AGRICULTURAL BIOLOGY,
2019, V. 54, № 4, pp. 820-831
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58:591.1:636.085.12:546.763

doi: 10.15389/agrobiology.2019.4.820eng
doi: 10.15389/agrobiology.2019.4.820rus

EFFECTS CAUSED BY DIFFERENT DOSES OF DIETARY CHROMIUM NANOPARTICLES FED TO BROILER CHICKENS

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by the Ministry of Science and Education of the Russian Federation for basic research according to the program of RAS Presidium (Agreement No. 075-02-2019-1847)
Received January 26, 2019

Abstract

Chromium is important chemical element for humans and animals which essentiality is manifested in reducing the amount of glucose and cholesterol in blood, reducing body fat. Factors which influence the absorption of chromium are source, size and composition of diet. Reducing size of Cr particles allows one to increase absorption. Replacement of traditional sources of microelements for organic and ultrafine metal forms is prospective due to their surface area, higher reactivity and bioavailability. In this paper, we show for the first time that Cr_2O_3 nanoparticles (NPs) at doses of 50 to 100 μg/kg of feed have no toxic effect, improve productive performance through stimulation of digestive enzymes and have positive effect on accumulation of the element in broiler chicken carcass. Our goal was to estimate effects of various doses of dietary chromium nanoparticles on the activity of digestive enzymes, biochemical blood parameters and gut microbiota in Arbor Aikres broiler chickens (*Gallus gallus*) (OAO Orenburg Poultry Farm, 2018). Five groups of chickens were formed, control and test groups 1, 2, 3, and 4 (n = 30 each) with live weight from 160 to 180 g. The control birds during experiment (0-14-21-42 days) received the basic diet, the birds of groups 1, 2, 3, and 4 additionally received 50, 100, 200 and 400 μg/kg feed of dietary Cr_2O_3 NPs (d = 91 nm; Platina LLC, Moscow, Russia). Addition of 200-400 μg/kg Cr_2O_3 NPs increased body weight and improved feed conversion by 3.1-3.9 and 7-11 %, respectively ($p \leq 0.05$), compared to control. Cr incorporation into carcass was 28.2 and 25.6 % higher when broilers were fed with NPs at 200 and 400 μg/kg, respectively, while this index in droppings was 15 % lower. Increased $Cr_{feed}/Cr_{droppings}$ (1.5-2.5) and $Cr_{feed}/Cr_{carcass}$ (4.6-6.4) values in the test groups indicate better absorption of chromium in the gastrointestinal tract. Cr_2O_3 NPs caused higher activity of endogenous transferases, the alanine aminotransferase and aspartate aminotransferase. Catalase and superoxide dismutase activity remained unchanged as well as concentration of malonic dialdehyde. That is, chromium acts as antioxidant, with up to 18 % increase ($p \leq 0.05$) in blood NO-metabolites. Cr_2O_3 NPs stimulate activity of blood enzymes: by 29.5 % (group IV, $p \leq 0.05$) on day 21 for amylase, by 19-30 % (group III and IV, $p \leq 0.05$) on days 21 and 42 for lipase, followed by a decrease in lipolytic activity by the end of the experiment in the test groups compared to day. NPs of 50 and 400 μg/kg suppressed amylase and activated lipase and protease in the duodenal chymeduction, with an increase in pH of the intestinal contents from 4.62 to 9.34 in all test groups. In droppings, digestive enzymes showed a reverse trend. Dietary Cr_2O_3 NPs at 50 μg/kg decreased the number of bifidobacteria, staphylococci and Salmonella in droppings, at 100 μg/kg increased the counts of enterobacteria, and at 400 μg/kg, on the contrary, reduced enterobacteria by 20 %, with simultaneous restriction Salmonella abundance in the cecum. Dietary Cr_2O_3 NPs decreased bifidobacteria. Thus, the dietary Cr_2O_3 NPs at 50-100 μg/kg has more pronounced positive effect and can be used as a chromium additive for poultry (for example, in premixes or vitamin-mineral complexes).

Keywords: chicken-broilers, antioxidant enzymes, Cr, productivity, concentration of Cr,

Chromium is an important chemical element for humans and animals, the essentiality of which, according to standards [1], is manifested in reducing the amount of glucose and cholesterol in the blood, reducing fat deposits, stimulation of muscular tissue formation [2]. Insufficient intake of chromium in the body, associated with the type and quality of nutrition, is accompanied by a slowdown in growth and deterioration of glucose tolerance [3]. Cr stimulates the insulin function by enhancing the function of the receptors of insulin-sensitive cells [4-6].

