

Original research

Morphological characteristics of the pheasant blood modulated with the exposure to natural adaptogen

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Received: 15 December 2022

Revised: 30 May 2023

Accepted: 05 June 2023

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Abstract. The study of the metabolic profile of farming pheasants exposed to the natural adaptogen «Humilid» that contains biologically active feed additive was carried out with using the birds of private farm. The results of present study have shown that treatment with «Humilid» induced positive dynamics in the bird growth and stimulated the development of pheasants from the 1st to the 35th day of age. According to the obtained results, an increase in the number of erythrocytes and hemoglobin content was observed, indicating the modulation of morphological parameters in the pheasant blood during the first 7 weeks of life. The detected hemoglobin content in blood of pheasants exposed to feed additive «Humilid» group was statistically higher for 7.1 % ($p < 0.001$) in compare to the control group. Similar to the increase in hemoglobin content, an observed rise in erythrocyte occurred in pheasant blood during the 21-day treatment with «Humilid». The increase in hemoglobin content was equal to 8.6 % ($p < 0.05$) in compare with control group. Furthermore, the results of present study evidence that the growth of hemoglobin content could increase the capacity of the blood oxygen absorbance and consequently provide most higher level of synthetic processes in the various tissues of the pheasants. Therefore, the application «Humilid» as a feed additive for farming pheasant health maintenance is accompanied by the resistance strengthening through the its adaptogenic, immunostimulatory and growth-stimulating features of this bioactive mixture. «Humilid» and similar feed additives are potent to develop a positive effect on the dynamics of growth and development of farming pheasants through the plural effect to normalize the body weight and blood physiological parameters.

Keywords: bird growth; homeostasis; pheasants; blood morphological characteristics

Морфологічні показники крові у мисливських фазанят на тлі використання природного адаптогену

Анотація. В умовах приватного господарства було проведено дослідження особливостей метаболічного профілю у мисливських фазанів на тлі використання природного адаптогену, а саме за дії застосування біологічно активної кормової добавки «Гумілід». У результаті чого, була відмічена позитивна динаміка росту та розвитку мисливських фазанят до 35 денного віку. За результатами морфологічних показників крові мисливських фазанят до 35 денного віку спостерігається збільшення кількості еритроцитів та гемоглобіну. На тлі дії кормової добавки гумінової природи «Гумілід» вмісту гемоглобіну у крові клінічно здорових мисливських фазанят дослідної групи був в середньому вірогідно вищий на 7,1 % ($p < 0,001$), ніж у птиці контрольній групі. При цьому, зростає середня концентрація гемоглобіну в еритроцитах (МНС) у віці 21 доби в середньому на 8,6 % ($p < 0,05$) відносно контролю, підвищуючи тим самим кисневу ємність крові для забезпечення більшого рівня синтетичних процесів у тканинах організму мисливських фазанят. Отже, вживання Гуміліду мисливським фазанятам забезпечує збільшення резистентності організму птиці, проявляючи свою адаптогенну, імуностимулюючу та рістстимулюючу властивість унаслідок накопичення та післядії. Кормова добавка позитивно впливає на динаміку росту і розвитку мисливських фазанят до 35 денного віку та в цілому нормалізує роботу організму та всіх його фізіологічних показників.

Ключові слова: ріст і розвиток птиці; гомеостаз; фазани; морфологічні показники крові

Cite this article: Haluzina, L. I., & Harashchuk, M. I. (2023). Morphological characteristics of the pheasant blood modulated with the exposure to natural adaptogen. *Theoretical and Applied Veterinary Medicine*, 11(2), 29–33. doi: 10.32819/2023.11015

Introduction

Recently breeding of pheasants for decorative purposes, replenishment of hunting grounds and obtaining dietary and special meat is becoming more and more widespread in Ukraine and in the whole world. Common or hunting pheasants (*Phasianus colchicus*) are most often selected for commercial purposes for breeding purposes. The hunting pheasant is a hybrid form that was obtained as a result of spontaneous crossing of different subspecies of the common pheasant. For decorative purposes, various species and subspecies of pheasants are chosen, including, for example, diamond, golden, silver, eared, horned, royal, blue, etc. (Barton et al., 2020, Song et al., 2020, Sauvala et al., 2021, Hua et al., 2022, Lu et al., 2022, Kivistö et al., 2023).

Biologically active feed additives of a humic nature have been used widely both in animal husbandry and poultry farming, as they have high biological activity and are environmentally friendly, and also contribute to increasing productivity and improving the quality of agricultural animal and poultry products (Stepchenko & Haluzina, 2011, Stepchenko et al., 2017, Disetlthe et al., 2018, Li et al., 2019).

