International Journal of GEOMATE, Sept., 2019 Vol.17, Issue 61, pp. 141-148
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.61.8217
Special Issue on Science, Engineering & Environment

FORECAST OF PRODUCTIVE AND BIOLOGICAL EFFECTS OF METAL NANOPARTICLES ACCORDING TO TOLERANCE INDEX

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ABSTRACT: In the experiment, different-sized NPs of the same elements are compared: iron nanoparticles sized 50.0±15 nm (Fe_aNPs) and iron nanoparticles sized 80.0±5 nm (Fe_bNPs); copper nanoparticles sized 55.0±15 nm (Cu_aNPs) and copper nanoparticles sized 103.0±2 nm (Cu_bNPs). Studies have shown that metal NPs with different physicochemical characteristics show different activity against *E. coli K12 TG1* and have different productive effects on broiler chickens. The experimentally established constants of NP concentrations that determine the "quenching" of bioluminescence are closely related to the presence of biological action of metals on the chicken model. A new criterion for predicting the productive effect of NPs is proposed - the tolerance index (TI). The balance of the toxic dose of metal-microelement (EC_{50} for *E. coli*) and the biological level of microelement consumption determines the biological meaning of TI for broiler chickens (mmol/kg $W^{0.75}$×24 hours). As follows from calculations for metal NPs having TI<1, the absence of a productive effect is characteristic, for metal NPs having TI>1, on the contrary, the growth-stimulating effect on the model of broiler chicken is characteristic. Signs of the presence of oxidative stress and cell cytolysis, revealed by the activity of alanine aminotransferases (ALT), aspartate aminotransferases (ACT), lactate dehydrogenase (LDG), gamma-glutamyl transferase (GGT), catalase (CT), total superoxide dismutase (T-SOD), malonic dialdehyde (MDA) were registered with the introduction of small-sized copper and iron nanoparticles with TI <1.

Keywords: Bioluminescence, Iron and Copper nanoparticles, E. coli K12TG1, Gallus gallus, Tolerance index.

1. INTRODUCTION

By various estimates, the total world market of nanotechnologies will be from 3 [1] to 3.4 trillion USD by 2020 [2]. Nanotechnologies can significantly affect the agro-industrial complex and make this industry more efficient and environmentally friendly [3]. Obviously, NPs will find wide application in crop production due to growth promoters [4], fertilizers, pesticides and herbicides [5]. In veterinary medicine, NPs will be widely used as antibiotics [6], in the production and use of vaccines [7], for the diagnosis of diseases [8].

It is expected to use a wide range of NPs for the creation of feed additives. The prospects of using NPs as sources of microelements [9], OVO additives [10], optimizers of the microbioma of animals, enhancers of immunity [11] and reproduction [12], growth promoters [13, 14], for correction of weight gain composition and improvement of feed efficiency [15, 16] have been shown.

Once in the digestive tract of broilers, nanoparticles give ions during digestion and they are further involved in metabolism. The main organs of biotransformation of nanoparticles are liver, spleen and kidneys. These organs, in general, are not used in human food. In the muscle tissue (meat) that a human consumes, nanoparticles do not accumulate. Thus, there is no reason to consider these nanoparticles environmentally hazardous.

Meanwhile, NPs of the same essential element, depending on the production technology, size, surface characteristics and other causes, exhibit different biological effects [17], which does not allow using them in practice without preliminary studies and continuous monitoring of production.

Studies on farm animals require a lot of time and considerable resources. In this regard, research using simple biological models including *E.coli* are promising. These bacteria are a widespread model in modern toxicology [18]. Especially, recombinant luminescent E. coli strains are popular, which provide detailed information on the biological activity of test compounds in real time [19]. Previously inducible luminescent bacteria of E. coli strains were widely used to assess the toxicity of a wide range of NPs [20-22].

In this regard, the study was aimed at a comparative study of the biological effects of metal nanoparticles in the inhibition test of bacterial bioluminescence (*E. coli*) and on the model of broiler chickens in order to develop a method for predicting the productive effect of nanoscale preparations.

