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Original Research Article

Changes in Biochemical Markers in Broiler Chickens Exposed to Gadolinium and Lanthanum Orthovanadate Nanoparticles

Key words

gadolinium orthovanadate nanoparticles;
lanthanum orthovanadate nanoparticles;
biochemical markers;
broiler chickens;
blood serum

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Abstract: In our research, we were interested in the presence of changes in the biochemical profile of the blood serum of broiler chickens under the influence of nanoparticles of gadolinium orthovanadate (NP $GdVO_4:Eu^{3+}$), lanthanum orthovanadate (NP $LaVO_4:Eu^{3+}$) and their mixture in therapeutic doses, which were established by us in previous studies on white rats. Day-old broiler chickens of the *Cobb 500* cross (n=150) were used as the object of study. Chickens of the experimental group I received NP $GdVO_4:Eu^{3+}$ for 10 days at a dose of 0.2 mg/L of drinking water, experimental group II – NP $LaVO_4:Eu^{3+}$ at a dose of 0.2 mg/L of drinking water, experimental group III – NP $GdVO_4:Eu^{3+}$ and NP $LaVO_4:Eu^{3+}$ at a dose of 0.2 mg/L of drinking water (on average, chickens received 0.09 (0.13-0.05) mg/kg body weight of NP) and chickens of the experimental group IV received with water the veterinary vitamin drug Devivit Complex to compare the antioxidant effect at a dose of 0.3 ml/L of drinking water, chickens of the control group received drinking water without additives. After 10 days, NP administration was stopped and the chickens were observed for another 5 days. The administration of these nanoparticles to broiler chickens for 10 days was found to lead to a decrease in lipid metabolism (total cholesterol and triglycerides), protein metabolism (uric acid) and lipid peroxidation (diene conjugates and malondialdehyde) against the background of activation of carbohydrate metabolism (increased glucose concentration) and activity of hepatospecific enzymes (alanine and aspartate aminotransferases) with a prolonged effect after discontinuation of administration. The data obtained show that rare earth element orthovanadates nanoparticles have antioxidant properties. These nanoparticles are promising candidates for use in feed additives and veterinary drugs with an adaptogenic effect.

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Introduction

TIn the current state of veterinary medicine, clinical and pathological parameters alone may not provide sufficient diagnostic information for many pathological conditions. Therefore, it is necessary to conduct in-depth studies on the biochemical processes that sustain living matter, as well as the changes that occur during pathology (1, 2).

Determining the quantitative content of various components in biological fluids and tissues of healthy animals, as well as their changes in diseases, allows laboratory tests to perform timely diagnostics (even in the absence of clinical manifestations of the disease), study the pathogenesis, and test the effectiveness of therapeutic measures and drugs. In addition, biochemical studies can be used to monitor the health status of animals and the adequacy of their feeding (3, 4, 5).

Scientific progress has led to the development of many new substances for various industries, including those that use nanotechnology. However, studying the direct effects of these substances on living organisms and assessing their safety and efficacy requires biochemical studies (6, 7, 8). Special attention is given to creating new antioxidant drugs for livestock (poultry) farming in order to meet the demands of intensive agricultural production under stressful conditions (9, 10).

Some nanomaterials have been proven to have antioxidant activity. The most studied ones are nanocerium (11), silica nanoparticles (12), polydopamine nanoparticles (13), and nanoantioxidants based on nanocomposites of polysaccharides and proteins (14, 15, 16). Nanoantioxidants have many advantages over traditional antioxidants. They have increased bioavailability, controlled release, and can be delivered directly to the site of action (17).

Nanoparticles of rare earth orthovanadates, specifically gadolinium and lanthanum, are potential antioxidant substances. In a subchronic toxicological experiment conducted on white rats under conditions of feeding stress, these nanoparticles demonstrated an adaptogenic effect. When administered in doses of approximately 0.03-0.15 mg/kg body weight, the concentration of primary and secondary lipid peroxidation products decreased, while the activity of hepatospecific enzymes normalized (18, 19). Nanoparticles were found to have a positive effect on the intestinal mucosa by activating its mechanical and immunological barrier (20). This discovery led to further research on the effect of these nanoparticles on poultry.

The study aimed to analyze the changes in biochemical markers in broiler chickens' bodies when exposed to gadolinium and lanthanum orthovanadate nanoparticles.

Materials and methods

The place of the experiment

The experiment was conducted on the basis of the vivarium of the State Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise in Kyiv, Ukraine (experimental chickens of all five groups were kept in identical conditions) and in the laboratory of toxicological monitoring of the National Scientific Center «Institute of Experimental and Clinical Veterinary Medicine» in Kharkiv, Ukraine.

