TRANSOVARIAL EFFECT OF THE MIXTURE OF METAL NANOPARTICLES (AG, CU, FE, MNO₂) ON BIOCHEMICAL INDICES OF BLOOD OF ONE-DAY-OLD CHICKENS COMPARED TO METAL SALTS

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Aim. Due to small sizes of metal nanoparticles (NPMe), after getting into the organism, they penetrate different internal organs, overcoming hematooencephalitic and placental barriers, and may cause different pathophysiological effects in the organism. Thus, the aim of our study was to study transovarial effect of NPMe mixture (Ag, Cu, Fe, MnO₂) on biochemical indices of blood of one-day-old chickens compared to salts of relevant metals.

Methods. The studies were conducted using Hisex White cross chickens (n = 28) with the sex ratio of roosters to hens of 1:6, which were fed for 37 days with the following additives: an experimental sample of NPMe mixture, containing nanoparticles of silver (31.5 ± 0.9 nm), iron (100.0 ± 10.0 nm), copper (70.0 ± 4.0 nm) and manganese peroxidase (50.0 ± 3.0 nm), in the doses of 0.3 and 4.0 mg/kg of bodyweight (experiments II and III); solution of salt mixture – AgNO₃, (CuSO₄·⁵H₂O), (MnSO₄·⁵H₂O) and (FeSO₄·⁷H₂O) (experiment I); distilled water (control). Starting with Day 30 of the experiment, eggs were collected from poultry in each group for a week and placed for further incubation. Blood samples were taken from hatched one-day-old chickens (n = 93) for biochemical studies. The registration of biochemical indices was conducted using spectrophotometer SHIMADZU UV-1800 (Japan).

Results. The experiments determined the mechanism of toxic effect of NPMe mixture after its administration to chickens in the dose of 4.0 mg/kg of bodyweight, which was manifested in decreasing the natural resistance (tendency to the increase in seromucoids – immunosuppressive proteins), excessive formation of toxic products of purine exchange (increase in the concentration of uric acid by 77.6 %; P < 0.05) and straining of enzyme systems of natural detoxication (increase in the activity of alanine aminotransferase and aspartate aminotransferase by 23.8 and 31.6 %; P < 0.05) with the development of oxidative stress (increase in the content of diene conjugates and malondialdehyde by 19.3 and 23.8 %; P < 0.01) in the organism of one-day-old chickens. The mechanism of pharmacological effect of NPMe mixture at its administration to chickens in the dose of 0.3 mg/kg of bodyweight lies in enhancing immune reactions (increasing the amount of general proteins and globulins by 14.4 and 12.0 %; P < 0.05) along with the antioxidant effect via the release of vitamins A and E from the endogenous depot (by 14.7 and 25.7 % above the control; P < 0.05) in the organism of one-day-old chickens.

Conclusions. In conditions of transovarial transmission, there is confirmed advantage of the effect of NPMe mixture (Ag, Cu, Fe, MnO₂) in the dose of 0.3 mg/kg of bodyweight of laying hens comparing to the effect of the analogue in the form of the mixture of metal salts, which may help in achieving a high percentage of liveability of young birds, forming defensive reactions in the organism of chickens and ensuring full realization of genetic potential in terms of meat or egg productivity of agricultural poultry. The mechanism of toxic effect of metal nanoparticles in the dose of 4.0 mg/kg of bodyweight was determined which will allow elaborating safe regulations for the application of non-organic nanomaterials in modern poultry breeding and timely management of the risks of their application.

Keywords: nanotoxicology, poultry breeding, metal nanoparticles, incubation, one-day-old chickens, embryotoxicity.

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INTRODUCTION

Physiologically substantiated (full-fledged) feeding ensures high performance of poultry and good incubation qualities of eggs. However, in addition to nutrients, substances of xenobiotic origin (residues of pesticides and veterinary medicinal preparations, mycotoxins, heavy metals, etc.) may penetrate the eggs from the organism of parents which may have negative effect on the embryogenesis and liveability of the obtained young birds [1, 2]. One of the factors of embryotoxicity risks, occurring in poultry breeding industry in Ukraine, is uncontrolled application of food additives, in particular, premixes with high content of microelements in non-organic form [3] which urges scientists to search for new safe and bioaccessible forms of essential microelements to be used in poultry breeding.

At present, metal nanoparticles (NPMe) meet the aforementioned criteria the most, and their effect on the organism of agricultural poultry is studied intensively but seems rather contradictory [4].

For instance, Ognik et al. (2019) determined that Mn in the form of NP-MnO, decreased the nitration of protein better than in the form of MnO in the organism of turkeys, but the decrease in Mn dose in the ratio from 100.0 to 50.0 mg/kg and then down to 10.0 mg/kg is unfavorable, as it increases the oxidation of proteins and DNA in cells proportionally, decreases the activity of antioxidant enzymes and increases the level of glutathione [5].

The correction of deficient main ratio of broiler chickens with NP-Cu increased the antioxidant potential of the organism and inhibited the peroxidase oxidation of lipids [6].

The studies of Gallocchio et al. (2017) demonstrated that the administration of NP-Ag with the average size of 20 nm to chickens resulted in metal accumulation only in the liver, about 5–20 % were in nanodisperse form, whereas no nanoparticles were found in the remaining edible tissues (muscles, kidneys and eggs) [7]. At the same time, Rezaei et al. (2018) demonstrated that the introduction of 12 mg/l NP-Ag into the drinking water for 30 weeks may lead to oxidative stress and damage to the liver of laying quail hens, which may be a prerequisite for impaired hepatic functions [8]. The results of investigations (Kulak et al. (2018)) show that regardless of the concentration, NP-Ag (5 nm) administration in the dose of 2.87–54.0 mg/bird did not have negative effect on the growth indices of chickens and did not cause Ag accumulation in the pectoralis. The administration of NP-Ag 2.87 mg/bird resulted in its accumulation in the wall of thin intestines and in the liver, and further increase in the dose enhanced Ag accumulation in these tissues. No Ag accumulation was observed in the heart of hens, until the dose amounted to 22.5 mg/bird [9].