2. MATERIAL AND METHODS

2.1 Production and Certification of NPs

NPs of iron and copper, produced by various technologies were used in the study. NPs were purchased from Advanced Powder Technologies (Russia) and the Institute of Energy Problems of Chemical Physics of RAS, (Russia). Material certification (Table 1) (particle size, polydispersity, volume, quantitative content of fractions, surface area) of the studied samples of NPs included: electron scanning, transmission, atomic force microscopy using LEX T OLS4100, JSM-7401F, JEM-2000FX (JEOL, Japan). Size distribution of particles was studied on a Brookhav en 90Plus/BIMAS Zeta PALS and Photocor Compact (Fotokor, Russia) NPs analyzer in lyzoles obtained by dispersing on an ultrasonic disperser UZDN-2T (Russia) under conditions f-35 kHz, N-300 W, A-10 μA, for 30 minutes.

Table 1 Physical and chemical characteristics of tested metal NPs

NPs	Size, nm	Chemical and phase composition	Z-potential, mV	Specific surface S_{sp},m^2/g
Cu_a	55 ±15	Cu^o99.7±2.5%	31±0.1	9±0.8
Cu_b	103 ±2	Cu^o96±4% Cu0 4,0±0.4%	25±0.5	8±0.5
Fe_a	50 ±15	Fe^o99.8±0.2%	13±0.5	7.7±0.7
Fe_b	80±5	Fe_3O_4, α - Fe_2O_3	15±0.2	32±2.3

2.2 Studies on *E. coli*

Estimation of toxic effects of the studied samples of metal NPs was carried out in a wide range of concentrations (4 M - 6×10^{-6} M). The genetically engineered luminescent strain *E. coli* K12 TG1, constitutively expressing the luxCDABE genes of the natural marine microorganism Photobacterium leiognathi 54D10, produced by Immunotekh NV (Russia, Moscow) in a lyophilized state was used as a bioengineering object under the commercial name «Ecolum».

The bacterial luminescence inhibition test was performed using a microplate spectrophotometer Infinite PROF200 (TECAN, Austria) dynamically recording the intensity of glow of the resulting mixtures within 180 minutes with intervals of 5 minutes. The results of the influence of NPs on the intensity of bacterial bioluminescence (1) were estimated using the formula:

$$I = \frac{Ic_{0min} \times It_{n\,min}}{Ic_{nmin} \times It_{\,0min}} \quad (1)$$

where Ic and It are luminescence intensity of the control and the tested samples at the 0^{th} and n^{th} minutes of measurement.

2.3 Studies on Gallus Gallus

Studies were carried out on broiler chickens "Smena-8" in the conditions of the vivarium. Feeding of poultry was carried out with complete feed fodders, compiled taking into account the recommendations [23]. For the experiment, 200 broiler chickens aged 1 day were selected (selection condition is a good development, differences in live weight not more than 5%). At selection, chickens were divided by sex. Based on data of individual daily weighing of chickens and accounting of feed costs by the method of pair analogs at 14 days of age, five groups (n = 40) were formed: one control (I) and four experimental (II, III, IV, V). Within the first 14 days, birds consumed the same diet, in the following (28 days) the chickens were transferred to an experimental diet. The diet of the control group was balanced by the estimated elements through additional sulfate administration, at a rate of 8 mg / kg iron and 1.7 mg/kg copper. In the diet of chickens of II and III groups, ferrous sulfate was replaced by Fe_a and Fe_b NPs, in the diet of IV and V groups copper sulfate was replaced by Cu_a and Cu_b in equivalent dosages. Lysozoles of NPs were prepared by ultrasonic treatment of aqueous suspensions of NPs. Then, lysozoles were introduced to the mixed fodder by stepwise mixing.

Blood samples were taken from birds in the morning, on an empty stomach, before slaughter at 21, 28, 35 and 42 days old from the axillary vein. Biochemical blood analysis was performed on an automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd, China) using commercial biochemical sets for veterinary DiAVetTest (Russia) and commercial biochemical kits Randox Laboratories Limited (UK).