Experimental bird, poultry keeping conditions and ration

Day-old broiler chickens of the *Cobb 500* cross (n=150) were used as the object of study. The birds were kept under optimal conditions: at a room temperature of (28 ± 4) °C with a relative humidity of (60-70) %; the day-night lighting cycle during the experiment was (15-9) h, and the vivarium room had 18 air volume changes per hour.

For feeding the chickens, we used the compound feed «PK 5 1-2 weeks Start Broiler», having previously determined the content of nutrients in it: the determination of crude protein content was carried out by the Kjeldahl method according to DSTU ISO 5983:2003, crude fiber - according to DSTU ISO 6865:2004, crude fat – according to DSTU ISO 6492:2003; vitamin content – according to DSTU 4687:2006, trace elements – according to DSTU EN 14082:2019. The research results are summarized in Table 1.

Table 1: Qualitative composition of the diet of broiler chickens (compound feed «PK 5 1-2 weeks Start Broiler»)

Indicator	Actually determined	Norm (21, 22)	± to the norm
Carbohydrates, g/100 g	57,18	Not standardized	–
Energy value, kcal	376,09	290,00	+ 86,09
Mass fraction of fat, %	6,69	Not standardized	–
Mass fraction of crude protein, %	21,79	21,00-22,00	Norm
Mass fraction of crude fiber, %	2,80	No more than 3.0	Norm
Vitamin B2, mg/kg	8,28	9,00	– 0,72
Vitamin A, IU/kg	7920,00	10000,00-13000,00	– 2080
Vitamin E, mg/kg	212,50	80,00	+ 132,50
Selenium, mg/kg	0,172	0,35	– 0,178
Copper, mg/kg	38,67	15,00	+ 23,67
Zinc, mg/kg	144,99	100,00	+ 44,99

The research program was reviewed and approved by the Bioethics Commission of the National Scientific Centre, Institute of Experimental and Clinical Veterinary Medicine in the current order. Animal experiments are in compliance

with the current legislation of EU (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 22 September 2010).

Experimental nanoparticles and comparator drug

In this work, we used experimental samples of gadolinium orthovanadate nanoparticles (NP $GdVO_4$:Eu^{3+}) (spindle-shaped geometry, size 8 × 25 nm) and lanthanum orthovanadate (NP $LaVO_4$:Eu^{3+}) (rod-shaped geometry, size 8 × 80 nm), with an initial concentration of 1.0 g/L (23, 24). The synthesis of gadolinium and lanthanum orthovanadate nanoparticles was carried out according to the method described in the scientific work of Klochkov et al. (23). The experimental samples of nanoparticles were synthesized and standardized according to their stability and size at the Department of Nanostructural Materials named after Yu. Malyukin of the Institute of Scintillation Materials of the National Academy of Sciences of Ukraine (Fig. 1).

Was used as a comparison drug veterinary vitamin preparation Devivit Complex (manufacturer LLC «DEVIE», Ukraine): one milliliter of the preparation contains active substances: vitamin A – 15000 IU, vitamin D_3 – 1000 IU, vitamin E – 20 mg, vitamins B_1 – 10 mg, B_2 – 0.5 mg, B_3 – 25 mg, B_5 – 35 mg, B_6 – 3.0 mg, B_{12} – 30 mg.

Experimental design

On the analog principle, 4 experimental and one control group of day-old broiler chickens (n=30) were formed: chickens of the experimental group I received NP $GdVO_4$:Eu^{3+} solution for 10 days at a dose of 0.2 mg/L of drinking water, experimental group II – NP $LaVO_4$:Eu^{3+} solution at a dose of 0.2 mg/L of drinking water, experimental group III – NP $GdVO_4$:Eu^{3+} and NP LaVO4: Eu3+ at a dose of 0.2 mg/L of drinking water (on average, chickens received 0.09 (0.13-0.05) mg/kg body weight of NP). The chickens of the experimental group IV received with water the veterinary vitamin drug Devivit Complex to compare the antioxidant effect at a dose of 0.3 ml/L of drinking water. The chickens of the control group received drinking water without additives. After 10 days, NP administration was stopped and the chickens were observed for another 5 days. The total duration of the experiment was 15 days.

During CO_2 anesthesia, 10 chickens from each group were euthanized 5 and 10 days after the start of administration and 5 days after the end of administration. Blood samples were taken from the chickens for further determination of biochemical parameters.