An important tool in regulating the metabolism of chromium received in the body is its ability to penetrate through the intestinal wall. This process is accelerated by reducing the size of chromium particles and the presence of digestive agents (vitamins, phytates, amino acids). Chromium has an extremely low digestibility, is poorly absorbed (25% for organic forms, 3% for inorganic), while the absorption of Cr^{3+} occurs mainly through the kidneys (80-95%) [7] with losses when deposited in the hair, excretion through the sebaceous glands and bile (45%) [8], indicating rapid absorption and recreation of Cr. Regardless of the increase in the dose of chromium in the diet (40-240 rg/day), the degree of its uptake remains constant – 0.4-2.0% [9]. Organic Cr has a more beneficial effect on poultry compared to inorganic forms due to increased absorption and bioavailability [10]. Consequently, the factors that determine the absorption of chromium are its source, particle size, and composition of the diet, while reducing the size of Cr particles allows increasing the rate of chromium absorption in the body.

The prospect of replacing traditional sources of microelements with organic and ultrafine forms of metals is determined by the high specific surface area of the latter, greater reactivity, and bioavailability. By taking into account small sizes and high penetrating power of nanoparticles (NPs), it is necessary to remember that each part of the gastrointestinal tract has a unique medium with a specific set of enzymes and pH [11]. Nanoparticles must be able to overcome these obstacles to exhibit biological activity in the small bowel [11].

The biological effect of chromium nanoparticles is associated with the participation in the metabolism of nucleic acids, an increase in the muscle area, the accumulation of chromium in tissues, and a decrease in the amount of fat [12, 13]. In poultry experiments, the addition of chromium to the diet increased the amount of protein in the chest and thigh muscles and reduced the cholesterol content in the muscles [14].

It should be noted that reports on the influence of various chromium sources on the activity of digestive enzymes in animals are rare, although the study of the possibility to use NPs as modulators of digestive enzymes activity seems promising. It is known that several regulatory centers specialized in relation to different effectors exist in enzyme molecules [15]. According to some researchers, rapid conformational transitions may occur as a result of the activity of modifiers [16]. Alternative forms of microelements can be a necessary tool in the management of digestive processes to improve nutrient conversion, the productivity and nutritional value of poultry products.

In this paper, we show for the first time that Cr_2O_3 nanoparticles (NPs) at doses of 50 to 100 μg/kg feed have no toxic effect, improve productive performance through stimulation of digestive enzymes and have a positive effect on the absorption of the element in broiler chickens' carcass.

The work objective was the biological certification of different doses of chromium nanoparticles in the diet of broiler chickens (*Gallus gallus*) on the activity of digestive enzymes, biochemical, and microbiological parameters.

Techniques. Investigations were carried out on broiler chickens of the Arbor Aikres cross (OAO Orenburg Poultry Farm, http://www.pfo56.ru, 2018). The experimental part of the work was carried out in accordance with the protocols of the Geneva Convention, the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009, good laboratory practice for preclinical studies in the RF (GOST 3 5100.4-96) and The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C., 1996). The birds were kept in KUN-05 cages with an area of 4,050 cm^2 (90×45×45 cm) and marked with plastic foot tags. On the basis of daily weighing by the method of pairs-analogs, 5 groups were formed: one control and four experimental (n = 30, weight from 160 to 180 g). The birds were fed 2 times a day with a diet prepared by taking into account the recommendations [17] in accordance with the need for different age periods.

The composition of the main diet (MD) in the starting and growth period was as follows: wheat grain (respectively 27.1% and 41.2%), corn (16% and 22%), soybean cake (25% and 15%), sunflower cake (18% and 8%), sunflower oil (5% and 2.8%), lysine monohydrochloride, 98% (0.35% and 0.17%), DL-methionine (0.10% and 0.13%), L-threonine (0.03% and 0.54%), kitchen salt (0.28% and 0.3%), monocalcium phosphate (0.7% and 0.7%), fodder chalk (0.5% and 0.4%), limestone meal (1.0% and 0.7%), premix (2%) (OOO Koudijs MKorma, Russia). Drinking was free. Weighing was carried out every week. The control birds received the main diet throughout the whole experiment. NPs of Cr_2O_3 (d = 91 nm, specific surface area 9 m^2/g, Z-potential 93±0.52 mV, Cr content 99.8%, produced by plasma chemical synthesis; Platinum, LLC, Moscow, Russia) were additionally introduced in the main diet of birds of the test groups during the experiment (14-42 days) in the following doses: Group I — 50, II — 100, III — 200 and IV — 400 rg/kg. Feed dosages were chosen by taking into account the previously obtained positive effect of chromium on the growth and biochemical parameters of broiler chickens [18, 19]. Mash was prepared with the step mixing method; NPs were introduced after dispersion in a saline solution (UZDN-2T, NPP Akadempribor, Russia; 35 kHz, 300 W, 10 μA, 30 min).

