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Morphology and phagocytic activity of white blood cells in the Pharaoh quail (*Coturnix coturnix Pharaoh*)

Morfologia i aktywność fagocytarna białych krwinek u przepiórki Faraon (*Coturnix coturnix Pharaoh*)

SUMMARY

The aim of our research was to analyse the morphological features of particular forms of white blood cells and to determine their diameters in males and females of Pharaoh quail. We also calculated the number of leucocytes, their percentage composition and absolute contents in the peripheral blood, and we estimated the phagocytic activity of heterophils in Pharaoh quail males and females.

The conducted experiments indicated that the Pharaoh quail white blood cells do not differ morphologically from the leucocytes in the Japanese breed. The size of the blood cells is similar to that in the Japanese quail. We also observed that the female white blood cells are smaller than those in males.

In females, both the number of leucocytes and of heterophils is bigger than in males, whereas the number of lymphocytes is nearly the same in both sexes.

In our studies we have observed that the phagocytic activity of heterophils in quail is lower than in mammals. We have also seen that female heterophils have a higher ability to reduce NBT and ingest latex particles, which indicates a higher immunity of this sex to unfavourable environmental factors.

STRESZCZENIE

Celem pracy było przeprowadzenie analizy cech morfologicznych i określenie średnicy poszczególnych form krwinek białych, oznaczenie liczby leukocytów, ich składu procentowego oraz bezwzględnej zawartości we krwi obwodowej oraz ocena aktywności fagocytarnej heterofili samców i samic przepiórki Faraon.

Przeprowadzone badania wykazały, że krwinki białe przepiórki Faraon nie różnią się pod względem cech morfologicznych od leukocytów ptaków rasy japońskiej. Także wielkość krwinek jest zbliżona do rozmiarów podanych dla przepiórki japońskiej. Stwierdzono również, że krwinki białe samic charakteryzują się mniejszymi rozmiarami w porównaniu z samcami.

U samic zarówno liczba leukocytów, jak i heterofili jest wyższa aniżeli u samców, natomiast liczba limfocytów niemal taka sama u przepiórek obu płci.

W przeprowadzonych badaniach ustalono, że aktywność fagocytarna heterofili przepiórki jest niższa w porównaniu ze ssakami. Ponadto stwierdzono, że większą zdolnością do redukcji NBT i fagocytowania ziaren lateksu charakteryzują się heterofile samic, co wskazuje na większą odporność ptaków tej płci na niekorzystne czynniki środowiskowe.

K e y w o r ds: quail, leucocytes, morphological features, phagocytic activity

INTRODUCTION

Avian blood, due to numerous features distinguishing it from mammalian blood, raised the interest of scientists as early as in the first years of the previous century (4, 9, 25). The usefulness of haematological data in the evaluation of organism's physiological condition was an impulse to a broad research on the blood morphology of domestic birds (8, 10, 24). Special attention was paid to hens' blood, less to other bird species, and the blood of quails, which are now the sixth usable bird species, has been relatively seldom examined. Moreover, the literature data concerning the number of leucocytes and the percentage composition thereof in quail are broad, and the data presenting sex dependent differences in the values of the above-mentioned parameters are often divergent. Some papers claimed a distinct dependence on birds' sex, while some other established nearly the same values in males and females. (2, 5, 12, 13, 20, 21, 23).

In the available literature there are merely two papers found, devoted to the morphological characteristics of white blood cells in quail, where Japanese breed birds were the material (1, 5). There is also just one paper evaluating the phagocytic ability of Pharaoh quail heterophils (7).

Given the above-presented data, we found it advisable to carry out the following research:

– an analysis of morphological features and determining the diameter of particular forms of white blood cells in Pharaoh quail,

– an estimation of the number of leucocytes, their percentage composition and absolute content in peripheral blood in both sexes of the birds,

– an evaluation of the phagocytic activity of heterophils in Pharaoh quail males and females.

MATERIAL AND METHODS

The blood was obtained from the Department of Biologically Based Animal Production of the Agricultural Academy in Lublin. It was taken from the wing vein of 30 Pharaoh quails (15 males and 15 females) (*Coturnix coturnix Pharaoh*), aged 4 months and weighing 170–210 g.