In the course of studies, the size of the pool of 24 chemical elements in the body of chickens was estimated. The slaughter was carried out at the end of the experiment - at the age of 42 days. The method of euthanasia was used: decapitation under nembutal anesthesia. Individually, for each animal, feather, skin, the flesh of carcass, internal organs, gastrointestinal tract, internal fat, blood, etc. was weighed. Then, the elemental composition of tissue homogenate was determined. The size of the pool of chemical elements in the body was established by summing the weight of elements in separate organs and tissues.

2.4 Elemental Analysis

The content of elements in the resulting ash was estimated using an Elan 9000 mass spectrometer (Perkin Elmer, USA) and an Optima 2000 V atomic emission spectrometer (Perkin Elmer, USA). The size of the pool of chemical elements in the body was established by summing the weight of elements in separate organs and tissues.

2.5 Statistical Analysis

Performing the research, we proposed a new evaluation criterion for metal NPs - potential preparations – micronutrients as a TI (2):

$$TI = \frac{EC_{50}}{C} \quad (2)$$

being: EC_{50} – concentrations of preparation that cause a 50% quenching of biosensor luminescence (*E. coli* K12 TG1 with cloned luxCDABE-genes of P. leiongnathi 54D10) compared to control, M; C-biotic level of consumption, mmol/kg W0,75×day; for copper it was 0.0048 mole/kg, for iron it was 0.052 mol/kg.

Data are expressed as mean values ± standard error of the mean. Statistical analysis was performed using Statistica 10.0 (StatSoft Inc., USA) and Microsoft Excel (Microsoft, USA). The significance of the group differences was estimated using Student's t-test with P≤0.05 considered as significant.

3. RESULTS AND DISCUSSION

3.1 Assessment of Biotoxicity of Nanoparticles Using Bacterial Luminescence Inhibition Test

The activity degree of NPs upon contact with *E. coli* K12 TG1 with cloned luxCDABE genes *P. leiongnathi* 54D10 is shown in Table 2.

Table 2 Toxicity degree* of different concentrations of NPs after 60 min of contact with *E. coli* K12 TG1 with cloned lux CDABE-genes *P. leiongnathi* 54D10.

Concentration, M	NPs			
	Cu_a	Cu_b	Fe_a	Fe_b
0,25	Tox	Tox	EC_{70}	EC_{30}
0.1	Tox	Tox	EC_{50}	NOEC
0.05	Tox	Tox	EC_{20}	NOEC
0.025	Tox	Tox	EC_{30}	NOEC
0.0125	Tox	EC_{70}	NOEC	NOEC
0.00625	EC_{70}	EC_{50}	NOEC	NOEC
0.003	EC_{50}	EC_{20}	NOEC	NOEC
0.0015	EC_{20}	NOEC	NOEC	NOEC

*Tox – concentrations causing 100 % luminescence quenching of biosensor; EC_{70}, EC_{50}, EC_{30}, EC_{20} – concentrations causing 70, 50, 30, 20 % luminescence quenching comparison with control; NOEC – concentrations with no effect [24].

The obtained results allowed to calculate the EC_{50} value - the molar concentration causing 50% inhibition of bacterial bioluminescence in comparison with the control after different exposure (Table 3).

Table 3 EC_{50} (M) values after contact of *E. coli* K12 TG1 test organism and cloned *luxCDABE*-genes P. *leiongnathi* 54D10 and the studied ultrafine particles

NPs	Duration , min		
	60	120	180
Cu_a	3×10^{-3} ±0.004	3×10^{-3} ±0.002	3×10^{-3} ±0.0001
Cu_b	6.25×10^{-3} ±0.003	6.25×10^{-3} ±0.001	6.25×10^{-3} ±.0002
Fe_a	5×10^{-2} ±0.00031	5×10^{-2} ±0.00031	5×10^{-2} ±0.00031
Fe_b	> 0.25	> 0.25	> 0.25

The peculiarities of the synthesis technology determined the same effect of 50 % bioluminescence inhibition at 60th min of contact for Cu_aNPs 0.003 M and Cu_bNPs 0.00625 M in nanoform.