Blood for analysis was taken before slaughter at 21- and 42-day age in the morning on an empty stomach from the axillary vein. Blood serum biochemical parameters were assessed (an automatic analyzer CS-T240, DIRUI Industrial Co., Ltd, China) using commercial veterinary kits DiaVetTest (OOO Diacon-Vet, Russia) and Randox Laboratories Limited (Randox Laboratories, Ltd., Great Britain).

The biomaterial was obtained after decapitation of broilers under Nembutal anesthesia on the 21st and 42nd days. Post-slaughter anatomical dressing of carcasses was carried out; the absolute and relative weight of internal organs was measured, followed by grinding and ashing (Multiwave 3000, Anton Paar, Austria). Microelement analysis was carried by the atomic emission spectrometry (Optima 2000 V, Perkin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA) methods according to the manufacturer's recommendations.

To assess the activity of digestive enzymes, the bowel was extracted immediately after surgical autopsy, the pancreas and duodenum were sampled in sterile tubes. The activity of pancreatic enzymes was measured (an automatic biochemical analyzer CS-T240, Dirui Industrial Co., Ltd, China) using commercial biochemical kits for veterinary DiaVetTest (OOO Diacon-Vet, Russia), protease activity by hydrolysis of casein [20].

The qualitative and quantitative composition of broilers' gut microbiocenosis was determined by the standard method [21]. Endo-agar (OOO SRCP, Russia) was used for enterobacteria with normal enzymatic activity and oppor-

tunistic lactose-negative enterobacteria, meat-and-peptone agar (MPA) (OOO SRCP, Russia) for aerobic flora, Rogosa agar (Himedia, India) for lactobacilli, Bifido agar (Himedia, India) for bifidobacteria, yolk-salt agar (YSA) (OOO SRCP, Russia) for counting staphylococci, BSA (Himedia, India) for pathogenic salmonella. The inoculations were incubated for 24-72 hours at 37 °C. The number of microorganisms of each group in 1 g of intestinal digesta (M) was calculated by the formula $M = N \times 10n$, where N is the number of colonies, n is the dilution. The final result per 1 g of the caecum digesta was expressed as CFU/g.

Statistical analysis was performed by the ANOVA method (Statistica 10.0 software, StatSoft, Inc., USA) and in Microsoft Excel. The statistical significance of differences between the compared indicators was estimated according to Student's *t*-test. The values at $p \leq 0.05$ were considered significant. The data are presented as mean values (*M*) and standard errors of means (±SEM).

Results. During the experiment, the feed consumption per 1 kg of live weight gain in the control was 1.85 kg, depending on the dose of Cr_2O_3 NPs, the difference with the control was from 8% to 16%. The poultry in the experimental Group III and Group IV was characterized by the highest growth rates (Table 1). A similar growth-stimulating effect was obtained by other authors when Fe, Cu, Zn NPs were tested as an additive [22, 23]. The data on the negative effect of the chromium preparation at a dose of 400 g/t on the productivity and safety of broilers [24] were obtained, while doses up to 1200 μg/kg had a positive effect on the live weight and efficiency of feed consumption [18, 19, 25].

