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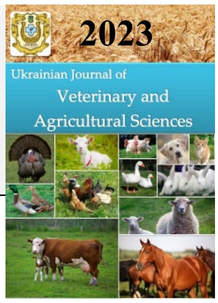

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Biochemical screening of Hisex brown cross chickens after multiple vaccinations

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Abstract

It is impossible to imagine the epizootic well-being of poultry without immunoprophylaxis. Today, there are many vaccines to prevent infectious diseases in poultry. Our work aimed to determine how repeated vaccination affects the biochemical parameters of poultry serum. The post-vaccination changes in the protein, lipid, and enzyme metabolism of the poultry of the experimental group, which received repeated vaccine prophylaxis, are highlighted in the work. The obtained blood serum samples on the 1st, 15th, 25th, 50th, 75th, 100th, and 120th days of the chickens' life were analyzed using a semi-automatic biochemical analyzer for the content of glucose, total protein, albumins, globulins, bilirubin, creatinine, uric acid, and alpha-amylase activity, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma glutamine transferase, and creatinine kinase. The conducted studies established an increase in indicators in the research group of 100-day-old chickens after the last vaccine prophylaxis complex compared to the control group, namely: hyperpotenemia by 8.6 %, hyperbilirubinemia by 19.6 %, hyperglycemia by 20.3 %, hyperenzymemia of alanine aminotransferase by 31 %, gamma glutamine transferase by 21.2 %, aspartate aminotransferase by 7.9 %, alpha-amylase by 3.9 %, alkaline phosphatase by 11.9 %, lactate dehydrogenase by 18.2 %. In connection with this, we can assume the stimulation of biochemical processes in the poultry's body against the background of vaccine prophylaxis.

Keywords: poultry; blood; enzymes; protein metabolism; vaccine prophylaxis.

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1. Introduction

The increase in the number of free economic zones on the territory of Ukraine made it possible to expand the capabilities of many agricultural complexes. In connection with the state of war in the country, industrial poultry farming suffered significant losses. At the same time, the meat and egg industry provides the population with high-quality and complete food products, which occupy almost the first place in the consumer's diet. Today, the demand for egg products has increased significantly due to the affordable price policy.

The industry provides the population with irreplaceable, organic, and energy-important food products for the human body.

Considering the growing demand for increasing the poultry population, preserving the maximum number of individuals and minimizing the death rate is necessary. To prevent the latter, multiple antigenic stimulation is used as the most important method of prevention of infectious diseases.

The primary physiological diagnostic of the clinical status of the bird is blood (Bashchenko et al., 2002). It allows to assess the body's internal state and reflects all internal processes (Martyshuk et al., 2022; Lesyk et al., 2022; Koreneva et al., 2023). Multiple immunizations are associated with high stress, which affects biochemical indicators, which makes it possible to qualitatively and quantitatively detect the effect of multiple vaccinations on the body of chickens.

Poultry farms widely use vaccines to control infectious diseases (Manelli et al., 2007). The use of vaccines aims to minimize and prevent clinical cases of disease at the farm level, thus maintaining and increasing production (Maragon et al., 2008).

Many veterinary pharmaceutical companies produce inexpensive vaccines that are cost-effective and effective (Wang et al., 2006), which for years have shown an accurate indicator of protecting poultry from mass mortality (Bessell et al., 2017).

The effectiveness of poultry vaccination depends primarily on the correctly selected vaccination scheme and its frequency (Campbell & Grohskopf, 2018). The multiple vaccination procedure is stressful, and its effect on the body can lead to metabolic disorders (Ezema et al., 2022).

Evaluation of the blood system of poultry is an important screening test to establish the clinical status of the bird, diagnose possible diseases and select medical treatment in the future (Ihedioha et al., 2011).

Laboratory hematological studies are used to assess the internal state of animals (Kral & Suchy, 2000; Olanrewaju et al., 2017).

The biochemical profile of the blood characterizes the processes of exchange, absorption, and excretion of substances in the poultry's body (Garraud et al., 2018). Changes in the levels of biochemical indicators of blood serum are the results of the work of internal organs and homeostasis in general (Olanrewaju et al., 2016).