Determining the effect of preparations of ultradisperse metal particles (Cu, Zn, Fe, CuZn alloy) on the microbiota composition of caecal cells of broiler chickens allowed obtaining the data which may be used to estimate the possibility of using NPMe in the ratios of poultry as microelement preparations for correction of dysbacteriosis and enhancing the use of feed energy [10], which is proven with the data, obtained by Sizentsov et al. (2018) regarding NP-Cu [11].

The results of the studies on the administration of NP-Au to hens (0.5–2.0 mg/kg of bodyweight for 8–14, 22–28 and 36–42 days) demonstrated that its effect on the immune system of poultry depended both on the dose and the duration of administration. Long-term administration of high doses of NP-Au had negative effect in the form of inflammatory reaction, whereas in the doses of 0.5 and 1.0 mg/kg of bodyweight/day it was recommended to avoid the development of the inflammatory reaction [12].

The investigations of Abedini et al. (2018) established that the addition of NP-ZnO to the ratio of hens had positive effect on the level of general proteins, albumins, glucose, activity of alkaline phosphatase, carbonic anhydrase and the level of Zn (P < 0.05). These data were used to make a conclusion on the reasonability of introducing NP-ZnO additive to the ratio which may enhance the performance of laying hens and become a more efficient source of zinc comparing to usual ZnO in the ratio [13].

However, little is known about the possibility for nanoparticles to accumulate in eggs and thus impact the development of poultry embryos via the organism of parents. There are articles about superficial treatment of incubation eggs and administration of nanoparticles in ovo. For instance, the administration of NP-Ag in ovo, conducted on Day 18 of the incubation in the dose of 15 μg/egg was efficient and modulated the immune response of broiler chickens after hatching, not affecting the hatchability, growth and other parameters of performance [14]. The treatment of eggs with NP-Cu in the dose of 50.0 mg/kg per one incubation day and setting after the release of NP-Cu 20.0 mg/kg of bodyweight with drinking water (for 35 days) resulted in the increase in the final bodyweight of boiler chick-
ens, average daily gain and feed conversion ratio [15]. The article of Mroczek-Sosnowska et al. also proves the positive effect of treating eggs with NP-Cu (2016) [16]. The effect of magnetic nanoparticles of iron oxide on incubation eggs in the dose of 200 μg/ml caused 100 % death of chicken embryos, and in case of applying the dosage range of 10–100 μg/ml, the histological studies determined degeneration of neurons in 50–60 % of samples [17].

Our previous studies established the mechanism of toxic effect of NPMe mixture (Ag, Cu, Fe, MnO₂) in the dose of 4.0 mg/kg of the bodyweight in the organism of laying hens, which was manifested in erythrope-nia, oligochromaemia, immunosuppression, hepatotoxic effect, spending their own antioxidant resources with partial formation of oxidative stress, enhancing the intensity of glomerular filtration, as well as increasing the release of metals from the organism and embryotoxic effect. The composition mixture of the abovementioned NPMe in the dose of 0.3 mg/kg of the bodyweight for 30 days had positive effect on the organism of chickens, incubation qualities of eggs, which was manifested with the enhanced breeding efficiency, breeding conditioned youngsters, mass of hatched chickens compared to metal salts [18, 19].

Thus, the aim of our study was to study transovarial effect of NPMe mixture (Ag, Cu, Fe, MnO₂) on biochemical indices of blood of one-day-old chickens compared to salts of relevant metals.

MATERIALS AND METHODS

The experiment in studying the effect of NPMe mixture on the development of chicken embryos using roosters (n = 4) and laying hens (n = 24) of Hi-sex White cross, 365 days old, with the weight of 1.2–1.6 kg was conducted in conditions of the vivarium of NSC IECVM. Four groups of poultry with the sex ratio of roosters to hens of 1 : 6 were formed by the principle of analogues.

The experimental samples of the following NPMe were used in the work: Argentum (the initial concentration – 86.4 μg/cc in terms of metal, the average size is 31.5 ± 0.9 nm), Ferrum (the initial concentration – 3174.0 μg/cc, the average size is 100.0 ± 10.0 nm), manganese dioxide (the initial concentration – 2785.0 μg/cc, the average size is 50.0 ± 3.0 nm), Cuprum (the initial concentration – 2678.0 μg/cc, the average size is 70.0 ± 4.0 nm) respectively.

The experimental samples of NPMe were synthesized by the method of chemical condensation, reducing relevant salts of metals in the aqueous medium, and standardized in terms of stability and size in the F. D. Ovcharenko Institute of Biocolloid Chemistry, NAS of Ukraine.

Argentum nanoparticles were synthesized via reducing silver nitrate (AgNO₃) in the interaction with 1 % tannin solution and 0.03 n potassium carbonate solution (K₂CO₃). Ferrum nanoparticles were obtained by reducing ferrum chloride (III) (FeCl₃) in the interaction with sodium borohydride. Nanoparticles of manganese peroxidase were synthesized by reducing potassium permanganate (KMnO₄) in the interaction with hydrogen peroxide (H₂O₂). Cuprum nanoparticles were synthesized by reducing cupric sulphate (CuSO₄ · 5H₂O) in the interaction with sodium borohydride. The synthesis was conducted in alkaline conditions of pH concentration. The solution of dextrin ((30,000–40,000) Da) in 0.9 % solution of sodium chloride was used as a stabilizer.

The average size of experimental NPMe samples was calculated by the method of laser correlation spectrometry (LCS). The measurements were conducted with laser correlation spectrometer Zetasizer-3 (Malvern Instruments Ltd, Great Britain).

The visualization of NPMe experimental samples was conducted by the transmission electronic microscopy using JEOL JEM-1230 Electron Microscope (Tokyo Boeki Ltd, Japan).

The abovementioned NPMe were used as a basis to produce the experimental sample of their mixture as a prototype of nano-nutraceutical to be used in poultry breeding, which contained colloid dispersions of nanoparticles of Argentum, Cuprum, Ferrum and Manganese peroxidase in the concentration of 100 μg/cc in terms of metal, respectively.

The NPMe mixture was composed based on the results of previous studies of nanoparticles in vitro, namely, these experimental samples did not have any genotoxic, mutagenic or membrane-toxic effect on isolated pro- and eukaryotic cells [20].