1. Growth rates and chromium concentration in biosubstrates and carcasses of Arbor Aikres cross broilers depending on the dose of Cr_2O_3 nanoparticles in the diet (*M*±SEM, *n* = 30, vivarium conditions, day 42)

Indicator	Group				
	control	I	II	III	IV
Initial weight, g	224±2.4	230±4.3	234±3.0	224±3.2	232±23.1
Final weight, g	2266±20.3	2366±40.1	2431±36.4	2533±59.8	2536±78.2
Feed consumption, g:					
total	3774.30	3649.80	3739.53	3855.70	3853.97
per 1 kg gain	1.85	1.71	1.70	1.67	1.55
Cr content, rg/kg:					
in the diet	540±1.5	587±1.5	637±2.5*	736±2.7*	937±2.4**
in droppings	433±3.5	373±4.6	466±6.1	363±4.2	366±3.8
in carcass	108±0.2	125±2.4**	129±4.1**	132±3.2*	145±2.8**
Cr ratio:					
feed/droppings	1.2	1.5	1.3	2.0	2.5
feed/carcass	5.0	4.6	4.9	5.5	6.4

N o t e. See the description of groups in the Techniques section.
*, ** Differences with the control are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

It is known that the biological role of minerals in the body depends on their biochemical availability. In the authors' experiment, the Cr content in the broiler carcass was by 28.2% and 25.6% higher than in the control at maximum doses of 200 and 400 μg/kg, with a 15% decrease in droppings. The coefficient $Cr_{feed}/Cr_{droppings}$ was the highest in groups with high chromium content in the diet, which indicates its best use. At the same time, the digestion from feed, expressed by the coefficient $Cr_{feed}/Cr_{carcass}$, was in the range of 4.6-6.4, which indicates the absence of agglomerations (formation of larger secondary particles) typical for nanoparticles entering the body in high doses, as well as the existence of a regulatory mechanism in chromium metabolism [23]. By taking into account that the absorption efficiency of 100-nm nanoparticles in bowel tissue cells is 15-250 times higher than that of larger microparticles [26], chromium deposition due to penetration into the cytoplasm [27] may be increased due to the deficiency of the transport protein transferrin and the formation of stable hard-to-adsorb hydrates in the duodenum, caecum, and colon [28].

The introduction of Cr_2O_3 NPs in the diet of broiler chickens was accompanied by the absence of oxidative stress, as indicated by the activity of catalase (CAT), superoxide dismutase (SOD) and the concentration of malondialdehyde (MDA) (Table 2) in blood. In particular, significant differences ($p \le 0.05$) with the control were typical for Group II (51.3%), the CAT content was consistently low and decreased in response to an increase in the dose of Cr_2O_3 NPs in the diet, with a decrease in SOD in all experimental groups. The absence of toxicity and the growth-stimulating effect of Cr_2O_3 NPs were determined by an increase in the amount of NO-metabolites in the experimental Group II and Group III by 18.8% and 9.2%, respectively, compared to control ($p \le 0.05$). The differences compared to the control did not exceed 5% in other groups.

2. Activity of catalase (CAT)**, superoxide dismutase** (SOD)**, the concentration of malondialdehyde** (MDA) **and NO-metabolites in blood of Arbor Aikres cross broilers depending on the dose of Cr_2O_3 nanoparticles in the diet** (M±SEM, n = 30, vivarium conditions, day 42)

Indicator	Group				
	control	I	II	III	IV
SOD, % of epinephrine autooxidation inhibition	568±68.6	405±57.0	277±18.6*	509±28.6	435±48.0
CAT, μmol $H_2O_2 \cdot l^{-1} \cdot min^{-1}$	2363±54.9	1050±82.8*	1871±51.38*	1510±47.4*	1116±64.1*
MDA, nmol/ml	0.65±0.260	0.36±0.006	0.41±0.120	0.29±0.090	0.18±0.040
NO-metabolites, μmol/l	59.8±2.53	61.4±3.42	73.6±3.62*	65.8±1.51*	62.9±1.83

N o t e. See the description of groups in the Techniques section.
* Differences with the control are statistically significant at $p \le 0.05$.

Differences in the mechanism of chromium NPs action in different dosages led to unequal changes in blood biochemical parameters (Table 3). The effect of Cr_2O_3 NPs was expressed in the stimulation of the activity of alanine-aminotransferase (AlAT) and aspartate aminotransferase (AsAT) on the 14th day. Thus, the AlAT activity in Group II and Group IV was almost 2 times higher than in the control ($p \le 0.05$). Significant differences were typical for all experimental groups for AsAT on the 21st day (see Table 3).