The enzymatic activity of the poultry blood system is quite sensitive to external processes (Gumus & Imik, 2016). The activity of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and gamma glutamine transferase in chickens are indicators of protein metabolism, and creatinine kinase characterizes the work of muscles and the heart. Total bilirubin indicates slowing metabolic processes in the bird's body with age (Zheng et al., 2021). In their studies, Puvadolpirod and Thaxton (2000) and Olanrewaju et al. (2006) describe the effect of the frequency of immunization on the level of glucose in the blood serum and claim that vaccination affects the growth of this indicator.

Thus, the study of the effect of multiple vaccinations on the enzymatic and protein parameters of blood serum of Hisex brown cross chickens, which were raised to 120 days of age, is relevant.

The **research aims** to establish the effect of multiple vaccinations on the biochemical parameters of poultry blood at different stages of immunization.

2. Materials and methods

For the experiment, two groups were selected according to the principle of analogs of Hisex brown cross chickens, grown in the conditions of the branch of “Solotvynska Poultry Factory” LLC “Zeleny Val” in the village of Stary Solotvyn, Berdychiv district, Zhytomyr region. The control group was not vaccinated; the chickens in the experimental group were vaccinated according to the plan of vaccinations for repairing young animals.

Blood tests were carried out in the educational-scientific clinical-diagnostic laboratory of the Polissia National University. The material was non-stabilized blood of chickens aged 1, 15, 25, 50, 75, 100, and 120 days selected from control and experimental poultry groups.

During the research, the “General Ethical Principles of Experiments on Animals” (Ukraine, 2001) were observed, which is consistent with the Law of Ukraine “On the Protection of Animals from Cruelty Treatment” dated 07.15.2021 No. 1684-IX and the Provisions of the “European Convention on the Protection of Animals that are used for experimental and other scientific purposes”.

Blood from the chickens of all research groups was collected using a puncture of the right jugular vein, as this method is an intravital method of blood collection and allows re-conducting research at other age stages. A total of 84 blood samples without anticoagulants were obtained. At the same time, sterile tubes of the vacutainer type with a coagulation activator and needles with a thickness of 0.25 mm were used. The test tubes were centrifuged at 4500 rpm for 4 min using a Hermle Z 300 centrifuge to obtain blood serum. Centrifuged serum in 500 µl was transferred using a single-channel dispenser “Satorius 100” – 1000 µl into disposable, sterile Eppendorf microtubes.

The blood serum of the birds was studied on the same day, so no additional manipulations were performed to preserve the samples. The concentration and activity of albumins and globulins, total protein, total bilirubin, creatinine, and uric acid were measured using a semi-automatic biochemical analyzer “Shem-7” (Erba, Czech Republic) and reagents “DAC” (Moldova) using biochemical reactions, according to the manufacturer's recommendations diagnostic sets. Enzymatic activity of blood serum, in particular, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatine kinase, gamma-glutamyltransferase, lactate dehydrogenase, alpha-amylase was determined by the kinetic method using the semi-automatic biochemical analyzer “Shem-7” (Erba, Czech Republic) and reagents “DAC” (Moldova). The analysis was performed in duplicate for each sample.

Statistical processing of the research results was done on a personal computer using variational statistical methods using the Statistica 6.0 program (StatSoft Inc., USA). Fisher's F-test assessed the reliability of the obtained data. The difference between the two values was considered significant at $P < 0.05$ and $P < 0.01$.

3. Results and discussion

With the help of organoleptic tests, it was established that the blood serum of a poultry without signs of destruction of erythrocytes with the release of hemoglobin has a light yellow tint. In the experimental and control groups, no extraneous impurities were recorded in the serum, and it was established that the color parameters of the blood serum did not differ practically.

On the 25th day of the poultry's life, the glucose level was 4.6 % higher than in one-day-old chickens of the experimental groups. Among the experimental groups, the highest glucose level was in 100-day-old poultry and was 10.18 ± 0.54 mmol/l. On the 120th day, the glucose level of the experimental group was 11.09 ± 1.24 mmol/l, which is 17.2 % more than in the control. There were no sharp jumps in the glucose level during the studies. It was established that the concentration in the blood serum was within the physiological parameters.