The solution of the mixture of salts of relevant metals in the ionic form was used as a comparing preparation in the experiments – AgNO₃, (CuSO₄ · 5H₂O), (MnSO₄ · 5H₂O) and (FeSO₄ · 7H₂O), the concentration of which equaled 100 μg/cc for each metal.

After feeding experimental chickens of all the groups with standard ratio for 15 days (the alignment period), the chickens of the control group had additional introduction of the physiological solution of sodium.
chloride into their combined feed, and the chickens of the experimental groups had the following additives to their combined feed for 37 days: group I – the solution of metal salt mixture (AgNO₃, CuSO₄ ∙ 5H₂O, MnSO₄ ∙ 5H₂O and FeSO₄ ∙ 7H₂O) in the dose of 0.3 mg/kg of the bodyweight, group II – NPMe mixture in the biotic dose (0.3 mg/kg of the bodyweight) and group III – NPMe mixture in the toxic dose (4.0 mg/kg of the bodyweight) respectively.

Starting with Day 30 of the experiment, eggs were collected from hens of each group for a week and put for further incubation. 93 chickens were hatched during incubation: 19 – from control poultry, 23 – experimental group I (mixture of metal salts), 24 – experimental group II (NPMe mixture, in the dose of 0.3 mg/kg of the bodyweight) and 27 – experimental group III (NPMe mixture, in the dose of 4.0 mg/kg of the bodyweight) respectively. After hatching, one-day-old chickens were clinically examined and weighed, after light chloroform anesthesia they were decapitated with subsequent collection of blood samples for biochemical studies. As the blood volume, obtained from chickens, was insignificant, up to 5 samples from one group were combined to receive reliable data of the sample.

The content of total proteins, albumins, fraction of total globulins, uric acid, creatinine, glucose was studied in the blood plasma of chickens along with the level of enzymatic activity of aspartate aminotransferase (AsAT, K.F. 2.6.1.1), alanine aminotransferase (AIAT, K.F. 2.6.1.2), alkaline phosphatase (AP, K.F. 3.1.3.1) using the set of reagents, manufactured by CORMAY (Poland) and R&D enterprise Felicit-Diagnostika (Ukraine).

The concentration of circulating immune complexes (CIC) of the average molecular mass was determined by the following algorithm. The method was based on selective precipitation of antigen-antibody complexes with 3.75 % solution of polyethyleneglycol M-6000 (PEG) with subsequent photometric determination of precipitate density at the wavelength (λ) of 450 nm. The tubes were added 0.3 ml of the investigated plasma, then 0.6 ml of 0.1 M borate buffer, mixed thoroughly and transferred 0.3 ml into 2 tubes. One of them was added 2.7 ml of 0.1 M borate buffer (control), and the other – 2.7 ml of 3.75 % solution of polyethyleneglycol M-6000 (experiment). The content of tubes was thoroughly mixed and kept for 60 min at room temperature. The optical density of the precipitate was then determined with spectrophotometer at the wavelength of 450 nm. After completing the series of measurements of the extinction of the investigated samples, the difference in the indices of optical density was calculated and the result was multiplied by 1,000. The quantitative values of CIC were obtained in 100 ml of blood plasma. The response was manifested in the units of optical density.

Seromucoids are a specific kind of glycoproteids, which get dissolved in chloric acid, but are unsolvable in phosphatotungstic acid. To determine them, other proteins were precipitated with chloric acid; adding phosphatotungstic acid to the supernatant liquid to precipitate seromucoids, the precipitate was washed, dissolving in the alkali, and the content of hexoses was determined by the reaction with orcin. The number of hexoses characterizes the general content of seromucoids. 0.1 ml plasma was added 1 ml of water and after mixing 2 ml of 0.6 n chloric acid was carefully added along the wall, mixed and kept for 10 min, then centrifuged for 10 min at 4,000 rpm, the supernatant liquid was poured into the clean tube, 2 ml of 5 % solution of phosphatotungstic acid were added, mixed and centrifuged. The supernatant liquid was removed, and the precipitate was added 2 ml of 96 % ethyl alcohol, mixed again and centrifuged for 10 min at 4,000 rpm. The supernatant solution was removed, the precipitate was dissolved in 1 ml of 0.1 n NaOH, with the addition of 8.5 ml of orcin reagent (sulphuric acid + orcin solution, 16 g/l 7.5 : 1). The determination was conducted spectrophotometrically, by the difference in optic density at the wavelength of 280 nm.

The intensity of the processes of peroxide oxidation of lipids (POL) in the blood plasma was determined by the level of formation of its products: primary ones – diene conjugates (DC) and final ones – malondialdehyde (MDA). The principle of this method lies in the fact that after the extraction of lipids with the mixture of heptane-isopropanol (1 : 1) from blood plasma, there is a registered absorption of conjugated diene structures at the wavelength of 233 nm and 247 nm in heptane extracts. Dry glass tubes with friction-fitted lids were added 0.1 ml of blood plasma, then 4.0 ml of fresh mixture of heptane and isopropanol (1 : 1). The “blank” assay was prepared separately using a similar method, but 0.1 ml of distilled water was added to the tube instead of blood plasma. The tubes were covered with glass friction-fitted lids, then shaken in the shaker for 15 min in order to improve the extraction of DC and MDA. To enhance the separation of heptane and isopropanol phases, the tubes were added 1.0
ml of distilled water with pH = 2, the mixture was thoroughly mixed and kept for 20 min. 1.0 ml of the supernatant was extracted with a pipettor from the upper layer and introduced to the measuring cell of the spectrophotometer, then 1.0 ml of ethanol-rectify was added. The “blank” sample was treated similarly to the experimental ones. The measurement of optic density was conducted using spectrophotometer against the “blank” sample: DC – at the wavelength of 233 nm, MDA – 247 nm. DC values were expressed in μmol/l, and MDA – in the units of specific absorption in 1.0 ml (ΔD/ml).