Research methods

The serum of experimental chickens was used to determine the content of total cholesterol (TC), total lipids (TL), triglycerides (TGL), total proteins, glucose, uric acid, and the activity level of indicator enzymes aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) – by conventional biochemical methods (27) using reagent kits produced by CORMAY (Poland) and SPE «Filisit-Diagnostics» (Ukraine), the intensity of lipid peroxidation (LPO) in blood serum was determined by the level of formation of its products: primary – diene conjugates (DC) and final – malondialdehyde (MDA) under conditions of extraction in a mixture of heptane-isopropanol (1:1) at wavelengths of 233 and 247 nm (28). The study was performed using a spectrophotometer (SHIMADZU UV-1800, Japan).

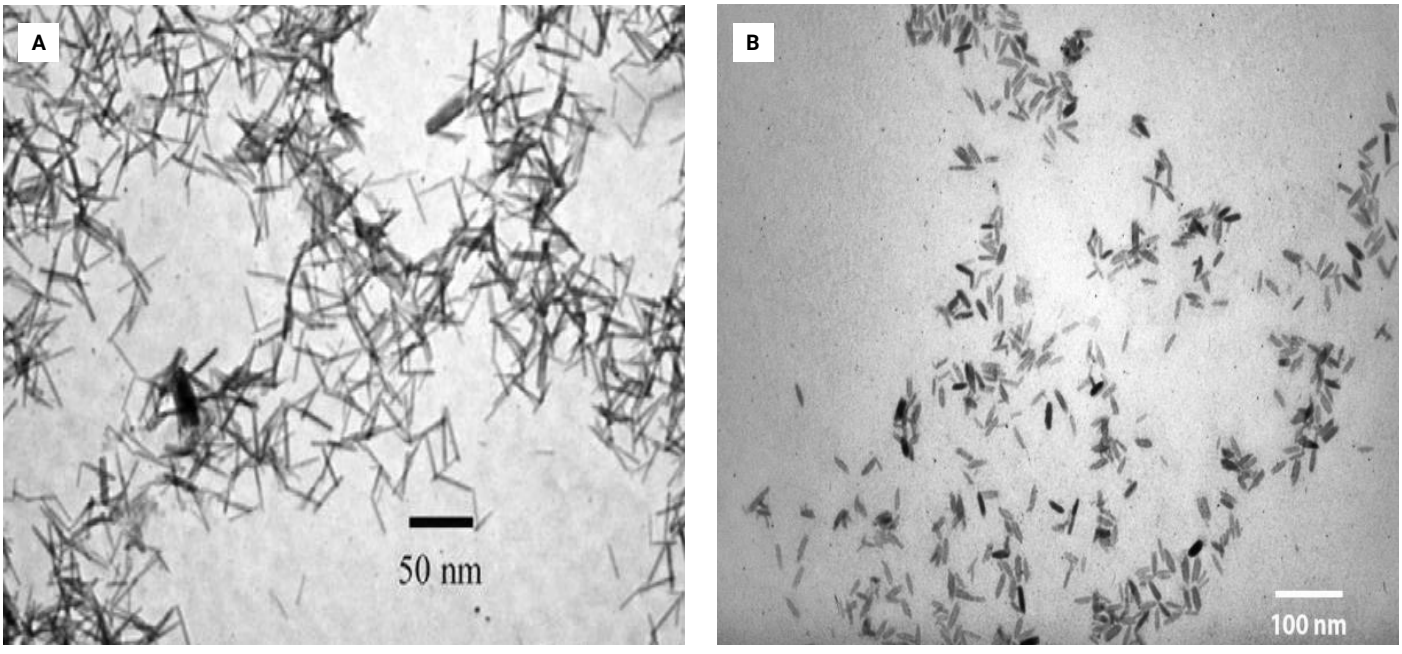

Figure 1: Photograph (transmission electron microscopy, TEM-125K, Selmi, Ukraine) of nanoparticles: A) $GdVO_4$:Eu^{3+}; B) $LaVO_4$:Eu^{3+} (25, 26).

Statistical analysis

The obtained results were processed by methods of variation statistics using the analysis of variance (ANOVA) software package StatPlus 7.6.5.0 (AnalystSoft Inc., USA). The reliability of the results was assessed by the Tukey's test (HSD difference of means) at a reliability level of 95.0% ($P < 0.05$)..