3. Biochemical blood parameters in broiler chickens of the Arbor Aikres cross on the 21st and 42nd days depending on the dose of Cr_2O_3 nanoparticles in the diet (M±SEM, n = 30, vivarium conditions)

Indicator	Group				
	control	I	II	III	IV
			Dat 21		
AlAT, U/l	7.3±0.50	8.3±2.40	14.2±1.00***	7.1±3.20	13.2±1.70**
AsAT, U/l	52.5±33.00	80.2±50.10*	190.4±76.90**	188.7±33.20**	106.4±20.00**
Glucose, mmol/l	14.0±0.40	14.8±0.80	14.4±1.60	15.3±0.50	14.8±0.50
Cholesterol, mmol/l	3.0±0.10	3.4±0.20	2.9±0.60	2.7±0.30	3.2±0.30
Triglycerides, mmol/l	0.6±0.20	0.6±0.20	1.6±0.20*	0.6±0.30	1.2±0.10*
Amylase, U/l	436±19.1	422±195.1	303±64.1	403±25.7	617±42.9*
Lipase, U/l	6.4±0.30	7.2±0.30	6.8±0.30	7.9±0.80	8.3±2.40
			Day 42		
AlAT, U/l	23.0±2.10	17.1±1.00	26.3±1.10	24.0±2.40	15.4±1.10
AsAT, U/l	70.5±6.10	152.0±8.50***	63.8±7.80	69.4±13.70	116.6±20.90**
Glucose, mmol/l	15.4±0.40	14.3±0.60	13.4±1.10	14.0±0.50	14.2±0.20
Cholesterol, mmol/l	3.8±0.30	3.3±0.10	3.0±0.30	3.1±0.30	3.9±0.40
Triglycerides, mmol/l	0.6±0.10	0.4±0.20	0.3±0.10	1.1±0.30*	2.1±0.70*
Amylase, U/l	173±5.6	180±5.6	157±10.4	166±1.2	186±1.2
Lipase, U/l	2.7±0.80	2.3±0.80	1.4±0.70	2.8±0.70	5.4±4.50**

N o t e. See the description of groups in the Techniques section. AlAT stands for alanine aminotransferase, AsAT stands for aspartate aminotransferase.
*, **, *** Differences with the control are statistically significant at $p \le 0.05$, $p \le 0.01$ and $p \le 0.001$, respectively.

By the end of the experiment, the blood AlAT activity (with a statistically insignificant difference with the control) decreased in the groups with the minimum and maximum loads of Cr_2O_3 NPs, while AsAT, on the contrary, increased by 53.7% and 39.6% ($p \le 0.05$). The high activity of endogenous trans-

ferases may be a sign of liver, kidney and pancreatic dysfunctions, but by taking into account the absence of inflammatory markers (SOD and CAT indicators), it is reasonable to assume that the chromium metabolic function is associated with fine mechanisms involved in stimulating the production of chromodulin (LMWCCr) [29]. Chromodulin accepts chromium molecules bound by biological molecules, including transferrin, and stimulates hepatoprotective activity [30]. Changes in the glucose and cholesterol indicators in the case of chromium introduction in the diet were not noted.

The concentration of triglycerides in the blood is a marker of energy and lipid metabolism. In broilers, receiving Cr_2O_3 NPs in the diet at a dose of 100 and 400 μg/kg, this indicator on the 21st day was higher by 61.6% and 48.5% respectively, compared to the control. The effect was prolonged by the end of the accounting period, which does not confirm the results of studies [31], where high cholesterol and triglycerides content was typical for chrome deficiency states. This ambiguous reaction of the organism may be related to both the high bioavailability of chromium nanoparticles and the restructuring of the enzymatic system [32].

The activity of amylolytic enzymes in the blood on the 21st day was the highest in Group IV, the difference with the control values was 29.5% ($p \leq 0.05$). In other groups, no significant deviations were found. Similar dynamics were typical for lipase. In the groups that received the highest doses of Cr_2O_3 NPs (200 and 400 μg/kg), the activity of this enzyme on the 21st and 42nd days was 19-30% higher than in the control. An increase in the blood enzyme activity may result from the synthesis or resynthesis of micronutrients, increased permeability of cell membranes, and translocation of digestive enzymes into the bloodstream [33].

A number of authors [33, 34] postulate that enzymes circulating with the bloodstream represent a repository for subsequent pancreatic recreation. The second mechanism of formation of pancreatic hydrolases in the blood is their resorption from the excretory ducts of the gland (the so-called escape of enzymes), the third mechanism is the resorption of enzymes from the small bowel [35].