The state of the antioxidant system (AOS) was determined by the level of enzymatic activity of catalase (K.F. 1.11.1.6). The method is based on the capability of hydrogen to form a stable dyed complex with ammonium molybdate with the maximal absorption at the wavelength of 410 nm. A glass tube was added 0.1 ml of blood plasma of chickens and then 3.9 ml of distilled water, mixed thoroughly and kept for 10–15 min (a working mixture). Two dry tubes (experimental and control) were added 2.0 ml of buffer-substrate mixture each (10.0 ml of tris-HCl buffer (pH = 7.4) + 30.0 ml of 0.04412 N of hydrogen peroxide solution), mixed thoroughly and incubated in a water bath at 37 ± 1°C for 10 min. The experimental tube was added 0.1 ml of the working mixture, mixed thoroughly and incubated in the water bath at 37 ± 1°C for 3 min (using a seconds counter). The reaction was stopped by the addition of 2.0 ml of 4.5 % solution of acid ammonium molybdate into the experimental tube. The control tube was simultaneously added at first 2.3 ml of 4.5 % solution of acid ammonium molybdate, then 0.1 ml of the working mixture. The optic density of the content of the experimental and control tubes was measured spectrophotometrically at the wavelength of 410 nm (a cell of 10 mm). A mixture, consisting of 1.0 ml of tris-HCl buffer, 3.0 ml distilled water and 0.1 ml of the working mixture, was used as a comparison solution. The activity of the enzyme was expressed in nmol of H₂O₂/sec mg of protein.

The level of total antioxidant activity (total AOA) of lipids, extracted from blood plasma, was determined by the degree of their capability to inhibit the accumulation of active products of peroxidation of thiobarbituric acid (TBA) in case of incubating plasma with the suspension of yolk lipoproteins (YLP). Two dry tubes were prepared per each investigated sample: No. 1 and No. 2, which were introduced the reagents in the following order:

- 0.15 ml of mother liquor of YLP (prepared with the yolk of chicken eggs, subjected to homogenization in the phosphate buffer (40 mM of potassium monophosphate solution + 105 mM potassium chloride solution) with pH = 7.45);
- 0.10 ml of blood plasma;
- 0.65 ml of phosphate buffer with pH = 7.45;
- 0.10 ml of 25 mM of ferrous sulphate solution in 0.002 N of chlorohydric acid solution.

After adding the ferrous sulphate solution, tubes No. 1 were immediately added 0.5 ml of 20 % trichloroacetic acid, then calamity was observed in the tubes.

Tubes No. 2 were placed into a hot air oven (37 ± 1°C) and incubated for 15 min. After the incubation, these tubes were also introduced 0.5 ml of 20 % trichloroacetic acid each.

The experimental samples were prepared along with the “blank” sample (tube No. 3) and two controls (tubes No. 4 and No. 5) using the similar procedure. Only the “blank” sample was not introduced the mother liquor of YLP and plasma, instead, 0.90 ml of phosphate buffer with pH = 7.45 was added. Control samples were introduced 0.75 ml of phosphate buffer with pH = 7.45 instead of plasma. The control samples were treated similarly to the experimental ones (tubes No. 5 were incubated in the hot air oven, and the reaction in tubes No. 4 was immediately stopped, adding 20 % of trichloroacetic acid.)

All the tubes were centrifuged at 900 g for 15 min. Then the supernatant was extracted from the tubes and transferred into previously signed new tubes. The tubes were added 1.0 ml of 0.5 % solution of 2-thiobarbituric acid and placed on the water bath (100 ± 1°C) for 15 min. After boiling, the tubes were cooled under the stream of cold water.

The absorption spectrum of TBA-active products was registered spectrophotometrically at the wavelength of 535 nm. The measurements of the optic density of experimental and control samples were conducted against the “blank” sample. AOA was expressed as the percentage of inhibition of oxidation for yolk lipoproteins after comparing the results of measurements in all the tubes.

The level of vitamin A was determined as follows: 0.2 ml of plasma was introduced into a glass tube with subsequent addition of 1.0 ml of ethanol-rectificate and mixed using a glass stirring rod. Then 2.0 ml of diethyl ether was added, shaken rigorously for 10 min and centrifuged at 900 g for 20 min. After centrifugation, the
upper fraction was extracted, and added 1.0 ml of diethyl ether. The measurements were spectrophotometrically conducted at the wavelength of 400 nm. The amount of vitamin A was estimated by the calibration curve per oil solution of the vitamin and expressed in mg/cu dm.

The principle of the method of determining the content of vitamin E in blood plasma of chickens lies in reducing tocopherol with ferric chloride and determining the newly formed bivalent iron in the form of pink-red complex with 2,2-bipyridyl. The centrifugation tubes were introduced 0.2 ml of blood plasma, added 2.0 ml of ethanol-rectification, 6.0 ml of diethyl ether, shaken rigorously for 3 min and centrifuged at 900 g for 5 min. The centrifugate was filtered into a flask via a filter with 15 g of anhydrous sodium sulphate, the filter was washed with diethyl ether to complete the volume up to 5.0 ml. The ether was evaporated on the rotation evaporator at (50 ± 1 °С). The dry residue was dissolved in ethanol and the volume was completed up to 4.0 ml in the measuring cylinder of 10 ml. The extract was added 1.0 ml of ferrous dipyridyl reagent (3 % solution of ferrous chloride in 1 % solution of 2,2-dipyridyl in ethanol) and placed in a dark place for 10 min. The measurements were spectrophotometrically conducted at the wavelength of 520 nm against the diethyl ether. The amount of vitamin Е was estimated by the calibration curve per oil solution of the vitamin and expressed in μmol/cu dm.

The registration of biochemical indices was conducted using spectrophotometer “SHIMADZU UV-1800” (Japan).

The statistical processing of the study results was conducted in Statistica 6.0 (StatSoft Inc., USA). The probability of the obtained results was estimated by Tukey test with Bonferroni correction.

### RESULTS

The biochemical studies of blood plasma of one-day-old chickens, hatched from the eggs of laying hens in conditions of long-term introduction of metal additives in different disperse forms established the following changes: no statistical changes in the level of proteins compared to the number of albumins were registered in blood plasma of chickens, hatched from the hens, which were given metal salts (experiment I) and NPMe mixture in the dose of 4.0 mg/kg of the bodyweight (experiment III), but the total number of globulins had a tendency towards decreasing compared to their control values (Table 1).