The leucocyte count was determined by the chamber method in the Natt-Herrick diluting solution (11). The percentage composition of leucocytes was calculated in smears stained in accordance with the Pappenheim method. The same preparations were used for measuring particular forms of leucocytes by means of the scale ocular.

On the basis of the number of leucocytes and their percentage composition we computed the absolute contents of particular forms of white blood cells and the leucocyte ratio (22).

The estimation of the phagocytic activity of heterophils was conducted using the nitrotetrazolium blue reduction test (NBT) (17, 18), determining the phagocytosis ratio with the use of latex, and computing the phagocytic index (19).

The obtained result were statistically analysed with the Student's t-test for independent data. The value $p < 0.05$ was assumed as statistically significant (15).

RESULTS

Characteristics of individual leucocyte forms

Heterophils, that is, neutrophilic granulocytes (neutrophils), in Pharaoh quail are round cells, whose cytoplasm is filled with specific granules in the shape of sharp, orange, light refracting bars or spindles. The heterophil nucleus is most often two-segmented, sometimes rod-shaped (Fig. 1A).

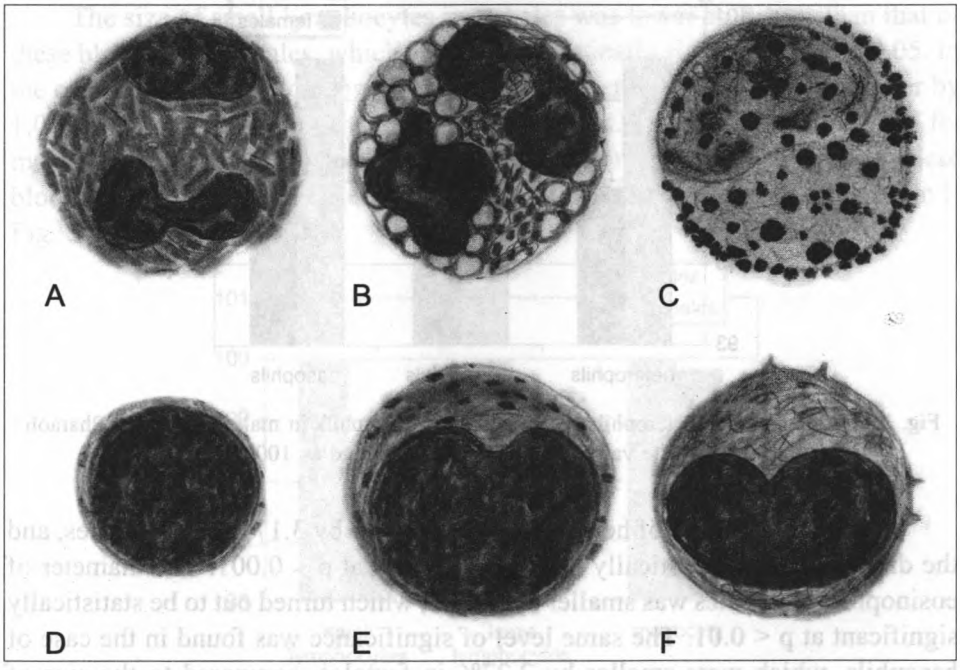

Fig. 1. Individual leucocyte forms: A – heterophil; B – eosinophil; C – basophil; D – small lymphocyte; E – large lymphocyte; F – monocyte

Eosinophils, that is, acidophilic granulocytes, are round cells, whose cytoplasm is filled with numerous, small, round eosinophilic granules and colourless, round vacuoles. Their nucleus is most often two-segmented, exceptionally three-segmented (Fig. 1B).

Basophils are round cells. In the colourless cytoplasm there are numerous, dark blue granules, whose number is sometimes so big that they cover a non-divided, peripherally placed nucleus (Fig. 1C).

Quail lymphocytes can be divided into small and large ones. Small lymphocytes are more numerous. Their nuclei are round, centrally placed and surrounded by a thin layer of light-blue basophilic cytoplasm. Large lymphocytes have a kidney- or egg-shaped, peripherally placed nucleus; their basic cytoplasm is more abundant than in small lymphocytes (Fig. 1 D and E).