Results

Biochemical studies of blood of broiler chickens revealed certain changes in the lipid profile, LPO indicators and enzymatic activity. Thus, the concentration of TC in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$, 0.2 mg/L of drinking water) on the 5th day of administration exceeded the control value ($P < 0.05$) by 10.2%, on the 10th day – decreased by 19.7% ($P < 0.05$) and remained 21.4% lower than the control 5 days after the end of administration. With the introduction of NP $LaVO_4:Eu^{3+}$, 0.2 mg/L of drinking water (experimental group II), the concentration of TC was lower than the control at all study periods ($P < 0.05$), which was 7.8% on the 5th day, 23.4% on the 10th day, and 31.4% 5 days after the end of the administration. During the period of administration (10 days) of both types of nanoparticles (experimental group III), the concentration of TC in the blood serum of chickens tended to decrease, and 5 days after the end of administration it significantly decreased compared to the control by 26.5% ($P < 0.05$). Similar was the dynamics of TC in the blood serum of chickens after administration of the vitamin preparation Devivit complex: a downward trend during the administration of the drug and a significant decrease compared to the control by 23.8% ($P < 0.05$) 5 days after the end of administration (Table 2).

The concentration of TL in the blood serum of chickens of experimental group I (NP $GdVO_4:Eu^{3+}$) on the 5th day of administration exceeded the control value ($P < 0.05$) by 6.0%, on the 10th day – decreased by 10.9% ($P < 0.05$), while 5 days after stopping the administration it again exceeded the control by 22.1% ($P < 0.05$). When NP $LaVO_4:Eu^{3+}$ was administered (experimental group II), the concentration of TL was higher than the control at all study periods ($P < 0.05$), which was 24.0% on the 5th day, 21.1% on the 10th day, and 12.7% 5 days after stopping the administration. After 5 days of administration of both types of nanoparticles (experimental group III, the concentration of TL in the blood serum of chickens significantly increased compared to the control by 18.0% ($P < 0.05$), after 10 days – it had only a tendency to increase and after 5 days after the end of administration it increased again compared to the control by 34.0% ($P < 0.05$). With the introduction of the vitamin drug Devivit complex, a significant decrease ($P < 0.05$) in the concentration of TL was observed only after 10 days of experiment by 8.9%, while at the first and last terms of the experiment no significant changes were observed (Table 2).

The concentration of TGL in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) significantly decreased compared to the control ($P < 0.05$): after 5 and 10 days of administration by 68.1 and 46.9%, respectively, and 5 days after stopping the administration – by 32.8%. A similar dynamics of TGL concentration was observed in the blood serum of chickens of the second experimental group (NP $LaVO_4:Eu^{3+}$): after 5 and 10 days of administration, the decrease ($P < 0.05$) was 31.9 and 62.5%, respectively, and 5 days after stopping the administration – 13.4% ($P < 0.05$). After 5 days of administration of both types of nanoparticles (experimental group III), the concentration of TGL in the blood serum of chickens significantly increased compared to the control by 13.0% ($P < 0.05$), after 10 days – decreased by 25.0% ($P < 0.05$) and 5 days after stopping the administration remained significantly lower than the control by 13.4% ($P < 0.05$). With the introduction of the vitamin preparation Devivit complex (experimental group IV), the concentration of TGL in the blood serum of chickens was lower than the control at all periods of the study ($P < 0.05$), which was 43.5% on the 5th day, 37.5% on the 10th day and 10.4% 5 days after the stopping of administration (Table 2).

A significant decrease ($P < 0.05$) in the concentration of diene conjugates was observed in the blood serum of chickens of all experimental groups throughout the study period. Thus, the concentration of DCs in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was lower than the control by 29.6, 59.1, and 56.7%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 34.8, 44.1, and 55.4% after 5 and 10 days of administration and 5 days after cessation of administration, respectively. In the III experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of DCs was 25.1% lower than the control after 5 days, 37.1% after 10 days, and 51.0% after 5 days after stopping the administration.

With the administration of the vitamin preparation Devivit complex (experimental group IV), the concentration of DCs in the blood serum of chickens was lower than the control by 44.5, 71.3 and 45.8%, respectively, after 5 and 10 days of administration and 5 days after cessation of administration (Table 3).