It should be noted that the tendency towards increasing the concentration of seromucoids, belonging to α-fetoproteins – proteins-immunosuppressors, by 8.9 and 3.6 % on average, was registered in blood plasma of chickens in these experimental groups. On the contrary, chronic alimentary intake of NPMe mixture in the biotic dose (experiment II) to the ratio of laying hens resulted in the increase of the content of total protein and the number of total globulins in blood plasma of chickens, the values of which exceeded the control ones by 14.4 and 12.0 % on average (P < 0.05) respectively.

After the alimentary introduction of the mixture of metals in macro- and nanodisperse forms (experiments I, II and III) to the organism of chickens, no statistically reliable deviations in the content of glucose and creatinine were registered (Table 2). The level of uric acid in blood plasma of chickens from poultry, which received NPMe mixture in a larger dose (experiment III, 4.0 mg/kg of the bodyweight), was reliably increased by 77.6 % on average compared to the control. Also, the tendency towards the increase in the level of creatinine was observed in blood plasma of poultry from

### Table 1. The level of protein profile indices and non-specific resistance in blood plasma of chickens, hatched from laying hens at chronic alimentary introduction of solutions of the mixture of metal salts and NPMe mixture (M ± m; n = 5)

| Indices                  | Control | Experimental |
|--------------------------|---------|--------------|
| Total proteins, g/cu dm  | 45.00 ± 3.02 | 44.60 ± 1.45 |
| Albumins, g/cu dm        | 10.25 ± 1.52 | 11.80 ± 0.82 |
| Total globulins, g/cu dm | 34.75 ± 2.10 | 32.80 ± 3.12 |
| CIC of mean molar mass, mg/cc | 0.16 ± 0.03 | 0.15 ± 0.02 |
| Sm, mg/cc                | 4.50 ± 0.22 | 4.90 ± 0.15 |

Note: * P < 0.05 – to the control.
this experimental group. These changes illustrate the disorders in the intensity of glomerular filtration in the kidneys of experimental chickens and are likely related to the rate of biotransformation (elimination) of the excessive amount of NPMe from the organism of poultry.

At the background of activating protein decomposition, an expressed straining of enzymatic systems of natural detoxication and POL system indices were registered in blood plasma of chickens in experimental group III.

Enhanced processes of natural detoxication in the liver of chickens of this group is evident in the increase in the activity of both transaminases – AlAT and AsAT and the decrease in AP activity, which was 23.8; 31.6 (P ≤ 0.05) and 9.0 % respectively on average, compared to the values of this activity in the blood of birds under investigation. The increase in AIAT activity (P ≤ 0.05) and insignificant activation of AsAT by 11.9 and 6.3 % on average was determined in blood plasma of chickens in experimental group I (Table 3).

The index of primary POL products – diene conjugates (DC) in blood plasma of chickens had the following dynamics: no reliable difference against the control was determined in experimental group I, whereas in experimental group II (biotic dose of NPMe) the content of DC was reliably lower, by 23.1 %, than the control, and in experimental group III (toxic dose of NPMe) – it exceeded control by 19.3 % (P < 0.05; P < 0.01) respectively (Table 4).

The index of final POL products – malondialdehyde (MDA) – in blood plasma of chickens from experimental groups I and II had no reliable differences from the control values, and in experimental group III – exceeded by 23.8 % (P < 0.01) respectively.

The level of catalase activity had reliable changes in groups. For instance, the chickens in experimental groups I and II had lower index values compared to its control level – by 11.5 and 10.9 % respectively, whereas in experimental group III they were 14.3 % higher (P < 0.001). The index of total AOA in blood plasma of chickens in experimental group I had no reliable differences from its control level, in experimental group II it exceeded the control by 10.2 %, and in group III – decreased by 7.8 % (P < 0.05) respectively.

The content of vitamins A and E in blood plasma of chickens in experimental group I (metal salts) had

| Poultry groups | Indices | Glucose, mmol/cu dm | Uric acid, μmol/cu dm | Creatinine, μmol/cu dm |
|----------------|---------|---------------------|-----------------------|-----------------------|
| Control        |         | 4.90 ± 0.21         | 218.00 ± 25.20        | 72.05 ± 6.12          |
| Experimental   |         |                     |                       |                       |
| I, Me salts, 0.3 mg/kg of the bodyweight | 5.20 ± 0.30 | 206.30 ± 15.10 | 72.51 ± 5.50 |
| II NPMe, 0.3 mg/kg of the bodyweight   | 5.10 ± 0.20 | 252.20 ± 31.60 | 71.80 ± 4.82 |
| III NPMe, 4.0 mg/kg of the bodyweight  | 4.70 ± 0.24 | 387.10 ± 28.10 ** | 80.02 ± 4.12 |

Note: * P < 0.05 – to the control.

| Poultry groups | Indices | AlAT, μmol/h cc | AsAT, μmol/h cc | AP, nmol/sec cu dm |
|----------------|---------|----------------|----------------|--------------------|
| Control        |         | 0.84 ± 0.06    | 1.90 ± 0.10    | 1,747.50 ± 57.10   |
| Experimental   |         |                |                |                    |
| I (metal salts, 0.3 mg/kg of the bodyweight) | 0.94 ± 0.06 ** | 2.02 ± 0.08    | 1,695.30 ± 114.90  |
| II (NPMe, 0.3 mg/kg of the bodyweight)   | 0.83 ± 0.02 | 2.00 ± 0.30    | 1,682.90 ± 73.30   |
| III (NPMe, 4.0 mg/kg of the bodyweight)  | 1.04 ± 0.05 ** | 2.50 ± 0.05 ** | 1591.00 ± 149.00   |

Note: * P < 0.05 – to the control.
no reliable changes from its control values, in experimental group II (biotic dose of NPMe) it exceeded the control by 14.7 and 25.7 % respectively, and in experimental group III (toxic dose of NPMe) the content of vitamin E was 22.7 % lower (P < 0.05) respectively, and that of vitamin A did not differ from the control.

The results, obtained in terms of the indices of the functional state of liver and the POL system, characterize the degree of manifestation of hepatotoxic effect of NPMe mixture in its toxic dose (experiment III; 4.0 mg/kg of the bodyweight) and are in agreement with the determined transformations in the proteinogram, the presence of immunosuppression in the organism of young birds under investigation and the development of the oxidative stress.