Monocytes are large, round, one-nucleus cells. They have a peripherally placed, egg- or kidney-like nucleus; the pale-blue, opulent cytoplasm has a follicle structure. On the periphery there can be seen cytoplasmic pseudopodia (Fig. 1F).

The average diameter of all the forms of white blood cells was smaller in females than in males (Table 1, Fig. 2 and 3).

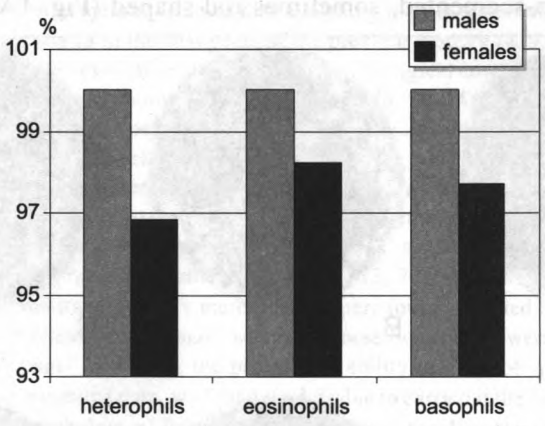

Fig. 2. The diameter of heterophils, eosinophils and basophils in male and female Pharaoh quail (the values in males were assumed as 100%)

In females, the size of heterophils was smaller by 3.17% than in males, and the difference was statistically highly significant at $p < 0.001$. The diameter of eosinophils in females was smaller by 1.77%, which turned out to be statistically significant at $p < 0.01$. The same level of significance was found in the case of basophils, which were smaller by 2.27% in females, compared to the size of these blood cells in males (Table 1, Fig. 2).

Table 1. The diameter of individual types of white blood cells in Pharaoh quail

Forms of leucocytes	Sex	Number of leucocytes	Diameter of leucocytes [μm]		P < *
			x ± SE	range	
Heterophils	♂	561	9.48 ± 0.037	7.6–12.8	0.001
	♀	584	9.18 ± 0.029	7.2–12.8	
Eosinophils	♂	33	9.60 ± 0.045	8.4–12.8	0.01
	♀	36	9.43 ± 0.040	8.8–11.2	
Basophils	♂	6	9.27 ± 0.093	8.4–10.8	0.01
	♀	5	9.06 ± 0.061	8.4–10.4	
Small lymphocytes	♂	843	6.42 ± 0.047	4.8–7.2	0.05
	♀	841	6.28 ± 0.051	4.8–7.2	
Large lymphocytes	♂	15	10.53 ± 0.072	9.9–13.8	ns
	♀	12	10.51 ± 0.061	8.4–13.6	
Monocytes	♂	34	11.33 ± 0.083	10.0–13.2	ns
	♀	31	11.21 ± 0.075	10.0–12.4	

The size of small lymphocytes in females was lower by 2.18% than that of these blood cells in males, which appeared statistically significant at $p < 0.05$. In the case of large lymphocytes, their average diameter in females was smaller by 1.06% than in males, and statistically insignificant. The average diameter of female monocytes was smaller only by 0.19% in comparison to the size of these blood cells in males. The noted difference was statistically insignificant (Table 1, Fig. 3).

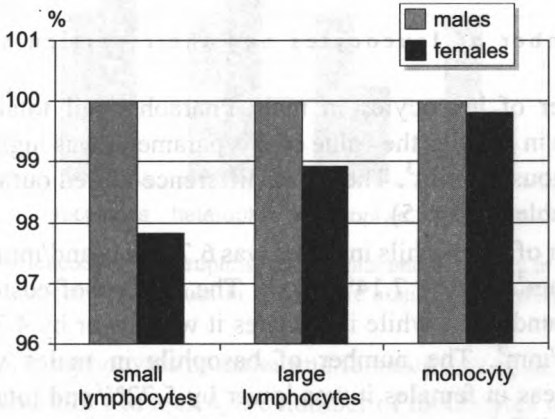

Fig. 3. The diameter of lymphocytes and monocytes in male and female Pharaoh quail (the values in males were assumed as 100%)

