ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect Of Ultrafine Particles Of Chromium On Growth Rates, Blood Biochemical Parameters And Activity Of Digestive Enzymes In Broilers Influence Of Ultra Disperse Cr Particles On The Organism Of Broiler Chickens.

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ABSTRACT

In study of 150 broiler chickens (Arbor Aires cross) was divided into five groups: control group, I - ultrafine particles (UFP) of Cr_2O_3 at dose of 50 µg/kg of feed, II - 100 µg/kg, III - 200 µg/kg and IV - 400 µg/kg. Growth-stimulating effect was confirmed by stimulation of protein metabolism (100 and 200 µg/kg), growth of NO-metabolites (200 µg/kg), triglycerides (100 µg/kg, 400 µg/kg). Functional significance of UFP Cr was expressed in activity of amylolytic enzymes in the blood plasma: on day 21 29.5% (400 µg/kg, $p \leq 0.05$), lipase activity on days 21 and 42 from 19% (200 µg/kg) to 30% (400 µg/kg) of UFP of Cr, on background of decrease in lipolytic activity on day 42. Activity of lipase in the duodenum was significantly increased in all experimental groups on day 21, and on day 42 - group 400 µg/kg. High protease activity at dose of 400 µg/kg for 21 days and for all groups, except 200 µg/kg on day 42. Significantly, activity of amylase increased at 100 µg/kg on day 21 and decreased at 200 µg/kg. On day 42 the activity at 50 µg/kg significantly increased and decreased at 200 µg/kg. Thus, was found that doses in the range of 100 and 200 mg/kg were characterized by pronounced positive effect.

Keywords: ultrafine chromium particles, broiler chickens, productivity, digestive enzymes

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ISSN: 0975-8585

INTRODUCTION

Chromium is essential element for carbohydrate, fat and lipid metabolism, participates in maintaining cholesterol homeostasis and exhibits antioxidant and growth-stimulating properties [1-3]. However, due to low chromium content in the components of the diet, its level is not regulated. In particular, chromium enhances the action of insulin by attaching it to sulfhydryl groups [4], as it was previously thought. Later it was found that there is a low-molecular Cr-binding substance (LMWCr), which activates phosphotyrosine phosphotase in isolated idipocyte membranes, which proves the fact of functional binding between the action of chromium and insulin [5].

Correction of the diet with chromium-containing products can reduce blood glucose, cholesterol and fat levels and stimulate the development of muscle tissue [6]. Thus, the participation of chromium in metabolic reactions of the body is undeniable, but the source of chromium can be a key factor affecting its bioavailability.

New prospects today open the development of nanotechnology, which brought new knowledge to many branches of science. The prospect of using nanomaterials is based on reducing the period of degradation of large-sized particles into small ones, which allows to pass unimpeded through the intestinal mucosa for absorption. It is known that organic forms of chromium have a higher bioavailability (10-25%) than inorganic sources (3%) [7-8]. Thus, decrease in the size of metals can significantly alter the absorption process by increasing bioavailability from the digestive tract. The prospective replacement of traditional sources of trace elements with ultradispersed forms of metals proves that the latter have a high specific surface area, greater reactivity and bioavailability.

Given the small size and high penetrating power of ultrafine particles, it must be remembered that each department of the gastrointestinal tract has a unique environment that includes its own set of enzymes and pH level. UFPs should be able to overcome these obstacles in order to manifest their biological activity in the appropriate place, that is, in the small intestine [9].

Scientists [10-11] found that supplementing the diet with UFP of chromium increases the muscle area, chromium content in the tissues, while reducing the fat ratio and the thickness of spinal fat in pigs. In bird experiments, the addition of chromium increases the percentage of protein in the muscles of the chest and thigh and reduces the level of cholesterol in the muscles [12]. Similar results were obtained by Domínguez-Vara et al [13] in studies on rats.

Nevertheless, reports on the effect of ultradisperse chromium on the activity of digestive enzymes in broilers are rare.

In this connection, a promising direction is to study the possibility of using UFP as modulators of digestive enzyme activity. This question is interesting by the existence [14], not of a single, but of several regulatory centers in molecules of digestive enzymes, specialized for different groups of effects. According to number of researchers [15], rapid conformational transitions of one molecule formula to another can occur as a result of the action of both organic and inorganic modifiers. This leads to changes in the level of enzyme activity. Another possible mechanism of interaction of metal with enzyme molecule is probably to bind metal ion to special contact pad (regulatory or allosteric center) spatially separated from the active center [16]. In this case, the metal component of certain enzymes (peptidase, alkaline phosphatase) is replaced by metal that is close in atomic structure.