Table 4 EC_{50} (M) values after contact of *E. coli* K12 TG1 cloned luxCDABE-genes *P. leiongnathi* 54D10 with test preparation

Substance	Duration of contact, min		
	60	120	180
$CuSO_4\times5H_2O$	4×10^{-5}± 0.000072	2×10^{-5}± 0.000043	6×10^{-6}± 0.000001
$FeSO_4\times7H_2O$	1×10^{-5}± 0.00008	1×10^{-5}± 0.000014	5×10^{-6}± 0.0000001

Biological evaluation of mineral salts used as control showed luminescence inhibition at all time stages of contact (60-180 min) (Table 4).

3.2 Growth Intensity of Broiler Chicken

The productive effect of the compared preparation of iron and copper NPs, estimated by a live weight increase of chickens, was different (Table 5).

Feeding with Fe_aNPs was accompanied by a decrease in live weight gain of broiler chickens during the experimental period by 8.4% (P≤0.01), Cu_aNPs by 12.4% (P≤0.01) in comparison with the control.

At the same time feeding with Fe_bNPs was accompanied by an increase in live weight gain by

5.1% (P≤0.05), the use of Cu_bNPs resulted in weight gain increase by 4.3% (P≤0.05) relative to the control.

Table 5 Dynamics of live weight of broiler chickens

Index	Con-trol	NPs administered with feed			
		Fe_a	Fe_b	Cu_a	Cu_b
Live weight, g	2470.0 ±32.6	2285.3 ±24.9*	2590.0 ±29.9*	2194.7 ±23.4*	2573.0 ±35.8*
Live weight gain, g	2238.9 ±21.23	2051.8 ±19.31	2353.9 ±18.23	1961.4 ±17.54	2334.9 ±20.21
Weight gain difference compared, %	0	- 8.4	5.1	-12.4	4.3

Note: * - a significant difference of the experimental groups with the control group (P≤0.05)

3.3 Biochemical Parameters of Blood Serum of Broiler Chickens

Data on bioindication on the model of *E. coli* have been confirmed in studies on poultry (Fig. 1).

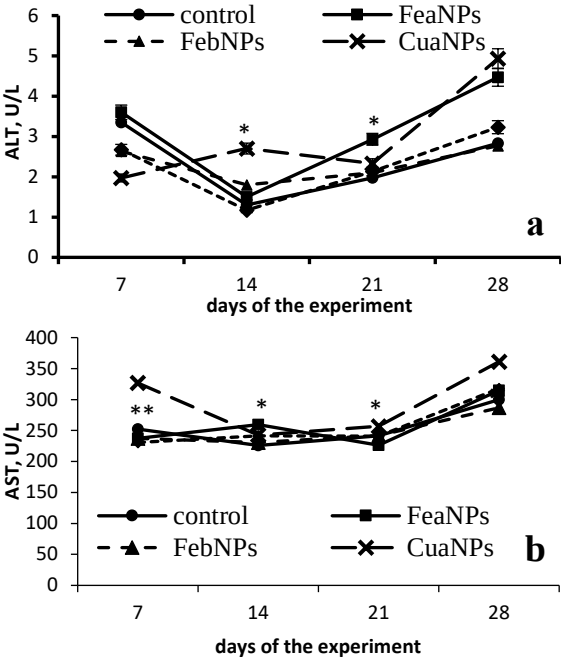

Fig. 1 Selected ALT (a) and AST (b) of broiler chickens (n=10); *- a significant difference of the experimental groups with the control group P≤0.05, ** - P≤0.01

The catalytic activity of aminotransferases as a consequence of metabolic shifts against the background of the introduction of NPs into the body of broiler chickens is different from the control values. Thus, the use of Fe and Cu_aNPs in feeding in contrast to their analogs was accompanied by a more significant increase in the activity of ALT and AST in blood serum.

We found no significant increase in the activity of serum GGT and LDH. LDH activity largely more than the control was recorded on the 21st and 28th days of the experiment against the background of feeding with small-sized Fe_aNPs sand Cu_aNPs (Fig. 2 a, b).

