart rate and haemoglobin saturation were assessed. The following blood parameters were determined: number of RBC and WBC, HGB content and HCT value. In addition, MCV, MCH and MCHC indices were calculated as well as the content (%) of individual leukocyte forms. Measured was also the length, width and circumference of erythrocytes. The measured heart rate (sexes pooled) ranged from 179 to 438 beats/min. Females had significantly higher (by 26.2 beats/min.) value of this trait in comparison with males. Blood of birds of both sexes was characterized by similar haemoglobin saturation (87%). MCV value in males was found by 3.35 fl higher than that in females. Eosinophiles content of blood was by 1.77 percent units higher in females which were also characterised by smaller and more elongated red blood cells. A significant positive relationship was also found between leukocyte and erythrocyte numbers, haemoglobin content and haematocrit value. Negative values of phenotype correlation coefficient were found between MCHC and erythrocyte number, haemoglobin content and haematocrit value. A lower MCV value and higher content of eosinophils of blood were identified in females which were also characterized by smaller and more elongated erythrocytes.

KEY WORDS: broiler chickens / haematological indicators / erythrocyte size / haemoglobin saturation

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Selection towards rapid growth and increased final body weight resulted in broiler breeders in the appearance of a number of metabolic diseases, the most important of which include sudden death syndrome (SDS) and ascites syndrome (HS). Both are directly associated with the failure of the functions of circulatory and respiratory systems in rapidly growing birds [Kranen *et al.* 1998, Imaeda 2000, Wideman 2001]. Ascites is related frequently to the phenomenon of hypoxemia resulting from reduced oxygen content of blood [Buys *et al.* 1999]. Moreover, interrelations were indicated in chicken between the frequency of ascites and haematocrit value [Shlosberg *et al.* 1998]. Lower blood oxygen tension (pO_2) as well as smaller haemoglobin saturation were both reported in fast growing broilers [Peacock 1990, Olkowski 1999]. Similarly, Julian and Mirsalimi [1992] reported a significantly negative correlation between chicken body weight and their blood saturation with oxygen. Pakdel *et al.* [2002] also showed the impact of birds' sex on the size and nature of ascites. These authors observed that male broilers were characterized by higher content of fluid in the abdomen as compared with females.

On the other hand, with the passage of the rearing period, maintenance conditions of broilers often deteriorate. Frequently, the layer of wet litter increases as well as the level of noxious gaseous admixtures in the chicken-house air. In addition, microbiological contamination of all facilities is also higher due to gradual multiplication of pathogenic microorganisms. Such conditions are stressful and affect the obtained production negatively [Jones 1996, Klecker *et al.* 2002] as well as the homeostasis of the organism through, among others, changes in haematological indices. Certain stress factors lead to reduction in erythrocyte number, haemoglobin content and haematocrit value [Yahav and Hurvitz 1996, Yahav 1999]. Frequently, total leukocyte numbers are found higher [Tymczyzna *et al.* 1996, Kontecka *et al.* 1999] and number of lymphocytes decline [Maxwell and Robertson 1995], whereas the ratio of heterophils to lymphocytes increases [McFarlane and Curtis 1989, Mitchell *et al.* after Maxwell 1993]. On the other hand, in another trial, broiler chickens exposed to elevated levels of ammonia in the environment showed higher numbers of erythrocytes, haemoglobin content and haematocrit value. Moreover, it was revealed that values of haematological indices and size of red blood cells may vary in relation to sex of birds [Pampori and Saleem 2007, Patodkar *et al.* 2008, Łukasiewicz and Michalczuk 2009] and, consequently, they should be considered and analysed separately.