The total protein content in the blood serum of 75-day-old chickens of the research group increased by 35.1% compared to 25-day-old chickens ($P < 0.05$). The highest rate was observed in 120-day-old chickens, which was 57.97 ± 1.25 g/l. At the same time, in experimental birds of 1, 15, 25, 50, 75, 100, and 120 days of age, a probable increase compared to the control of total protein was noted by 4.5 %, 6.5 %, 2.85 %, 6.4 %, 7.6 %, 8.6 %, 12.2 %, respectively (Table 1).

According to the results of our research, the maximum indicator of the content of globulins is noted already on the 50th day, which indicates the activation of the synthesis of globulins due to the increase in the load on the immune system of the bird after vaccination. It should be emphasized that in the experimental group of chickens of the following age period (75 days), there is an increase in the albumin fraction by 33.2 % compared to one-day-old chickens (P < 0.01), which indicates better assimilation of protein components of feed during the period of active growth against the background of vaccination and effects of stress factors. On the 100th day, the albumin fraction increased significantly due to the decrease in globulins and the formation of the immunogenesis system, which amounted to 19.2 g/l, 35.5 %, and 3.1 % more than on the 25th and 50th days (Table 1).

Table 1
Biochemical indicators of protein and carbohydrate metabolism of chickens (M ± m)

Group of animals, n = 6	Indicators					
	Total protein	Albumins	Glucose	Bilirubin	Creatinine	Uric acid
1 day						
Control	35.95 ± 0.58	13.02 ± 0.23	7.68 ± 0.27	1.63 ± 0.10	72.61 ± 1.11	0.16 ± 0.08
Experimental	37.60 ± 0.76	14.13 ± 0.26	8.26 ± 0.2	1.64 ± 0.12**	81.8 ± 3.4*	0.16 ± 0.02
15 days						
Control	39.60 ± 0.46	13.14 ± 0.23	7.9 ± 0.08	1.83 ± 0.07	84.18 ± 1.54	0.16 ± 0.07
Experimental	42.17 ± 0.54	14.7 ± 0.17	8.52 ± 0.22*	2.34 ± 0.15	92.42 ± 1.04**	0.18 ± 0.02
25 days						
Control	42.52 ± 0.88	13.94 ± 0.21	7.68 ± 0.21	1.76 ± 0.03	93.05 ± 0.83	0.17 ± 0.03
Experimental	42.70 ± 0.90	15.49 ± 0.23	8.72 ± 0.22**	2.4 ± 0.19*	98.78 ± 2.91	0.20 ± 0.72
50 days						
Control	52.04 ± 0.75	15.86 ± 0.28	7.99 ± 0.14	2.11 ± 0.09	103.61 ± 1.65	0.18 ± 0.23
Experimental	55.39 ± 1.08*	18.57 ± 0.36	8.82 ± 0.07**	3.01 ± 0.24*	121.8 ± 3.13**	0.20 ± 0.02
75 days						
Control	53.63 ± 1.18	16.18 ± 0.21	8.22 ± 0.1	2.34 ± 0.18	120.46 ± 0.44	0.18 ± 0.29
Experimental	57.69 ± 0.92*	18.82 ± 0.26**	8.54 ± 0.19	3.26 ± 0.23**	131.6 ± 2.89**	0.22 ± 0.07
100 days						
Control	53.38 ± 0.65	17.02 ± 0.27	8.46 ± 0.13	2.50 ± 0.17	120.47 ± 3.65	0.23 ± 0.82
Experimental	57.98 ± 0.54**	19.16 ± 0.34	10.18 ± 0.22**	2.99 ± 0.31*	128.85 ± 3.33	0.25 ± 0.12
120 days						
Control	52.77 ± 0.58	17.53 ± 0.28	9.46 ± 0.25	2.14 ± 0.09	117.79 ± 4.0	0.24 ± 0.42
Experimental	59.21 ± 1.57**	19.3 ± 0.20	11.09 ± 0.51**	2.32 ± 0.06*	130.23 ± 3.05*	0.35 ± 0.11

* P < 0.05
** P< 0.001 regarding control

It is known that the final products of nitrogen metabolism in poultry are uric acid and creatinine; their concentration is an integral indicator of the physiological state of the excretory system. As a result of changes in the biochemical parameters of the blood serum of chickens of the research group for vaccine prophylaxis, it was established that in 15-day-old poultry, the level of uric acid increased by 12.5 % (P < 0.05), creatinine by 14 % (P < 0.05), compared to one-day. Later, at 50 days, the uric acid and creatinine level in vaccinated chickens was 0.20 ± 0.02 mmol/l and 121.8 ± 3 μmol/l, 11.1 % and 17.5 % more than in the control. In addition, an increase in uric acid by 8.7% and 45.8 % was observed in the vaccinated group compared to non-vaccinated poultry of 100 and 120 days of age. Increased uric acid and creatinine in the blood serum of chickens indicate the activation of metabolic processes in the animal's body during the inductive phase after vaccination (Table 1).