Similarly to DCs, a decrease in the concentration of malondialdehyde in the blood serum of chickens of all experimental groups was found throughout the study period. Thus, the concentration of MDA in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was lower than the control by 6.4, 47.7 and 34.6%, respectively ($P < 0.05$). In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 8.0,

Table 2: Dynamics of lipid metabolism in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Total cholesterol (TC), mMol/L			
Control	5,28±0,08	5,17±0,09	5,13±0,08
Experimental group I (NP $GdVO_4:Eu^{3+}$)	5,82±0,10*	4,15±0,08*	4,03±0,08*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	4,87±0,10*	3,96±0,07*	3,52±0,09*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	4,98±0,11	4,82±0,09	3,77±0,08*
Experimental group IV, Devivit Complex	5,17±0,09	5,01±0,11	3,91±0,09*
Total lipids (TL), g/L			
Control	3,17±0,03	3,03±0,04	2,44±0,02
Experimental group I (NP $GdVO_4:Eu^{3+}$)	3,36±0,03*	2,70±0,04*	2,98±0,03*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	3,93±0,03*	3,67±0,02*	2,75±0,04*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	3,74±0,02*	3,17±0,03	3,27±0,07*
Experimental group IV, Devivit Complex	3,21±0,03	2,76±0,04*	2,45±0,03
Triglycerides (TGL), mMol/L			
Control	0,69±0,02	0,64±0,02	0,67±0,01
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,22±0,01*	0,34±0,01*	0,45±0,01*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,47±0,02*	0,24±0,01*	0,50±0,01*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,78±0,02*	0,48±0,01*	0,58±0,01*
Experimental group IV, Devivit Complex	0,39±0,01*	0,40±0,01*	0,60±0,01*

Note * p<0.05 – compared to the control group

17.8 and 34.6% after 5 and 10 days of administration and 5 days after stopping the administration, respectively ($P < 0.05$). In the third experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of MDA tended to decrease after 5 days, after 10 days it significantly decreased by 22.5% ($P < 0.05$) and 5 days after stopping the administration – by 29.2% ($P < 0.05$). In the case of the vitamin preparation Devivit complex (experimental group IV), the concentration of MDA in the blood serum of chickens was lower than the control ($P < 0.05$) by 32.0, 47.3 and 28.0%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 3).

The ALT activity in the blood serum of chickens of the experimental group I (NP $GdVO_4:Eu^{3+}$) had no significant deviations from the control group during the entire study period. In the second experimental group (NP $LaVO_4:Eu^{3+}$), after 5 days of administration, an increase in enzyme activity by 12.7% ($P < 0.05$) was observed, after 10 days no deviations from the control were found, and 5 days after the administration was stopped, an increase in ALT by 25.9% ($P < 0.05$) was again detected. After 5 days of administration of both types of nanoparticles (experimental group III), no significant changes in ALT activity were observed, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity exceeded the control values by 12.3 and 17.2% ($P < 0.05$). With the administration

Table 3: Dynamics of lipid peroxidation indicators and aminotransferase activity in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Diene conjugates DC, μMol/L			
Control	12,37±0,29	13,50±0,28	15,86±0,27
Experimental group I (NP $GdVO_4:Eu^{3+}$)	8,71±0,18*	5,52±0,14*	6,86±0,16*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	8,06±0,22*	7,55±0,21*	7,08±0,18*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	9,26±0,19*	8,49±0,32*	7,77±0,21*
Experimental group IV, Devivit Complex	6,86±0,21*	3,88±0,18*	8,60±0,17*
Malondialdehyde (MDA), ΔD/mL			
Control	2,50±0,04	2,58±0,03	3,18±0,06
Experimental group I (NP $GdVO_4:Eu^{3+}$)	2,34±0,03*	1,35±0,03*	2,08±0,05*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	2,30±0,03*	2,12±0,03*	2,08±0,05*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	2,38±0,03	2,00±0,03*	2,25±0,03*
Experimental group IV, Devivit Complex	1,70±0,04*	1,36±0,03*	2,29±0,03*
Alanine aminotransferase activity, mmol/h×L			
Control	0,55±0,01	0,57±0,01	0,58±0,01
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,55±0,01	0,55±0,01	0,60±0,01
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,62±0,01*	0,58±0,01	0,73±0,01*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,57±0,01	0,64±0,01*	0,68±0,01*
Experimental group IV, Devivit Complex	0,58±0,01	0,59±0,01	0,59±0,01
Aspartate aminotransferase activity, mmol/h×L			
Control	5,70±0,15	6,10±0,09	6,30±0,12
Experimental group I (NP $GdVO_4:Eu^{3+}$)	5,80±0,11	5,60±0,10*	5,80±0,11*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	5,80±0,12	5,80±0,11	6,40±0,10
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	5,90±0,11	5,80±0,08	5,90±0,12
Experimental group IV, Devivit Complex	6,00±0,14	6,80±0,17*	6,80±0,11*
De Ritis ratio, AST/ALT			
Control	10,4	10,7	10,9
Experimental group I (NP $GdVO_4:Eu^{3+}$)	10,5	10,2	9,7
Experimental group II (NP $LaVO_4:Eu^{3+}$)	9,4	10,0	8,80
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	10,4	9,1	8,70
Experimental group IV, Devivit Complex	10,3	11,5	11,5

