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Biochemical analysis of Hisex brown cross chickens after booster shots

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Abstract

The epizootic health of chickens is unthinkable without monoprophylaxis. There are a variety of vaccinations available for use in modern poultry health management. The purpose of this study was to evaluate the effects of several vaccinations on the biochemical characteristics of serum from chickens. Changes in protein, lipid, and enzyme metabolism after repeated vaccine prophylaxis in the experimental group of fowl are discussed. The levels of glucose, total protein, albumins, globulins, bilirubin, creatinine, uric acid, alpha-amylase activity, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase, and creatinine were determined in blood serum samples taken from chickens on days 1, 15, 25, In this context, it is reasonable to believe that, against the backdrop of vaccination prophylaxis, biochemical activities in the chicken's body are being stimulated.

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

Introduction

Many agricultural complexes were able to boost their production capacity as a result of Ukraine's expansion in the number of free economic zones. Due to the ongoing conflict, industrial poultry farming has taken a major hit. Meat and eggs are staples in most people's diets, and the business that produces them offers them at a high standard and in entire form. The current policy of low prices has led to a dramatic rise in the demand for egg products.

The business supplies the populace with essential, organic, and energy-based food items.Due to rising demands, the poultry population must be protected as much as possible while limiting mortality. The latter is why repeated antigenic stimulation is so crucial in stopping infectious diseases.

According to research by Bashchenko et al. (2002), blood is the major physiological diagnostic of the bird's clinical status. It reflects all internal processes and allows evaluation of the status of the organism internally (Martyshuk et al., 2022; Lesyk et al., 2022; Koreneva et al., 2022). It is feasible to subjectively and quantitatively determine the impact of numerous vaccines on the body of hens since they are linked to excessive stress, which in turn changes biochemical markers.In order to prevent the spread of illness, vaccinations are often used in poultry farms (Manelli et al., 2007). Vaccines are used to limit the spread of illness and keep farms operating at peak efficiency (Maragon et al., 2008).Vaccines developed by a plethora of veterinary pharmaceutical firms are both inexpensive and effective (Wang et al., 2006), and they have reliably prevented widespread mortality in chicken for many years (Bessell et al., 2017).The success of a vaccination program for poultry hinges largely on the scheme chosen and the frequency with which it is administered (Campbell & Grohskopf, 2018). Multiple vaccinations are a demanding process with potential adverse health consequences (Ezema et al., 2022).Clinical state, illness diagnosis, and treatment planning may all be improved with the use of a simple blood test on chickens (Ihedioha et al., 2011).

It is possible to evaluate an animal's health by looking at their blood in the lab (Kral & Suchy, 2000; Olanrewaju et al., 2017).

The purpose of this study is to determine how various immunization schedules affect the biochemical characteristics of chicken blood.

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1. 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 Poul- try 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 Uni- versity. 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 Protec- tion of Animals from Cruelty Treatment" dated 07.15.2021 No. 1684-IX and the Provisions of the "European Conven- tion on the Protection of Animals that are used for experi- mental and other scientific purposes".Blood from the chickens of all research groups was col- lected using a puncture of the right jugular vein, as this method is an intravital method of blood collection and al- lows re-conducting research at other age stages. A total of84 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 dis-posable, sterile Eppendorf microtubes.

The blood serum of the birds was studied on the same day, so no additional manipulations were performed to pre- serve the samples. The concentration and activity of albu- mins and globulins, total protein, total bilirubin, creatinine, and uric acid were measured using a semi-automatic bio- chemical 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, ala-nine aminotransferase, alkaline phosphatase, aspartate ami- notransferase, 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 duplicatefor each sample.

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

2. Results and discussion

With the help of organoleptic tests, it was establishedthat the blood serum of a poultry without signs of destruc- tion 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 exper- imental groups. Among the experimental groups, the highestglucose 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 establishedthat 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% com- pared 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 emphasizedthat 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 for- mation of the immunogenesis

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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 according control

It is known that the final products of nitrogen metabo- lism in poultry are uric acid and creatinine; their concentra- tion 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 addi-tion, an increase in uric acid by 8.7% and 45.8 % was ob- served 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 activa-tion 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 mac- roelements and hormones to bilirubin. Thus, in the experi- mental 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 re-generate and decay; in particular, the bilirubin level dependson the amount of hemoglobin metabolism.

To obtain more specific information about the origin of hyperglobulinemia and to find out the location of the patho- logical 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 bili- rubin 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 en- zyme 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 en- zymes 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

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± 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 ± 7.72un/l, 237.42 ± 3.94 un/l, ALT 8.21 ± 0.26 un/l, 8.31 ± 0.45un/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 se- rum occurs during animals' intensive growth and develop- ment. In poultry, the activity of this enzyme increases duringthe formation of the egg-forming function. It should be noted that the physiological norm of this enzyme was ex- ceeded by 11.9 % in 100-day-old chickens of the experi-mental group compared to the control. Thus, the LF content on day 75 was 20.2 % higher than on day 25 of the experi- mental 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 benoted that there was a significant increase in alpha-amylase activity in the birds of the three experimental groups, name-ly at 75, 100, and 120 days. The highest level of this enzymewas 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 exper- imental group. In the blood serum of the experimental groupon day 120, the enzyme content was 10.6 % higher than in the control group.

Lactate dehydrogenase indicates the growth of the mus- cular component of the body, the work of the skeletal mus- cles, and the lymphatic tissue of the bird. A significant in- crease 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 in- crease 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 increatinine kinase of 100-day-old chickens of the experi- mental group by 25.7% compared to the control could indi-cate 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 indica- tors in the blood of birds after multiple vaccinations.

3. 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 physio- logical 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 bio- chemical 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 presentedwork.

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