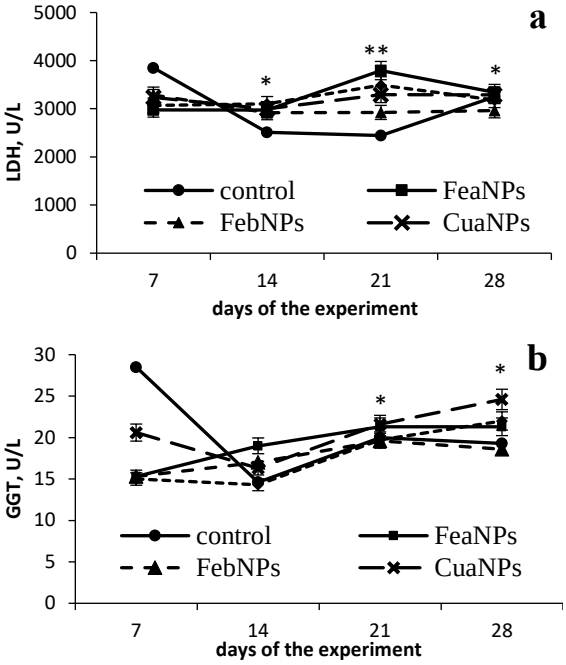

Fig. 2 Selected LDG (a) and GGT (b) of broiler chickens (n=10); *- a significant difference of the experimental groups with the control group P≤0.05.

Such dynamics of GGT and LDH activity may indicate the destruction of membranes of a small population of cells and weak induction of microsomal oxidation under the influence of metal NPs of variable valence [25].

At the same time, increasing exposure duration up to 28 days, the activity of GGT against the background of feeding with Cu_aNPs increased by 27% (P≤0.05), while feeding with Fe_aNPs - by 10.3% (P≤0.05), which can be estimated as a sign of oxidative stress, induced by small-sized NPs [26].

3.4 Prooxidant and Antioxidant Activity of Blood Broiler Chickens

The dynamics of the activity of CT and T-SOD indicated the manifestation of oxidative stress during feeding with Fe_aNPs and Cu_aNPs (Table 6, 7).

Table 6 Prooxidant and antioxidant activity of blood broiler chickens cross Smena 8 (n=10).

Days	Control	NPs	
		Fe_a	Fe_b
	CT, μm H_2O_2/l×min		
7	9160.3 ±89.48	9193.2 ±278.45	8011.2 ±68.07
14	7516.1 ±98.06	11688.5 ±233.16***	6480.3 ±66.35
21	8162,7 ±54.39	15726.1 ±323.75**	4566.4 ±67.33
28	5431.4 ±95.90	15792.6 ±154.01**	4234.4 ±72.39
	T-SOD, %		
7	641.6 ±22.59	724.4 ±42.89	740.4 ±19.51
14	323.3 ±17.43	654.9 ±23.93	656.0 ±39.65
21	1291.3 ±46.59	883.4 ±45.57	944.9 ±26.24
28	869.4 ±43.35	409.0 ±29.28**	646.0 ±3320
	MDA, μm/l		
7	0.11±0.068	0.15±0.026*	0.12±0.039
14	0.11±0.006	0.14±0.036	0.12±0.011
21	0.11±0.003	0.17±0.007**	0.13±0.006
28	0.12±0.011	0.12±0.011	0.12±0.011

Note:*- a significant difference of the experimental groups with the control group P≤0.05, ** - P≤0.01, *** - P≤0.001

Table 7 Prooxidant and antioxidant activity of blood broiler chickens cross Smena 8 (n = 10).

Days	Control	NPs	
		Cu_a	Cu_b
	CT, μm H_2O_2/l×min		
7	9160.3 ±89.48	8897.7 ±75.94	10473.7 ±142.77
14	7516.1 ±98.06	17923.4 ±156.65**	12423.4 ±179.18*
21	8162,7 ±54.39	3951.9 ±57.14	4581.4 ±90.45
28	5431.4 ±95.90	6336.7 ±67.30	5483.1 ±98.98
	T-SOD, %		
7	641.6 ±22.59	582.7 ±3388	684.2 ±20.26
14	323.3 ±17.43	763.2 ±29.43*	742.2 ±58.09
21	1291.3 ±46.59	2671.3 ±120.79**	1194.6 ±99.10
28	869.4 ±43.35	252.8 ±14.14**	508.0 ±27.36*
	MDA, μm/l		
7	0.11±0.068	0.18±0.040	0,10±0.034
14	0.11±0.006	0.14±0.029	0,14±0.019
21	0.11±0.003	0.14±0.013	0,16±0.028
28	0.12±0.011	0.13±0.011	0.11±0.013

Note:* - a significant difference of the experimental groups with the control group P≤0.05, ** - P≤0.01

Peaks of CT indices were recorded after feeding with Fe_aNPs (14th, 21st and 28th days, with a difference with the control by 1.5 (P <0.001), 1.9 (P <0.01), 2.9 times (P<0.01). The difference in CT activity when feeding with large NPs (Fe_b, Cu_b) was less pronounced, and at some points below control values.