Humic preparations are used in animal husbandry not only as feed additives, but also to increase the non-specific resistance of animals and poultry to various diseases (Haluzina, 2017, Domínguez-Negrete et al., 2019, Nova et al., 2021, Ahfeethah et al., 2023)

Humic substances as feed additives are used in poultry farming to increase the growth and development of young birds, improve the egg production of laying hens and the biological qualities of hatching eggs (Domínguez-Negrete et al., 2021, Hudák, et al., 2021, Ozerov et al., 2022, Proskina et al., 2023, Yurchenko et al., 2023). The results of study demonstrated that adding sodium humate to the diet of chickens, either through a drinking solution or by incorporating humate powder into dry feed, increase growth by 9-14 % (Semjon et al., 2020). Furthermore, the survival rate of young birds improved from 78 96 % in the control group to 98-100 % in the group that received humates. Due to humates, the growth of ducklings increases by 24-43 %. In addition, under the influence of biologically active humic substances, the indicators of hemoglobin content, vitamin A, and nucleic acids are enhanced in young birds (Broshkov et al., 2017, Utkina et al., 2019, Ahmed et al., 2021, Xu et al., 2023).

Adaptogenic, hepatoprotective, antitoxic, immunostimulating, and growth-stimulating properties are well-known characteristics of humic substances (Terry et al., 2018, Samiee-Rad et al., 2022, Maguey-González et al., 2023). The goal of our work was to establish the peculiarities of the metabolic profile of hunting pheasants within the Private Joint Stock Company "Agro-Soyuz" in the Synelnykivsky District of the Dnipropetrovsk Region.

Present study was aimed to examine the impact of natural adaptogen contained humic compounds "Humilid" on the morphological parameters of the blood of hunting pheasants.

Materials and methods

The experimental procedures of the work was carried out in the conditions of PJSC "Agro-Soyuz" of the Synelnykivsky district of the Dnipropetrovsk region on the basis of the pheasant breeding complex. The analysis of biological material from the pheasants in the experimental groups was carried out at the Research Center for Biosafety and Environmental Control of Agricultural Resources at Dnipro State Agrarian and Economic University.

Pheasant chicks from 1 day to 35 days of age were used for the present study. Clinic healthy birds were divided into two groups: control and experimental, in which the feed additive "Humilid" was used, (n = 50). The research scheme is shown in Table 1.

The conditions of housing and feeding were the same in both groups. Pheasants had free access to water and feed. The scientific and economic experiment lasted for 35 days. To the pheasants of the experimental group, a biologically active feed additive of humic nature "Humilid" (TC U 15.7-00493675-004:2009) was added to the water at the optimal dose (Stepchenko & Haluzina, 2013), depending on the average body weight of the pheasants.

Biological material from the pheasants of the experimental groups was collected for biochemical studies on the 14th, 21st and 35th day of the experiment. All manipulations with the bird involved in the experiment were carried out in accordance with the "Provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experiments", adopted by the First National congress on bioethics (Kyiv, 2001).

In samples of the experimental blood stabilized with heparin, the following parameters were determined: hemoglobin content (hemoglobin cyanide method with acetone cyanhydrin), hematocrit index – by the unified micromethod modified by J. Todorov, the number of erythrocytes and leukocytes – by counting with the hemocytometer; the leukogram was determined by the generally accepted method in fixed blood smears stained according to Romanovsky–Giemsa, indices of red blood cells (mean concentration hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC)) – by cell calculation.

Biochemical studies of blood serum of pheasants of both groups were performed using the automatic biochemical analyzer "Miura 200" (Italy) using High Technology reagent kits (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain) at the Research Center for Biosafety and Environmental Control of Agricultural Resources of AIC the Dnipro State Agrarian and Economic University.

The body weight of the bird was measured individually using FR-H-150 scales and a Professional digital tabletop scale 500g/0.01g. The number of birds in groups was taken into account daily for the purpose of determining survival.

The obtained results were estimated statistically with using the Student's t-test, determining the p significant differences between

Table 1 –Research scheme

Age period, days	Group of pheasants	
	Control	Experimental
1 – 7		MCF
8 – 14	MCF	MCF + "Humilid" (0.005 g/kg body weight of pheasants)
15 – 21		MCF
22 – 28	MCF	MCF + "Humilid" (0.005 g/kg body weight of pheasants)
29 – 35		MCF

Note: MCF – the main compound feed.