Although, in the literature there are few articles in which the haematological indices of broilers were analyzed based on gender [Pakdel *et al.* 2005], the performed studies in domestic chicken indicate that the males were characterized, among others greater number of erythrocytes and leukocytes in the blood and increased haemoglobin content [Sharmin and Myenuddin 2004, Simaraks *et al.* 2004, Peters *et al.* 2011]. Most producers of broilers kept males and females together. But we know that the cocks are characterized by faster growth, thereby achieving higher slaughter body weight at the same time of rearing. Therefore, changes in haematological indices as well as chicken

blood oxygenation can be treated as indicators of deterioration of their environment and appearance of metabolic diseases.

The objective of this study was to analyse and compare haematological indices, blood saturation with oxygen and red blood cell dimensions of broiler chickens of both sexes maintained under productive conditions and to evaluate interrelations between these traits.

Material and methods

Investigations were conducted on a farm, in commercial conditions, in a chicken-house of 780 m² area. Density was approx. 18 birds/m². The material comprised broiler chickens (Ross 308) kept under standard environmental conditions common for chicken broiler farms. The initial chicken-house temperature (on the day of settling the chicks) amounted to about 35°C and was gradually reduced to 18°C on day 35 of rearing. Relative air humidity in the trial facility was kept at 65%. Chickens were fed *ad libitum* a complete diets STARTER (from day 0 to 12); GROWER (from day 13 to 36) and FINISHER (from day 37 to 44). STARTER and GROWER mixture contained 12.3; 12.9 MJ/kg ME and 22.0; 20.2% crude protein, respectively. The level of metabolize energy and crude protein in FINISHER diet was 13.2 MJ/kg and 18.7%. Birds had free access to water.

On day 44 of rearing, blood from the subcutaneous elbow vein (*vena ulnaris cutanea*) was withdrawn once from 60 randomly selected chickens (30 females and 30 males). Before blood collection, chickens were weighed – BW (g) and their heart rate – HR (BPM) and haemoglobin saturation – HS (%) measured using a pulse-oximeter Vet/Ox® G2Digital™ Monitor. For this purpose, after catching, the bird was immobilized and then connected to a sensor which monitored blood saturation with oxygen. The following blood parameters were determined: number of erythrocytes – RBC (T/l) and leucocytes – WBC (G/l), haemoglobin content – HGB (g/l) and haematocrit value – HCT (l/l). In addition, mean corpuscular volume – MCV (fl), mean corpuscular haemoglobin – MCH (pg) and mean corpuscular haemoglobin concentration – MCHC (g/l) indices were calculated. Haematological assays were carried out with a Cell-Dyn 3500 Abbott Company apparatus taking into consideration the parameters describing the red-blood cell system as well as the total leukocyte number (without division into fractions). Leukocyte were assessed calculating their numbers by the chamber method. The prepared blood smears were stained using the Pappenheim method [Bomski 1989] and then 100 consecutive leukocytes were counted under the microscope differentiating them into granulocytes (heterophils, eosinophils and basophils) and agranulocytes (lymphocytes and monocytes) in order to calculate their percent content. In the case of the blood red cell system, the results obtained using the haematological apparatus were verified by performing mechanical determination of the haematocrit value. Erythrocytes were measured under the microscope at 1500 x magnification employing a computer microscope image analysis (MultiscanBase

v. 8.08). Approximately 50 erythrocytes were measured in each preparation corresponding to one bird. The following measurements were taken: length (μm), width (μm), circumference (μm) and surface area (μm^2). In addition, erythrocyte shape index (%) – an indicator introduced to allow determination of erythrocyte shape (oblong, oval) – was calculated. Measurements were performed after loading of the preparation image into the MultiscanBase programme followed by filtration and contrasting. Then the procedure of automatic measurement of erythrocytes was activated. The erythrocyte shape index was calculated from the following formula:

$$\text{Shape index (\%)} = \text{erythrocyte width (\mu m)} \times 100 / \text{erythrocyte length (\mu m)}$$

Statistical evaluations were conducted using the SAS[®] v. 9.1 package. Significance of differences between males and females with regard to the body weight on day 44 of life, heart rate, haemoglobin saturation and blood morphological characteristics were evaluated employing the Student t-test. Pearson's phenotype correlation coefficients among chosen traits of broilers were calculated for sexes pooled.