The pharmacological effect of NPMe mixture on the organism of chickens in the biotic dose (0.3 mg/kg of the bodyweight) had an advantage over the effect of its analogue in the form of the mixture of metal salts and lied in the adaptogenic effect, namely, enhancing immune reactions along with the antioxidant effect due to the release of vitamins A and E from the endogenous pool in the organism of chickens.

**DISCUSSION**

It should be noted that we have not found any scientific data about the combined effect of NPMe on a living organism or about their transovarial transmission. However, the data obtained were in agreement with the results of many scientists regarding the effect of one NPMe kind on the organism of both laboratory animals and poultry and its embryos.

For instance, A.O. Pryskoka investigated the impact of multiple intravenous administrations of NP-Ag in different doses (1.6; 8.0 and 16.0 mg/kg of the bodyweight) on biochemical indices of blood serum of mice. A statistically reliable dose-dependent increase in the activity of AIAT and a decrease in the activity of AsAT, and as a result, a decrease in the ratio of their activity indices was determined. A decrease in concentrations of total bilirubin and creatinine was noted in blood serum of mice, administered NPMe as 16.0 mg/kg of the bodyweight [21]. Sarhan & Hussein (2014) abdominally administered 0.5 ml of distilled water, containing NP-Ag in the dose of 2,000 mg/kg of bodyweight with the subsequent second injection 48 h later, to white rats of the experimental group. Control rats were administered a double dose of distilled water. Blood samples were taken on day 3 of the experiment. The results demonstrated a considerable increase in the level of creatinine, urine and activation of both aminotransferases [22]. To determine potential systemic toxicity of NP-Ag in the amounts of 20 and 100 nm, rats were intravenously administered solutions of NPMe in the dose of 6.0 mg/kg of the bodyweight for 28 days. The increase in enzymatic activity of AP, AIAT and AsAT was determined in the results, which indicated a liver damage. Here the most evident toxic effect was almost complete inhibition of the activity of mononuclear cells in the spleen, and such immunosuppression features as

**Table 4.** The level of indices of peroxide oxidation of lipids and its antioxidant regulation in blood plasma of chickens, hatched from laying hens at chronic alimentary introduction of solutions of the mixture of metal salts and NPMe mixture (M ± m; n = 5)

| Poultry groups | POL/AOS indices |
|----------------|----------------|
|                | DC, µmol/cu dm | MDA, ΔD/cc | Catalase activity, nmol of H₂O₂/sec mg of protein | Total AOA, % of inhibition | Vitamin E, µmol/cu dm | Vitamin A, mg/cu dm |
| Control        | 21.96 ± 0.75   | 1.43 ± 0.06 | 34.72 ± 0.84 | 55.57 ± 0.91 | 16.74 ± 0.71 | 0.74 ± 0.04 |
| Experimental I| 20.73 ± 0.86   | 1.37 ± 0.05 | 30.74 ± 0.29** | 56.45 ± 0.96 | 18.48 ± 0.90 | 0.81 ± 0.03 |
| II (NPMe, 0.3 mg/kg of the bodyweight) | 16.89 ± 0.37*** | 1.43 ± 0.07 | 30.94 ± 0.72** | 61.26 ± 0.65** | 19.20 ± 0.54** | 0.93 ± 0.01** |
| III (NPMe, 4.0 mg/kg of the bodyweight) | 26.20 ± 0.35** | 1.77 ± 0.02** | 39.67 ± 0.27*** | 51.22 ± 0.72** | 12.94 ± 0.34** | 0.76 ± 0.05 |
I.R. Shamsutdinova et al. studied the effect of NP-Ag on the organism of rats: the animals in experimental groups were fed with aqueous dispersion of NP-Ag added to drinking water in the daily dose of 4.25; 6.61 and 12.81 mg/kg of the bodyweight for 30 days. The investigations established that oral introduction of aqueous NP-Ag dispersion into the organism of rats had more significant effect on the level of AsAT activity in blood plasma, supernatant of liver and kidneys compared to AIAT, which confirms their participation in the functioning of mitochondria and energy exchange; enzymatic disorders are more expressed in the supernatant of liver compared to the kidneys, which is related to a greater participation of the organ in metal elimination; changes in the activity of aminotransferases in blood, supernatant of liver and kidneys are dose-dependent; the introduction of aqueous NP-Ag dispersion in the daily dose of 12.81 mg/kg had membranotoxic effect on kidney cells [24]. When feeding broiler chickens with NP-Ag (14 ± 0.8 nm) in the doses of 4.0; 8.0 and 12.0 mg/kg of feed for 21 days, the increase in the concentration of triglycerides, lipoproteins of low and very low density and uric acid (P ≤ 0.05) was established in the blood serum of poultry [25], whereas the administration of the same NP-Ag in the doses of 20.0; 4.0 and 60.0 mg/kg of feed demonstrated a considerable increase in MDA content, the activity of catalase and superoxide dismutase (P < 0.01) along with the decrease in the concentration of total proteins, albumins, total cholesterol and enzymatic activity of AIAT and AsAT (P < 0.05) [26].

A.A. Strode studied NP-Cu with the diameter of 50–60 nm. Aqueous suspension was administered subcutaneously to white outbred mice once a day in the dose of 0.05 mg/kg for three days. Then the level of enzymatic activity of AIAT, AsAT and KFK was determined in blood serum of animals. It was determined that NP-Cu did not have considerable effect only on the activity of AIAT, the activity of other investigated enzymes increase at the effect of NPMe. The activity of AsAT increased 2.5 times, and the activity of KFK – 2.1 times compared to the indices of the control group. The increase in the activity of enzymes with intracellular localization in blood serum is a marker of cytolysis. Thus, in case on subcutaneous administration, NP-Cu manifested its damaging effect of the cardiac muscle cells, as the maximal activity of KFK and AsAT was manifested in myocytes [27].