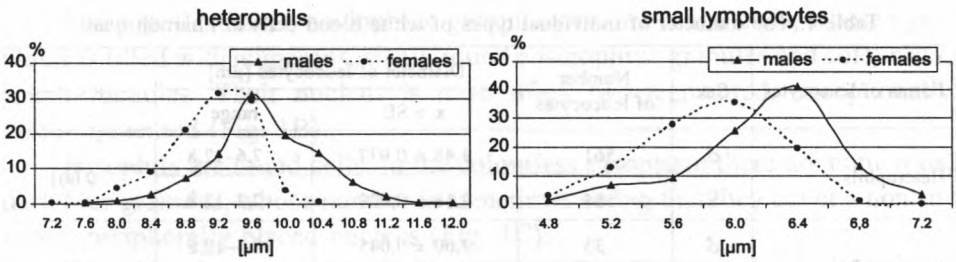

Fig. 4. The anisocytosis graphs of heterophils and small lymphocytes in male and female Pharaoh quail

In Figure 4 we have presented the anisocytosis graphs for the two most numerous forms of white blood cells-heterophils and small lymphocytes.

The diameter of the biggest number of heterophils in males was 9.6 µm. Blood cells of this size constituted 31.07%. There were only 0.18% of the smallest heterophils with 7.6 µm in diameter, and 0.18% of the biggest ones with the diameter reaching 12.8 µm. In females, the biggest number of heterophils (31.34%) was 9.2 µm in diameter; the smallest ones (0.17%) were 7.2 µm, while the biggest blood cells (0.51%) reached 10.4 µm.

The largest number of small lymphocytes in males had the diameter of 6.4 µm, and they comprised as much as 41.11% of this form of white blood cells. The smallest lymphocytes of 4.8 µm in diameter were scarce – they amounted to 1.30%. The blood cells with the biggest diameter (7.2 µm) made up 3.30%. In females, the diameter of the majority of lymphocytes was 6.0 µm. They constituted 35.67% of this blood cell form. The smallest blood cells sized 4.8 µm constituted 2.85%, and the biggest ones of 7.2 µm in diameter – only 0.12%.

The number of leucocytes and their particular forms

The number of leucocytes in male Pharaoh quail totalled 17.96 thousand/mm^3, while in females the value of this parameter was higher by 2.56% and reached 18.42 thousand/mm^3. The noted difference turned out to be statistically insignificant (Table 2, Fig. 5).

The number of heterophils in males was 6.72 thousand/mm^3, and in females 7.20 thousand/mm^3, that is, 7.14% more. The number of eosinophils in males was 0.426 thousand/mm^3, while in females it was lower by 4.33% and totalled 0,408 thousand/mm^3. The number of basophils in males was 0.070 thousand/mm^3, whereas in females it was lower by 5.72% and totalled 0,066 thousand/mm^3. Sex difference proved statistically insignificant in the case of the above-mentioned forms of blood cells (Tab. 2, Fig. 5).

Table 2. The number of leucocytes and their individual forms in Pharaoh quail

Parameters	Sex	Number of leucocytes [thousand/mm^3]		p < *
		x ± SE	range	
Leucocytes	♂	17.96 ± 0.643	13.97–22.58	ns
	♀	18.42 ± 0.498	15.05–21.28	
Heterophils	♂	6.72 ± 0.285	4.89–9.13	ns
	♀	7.20 ± 0.284	5.27–8.75	
Eosinophils	♂	0.426 ± 0.068	0.140–0.903	ns
	♀	0.408 ± 0.053	0.168–0.813	
Basophils	♂	0.070 ± 0.023	0–0.226	ns
	♀	0.066 ± 0.027	0–0.213	
Lymphocytes	♂	10.35 ± 0.451	7.85–13.10	ns
	♀	10.35 ± 0.292	8.37–11.63	
Monocytes	♂	0.392 ± 0.054	0–0.816	ns
	♀	0.380 ± 0.039	0.156–0.638	