The administration of Cu_aNPs caused a surge in CT activity on the 14th day of the experiment (difference with the control by 2.4 times) by 21 and 28 days the activity of CT was below the control values. Apparently, in response to increased lipid peroxidation and accumulation of hydrogen peroxide and other products of oxidative stress, CT is activated, which metabolizes them and prevents their accumulation in cells.

3.5 Elemental Status of Broiler Chickens

The use of metal NPs as microelement preparations did not equally affect the exchange of some optimized elements. An increase of iron in chickens after feeding with Fe_bNPs was 37.2% (P≤0.05), while using Fe_aNPs was only 2.3%. Feeding with Cu_aNPs was accompanied by an increase in the content of copper in the body by 51.4% (P≤0.05), Cu_bNPs by 65.5% (P≤0.01).

The use of similar nanoparticles was accompanied by various changes in the elemental status of chickens. Thus, feeding with Feb NPs leads to an increase in the deposition of iron in the body by 34% (P≤0.01) compared with the level achieved by feeding with Fe_aNPs.

The similar copper NPs show the same trend. Thus, the deposition of copper during the consumption of Cu_bNPs is higher by 9.3% (P≤0.05) in comparison with Cu_aNPs.

4. DISCUSSION

The preparation of nanoforms is considered as one way to increase the bioavailability of food components [27], including trace elements [28], [29]. This determines the interest in creating new food and feed additives with nano-sized components [30].

Meanwhile, the unique prospects for using NPs are largely related to their extraordinary biological properties. The small size, ability to penetrate into tissues and organs, high surface area [31] form previously unknown biological effects, including toxicity. Therefore, as new technologies of nanoparticle synthesis are developed, and because of the high variability of properties of the same substances [32], [17], the need to create effective methods for predicting biological effects of NP by simple and accessible methods becomes evident. It includes maximal replacement of tests with animals used [33]. Therefore, methods using various test organisms are increasingly used [34].

One of the promising are tests of bacterial bioluminescence inhibition [35], it is confirmed by toxicity tests for a wide range of chemicals [36], [37] and environmental samples, including wastewater, waste, soil, etc. [38].

Assessment of the experimental material accumulated in the course of our work has made it possible to establish various effects of NP preparations of the same metal. This was expressed both in changes of biological activity in microorganism strain and in various productive effects on the model of chickens. In particular, Cu_aNPs with a size of 55 nm caused a 100% quenching in the dilution series to a concentration of 0.0125 M, 50% and 20% at concentrations of 0.003 and 0.002 M, while Cu_bNPs with a 103 nm had a smaller toxicity, providing 100% inhibition of bioluminescence in the concentration range from 4 to 0.025 M. The dilutions that did not have a significant effect on bacterial luminescence, were characterized as biotic doses, and began at 0.00078 M and below.

It should be pointed out that Fe_bNPs and Fe_aNPs have differences in biological activity already observed at the 60th minute of contact, with a similar trend after prolongation of contact up to 120 and 180 min.

The *E. coli* K12TG1 test proved to be promising for predicting the productive effect of trace element preparations on the model of broiler chickens. In particular, using iron nanoparticles we showed that Fe_aNPs demonstrated EC_{50} at a dose of 0.05 M on the model of *E. coli*. At the same time, on the model of broiler chickens, Fe_aNPs had no increase in bird growth rate, etc. At the same time, Fe_bNPs, demonstrating EC_{50} at a dose of 0.25 M on the model *E. coli*, in contrast, had a growth-stimulating effect on *Gallus gallus*.