Note * $p<0.05$ – compared to the control group

Table 4: Dynamics of key indicators of protein and carbohydrate metabolism in the blood serum of broiler chickens receiving different doses of antioxidant drugs with drinking water (M±m, n=10)

Poultry groups	Research periods, days		
	5 days	10 days	5 days after stopping the administration
Total proteins, g/L			
Control	43,68±0,63	43,91±0,44	42,65±0,34
Experimental group I (NP $GdVO_4:Eu^{3+}$)	43,56±0,48	43,34±0,42	42,94±0,40
Experimental group II (NP $LaVO_4:Eu^{3+}$)	42,88±0,47	44,36±0,28	43,28±0,32
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	43,79±0,37	43,68±0,41	43,68±0,35
Experimental group IV, Devivit Complex	43,00±0,51	45,27±0,24	43,28±0,26
Glucose, mmol/L			
Control	11,54±0,17	11,18±0,22	11,18±0,28
Experimental group I (NP $GdVO_4:Eu^{3+}$)	12,99±0,19*	13,28±0,28*	12,77±0,27*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	12,93±0,25*	13,43±0,24*	12,25±0,21*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	12,60±0,22*	13,75±0,25*	11,96±0,20
Experimental group IV, Devivit Complex	12,35±0,12*	13,16±0,28*	12,43±0,28*
Uric acid, mmol/L			
Control	0,53±0,016	0,66±0,014	0,73±0,011
Experimental group I (NP $GdVO_4:Eu^{3+}$)	0,52±0,014	0,61±0,015*	0,46±0,01*
Experimental group II (NP $LaVO_4:Eu^{3+}$)	0,47±0,015*	0,60±0,013*	0,51±0,009*
Experimental group III (NP $GdVO_4:Eu^{3+}$ and $LaVO_4:Eu^{3+}$)	0,44±0,012*	0,53±0,01*	0,49±0,01*
Experimental group IV, Devivit Complex	0,47±0,014*	0,63±0,01*	0,61±0,009*

Note * p<0.05 – compared to the control group

of the vitamin preparation Devivit complex (experimental group IV), ALT activity had no significant deviations from the control group during the entire study period (Table 3).

The activity of AST in the blood serum of chickens of experimental group I (NP $GdVO_4:Eu^{3+}$) after 5 days of administration did not have a significant deviation from the control, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity was lower than the control by 8.2 and 7.9% ($P < 0.05$). With the administration of NP $LaVO_4:Eu^{3+}$ (experimental group II) and NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$ (experimental group III), AST activity did not have significant deviations from the control group during the entire study period. And in the case of vitamin preparation Devivit complex (experimental group IV), AST activity in the blood serum of chickens after 5 days of administration had no significant deviation from the control, while after 10 days of administration and 5 days after stopping the administration, the enzyme activity exceeded the control values by 11.5 and 7.9% ($P < 0.05$) (Table 3).

The concentration of total proteins did not have significant deviations in all groups during the experiment, but during the period of administration in the first experimental group (NP $GdVO_4:Eu^{3+}$) a downward trend was recorded, in experimental groups II and IV (NP $LaVO_4:Eu^{3+}$ and Devivit complex) level of TP tended first to decrease, and then to increase, in experimental group III (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$) the level of TP was close to the control. It should

be noted that 5 days after the stopping the administration, the levels of TP in the blood serum of chickens of all experimental groups tended to increase (Table 4).

A significant increase ($P < 0.05$) in glucose concentration was observed in the blood serum of chickens of all experimental groups during the entire period of the study. Thus, the concentration of glucose in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 and 10 days of administration and 5 days after stopping the administration was higher than the control by 12.6, 18.8 and 14.2%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the excess was 12.0, 20.1, and 9.6% after 5 and 10 days of administration and 5 days after stopping the administration, respectively. In the third experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the glucose concentration was 9.2% higher than the control after 5 days, 23.0% higher after 10 days, and 7.0% higher 5 days after stopping the administration. When administering the vitamin preparation Devivit complex (experimental group IV), the concentration of glucose in the blood serum of chickens was higher than the control by 7.0, 17.7 and 11.2%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 4).