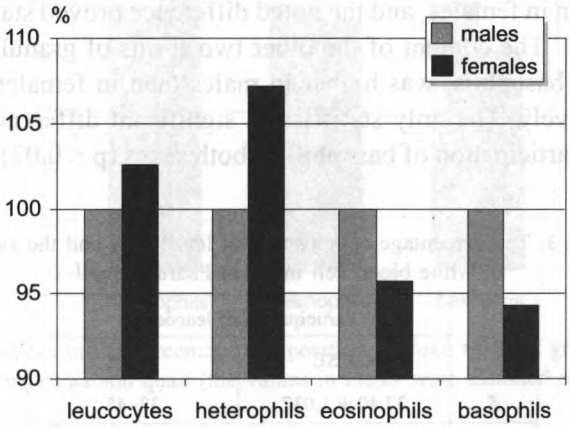

Fig. 5. The number of leucocytes, heterophils, eosinophils and basophils in male and female Pharaoh quail (the values in males were assumed as 100%)

The number of lymphocytes in males and females was equal and in both totalled 10.35 thousand/mm^3. In males, the number of monocytes was 0.392 thousand/mm^3. In females, the number of these blood cells was lower by 3.16%, compared to the value established for males, and it totalled 0.380 thousand/mm^3.

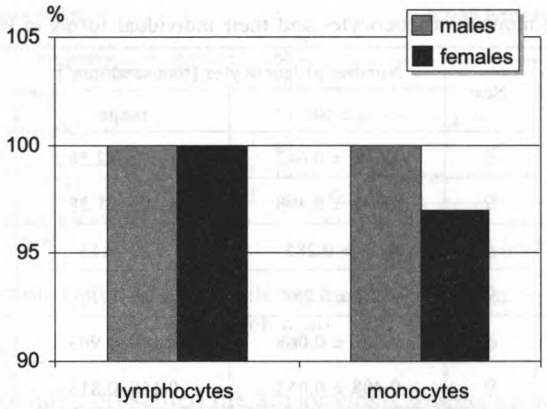

Fig. 6. The number of lymphocytes and monocytes in male and female Pharaoh quail (the values in males were assumed as 100%)

The observed difference between the sexes turned out to be statistically insignificant (Table 2, Fig. 6).

The percentage composition of leucocytes

The percentage participation of heterophils in Pharaoh quail males was by 4.28% lower than in females, and the noted difference proved statistically significant at $p < 0.05$. The content of the other two forms of granulocytes, namely, eosinophils and basophils, was higher in males than in females by 5.91% and 21.21% respectively. The only statistically significant difference was between the percentage participation of basophils in both sexes ($p < 0.02$) (Table 3, Fig. 7 and 8).

Table 3. The percentage composition of leucocytes and the values of white blood cell index in Pharaoh quail

Parameters	Sex	Participation of leucocytes		p < *
		x ± SE	range	
Heterophils [%]	♂	37.40 ± 1.037	32–45	0,05
	♀	39.07 ± 0.871	33–46	
Eosinophils [%]	♂	2.33 ± 0.335	1–5	ns
	♀	2.20 ± 0.262	1–4	
Basophils [%]	♂	0.40 ± 0.131	0–1	0,02
	♀	0.33 ± 0.126	0–1	

Lymphocytes [%]	♂	57.53 ± 1.004	50–63	ns
	♀	56.33 ± 0.926	50–64	
Monocytes [%]	♂	2.27 ± 0.329	0–5	ns
	♀	2.07 ± 0.204	1–3	
White blood cell index	♂	0.67 ± 0.036	0.51–0.89	ns
	♀	0.72 ± 0.019	0.54–0.92	

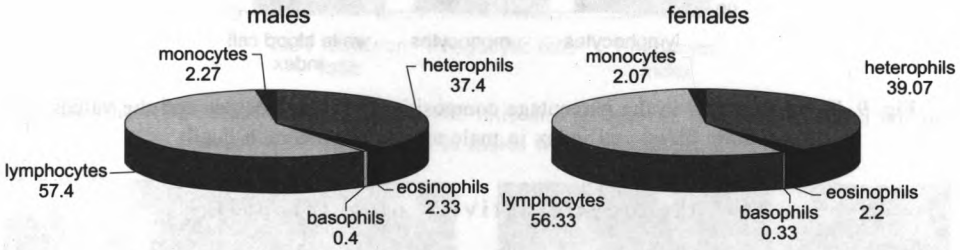

Fig. 7. The percentage composition of leucocytes in male and female Pharaoh quail