Thus, the search for alternative forms of trace elements is indispensable tool for managing the process of digestion, increasing the conversion of nutrients in the body, to achieve better absorption, productivity and nutritional value of poultry products.

The purpose of this study was to study the effectiveness of using different doses of UDC of chromium on the activity of digestive enzymes of duodenal chyme, the biochemical and growth parameters of broiler chickens.

ISSN: 0975-8585

MATERIALS AND METHODS

Animals and feed

For research, broiler chickens of the cross Arbor Aykres at the age of 7 days purchased at JSC "Orenburg Poultry Farm" (www.pfo56.ru).

The experimental part of the work was carried out in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (National Standard of the Russian Federation GOST R 53434-2009) with standard procedures for the operation of bioobjects. The experiments were conducted in accordance with the requirements of humane treatment of animals. Poultry maintenance and procedures during the execution of the experiments met the requirements of the instructions and recommendations prescribed by the national regulations (Order of the Ministry of Health of the USSR 755 of 12.08.1977) and "The Guide for Care and Use of Laboratory Animals".

The animals were kept in the vivarium of the Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences in individual cells KUN-05 with useful area of 4050 cm^2 (90×45×45 cm) made of galvanized welded wire mesh and galvanized iron sheet. Cells are equipped with automatic 2 nipple drillers with polypropylene hose, feeder (length - 90 cm), galvanized pallet.

Feeding of the poultry was carried out 2 times a day balanced according to the recommendations of VNITIP (2011), NRC, (1994) mixed fodders (Table 1).

Table 1: Basic dietary composition of broiler chickens

Ingredients, %	Starting (0-3 weeks)	Growth (3-5 weeks)
Wheat	27,1 %	41,2 %
Corn	16,0 %	22,0 %
Soybeanmeal, 40%	25,0 %	15,0 %
Sunflowermeal, 38%	18,0 %	8,0 %
Fishmeal, 59%	4,00 %	6,00 %
Sunfloweroil	5,0	2,8 %
Lysinemonochlorohydrate 98%	0,24 %	0,11 %
Dl-methionine 98,5%	0,10 %	0,13 %
L-threonine 98%	0,03 %	0,54 %
Cookingsalt	0,28 %	0,30 %
Monocalciumphosphate	0,7 %	0,7 %
Chalkaft	0,5 %	0,4 %
Limestoneflour, 35%	1,0 %	0,7 %
Foodsoda (sodiumbicarbonate)	0,05 %	0,10 %
Vitaminpremix*	1,0 %	1,0 %
Mineralpremix*	1,0 %	1,0 %
CalculatedValues:		
Crudeprotein (%)	23,98	21,0
ME (Kcal/kg)	2860	2900
Calcium (%)	1	0,9
Availablephosphorus (%)	0,45	0,4
Lysine (%)	1,36	1,12
Met + Cys (%)	0,9	0,7
Threonine (%)	0,9	0,8
Valueofanalysis:		
Crudeprotein (%)	23,3	20,9
Cr (ppb)	770	650
* Premixforvitaminsperkgofration: vitamin A, 12500 ME; vitaminD_3 , 3125 ед. vitamin E, 37,5 ME; vitamin K_3 , 6,25 мг; vitamin B_1, 3,75 мг; vitamin B_2 , 12,5 мг; vitamin B_6 , 10,0 мг; Panto-thenate, 18,8 мг; Niacin, 50		

мг; Biotin, 0,06 мг; folicacid, 1,25 мг; vitamin B_{12} , 0,05 мг.

*Mineral premix per kg of ration:Cu ($CuSO_4 \times H_2O$, 25,45% Cu) 6 mg; Fe ($FeSO_4 \times 7H_2O$, 20,29% Fe) 50 mg; Mn ($MnSO_4 \times H_2O$, 32,49% Mn), 40 mg; Zn (ZnO, 80,35% Zn) 60 mg; Se ($NaSeO_3$, 45,56% Se) 0,075 mg.