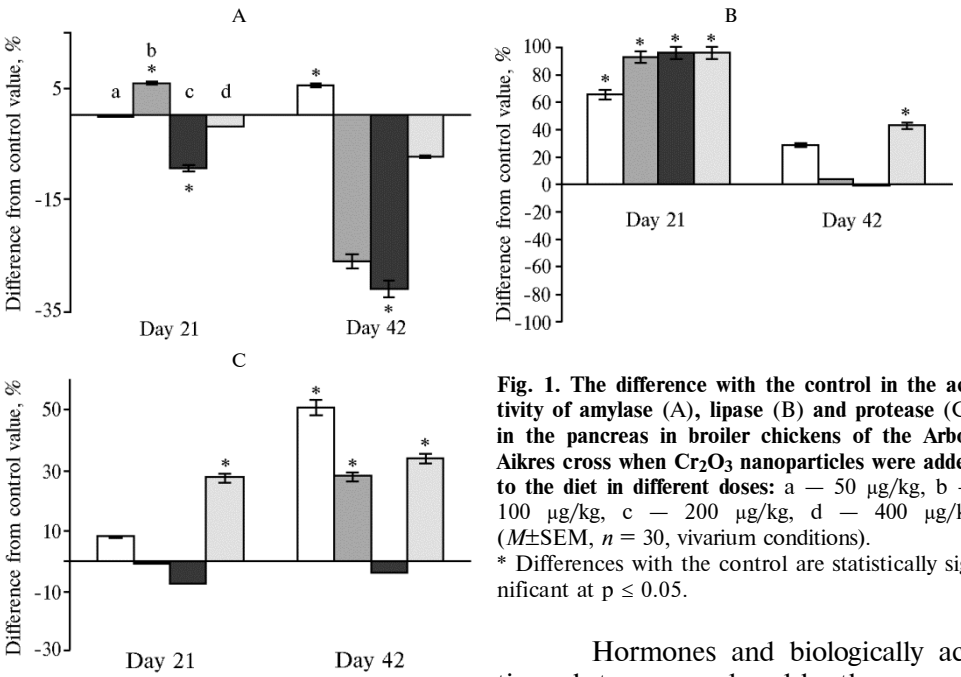

Fig. 1. The difference with the control in the activity of amylase (A)**, lipase** (B) **and protease** (C) **in the pancreas in broiler chickens of the Arbor Aikres cross when Cr_2O_3 nanoparticles were added to the diet in different doses:** a — 50 μg/kg, b — 100 μg/kg, c — 200 μg/kg, d — 400 μg/kg ($M \pm SEM$, $n = 30$, vivarium conditions).
* Differences with the control are statistically significant at $p \leq 0.05$.

Hormones and biologically active substances produced by the pancreas are necessary for the effective absorption of nutrients through the bowel mucosa. Pancreatic enzymes were character-

ized by an increase in lipase activity on the 21st day (up to 96% in all experimental groups). The peak activity of amylase and protease corresponded to Cr NPs doses of 100 and 400 μg/kg ($p \leq 0.05$). The dose-dependent difference on the 42nd day of the experiment was expressed in amylase inhibition in the dose range from 100 to 400 μg/kg with an increase in lipase and protease activity at low and high concentrations of Cr NPs (Fig. 1).

In this age period (on the 42nd day of the experiment), functional stress of enzyme incretion under the influence of Cr NPs is due to a decrease in the activity of amylase and lipase in the pancreas against the background of increased proteolytic activity. The readsorption of enzymes into the blood and then into the lumen of the small bowel is considered as a possible adaptation of entero-pancreatic regulation in different age periods [36]. This mechanism is probably related to the observed change in enzyme activity in the duodenum to values diametrically opposite to the corresponding indicators in the pancreas.

Thus, it has been shown that broiler chickens that consumed Cr NPs in the diet have dose-dependent multidirectional changes in the activity of digestive enzymes in the pancreas at different age periods. Our data on the modification of enzymatic activity in the presence of nanoparticles is consistent with the results obtained by other authors [37-39]. In their experiments, the formation of chymotrypsin complex with selenium NPs contributed to the pH shift of hydrolytic activity to the alkaline side with a simultaneous increase in the maximum enzymatic activity in comparison with the free enzyme.