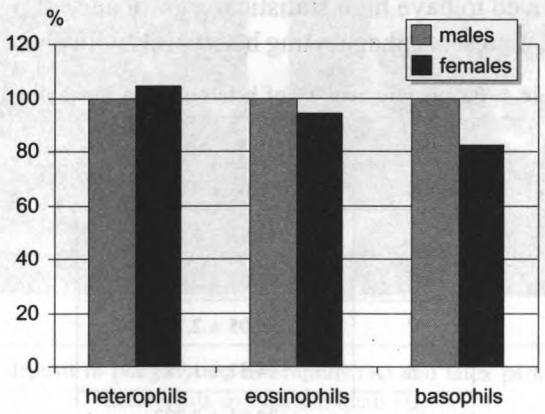

Fig. 8. The differences in the percentage composition of three types of granulocytes in male and female Pharaoh quail (the values in males were assumed as 100%)

The content of agranulocytes, that is lymphocytes and monocytes, was higher in males than in females by 2.13% and 9.66%, respectively. The differences between the sexes were statistically insignificant (Table 3, Fig. 7 and 9).

The value of the white blood cell index in males was lower by 6.95%, compared to that in females. The noted difference did not attain statistical significance (Table 3, Fig. 9).

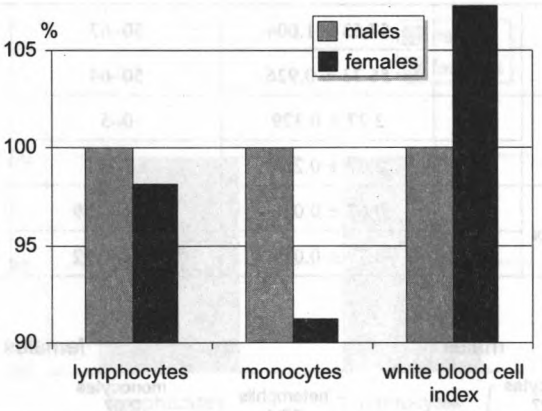

Fig. 9. The differences in the percentage composition of agranulocytes and the values of white blood cell index in male and female Pharaoh quail

The phagocytic activity of heterophils

The NBT reduction ratio marked for heterophils in female Pharaoh quail was by 35.97% higher than that of males. The difference between the values of this parameter proved to have high statistical significance at $p < 0.001$ (Table 4, Fig. 10). Figure 11A presents phagocyting heterophils with phormazane deposits.

Table 4. Phagocytic activity of heterophils in Pharaoh quail

Parameters [%]	Sex	x ± SE	p < *
NBT reduction ratio	♂	11.08 ± 0.688	0,001
	♀	13.95 ± 1.112	
Phagocytic ratio	♂	65.02 ± 5.464	0,001
	♀	75.05 ± 2.701	
Phagocytic index	♂	14.57 ± 1.783	0,001
	♀	20.03 ± 3.227	

The value of phagocytic ratio in female Pharaoh quails was higher by 15.43% than in males. The observed difference turned out to be of high statistical significance at $p < 0.001$ (Table 4, Fig. 19). Figure 11B shows phagocyting heterophils with latex particles.

Similarly to the NBT reduction ratio and phagocytic ratio, the phagocytic index was higher in females than in males. The observed difference was 37.47% and it was highly significant at $p < 0.001$ (Table 4, Fig. 10).

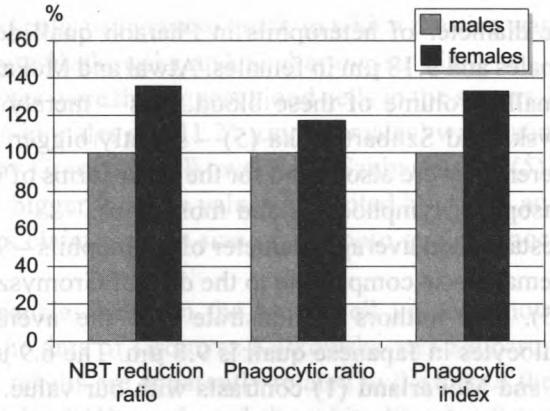

Fig. 10. The NBT reduction ratio, phagocytic ratio and phagocytic index in male and female Pharaoh quail (the values in males were assumed as 100%)