Therefore, protein exchange is accompanied by changes in the intensity of synthesis, first of all, of globulin fractions of blood serum and an increase in protein fractions with age due to vaccination.

Albumin fractions ensure the transfer of micro- and macroelements and hormones to bilirubin. Thus, in the experimental group, we observed an increase in total bilirubin by 35.9 %, 42 %, and 37.8 % on the 25th, 50th, and 75th days compared to the control. In the 100-day-old chickens of the experimental group, the level of bile pigment was 81 % more than in the one-day-old control group. In 120-day-old chickens of the control group, the indicator was 8.8 % less than in the experimental group. Blood proteins tend to regenerate and decay; in particular, the bilirubin level depends on the amount of hemoglobin metabolism.

To obtain more specific information about the origin of hyperglobulinemia and to find out the location of the pathological process in the biliary tract, it is recommended to determine the activity of gamma glutamine transferase (GGT), the increase of which indicates the pathology of the intrahepatic bile ducts.

A change in the activity of GGT in the blood serum of chickens confirms a violation of the metabolism of the bilirubin fraction. Thus, in vaccinated chickens 25 days old, an increase in GGT activity by 32 % was observed compared to the control group. On the 100th and 120th day of the life of the chickens of the research group, the content of this enzyme was higher by 21.2 % and 31.5 % compared to the control. The obtained data confirm increased GGT activity in chickens' blood serum due to the body's stress during multiple vaccinations (Table 2).

A change in the activity of enzymes in the hepatobiliary system indicates pathological processes in exchanging vital substances for the bird's body. Intracellular enzymes ALT and AST are found in high concentrations in the liver, heart, muscles, and red blood cells (Dunets & Slivinska, 2018). It should be noted that an increase in the activity of both enzymes was observed in the blood serum of birds. Thus, the activity of ALT was 6.44 ± 0.23 U/l, 7.99 ± 0.36 un./l, and

AST was 179.11 ± 3.9 un./l, 199.21 ± 10.23 un./l, in 50 and 75-day-old chickens of the research group. Hyperenzynemia was observed in chickens of the experimental group on days 100 and 120. Thus, the AST indicator was 201.42 ± 7.72 un/l, 237.42 ± 3.94 un/l, ALT 8.21 ± 0.26 un/l, 8.31 ± 0.45 un/l, respectively. In the blood serum of all experimental groups of different age periods, an increase in AST activity by 31.9 %, 27.7 %, 22.3 %, 10.7 %, 26.4 %, 28.8 %, 30.1 %, and ALT by 10.3 %, 30.3 %, 37.5 %, 56.7 %, 8 %, 31 %, 18.7 %, compared to the control (Table 2).

An increase in alkaline phosphatase activity in blood serum occurs during animals' intensive growth and development. In poultry, the activity of this enzyme increases during the formation of the egg-forming function. It should be noted that the physiological norm of this enzyme was exceeded by 11.9 % in 100-day-old chickens of the experimental group compared to the control. Thus, the LF content on day 75 was 20.2 % higher than on day 25 of the experimental group. On the 120th day of the study, the LF index in the chickens of the experimental group was 110.6 units/l, which is 5.1 % more than in control.