The concentration of uric acid in the blood serum of chickens of all experimental groups during the entire period of research was lower ($P < 0.05$) than the control values. Thus, the concentration of uric acid in the blood serum of chickens of the first experimental group (NP $GdVO_4:Eu^{3+}$) after 5 days of administration tended to decrease, after 10 days of administration and 5 days after stopping the administration was lower than the control by 7.6 and 37.0%, respectively. In the second experimental group (NP $LaVO_4:Eu^{3+}$), the decrease was 11.3, 9.1, and 30.1% after 5 and 10 days of administration and 5 days after stopping the administration, respectively. In the III experimental group (NP $GdVO_4:Eu^{3+}$ + NP $LaVO_4:Eu^{3+}$), the concentration of uric acid was 17.0% lower than the control in 5 days, 19.7% lower in 10 days, and 32.9% lower in 5 days after the administration was stopped. When administering the vitamin preparation Devivit complex (experimental group IV), the concentration of uric acid in the blood serum of chickens was lower than the control by 11.3, 4.5 and 16.4%, respectively, after 5 and 10 days of administration and 5 days after stopping the administration (Table 4).

Discussion

Today, broiler chickens reach slaughter weight twice as fast as 60 years ago. Such changes are associated with hyperphagia and excessive fat deposition, mainly in the body of broilers, which require restricted feed and feeding regimens. Obesity in broilers disrupts reproductive functions and sexual activity and, as a result, changes their productivity: it reduces the yield and quality of meat, which together leads to a loss of profit (29). At the same time, changes in adipocyte differentiation, lipid synthesis, lipolysis, β-oxidation of fatty acids and lipid content were detected in the adipose tissue of chicken embryos from the 12th to the 9th day before hatching, with the number of mitochondrial copies and β-oxidation of fatty acids increasing after hatching, indicating an important role of subcutaneous adipose tissue in providing energy to the poultry (30).

That is why a lot of scientific literature is devoted to lipid metabolism in broiler chickens, especially its control by biochemical markers of blood serum. Thus, the concentration of certain components in the blood serum (plasma) of broiler chickens: TC (31) and TGL (32) mainly decreased in the dynamics of the use of vitamin and mineral preparations, and in the case of the use of cerium dioxide nanoparticles (33), the concentration of TL increased, which is consistent with the results of our studies.

Lipid metabolism is closely related to the antioxidant defense system of broiler chickens and lipid peroxidation (34). Diene conjugates are the primary products of lipid peroxidation, which are toxic metabolites that can damage lipoproteins, proteins, nucleic acids, and enzymes. During the free radical oxidation of arachidonic acid, hydrogen is detached in the alpha position in relation to the double bond, which leads to its movement with the formation of diene conjugates, which are subsequently metabolized into secondary malondialdehyde. The body's antioxidant defense system blocks these compounds, interrupting the chain reaction (35, 36).

The administration of gadolinium and lanthanum orthovanadates nanoparticles led to a decrease in the concentration of primary and secondary lipid peroxidation products (DC and MDA) in the blood serum of experimental chickens, as well as with the administration of a vitamin preparation, indicating their antioxidant effect, with a prolonged effect (a decrease was also observed 5 days after the stopping the administration of nanoparticles).

The liver plays a significant role in neutralizing toxicants, including LPO products. The degree of liver damage is characterized by alanine and asparagine transaminases. However, in poultry, the activity of AST is ten times higher than that of ALT. This can be explained by the fact that the reactions of transamination with alanine, catalyzed by ALT, play a less important role in the metabolism of amino acids in chickens than those with AST. AST is a central component of metabolism, providing substrates for the tricarboxylic acid cycle. Therefore, it participates in the regulation of energy production in oxidative phosphorylation. In poultry metabolism, AST serves as a marker of the intensity of the catabolic vector (3, 4).

Our study found that chickens that received orthovanadate nanoparticles had a decrease in serum AST and De Ritis ratio (AST to ALT ratio) compared to the control group and

the vitamin drug group, where both indicators increased during the experiment. The change in aminotransferase activity is attributed to the hepatoprotective effect of nanoparticles, as established in previous studies (37, 38).