It should be noted that our data are consistent with earlier studies [39] with more than four dozen chemicals used as samples, and it was demonstrated that EC_{50} for *P. Phosphoreum* inhibition of bioluminescence correlates (p2=0.20-0.79) with acute toxicity data for daphnia, fish, animals and human cell lines, rodents, dogs and humans. Thus, an increase in the activity of ALT and AST in the blood of experimental animals receiving NPs indicates the presence of cytolysis processes. The increase of MDA is registered against the background of the consumption of Fe_aNPs. It can be caused either by an increase in the formation of peroxides and a decrease in the activity of antioxidant defense enzymes, in response to the chronic effects of low-frequency metals [40]. The use of Fe_aNPs and Cu_aNPs was accompanied by an increase in the content of essential and conditionally essential microelements in the poultry organism to 1.96 and 2.14 μmol/g, which exceeded these values in groups receiving Feb and Cub by 17.7% ($P \leq 0.05$) and 45.9% ($P \leq 0.01$), respectively. A similar difference in the content of macronutrients in the body was 17.9% ($P \leq 0.05$) and 40.4% ($P \leq 0.05$), respectively. At the same time, feeding Fe_aNPs and Cu_aNPs was accompanied by a decrease in the content of toxic elements in the body to 0.0396 and 0.0577 μmol/g, or 32.1% ($P \leq 0.05$) and 8.7%, respectively.

Analysis of the experimental material let us propose a new criterion for predicting the productive effect of NPs - the tolerance index (TI). The biological significance of TI is determined by the proportionality of toxic dose of the metal-trace element (EC_{50} for *E. coli*) and the biological level of consumption by broiler chickens. As follows from our calculations of metal NPs with a value of TI<1, there is no productive effect on the model of broiler chickens, which is probably determined by toxicity. At the same time, on the contrary, the growth-stimulating effect is characteristic of metal NPs with TI>1. In particular, TI of Fe_aNPs was 0.96, while TI Fe_bNPs was greater than one (4.8). A similar dynamics of TI was demonstrated by copper NPs. Thus, TI of Cu_aNPs= 0.63, TI of Cu_bNPs = 1.3.

The tolerance index can be used to predict the productive effect of metal NPs. In fact, expressing the productive effect (Δm) after the use of NP preparation (the difference in live weight gain of the control and experimental groups) through the tolerance index can be expressed in two equations of linear dependence.

(FeNPs) $\Delta m = 3.52\ TI - 11.8$

(CuNPs) $\Delta m = 25.7\ TI - 29.1$

Obviously, the coefficients 3.52 and 25.7 correspond to the tangent of the slope of the direct dependence of Δm from TI to the abscissa axis. The biological significance of these values is the "speed" of the increase of NP effects after feeding to animals. It can be assumed that the difference in the rate of increase of the effect from the use of CuNPs and FeNPs is 7.3 times. This is similar to the difference in the value of biogenic standards of consumption of these two metals (each copper atom in the bird's diet, prepared according to the modern standards, should contain 7 to 8 iron atoms).

5. CONCLUSIONS

The productive effect of metal nanoparticle preparations produced by various technologies can be predicted by calculating the tolerance index - EC_{50} inhibition ratio of *E. coli* bioluminescence and the biological level of micronutrient consumption by broiler chickens. The balance of the toxic dose of metal-microelement (EC_{50} for *E. coli*) and the biological level of microelement consumption determines the biological meaning of TI for broiler chickens (mmol/kg $W^{0.75}$×24 hours). As follows from calculations for metal NPs having TI<1, the

absence of a productive effect is characteristic, for metal NPs having TI>1, on the contrary, the growth-stimulating effect on the model of broiler chicken is characteristic. Signs of the presence of oxidative stress and cell cytolysis, revealed by the activity of alanine aminotransferases (ALT), aspartate aminotransferases (ACT), lactate dehydrogenase (LDG), gamma-glutamyl transferase (GGT), catalase (CT), total superoxide dismutase (T-SOD), malonic dialdehyde (MDA) were registered with the introduction of small-sized copper and iron nanoparticles with TI <1.

6. ACKNOWLEDGMENTS

The research was performed with the financial support of the Russian Science Foundation, project No. 14-16-00060 P

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