Results and discussion

Table 1 presents mean body weights and blood indicators in chickens. Means for body weight (sexes pooled) occurred close to the accepted standards which, on day 44 of life, amounted to 3064 g for males and 2596 g for females [Aviagen 2007]. Besides, the chickens weight on day 44 of life was related to sex. Males were significantly heavier (by 619 g) than females. The measured heart rate (sexes pooled) ranged from 179 to 438 beats/min. Females had significantly higher (by 26.2 beats/min.) value of this trait in comparison with males. In investigations carried out on 6-week old chickens and using for the assessment of haemoglobin saturation a pulse oximeter

Table 1. Means and standard errors (SEM) for body weight and selected blood indicators in broiler chickens at the age of 44 days

Trait	Sex							
	males				females			
	mean	SEM	min.	max.	mean	SEM	min.	max.
Body weight (g)	3120*	35.76	2207	3551	2501*	33.16	1914	2834
Heart rate (BPM)	345.7*	3.84	179.0	417.0	371.9*	3.99	222.0	438.0
Hemoglobin saturation (%)	87.20	0.88	74.00	96.00	87.10	1.05	79.50	96.50
WBC (G/l)	32.60	1.66	16.90	52.60	34.90	2.15	18.20	59.60
RBC (T/l)	2.48	0.05	2.06	3.17	2.53	0.09	1.12	3.40
HGB (g/l)	127.13	2.48	109.00	152.00	124.70	3.60	67.40	157.00
HCT (L/L)	0.36	0.007	0.30	0.45	0.35	0.012	0.15	0.46
MCV (fl)	144.00*	0.74	137.00	149.00	140.65*	0.88	133.00	148.00
MCH (pg)	51.25	0.28	47.80	53.50	49.88	0.94	32.30	60.10
MCHC (g/l)	356.00	1.83	338.00	372.00	355.09	6.86	240.00	451.00

*Within rows means bearing asterisks differ significantly at $P \leq 0.05$.

similar to the one employed in this study, Julian and Mirsalimi [1992] found a lower value of the trait in heavier, fast-growing birds weighing approximately 3500 g. Lighter, slow-growing birds (mean life body weight = 2285 g) were characterized by higher haemoglobin saturation (91.6 against 86.0%). Furthermore, the above authors demonstrated a highly negative correlation between this trait and body weight of chickens. In addition, they determined the heart rate of the birds amounting on the average to 250 BPM and higher. The results of our own investigations concerning haemoglobin saturation were within the interval determined by the above-mentioned authors, being also close to those obtained by Roush and Wideman [2000]. However, heavier males and lighter (by about 620 g) females were characterized by very similar values of the analysed trait (87.1%). Nowaczewski *et al.* [2011], reported similar values of haemoglobin saturation for broiler chicken females and males kept on three different types of litter.

There are only few literature reports characterizing haematological indices in broiler chickens separately for each sex. No differences were found between sexes in leukocyte and erythrocyte numbers as well as haemoglobin content and haematocrit value, although there was a trend for a slightly higher value of the two former traits in females. A reverse trend was identified in haemoglobin content – females were characterized by lower value of the trait being, however, nonsignificantly different from males. When analysing indicators associated with erythrocytes, significant differences between sexes were recorded only in MCV where the value was by 3.35 fl higher in males. Pampori and Saleem [2007] recorded, among others, a greater number of erythrocytes, but a smaller number of leukocytes in males. Moreover, similarly as in the present study, the males showed a significantly higher (by about 5.0 fl) value of the MCV. On the other hand, Yalçın *et al.* [2004] failed to find any effect of the sex of Ross 308 chicken broilers of different ages on blood haemoglobin content.