The liver synthesizes various plasma proteins and lipoproteins, as well as low molecular weight biochemically active substances such as creatine, 25-oxycholecalciferol, and heme. Additionally, it produces cholesterol and the end product of nitrogen metabolism, urea (uric acid in poultry) (39, 40). In our study, the concentration of total protein remained unchanged throughout the experiment. However, the concentration of uric acid in the blood serum of chickens decreased ($P < 0.05$) after administration of gadolinium and lanthanum orthovanadates nanoparticles, their mixture, and a vitamin drug. This result is consistent with the findings of Tsekhmistrenko et al., 2020 (38) in the case of the use of nanoparticles of another rare earth element (cerium dioxide) and indicates the activation of protein metabolism. And the concentration of uric acid in the blood serum of poultry decreased under the influence of antioxidant drugs (41, 42, 43).

Rare earth elements have similar biochemical properties to calcium and can replace it in reactions (44). This can affect phosphorus metabolism and, consequently, energy (carbohydrate) metabolism. For instance, when broiler chickens were fed a diet with mineral supplements replaced by 40%, 60%, 80%, and 100% with dicalcium phosphate nanoparticles, glucose concentration increased at 60% replacement but decreased at 40%, 80%, and 100% replacement (45). This partially aligns with our findings on the administration of gadolinium and lanthanum orthovanadate nanoparticles, which resulted in increased serum glucose concentrations in all experimental groups at all study periods.

Conclusions

Nanoparticles of orthovanadates of rare earth elements (gadolinium and lanthanum) show promise as candidates for inclusion in feed additives and veterinary drugs with adaptogenic action, since their administration to broiler chickens for 10 days at a dose of 0.09 mg/kg of body weight (0.2 mg/L of drinking water) leads to a decrease in certain biochemical markers of lipid metabolism (TC and TGL), protein metabolism (uric acid) and lipid peroxidation against the background of activation of carbohydrate metabolism and activity of hepatospecific enzymes. The introduction of nanoparticles (both in mono-solutions and in a mixture) along with a vitamin preparation-comparison confirms their adaptogenic effect on the body of experimental chickens and indicates a prolonged effect (the above trends persisted 5 days after the cessation of administration), but gadolinium orthovanadate nanoparticles showed the best results.

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Spremembe biokemičnih označevalcev pri pitovnih piščancih, izpostavljenih nanodelcem gadolinijevega in lantanovega ortovanadata

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Izvleček: V raziskavi nas je zanimala prisotnost sprememb v biokemičnem profilu krvnega seruma piščancev brojlerjev pod vplivom nanodelcev gadolinijevega ortovanadata (NP $GdVO_4:Eu^{3+}$), lantanovega ortovanadata (NP $LaVO_4:Eu^{3+}$) in njihove mešanice v terapevtskih odmerkih, ki smo jih ugotovili v prejšnjih študijah na belih podganah. Raziskava je bila izvedena na enodnevnih brojlerjih *Cobb 500* cross (n = 150). Piščanci eksperimentalne skupine I so 10 dni prejemali NP $GdVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode, piščanci eksperimentalne skupine II – NP $LaVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode, piščanci eksperimentalne skupine III pa NP $GdVO_4:Eu^{3+}$ in NP $LaVO_4:Eu^{3+}$ v odmerku 0,2 mg/l pitne vode (v povprečju so piščanci prejemali 0,09 (0,13–0,05) mg/kg telesne mase NP). Piščanci poskusne skupine IV so z vodo prejemali veterinarski vitaminski pripravek Devivit Complex v odmerku 0,3 ml/l pitne vode za primerjavo antioksidativnega učinka, piščanci kontrolne skupine pa pitno vodo brez dodatkov. Po 10 dneh smo prenehali dajati NP in piščance opazovali še 5 dni. Ugotovili smo, da je 10-dnevno dajanje omenjenih nanodelcev piščancem brojlerjem povzročilo zmanjšanje presnove lipidov (skupnega holesterola in trigliceridov), presnove beljakovin (sečne kisline) in peroksidacije lipidov (dienskih konjugatov in malondialdehida) ob aktivaciji presnove ogljikovih hidratov (povečani koncentraciji glukoze) in aktivnosti hepatospecifičnih encimov (alanina in aspartat aminotransferaze) s podaljšanim učinkom po prekinitvi dajanja. Pridobljeni podatki kažejo, da imajo nanodelci ortovanadatov redkih zemeljskih elementov antioksidativne lastnosti, zato so obetavni kandidati za uporabo v krmnih dodatkih in veterinarskih zdravilih z adaptogenim vplivom.

Ključne besede: nanodelci gadolinijevega ortovanadata; nanodelci lantanovega ortovanadata; biokemični označevalci; piščanci brojlerji; krvni serum