Table 2 – Morphological parameters of the blood of hunting pheasants treated with "Humilid" (M ± m, n = 5)

Parameters	Pheasant groups					
	C		E		C	
	E		C		E	
	Age of pheasants, days					
	14		21		35	
Hemoglobin, g/L	126.00 ± 1.70	137.00 ± 1.30***	129.00 ± 1.732	134.20 ± 1.241*	122.80 ± 1.594	135.60 ± 1.860***
Hematocrit, %	34.50 ± 0.50	36.20 ± 0.86	39.64 ± 1.300	37.60 ± 0.510	33.42 ± 0.504	36.00 ± 0.680*
Erythrocytes, T/l	3,94 ± 0.14	4.06 ± 0.14	3,98 ± 0.215	3.82 ± 0.220	3.96 ± 0.093	4.04 ± 0.150
MCH, pg	32.13 ± 1.19	33.90 ± 1.20	32.72 ± 1.41	35.60 ± 2.03	31.07 ± 0.80	33.71 ± 1.06
MCHC, %	36.54 ± 0.45	37.90 ± 0,54	32.64 ± 0.76	35.72 ± 0.60*	33.71 ± 0.99	33.71 ± 0.57
MCV, fl	87.93 ± 2.94	89.56 ± 3.53	100.34 ± 4.43	99.74 ± 5.90	84.54 ± 1.99	89.40 ± 2.25
Leukocytes, g/L	16.56 ± 0.46	18.24 ± 0.84	19.38 ± 1.916	20.02 ± 2.415	19.26 ± 1.098	19.14 ± 1.627

Notes: C – control group, E – experimental group; * – p<0,05; *** – p<0,001 – in comparison to the control group.

the investigated indicators of the pheasants in the control and experimental groups. The results of average values were considered statistically reliable at p<0.05*, p<0.01**, p<0.001***.

Results

The results of the morphological blood parameters in control and exposed to feed additive hunting pheasants groups are present in Table 2.

Obtained results showed that against the background of the impact of the "Humilid" humic feed additive at the age of 14 days, the hemoglobin content in the blood of clinically healthy hunting pheasants of the experimental group was higher than in the control group of birds by an average of 8.73 % (p<0.001). This trend in hemoglobin content in the blood of experimental pheasants is observed in the future as well. Thus, at the age of 21 and 35 days, the hemoglobin content of hunting pheasants of the experimental group was higher on average by 4.03 % (p<0.05) and by 10.4 % (p<0.001), respectively, than in the control bird.

According to other morphological blood parameters in hunting pheasants of the control and experimental groups at the ages of 14, 21, and 35 days, no significant changes were observed. Thus, the hematocrit index in the blood of the control and experimental groups of pheasants at the age of 14 and 21 days probably did not differ. This indicator was on average 34.50 and 36.20, respectively, at the age of 14 days and 39.64 and 37.60 at the age of 21 days in the control and experimental groups. (Kowalski et al., 2016, Fernye et al., 2019, Gugala et al., 2019, Williams et al., 2022) However, at

the age of 35 days, the hematocrit index in the experimental bird was higher on average by 7.2 % (p<0.05) than in the control bird.

No significant changes were observed in the number of erythrocytes in the blood of the experimental groups of hunting pheasants aged 14, 21 and 35 days. Thus, at the age of 14, 21, and 35 days, this indicator averaged 3.94 and 4.06; 3.98 and 3.82; 3.96 and 4.04 T/l, respectively, in control and experimental bird groups.

These parameters as the mean concentration hemoglobin (MCH), the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) in all experimental groups of hunting pheasants during their growth probably did not differ from each other and on average were: MCH at the age of 14 days - 32.13 and 33.90 pg, MCHC – 36.54 and 37.90 %, MCV – 87.93 and 89.56 fl, respectively, in the control and experimental groups of birds. At the age of 21 days, MCH – 32.72 and 35.60 pg, MCV – 100.34 and 99.74 fl, respectively, in the control and experimental groups of birds, and according to the indicator of the average concentration of hemoglobin in the erythrocyte (MCHC) in experimental pheasants of this age, this indicator was higher by 8.6% (p<0.05). At the age of 35 days, MCH – 31.07 and 33.71 pg, MCHC – 33.71 and 33.71 %; MCV – 84.54 and 89.40 fl, respectively, in control and experimental birds.

The number of leukocytes in the blood of the experimental groups of hunting pheasants aged 14, 21 and 35 days did not present any significant changes either. Thus, this parameter was on average 16.56 and 18.24 in the groups at 14 days of the age respectively in the blood of the control and experimental groups. Similar to 14 days groups, there were no detected significant changes between the

Table 3 – Leukocyte formula of the blood of hunting pheasants (M ± m, n = 5), %

Parameters	Pheasant groups					
	C		E		C	
	E		C		E	
	Age of pheasants, days					
	14		21		35	
Basophils	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.40 ± 0.245	0.40 ± 0.245
Eosinophils	1.80 ± 0.490	2.00 ± 0.447	2.20 ± 0.583	1.80 ± 0.374	2.40 ± 0.245	2.00 ± 0.316
Heterophiles	56.80 ± 1.068	54.60 ± 1.4000	57.00 ± 1.225	55.60 ± 1.939	54.00 ± 1.304	56.00 ± 0.949
Lymphocytes	35.60 ± 1.435	36.80 ± 0.800	36.60 ± 1.288	37.40 ± 2.159	35.20 ± 1.241	36.60 ± 1.691
Monocytes	5.80 ± 0.583	6.60 ± 0.872	4.20 ± 0.860	5.20 ± 0.860	4.80 ± 0.663	4.40 ± 0.748

Notes: C – control group, E – experimental group.

groups of 21 days of life – 19.38 and 20.02, as well as between the groups of 35 days of life – 19.26 and 19.14 g/L respectively in the blood of the control and experimental bird groups (Table 3).