Table 2
The activity of enzymes of the hepatobiliary system of Hisex brown cross chickens (M ± m)

Group of animals, n = 6	Indicators				
	LF	AST	GGT	ALT	Alpha amylase
1 day					
Control	59.95 ± 2.56	100.12 ± 4.37	9.05 ± 0.17	3.36 ± 0.27	543.66 ± 12.65
Experimental	62.87 ± 3.07	110.42 ± 6.77	9.15 ± 0.2	4.43 ± 0.37	595.55 ± 18.65*
15 days					
Control	64.22 ± 1.65	109 ± 4.52	13.93 ± 0.52	4.01 ± 0.06	610.98 ± 14.04
Experimental	76.42 ± 2.22**	142 ± 7.42**	14.45 ± 0.35	5.12 ± 0.22**	633.76 ± 18.12*
25 days					
Control	79.17 ± 0.86	168.37 ± 3.7	19.22 ± 1.09	5.91 ± 0.15	750.62 ± 26.63
Experimental	84.4 ± 4.14	122.43 ± 4.53**	25.55 ± 1.47	7.23 ± 0.33**	789.94 ± 30.84**
50 days					
Control	88.2 ± 0.75	114.3 ± 4.5	23.64 ± 0.57	5.82 ± 0.23	821.08 ± 19.99
Experimental	92.14 ± 0.69**	179.11 ± 3.9**	24.88 ± 0.69**	6.44 ± 0.23	890.76 ± 25.66**
75 days					
Control	92.39 ± 0.81	184.37 ± 8.44	27.94 ± 0.41	6.32 ± 0.19	1300.96 ± 65.97
Experimental	101.45 ± 3.18*	199.21 ± 10.25**	29.88 ± 0.38**	7.99 ± 0.36**	1418.43 ± 30.86**
100 days					
Control	94.72 ± 1.51	153.72 ± 7.11	25.08 ± 0.7	6.37 ± 0.20	1659.73 ± 45.98
Experimental	106.02 ± 3.95*	201.42 ± 7.42**	30.40 ± 1.39**	8.21 ± 0.26**	1725.8 ± 21.96**
120 days					
Control	105.23 ± 2.46	200.01 ± 5.94	25.15 ± 0.64	6.38 ± 3.5	2019.17 ± 61.9
Experimental	110.60 ± 3.28	237.42 ± 3.94**	31.25 ± 0.73**	8.31 ± 0.45**	2234.41 ± 40.53**

* P < 0.05
** P< 0.001 regarding control

The condition of the pancreas of birds was determined by the activity of the enzyme alpha-amylase in blood serum, which breaks down complex carbohydrates. It should be noted that there was a significant increase in alpha-amylase activity in the birds of the three experimental groups, namely at 75, 100, and 120 days. The highest level of this enzyme was on day 75 and was 1418.43 ± 30.86, which is 9.3 % more than in the control group. The alpha-amylase content on day 120 was 57.7 % higher than on day 75 of the experimental group. In the blood serum of the experimental group on day 120, the enzyme content was 10.6 % higher than in the control group.

Lactate dehydrogenase indicates the growth of the muscular component of the body, the work of the skeletal muscles, and the lymphatic tissue of the bird. A significant increase in this enzyme was observed in experimental groups starting at 50 days of age. The activity of this enzyme was higher in the 50-day-old experimental group by 21.6 % compared to the 25-day-old group. On the 75th, 100th, and 120th day, the LDH indicator was 8.8 %, 18.3 %, and 34.3 % higher than in the control group.

Creatinine kinase is a necessary enzyme for the bird's body to provide energy for muscle contraction. In 50-day-old chickens of the experimental group, a tendency to increase the concentration of creatinine kinase in blood serum by 49.7 % was observed compared to one-day-old chickens. In 75-day-old vaccinated chickens, a continued increase in the concentration of this enzyme by 57.5 % compared to the previous age group was found. A significant increase in creatinine kinase of 100-day-old chickens of the experimental group by 25.7% compared to the control could indicate a critical period of muscle tissue growth and multiple poultry vaccinations (Table 2).

The results of our research indicate the presence of age-related dynamics of changes in serum biochemical indicators in the blood of birds after multiple vaccinations.

4. Conclusions

It has been established that multiple vaccinations do not harm the bird's body. This is confirmed by the fact that the biochemical profile of blood serum was within the physiological norm.

However, some serum parameters tended to increase. In particular, glucose increased by 20.3 %, ALT by 31 %, AST by 7.9 %, creatinine kinase by 25.7 %, uric acid by 8.7 %, and creatinine by 6.7 % compared to the control group on day 100 of the study. Multiple vaccination affects the biochemical parameters of the bird's blood, namely the increase in protein profile and enzyme activity.

Conflict of interest

The authors report no conflict of interest in the presented work.

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