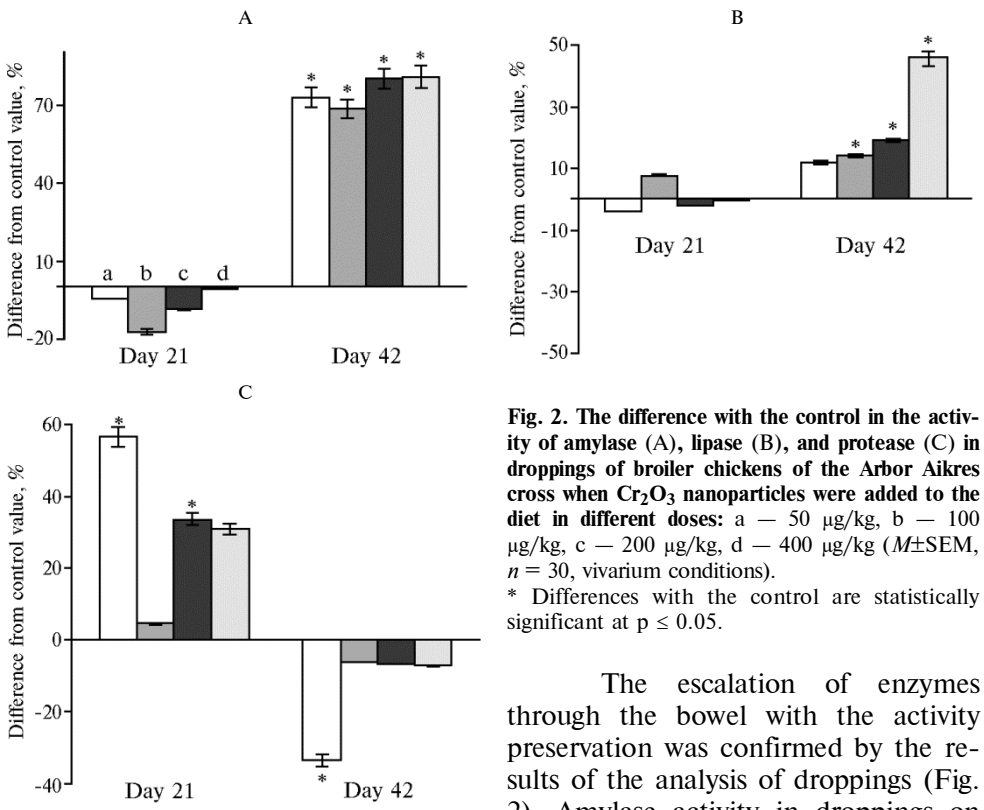

Fig. 2. The difference with the control in the activity of amylase (A)**, lipase** (B)**, and protease** (C) **in droppings of broiler chickens of the Arbor Aikres cross when Cr_2O_3 nanoparticles were added to the diet in different doses:** a — 50 μg/kg, b — 100 μg/kg, c — 200 μg/kg, d — 400 μg/kg (M±SEM, $n = 30$, vivarium conditions).
* Differences with the control are statistically significant at $p \leq 0.05$.

The escalation of enzymes through the bowel with the activity preservation was confirmed by the results of the analysis of droppings (Fig. 2). Amylase activity in droppings on the 21st day decreased, but increased by the end of the accounting period. Lipase activity did not change in the first period and increased by 70-75% on the 42nd day ($p \leq 0.05$). Protease activity, on the contrary, was higher than control on the 21st day, followed by a decrease by the end of the experiment. On the 21st day, the control group had the lowest pH

value of intestinal digesta, while in other groups, this value varied within the pH range of 4.62-7.53. Only in the group received 400 μg/kg of Cr_2O_3 NPs the acidity index on the 42nd day was pH 9.34 and differed from the control. Preserving and increasing the activity of amylase and lipase at this pH may be the example of adaptation of enzymes, as well as their stabilization by nanoparticles. The decrease in amylase activity in Group IV probably indicates the sensitivity of this enzyme to changes in the acid-base balance of the medium.