Males and females failed to differ significantly regarding the share of individual forms of leukocytes (Tab. 2) with the exception of eosinophils whose contents were found higher of female blood. The difference amounted to 1.77 per cent points. The maximum proportion of eosinophils in females reached 11%. Similar results,

Table 2. Leukocytes and H:L ratio in broiler chickens at the age of 44-days

Trait	Sex							
	males				females			
	mean	SEM	min.	max.	mean	SEM	min.	max.
Eosinophils (%)	2.48*	0.38	0.00	6.00	3.92*	0.61	0.00	11.00
Basophils (%)	3.93	0.5	0.00	8.74	3.53	0.5	0.00	8.08
Heterophils (%)	33.09	2.14	18.40	56.00	32.35	2.26	11.00	61.00
Lymphocytes (%)	58.90	1.92	40.00	73.00	58.55	2.24	36.00	77.48
Monocytes (%)	1.63	0.21	0.00	3.00	1.71	0.41	0.00	7.76
H:L ratio (1/1)	0.60	0.06	0.27	1.40	0.56	0.06	0.14	1.21

*Within rows means bearing asterisks differ significantly at $P \leq 0.05$

including lack of significant differences between males and females with respect to the content of eosinophils were reported by Yalçin *et al.* [2004] and Pampori and Saleem [2007]. In the present study the mean content of eosinophils in the examined chickens higher than that found in the literature [Yalçin *et al.* 2004, Witkowska *et al.* 2007] and ranging from 1.8 to 2.8% can be attributed to the elevated level of that type of cells (6-11 %) observed in some individuals. This, in turn, can indicate an impact on these birds of some stress or other factors stimulating them to increase eosinophil production. Maxwell [1987] reported increased levels of eosinophils in ducks (up to 8-12 %) following injection of horse blood serum. Also Kontecka *et al.* [1999] found elevated proportions of eosinophils in ducks exposed to stress caused by limited access to drinking water. In broiler chickens, a similar effect was achieved three hours after ochratoxin administration which caused a 3.7 per cent points increase in the level of eosinophils [Moura *et al.* 2004]. On the other hand, Nowaczewski *et al.* [2006] found no significant differences between pheasants fed complete diet with or without vitamin C supplementation (anti-stress agent) in the number of leukocytes and agranulocytes and granulocytes content.

Significant differences between sexes were identified in the dimensions of erythrocytes (Tab. 3) which in males had larger width, surface area and circumference. In addition, they were more round in males as their erythrocyte index was by 1.11 per cent points higher in males. In the literature, only one study was found presenting results dealing with erythrocyte measurements of chickens separately of sexes [Pampori and Saleem 2007]. The above-mentioned authors also found larger erythrocytes (mean length = 13.86 μm ; mean width = 8.37 μm) in males than in females of native adult chickens of Kashmir.

Table 3. Erythrocyte size in chicken broilers at the age of 44 days

Erythrocyte trait	Sex							
	males				females			
	mean	SEM	min.	max.	mean	SEM	min.	max.
Length (μm)	11.93	0.03	8.69	14.51	11.92	0.03	9.35	14.19
Width (μm)	7.13*	0.01	5.82	9.37	7.00*	0.02	5.61	8.66
Area (μm^2)	66.06*	0.20	47.47	82.29	65.27*	0.24	45.74	88.19
Circumference (μm)	33.50*	0.07	26.99	42.80	32.80*	0.07	26.98	38.43
Index (%)	60.10*	0.23	56.10	69.30	58.99*	0.22	54.70	64.10