Therefore, the analysis of obtained results on the leukocyte blood formula have no shown the statistically significant changes between the control and the experimental groups of hunting pheasants during their growth up to 35 days of age. Taking into the account observed in present study results on the blood leukocyte formula, the characteristics for pheasant immunity could be limited with aforementioned data. Besides, obtained results have no detected statistical changes in certain forms of leukocytes in respect to comparative analysis of control and experimental groups in the age periods of 14, 21, 35 days.

Discussion

The study of indicators of the blood, which is a labile system, of hunting pheasants in the dynamics of growth up to 35 days of age is a sufficiently informative study. As is commonly understood, blood upholds the stability of its composition, a crucial factor for the functional unity and normal operation of the entire organism as a unified system.

The hematocrit indicator indicates the percentage ratio of plasma volumes and formed blood elements. It is known that the physiological norm of this parameters for poultry is on average 37.0 % (Hrabčáková et al., 2014, Flis et al., 2019, Damaziak et al., 2020, Lacková et al., 2022) The results of our research, the hematocrit index in the blood of control and experimental birds in the age periods of 14, 21 and 35 days was from 33.42 to 39.64 %.

According to the results of morphological indicators of blood, the feed supplement "Humilid" stimulates the processes of erythropoiesis (table 2). Due to the drinking of Humilid pheasants, an increase in the hemoglobin content at the age of 35 days is observed on average by 9.4 % ($p < 0.001$) compared to the control bird.

At the same time, the mean corpuscular hemoglobin concentration (MCHC) at the age of 21 days increases by an average of 8.6 % ($p < 0.05$) relative to the control, thereby increasing the oxygen capacity of the blood to ensure a higher level of synthetic processes in the organism tissues of hunting pheasants.

Obtained in our study results showed that the leukocyte formula of the blood of hunting pheasants does not change under the influence of the Humilid feed additive. Thus, basophils were absent in the blood of the experimental groups of hunting pheasant chicks at the age of 14 and 21 days, at the age of 35 days the number of basophils was 0.40 % on average in the blood of control and experimental birds.

The number of eosinophils in the blood of control and experimental groups of hunting pheasants chicks at the age of 14, 21 and 35 days was on average 1.8 and 2.00; 2.20 and 1.80; 2.40 and 2.00 % respectively from the total number of leukocytes. The number of heterophils in the blood of control and experimental groups of hunting pheasants chicks at the age of 14, 21 and 35 days averaged 56.80 and 54.60; 57.00 and 55.60; 54.00 and 56.00 % respectively. Regarding the number of lymphocytes in the blood of the control and experimental groups of hunting pheasants chicks, at the age of 14, 21 and 35 days, it averaged 35.60 and 36.80; 36.60 and 37.40; 35.20 and 36.60 % respectively. The number of monocytes in the blood of the control and experimental groups of hunting pheasants chicks at the age of 14, 21 and 35 days was on average 5.80 and 6.60; 4.20 and 5.20; 4.80 and 4.40 %, respectively. The absence of changes in the blood leukocyte formula of hunting pheasants chicks under the influence of the "Humilid" feed additive indicates the absence of a toxic effect of the "Humilid" additive on the organism of the experimental bird. The absence of changes in the number of leukocytes, in our opinion, indicates the absence of a toxic effect of the "Humilid" additive on the organism of the experimental bird. (Mushtaq et al., 2021, Maguey-González et al., 2022, López-García et al., 2023, Xie et al., 2023)

Therefore, the supplementation of a biologically active feed additive of humic nature "Humilid" in the main diet of hunting pheasants chicks up to the age of 35 days has a positive effect on the morphological parameters of the blood of pheasants.

Conclusions

Supplementation of feed additive "Humilid" modulates increase in hemoglobin content in the blood of hunting pheasants chicks for 7.1 % ($p < 0.001$) in compare with untreated control group. This can be explained by the fact that a more active process of hemoglobin synthesis takes place in the blood of the pheasants of the research group, thereby affecting the oxygen saturation of the organism and the release of carbon dioxide. Besides, the mean corpuscular hemoglobin concentration (MCHC) at the age of 21 days increases by an average of 8.6 % ($p < 0.05$) relative to the control, thereby increasing the oxygen capacity of the blood to ensure a higher level of synthetic processes in the tissues of the organism of hunting pheasants.

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