A.A. Slobodskov studied the effect of intramuscular administration of NP-Cu on biochemical indices of blood of pregnant female rats. It was determined that the administration of NP-Cu in the dose of 2.0 mg/kg of the bodyweight resulted in the termination of pregnancy, and at the concentration of 0.5 and 1.0 mg/kg of bodyweight the pregnancy was not terminated: on day 20 the fetuses were viable without any congenital anomalies. All the experimental groups had a decrease in total protein and bilirubin in blood serum. The highest decrease was noted at the administration of NP-Cu in the concentration of 2.0 mg/kg (total proteins decreased 1.5 times, bilirubin – 2.5 times). A considerable decrease in the level of triglycerides and cholesterol, 8 and 2 times on average to the control, was determined in the same group. The administration of NP-Cu in the concentration of 0.5 and 1.0 mg/kg of bodyweight resulted in the increase in the level of alpha-amylose, which indicated the damage to pancreas tissue [28]. The investigations of Ognik et al. (2019) determined that 4-week-long administration of NP-Cu with feeds to rats in the doses of 6.5 and 3.25 mg/kg of the bodyweight resulted in the increase in ceruloplasmin activity and the content of DC and MDA and the decrease in catalase activity and the level of total glutathione [29]. However, the study of the effect of NP-Cu on the development of chicken embryos at the administration of their solution in the dose of 0.3 cc into the air pouch determined the inhibition of lipid oxidation process in embryos [30]. A decrease in the concentration of glucose and cholesterol in blood serum was determined in case of a similar way of administering NP-Cu into incubation eggs in the dose of 50 mg/kg of the bodyweight, after hatching of chickens and their 42-day-long breeding [31].

M.K. Sljunjaeva investigated NP-Fe powder, the diameter of particles in which was 50–60 nm. NP-Fe was administered to male mice subcutaneously in the form of suspensions with physiological solution once a day for three days (a daily dose was 50.0 mg/kg of the bodyweight). The following changes were revealed by the conducted studies. The activity of AsAT in all the experimental animals increased 8.8 times, the activity of AIAT – 18 times compared to the control group, and the activity of KFK increased 2.9 times in the experimental group. The maximal activity of AsAT and KFK was noted in the cardiac muscle, and AIAT demonstrated maximal activity in liver cells, therefore, based on the results obtained, the author assumed that at subcutaneous administration NP-Fe had hepatotoxic
effect and damaged myocardium cells [32]. The intravenous administration of NP-Fe$_2$O$_3$ to white rats in the doses of 7.50; 15.0 and 30.0 mg/kg of the bodyweight once a week for 28 days demonstrated that Fe$_2$O$_3$NP doses of 7.50; 15.0 and 30.0 mg/kg of the bodyweight have dose-dependent inhibition (P < 0.05) of antioxidant enzymes along with the increase in the level of MDA (P < 0.05). The authors made a conclusion that NPMe triggered an inflammatory reaction, which caused the oxidative stress and might have a negative effect on cellular functions [33]. The investigations with Wistar rats, administered nanoparticles of iron oxide in the doses of 50 and 150 μg/kg of the bodyweight for 15 days, determined the increase in the activity of alkaline phosphatase AlAT and AsAT, which demonstrated the toxic effect of nanoparticles on the liver [34]. The studies of NP-Fe were also conducted with agricultural poultry, but no negative effect of NP-Fe on the organism of chickens was revealed [35].

Je. F. Mamedov and Ju. I. Popova conducted the experiment with male mice. The animals were divided into 4 experimental groups and 1 control. The animals of the experimental groups were administered the investigation concentrations of NP-Mn powder in the form of suspensions on vegetable oil for 6 days: group I – 0.05 mg/kg, group II – 1.25 mg/kg, group III, 2.50 mg/kg, group IV – 5.0 mg/kg of the bodyweight. Je. F. Mamedov did not determine any changes in the indices of carbohydrate exchange: the deviations in the concentration of glucose, lactate and pyruvate did not exceed 5 % to the control for animals of all the groups. The changes in cholesterol concentration in blood serum under the effect of NP-Mn were also insignificant at their introduction in the concentrations of 1.25 and 5.0 mg/kg of the bodyweight. The study of protein exchange determined a reliable decrease in the concentration of total protein in blood in case of administering NP-Mn in all the concentrations, especially low values of proteins were noted in case of introducing nanoparticles in the concentrations of 1.25 and 2.5 mg/kg of the bodyweight (1 and 1.7 times respectively), here the decrease was observed due to the albumin fraction. The level of urea was also lower for animals of all the experimental groups compared to the control. Based on the conducted studies, the author made a conclusion that oral administration of NP-Mn in the concentrations of 0.05–5.0 mg/kg of the bodyweight was less active in terms of carbohydrate and lipid exchange but induced changes in protein exchange. Yu. I. Popova determined the increase in the activity of blood enzymes – AsAT, AIAT, LDH, KFK and GGT when NP-Mn was administered in all the investigated concentrations. A considerable excess in AsAT enzymatic activity was observed in case of administering NP-Mn in the concentration of 0.05 mg/kg of the bodyweight – 7 times on average, and that of 1.25 mg/kg – 3.8 times respectively. The level of AIAT activity increased in case of administering NP-Mn in all the investigated concentrations – 2.0–2.3 times on average [36, 37].

Therefore, both toxic and pharmacological effect of NPMe on a living organism depends on many factors: administration method, dose, physiological condition of animals, their form and aggregate state and many others, which requires more detailed studies. However, our studies and the investigations of other scientists give grounds for the assertion that NPMe are promising in veterinary nutritional science, as most risks of their application (embryonic mortality, hepatotoxic-, nephro-, cardiotoxic effect, development of oxidative stress and immunosuppression) have already been determined, and thus may be controlled.

CONCLUSION

Our results expand current knowledge about the effect of NPMe mixture (Ag, Cu, Fe, MnO$_2$) on the organism of agricultural poultry due to the determination of their transovarial effect of different character, namely,

- determination of the mechanism of toxic effect of NPMe at the administration to chickens in the dose of 4.0 mg/kg of the bodyweight which lies in decreasing natural resistance, excessive formation of toxic products of purine exchange and straining enzymatic systems of natural detoxication along with the development of oxidative stress in the organism of one-day-old chickens;

- determination of the mechanism of pharmacological effect of NPMe mixture at the administration to chickens in the dose of 0.3 mg/kg of the bodyweight, which lies in enhancing immune reactions along with the antioxidant effect via evident release of fat-soluble vitamins A and E from the endogenous pool in the organism of one-day-old chickens.