One of the reasons for the decrease in the intestinal digestion function in birds is the excessive growth of microbial flora in the intestinal lumen, which leads to a decrease in the promotion of chyme and premature deconjugation of primary bile acids [40]. Excessive microbial flora may cause damage to the small bowel epithelium since the metabolites of some microorganisms are cytotoxic. Determining the number of microorganisms in the caecum of broilers is an important step in monitoring the viability of the organism [41]. There was a decrease in total number of microorganisms (by 88.4%) ($p \leq 0.05$), enterobacteria and bifidobacteria (by 28.0 and 65.4%, respectively) ($p \leq 0.05$) in the caecum of broilers on the 21st day in the group that received 400 μg/kg of Cr in feed, and the counts of salmonella increased (by 21.7%, $p \leq 0.05$) (Table 4). At a dose of 50 μg/kg, the number of bifidobacteria and lactobacilli decreased (by 35.6 and 53.8% respectively) ($p \leq 0.05$), a dose of 100 μg/kg increased the number of enterobacteria, but reduced the number of salmonellae, 200 μg/kg contributed to the growth of the number of staphylococci, enterobacteria, salmonella, while reducing the number of bifidobacteria and lactobacilli. The number of bifidobacteria in Group I continued to decline with a simultaneous reduction in the number of staphylococci and salmonella on the 42nd day. The number of enterobacteria increased and bifidobacteria decreased in Group II. The representation of bifidobacteria decreased in Group III, the representation of enterobacteria, bifidobacteria, and salmonella decreased in Group IV on the 42nd day (as well as on the 21st day).

4. The number of different groups of microorganisms in the caecum of broiler chickens of the Arbor Aikres cross on the 21st and 42nd days depending on the dose of Cr_2O_3 nanoparticles in the diet ($M \pm$SEM, $n = 30$, vivarium conditions)

Group	Total microbial number	Staphylococci	Enterobacteria	Salmonellae	Bifidobacteria	Lactobacteria	Cellulose-fermenting bacteria
				Day 21			
Control	37.6±3.20	0.5±0.10	8.9±0.60	15.0±0.70	6.2±0.40	1.4±0.20	0.9±0.20
I	40.0±5.10	0.9±0.10	11.7±1.20	16.5±0.80	4.0±0.30*	0.6±0.09*	1.1±0.40
II	44.8±2.30	1.3±0.20	12.8±1.20*	10.3±0.30*	6.6±0.50	0.9±0.20	0.8±0.20
III	42.6±3.10	2.3±0.20*	15.1±2.10*	20.2±1.40*	3.0±0.20*	0.2±0.01*	1.1±0.30
IV	4.1±0.60*	0.9±0.10	6.4±0.30*	19.2±1.00*	2.1±0.20*	1.7±0.40	0.5±0.10
				Day 42			
Control	48.6±4.10	3.1±0.60	15.1±0.60	2.5±0.30	31.0±2.60	67.0±5.90	2.1±0.30
I	56.1±4.90	1.2±0.10*	18.6±1.30	0.9±0.10*	20.0±2.90*	52.3±4.80	1.8±0.40
II	46.6±3.80	2.2±0.20	19.2±1.10*	0	20.7±2.50*	56.3±5.50	1.9±0.20
III	60.6±5.80	2.0±0.10	17.4±1.90	3.5±0.60	18.7±2.70*	52.3±5.30	2.3±0.30
IV	62.2±5.20	1.8±0.10	12.3±0.80*	0.7±0.20*	17.7±3.10*	62.2±6.80	2.2±0.40

Note. See the description of groups in the Techniques section.
* Differences with the control are statistically significant at $p \leq 0.05$.

In this context, the observed "enzymatic release" indicates violations in the microbial ecology of the bowel, which happens due to a number of reasons: hyperperistalsis, the altered composition of chyme entering the large bowel, hyperproduction of alkaline secretions in the case of pathologies [39]. It is known that under the influence of microbial proteolytic enzymes, the droppings pH shifts towards alkaline values, contributing to the preservation of enzyme activity in the bowel [38, 42, 43].

The multiple physiological effects of Cr NPs observed earlier in rats [44] and, in the present study, in broiler chickens are related to the physicochemical properties of nanoparticles facilitating their interaction with biological objects [45].

So, Cr_2O_3 nanoparticles in the diet are not toxic, as indicated by the absence of changes in the activity of catalase, superoxide dismutase and in the accumulation of malondialdehyde. In this case, chromium acts as an antioxidant, increasing the concentration of NO-metabolites in the blood. The biological role of chromium nanoparticles when introduced in the diet of broiler chickens at doses of 50 and 100 μg/kg is manifested in stimulating growth, production of digestive enzymes and reducing feed costs. At high concentrations of NPs in different age periods (Day 21 and Day 42), the partial suppression of digestive enzymes absorption into the blood and their activity in the bowel (amylase) occurs while maintaining activity in droppings (amylase, lipase) due to the weakening growth of microorganisms (bifidobacteria, staphylococci, and salmonella) and the shift of pH to the alkaline side.

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