*Within rows means bearing asterisks differ significantly at $P \leq 0.05$

Table 4 shows the coefficients of phenotype correlations among the examined traits. No correlations were found between the body weight of broilers and their blood traits except of the MCV index ($r_p = 0.351$). Similarly, no relationship was found between the heart rate of birds and haemoglobin saturations and other blood indices and size of erythrocytes. A significant, positive correlation was identified between the number of leukocytes and the number of erythrocytes, haemoglobin content and

haematocrit value (r_p = from 0.351 to 0.426). Correlations occurring among the examined traits in this study present interesting negative relationships between MCH and MCHC and number of erythrocytes, haemoglobin content and haematocrit value. Theoretically, an increase in the number of erythrocytes of the same volume (MCV) always leads to the increase in haematocrit value; hence the MCHC index should decline, assuming that the content of haemoglobin remains unchanged. Therefore, elevated content of haemoglobin should lead to the increase in MCHC, provided the number of erythrocytes and their volume (MCV), and hence haematocrit, do not change. An attempt at elucidating the results of this study can be a situation in which the increase in the number of erythrocytes (at constant MCV) and hence growth of haematocrit in chickens was higher than the increasing haemoglobin content. This continued to reduce MCHC and caused the mentioned negative relationships to occur. In other words, chickens that were characterized by greater numbers of red blood cells (and hence, higher haematocrit value) could have erythrocytes of lower haemoglobin content although its amount in blood was higher. This phenomenon, in turn, can be explained in many different ways. It

can be assumed that normally, these chickens produce more cells because they are perhaps characterized by a lower efficiency of incorporating haemoglobin into cells. Such birds can also be characterized by a greater demand for oxygen which could, in part, explain the occurrence in current chicken hybrids of problems associated with

Table 4. Coefficients of phenotypic correlation between body weight, heart rate, haemoglobin saturation and blood traits in broiler chickens at the age of 44-days

	HR	HS	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	EA	EC	EL	EW	EX
BW (g)	-0.311	-0.014	-0.140	-0.047	0.128	0.027	0.351**	0.243	0.084	0.061	0.171	-0.101	0.272	0.237
HR (BPM)		0.215	0.185	0.073	0.103	0.047	-0.104	0.033	0.094	0.208	0.145	0.229	0.020	-0.149
HS (%)			0.276	-0.071	-0.188	-0.096	-0.119	-0.160	-0.114	0.007	0.034	0.032	-0.017	-0.021
WBC (G/l)				0.351**	0.426**	0.384**	0.198	-0.103	-0.184	0.259	0.178	0.220	0.099	-0.077
RBC (T/l)					0.830**	0.977**	0.047	-0.691**	-0.726**	0.179	0.111	0.185	0.031	-0.112
HGB (g/l)						0.875**	0.322**	-0.191	-0.333**	0.336*	0.300	0.372**	0.047	-0.234
HCT (l/l)							0.256	-0.597**	-0.724**	0.254	0.175	0.215	0.104	-0.085
MCV (fl)								0.293*	0.900**	0.407**	0.353**	0.191	0.367**	0.104
MCH (pg)										0.039	0.097	0.087	-0.039	-0.091
MCHC (g/l)										-0.134	-0.061	0.009	-0.203	-0.143

BW – body weight, HR – heart rate, HS – haemoglobin saturation, WBC – white blood cells, RBC – red blood cells, HGB – haemoglobin content, HCT – haematocrit value, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, EA – erythrocyte area, EC – erythrocyte length, EL – erythrocyte width, EW – erythrocyte width, EX – erythrocyte index.

*P≤0.05; **P≤0.01.

the functionality and efficiency of the circulatory system faced with very rapid body weight gain. It is also possible that the “haemoglobin quality” in these chickens was worse and, therefore, they produced more of it. The studies carried out by Maxwell *et al.* [1990] also indicate lower (albeit non-significantly) MCH and MCHC indices in chickens reared under conditions of reduced content of oxygen and which were characterized by significantly higher numbers of erythrocytes and haemoglobin content. The analysis of the erythrocyte shape revealed a positive correlation between the length and surface area of erythrocytes and content of haemoglobin.

The research presented in this paper are primarily cognitive value. Although, there was no significant differences between males and females with regard to the majority of blood indices and oxygen saturation of haemoglobin, the results indicate how the organism of broiler chickens (specific hybrid), through the blood parameters, reacts to intensive farming conditions. Besides, the results expand and supplement the existing knowledge (in literature) in this field.

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