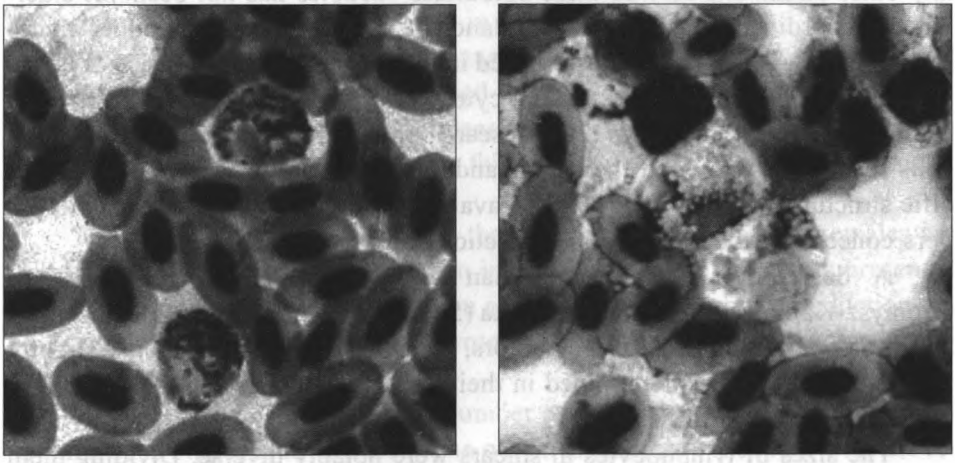

Fig. 11. Phormazane deposit in phagocyting heterophils (A) and latex particles in phagocyting heterophils in Pharaoh quail (B)

DISCUSSION

The characteristic features of white blood cells in Pharaoh quail, established in the present paper, are equal to those described by Gromysz-Kałkowska and Szubartowska (5) in Japanese quail.

However, compared to Japanese quail, there were certain differences regarding the diameter of particular leucocyte forms, which we indicated in our research.

The average diameter of heterophils in Pharaoh quail determined herein was 9.48 μm in males and 9.18 μm in females. Atwal and McFarland (1) demonstrate a much smaller volume of these blood cells – merely 7.7μm, whereas Gromysz-Kałkowska and Szubartowska (5) – slightly bigger, i.e. 9.8 μm.

Similar differences were also found for the other forms of white blood cells – eosinophils, basophils, lymphocytes and monocytes.

The herein established average diameter of eosinophils – 9.60 μm in males and 9.43 μm in females – is comparable to the data of Gromysz-Kałkowska and Szubartowska (5). The authors demonstrate that the average diameter of acidophilic granulocytes in Japanese quail is 9.8 μm. The 6.9 μm diameter presented by Atwal and McFarland (1) contrasts with our value.

The features of quail eosinophils are worth pointing out. Their vacuoles are specific only for this bird species. This kind of structures has not been found in any other birds. The role of these colourless vacuoles has not been yet elucidated. According to Atwal and McFarland (1), they do not contain either glycogen or lipids. A cytochemical assay used in order to reveal the presence of lipase failed, too. It has been suggested that cytoplasmic vacuoles in quail eosinophils contain secondary lysosomes (26). Research on the ultrastructure of these blood cells seems likely to elicit the details and, possibly, the functions of these specific structures. Unfortunately, in the available literature we have found no papers concerning the structure and functions of quail eosinophils.

A description of basophils can only be found in the work of Gromysz-Kałkowska and Szubartowska (5). The diameter of these blood cells in Japanese quail, according to the authors, equals 9.4 μm, which is only slightly bigger than the values determined in their present research, that is, 9.27 μm in males and 9.06 μm in females.

The sizes of lymphocytes in smears were notably diverse. Dividing them into small and large ones was based on the amount of cytoplasm surrounding the nucleus as well as on the size of the nucleus. These criteria were adopted from Lucas and Jamroz (10), according to whom the division into small and large lymphocytes should be based on the size of the nucleus, rather than on the diameter of the whole cell. The authors observed that lymphocytes often discard cytoplasmic vesicles; therefore a cell with a medium-sized nucleus could be wrongly classed as a small lymphocyte.

The average diameter of small lymphocytes – 6.42 μm in males and 6.28 μm in females – was only slightly bigger than that described by Gromysz-Kałkowska and Szubartowska (5) for Japanese quail, which was 6.1 μm. It also fitted in the range established by Atwal and McFarland (1).