The data obtained demonstrate the advantage of the effect of NPMe mixture (Ag, Cu, Fe, MnO$_2$) over the effect of the analogue in the form of the mixture of metal salts, which may help in achieving a high percentage of liveability of young birds, forming defensive reactions in the organism of chickens and ensuring full realization of genetic potential in terms of meat or egg productivity of agricultural poultry.
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The experimental studies using poultry were approved and confirmed by the bioethics commission of the National Scientific Center “Institute of Experimental and Clinical Veterinary Medicine” of NAAS and conducted with the consideration of the recommendations of the European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), norms of breeding, keeping and feeding.

The authors state the absence of conflict of interests while conducting this work. The work was not financed from additional sources.

Transvanirialna dii sumish nаночастицок металлів (Ag, Cu, Fe, MnO2) na biохімічні показники крові добових курчат у порівнянні з солями металів

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Мета. Наночастики металлів (NPMe), потрапляючи в організм, завдають своїм малым розмірам, проникаючи у різні внутрішні органи, додаючи гематоенцефалічний та плацентарний концентраціонні впливи, і можуть викликати в організмі різні патофізіологічні ефекти. Тому, метою нашого дослідження стало вивчення трансваніріальної дії суміші NPMe (Ag, Cu, Fe, MnO2) на біохімічні показники крові добових курчат у порівнянні з солями відповідних металів.

Методи. Дослідження були проведено на птиці кросу Хайексе Уайт (n = 28) зі статевим співвідношенням північ до курей 1:6, яким протягом 37 діб вводили з кормом: дослідний зразок суміші NPMe, який містив наночастики срібла (31,5 ± 0,9 нм), заліза (100,0 ± 10,0 нм), міді (70,0 ± 4,0 нм) і двокису марганцю (50,0 ± 3,0 нм), у дозах 0,3 і 4,0 мг / кг маси тіла (II і III дослід); розчин суміші солей – AgNO3, (CuSO4 ∙ 5H2O), (MnSO4 ∙ H2O) і (FeSO4 ∙ 7H2O) (I дослід); дистилковану воду (контроль). Починаючи з 30-ї доби експерименту від птиці кожної групи протягом тижня збирали яйця та закладали їх на інкубацію. Від виведених добових

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Ключові слова: нанотоксикологія, птахівництво, наночастики металів, інкубація, добові курчат, ембріотоксичність.

Трансваніріально дія смеси наночастиц металлів (Ag, Cu, Fe, MnO2) на біохімічні показники крові сутокних цыплят в сравнении с солями металлов

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Цель. Наночастицы металлов (NPMe), попадая в организм, благодаря своим малым размерам проникают в
Трансовариальная эффективность смеси металлических наночастиц (Ag, Cu, Fe, MnO₂)

различные внутренние органы, преодолевая гематоэнцефалический и плацентарный барьеры, и могут вызвать в организме различные патофизиологические эффекты. Поэтому, целью нашего исследования стало изучение трансовариального действия смеси НПМе (Ag, Cu, Fe, MnO₂) на биохимические показатели крови суточных цыплят по сравнению с солями соответствующих металлов. Методы. Исследования были проведены на птице кросса Хайскес Уайт (n = 28) с половым соотношением петухов к курам 1 : 6, которым в течение 37 суток вводили с кормом: опытный образец смеси НПМе, содержащей наночастицы серебра (31,5 ± 0,9 нг), железа (100,0 ± 10,0 нг), меди (70,0 ± 4,0 нг) и двуокиси марганца (50,0 ± 3,0 нг), в дозах 0,3 и 4,0 мг/кг массы тела (II и III опыт); раствор смеси солей металлов – AgNO₃, (CuSO₄ · 5H₂O), (MnSO₄ · H₂O) и (FeSO₄ · 7H₂O) (I опыт); дистиллированную воду (контроль). Начиная с 30-х суток эксперимента, от птицы каждой группы в течение недели собирали яйца и закладывали их на инкубацию. От выведённых суточных цыплят (n = 93) отбирали пробы крови для биохимических исследований. Регистрацию биохимических показателей осуществляли на спектрофотометре SHIMADZU UV-1800 (Япония). Результаты. В результате установили механизм токсического действия смеси НПМе при введении курам в дозе 4,0 мг/кг массы тела, который заключается в снижении естественной резистентности (тенденция к повышению серомукоида – белков иммуносупрессоров), избыточном образовании токсических продуктов пуринового обмена (повышение концентрации мочевой кислоты на 77,6 %; Р < 0,05) и напряжении энзимных систем естественной детоксикации (повышение активности аланинамино-трансферазы и аспартатамино-трансферазы на 23,8 и 31,6 %; Р < 0,05) с развитием окислительного стресса (повышение содержания диеновых кольцеватогов и малого диальдегида на 19,3 и 23,8 %; Р < 0,01) в организме суточных цыплят. Механизм фармакологического действия смеси НПМе при введении курам в дозе 0,3 мг/кг массы тела заключается в усилии иммунных реакций (повышение количества общих протеинов и глобулинов на 14,4 и 12,0 %; Р < 0,05) наряду с антиоксидантным воздействием через восстановление из эндогенного депо витаминов A и Е (на 14,7 и 25,7 % выше относительно контроля; Р < 0,05) в организме суточных цыплят. Выводы. В условиях трансовариальной передачи подтверждено преимущество влияния смеси НПМе (Ag, Cu, Fe, MnO₂) в дозе 0,3 мг/кг массы тела кур-несушек над действием аналога в форме смеси солей соответствующих металлов, что может способствовать получению высокого процента сохранности молодняка птицы, формированию защитных реакций в организме цыплят и обеспечить полноценную реализацию генетического потенциала по мясной или яичной продуктивности птицы. Установлен механизм токсического действия наночастиц металлов в дозе 4,0 мг/кг массы тела, что позволит разработать безопасные регламенты применения наноматериалов неорганического происхождения в современном птицеводстве и своевременно управлять рисками их применения.

Ключевые слова: нанотоксикология, птицеводство, наночастицы металлов, инкубация, суточные цыплята, эмбриотоксичность.

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