The less numerous large lymphocytes had the average diameter of 10.53 μm in males and 10.51 μm in females of Pharaoh quail, and they were smaller than

this form of blood cells in Japanese quail, in which the size was 11.8 μm, as presented by Gromysz-Kałkowska and Szubartowska (5).

The monocytes were the biggest blood cells in the smears. Their average diameter (11.33 μm in males and 11.21 μm in females) was distinctly smaller than that established by Gromysz-Kałkowska and Szubartowska (5) – 12.6 μm; at the same time it was bigger than the values presented by Atwal and McFarland (1). The latter authors claim that the size of Japanese quail monocytes ranges from 6.0 to 8.4μm.

The discrepancies between the blood cell volume shown by Atwal and McFarland (1), the data of Gromysz-Kałkowska and Szubartowska (5) and the herein presented results are apparently related to the age of the examined birds. Atwal and McFarland (1) conducted the white blood cell tests in very young birds aged up to the 50th day of life, while Gromysz-Kałkowska and Szubartowska (5) examined 6-month-old individuals. The present study was carried out on 4-month-old quails, as mentioned in Material and Methods.

As reported in the paper of Kaczanowska et al. (6), in the early stage of quails' life when the chicks are exposed to various kinds of unfavourable environmental factors, there occurs a release of small-sized heterophils from the bone marrow. It can be assumed that this regularity affects the other forms of white blood cells as well.

The conducted research showed that the white blood cells in females are characterised by smaller volumes than in males. Based on the above presented data of Kaczanowska et al. (6), one can assume that females in reproduction period, similarly to chicks, are better protected against disadvantageous environmental factors.

We have also observed that the number of leucocytes as well as heterophils in females is bigger than in males. The obtained data are consistent with the results of Kaczanowska et al. (6). Other authors did not mention this regularity.

The sex differentiation of the values of the discussed white blood cell parameters are related, as it seems, to the process of laying eggs by females. Kaczanowska et al. (6) clearly emphasise that in the experiments they only used egg-laying females, and it is these authors that present the highest values for leucocytes and heterophils. The majority of the females in our research were also laying eggs. According to Witkowski and Paleolog (27), the process of producing and laying eggs generates stress conditions in the organism, resulting in leucocytosis accompanied by an increased level of heterophils.

The increased number of leucocytes and heterophils in mature females is presumably related to the influence of estrogens and activation of pituitary-adrenal axis. The influence of estrogens on granulopoiesis is confirmed by the increased adrenal cortex in maturing females (13). A high level of estrogens stimu-

lates the release of mineralocorticoids and glycocorticoids from the adrenal glands. Mineralocorticoids cause hypertrophy of granulopoietic tissue in the bone marrow, while glycocorticoids depress the bone marrow barrier to heterophils (6).

Assuming that laying eggs by females is stressogenic, one could expect a lower number of lymphocytes in birds of this sex. However, the results of the present research showed an almost identical number of these blood cells in males and females. A slower involution of lymphoidal organs in females seems to be the reason for the lack of sex differentiation. We observed that, as early as since the third week of quails' life, the thymus and the bursa of Fabricius have a bigger weight in females than in males (3).

The analysis of phagocytic activity of quail heterophils carried out in the present study revealed that, compared to mammals, birds are characterised by lower values of the NBT reduction ratio, phagocytic ratio and phagocytic index. That is to say, the value of NBT reduction ratio was 11.08% in Pharaoh quail males and 13.95% in females, while in cow the granulocyte ability of NBT reduction expressed by the NBT cell percentage reached 26% (14). Similarly, Kaczanowska-Taraszkiewicz and Gromysz-Kałkowska (7) showed in the NBT test that the ability of phagocytes to produce peroxide anion was higher in rats than in quails. The authors also observed that the percentage of cells with ingested latex particles was visibly higher in rats than in quails.

In the present study we have determined a higher phagocytic activity of heterophils in females. The obtained results confirm the formerly suggested higher immunity of this sex to disadvantageous environmental factors. The high immunity of females emerges, apparently, as a result of their biological role, namely, of ensuring species survival.

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