There was plenty of drinking. The temperature regime was carried out with the help of a thermostat for internal premises RTR-B to maintain the set temperature, with an accurate temperature adjustment from +15 to +25° C (error - no more than 1 ° C). Lighting mode - 12 h light/12 h dark. The humidity of the room was 60%. The air quality was assessed for several components: oxygen content - 18%, carbonic acid - 0.15%, ammonia - 7 mg/m^3 and hydrogen sulfide - 2 mg/m^3.

150 chickens of 7 days of age were used for the experiment. The bird was marked with plastic foot tags. 5 groups were formed on the basis of daily weighing of accounting and accounting for the multinutrient composition of the compound by the method of para- analogues: one control and four experimental (n = 25, weight 160 to 180 g).

The control bird received OD throughout the experiment, in which chromium was included as chromium chloride ($CrCl_3 \times 6H_2O$ containing 19.5% chromium) (Reahim JSC, Russia). Bird of experimental groups during the experiment (14-42 days) replaced chromium chloride with UFP Cr2O3 (LLC Platinum, Moscow, method for obtaining plasma-chemical synthesis, d = 91 nm, specific surface area 9 m2 / g, Z-potential 93 ± 0.52 mV, 99.8% Cr) group I at a dose of 50 μg/kg of feed, II - 100 μg /kg, III - 200 μg/kg and IV - 400 μg/kg.

Material testing of UDC (particle size determination, polydispersity, bulk, fraction content, surface area) included electron scanning, translucent and atomic force microscopy using LEX T OLS4100, JSM 7401F and JEM-2000FX (JEOL, Japan). The particle size distribution was examined on a Photocor Compact analyzer (Fotocor LLC, Russia). UFP preparations were dispersed in physiological saline using UZDN-2T (NPP Akadempribor", Russia) (35 kHz, 300 W, 10 μA, 30 min).

Dosages from 50 to 400 μg/kg of feed are selected taking into account the previously obtained positive effect of chromium influence on the growth and biochemical parameters of broiler chickens [17]. In particular, [18] found that the addition of Cr increased the mass of pectoral muscles, with low fat and cholesterol. [19] found that with additional inclusion of Cr at a dose of 300 μg / kg of feed, the weight of the carcass increased.

Mixed feed was prepared by the stepwise mixing method, UDC was injected after 30 min of dispersion in distilled water with USDN-2T ("NPP Akadempribor", Russia) (35 kHz, 300 W, 10 μA, 30 min).

OBSERVATION AND AUTOPSY

Control over the growth of individuals was carried out at the beginning and after 3 and 5 weeks of the experiment before feeding. Based on the weightings, the absolute and average daily growth was calculated, and the growth dynamics of the experimental animals was studied.

Daily feed intake was recorded to calculate feed intake and feed conversion ratio.

Biomaterial for the study was obtained after decapitation of broilers (5 individuals in each variant of the experiment and in control on days 21 and 42.

Then post-mortem anatomical cutting of carcasses was carried out according to the [20], during which absolute and relative masses of internal organs were measured.

Morphological and biochemical composition of blood

At the end of the experiment, broiler blood samples were collected from the wing vein (feed removed 12 hours before blood collection) and centrifuged at 2000 g, 25 °C for 10 min. The serum was then harvested and stored at 20 ° C until analysis.

Blood sampling in birds was carried out in the morning, on an empty stomach, before slaughter at 21 and 42 days old from the axillary vein, for morphological examination - in vacuum tubes with an anticoagulant (EDTA), for evaluation of biochemical indicators - in vacuum tubes with a coagulation activator (thrombin).

The morphological parameters were determined using the automatic hematological analyzer model URIT-2900 Vet Plus ("URIT Medial Electronic Co., Ltd", China). Biochemical studies of blood serum were performed on an automatic analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) using commercial kits for veterinary DiAvTest (Russia) and Randox Laboratories Limited (United Kingdom).

The level of NO-metabolites in blood plasma and tissues was determined spectrophotometrically using the Griss reagent on the Infinite PRO 200 microtiter plate analyzer (TECAN, Austria) at a wavelength of 540 nm [21]. Laboratory studies were conducted in the laboratory "Agroecology of Technogenic Nanomaterials" of the Test Center of the FSC of the BST RAS, (accreditation certificate RA. RU.21PF59 dated 02.12.15).

Determination of digestive enzymes in the duodenum and blood

After opening 12 duodenum were recovered into sterile tubes 12 and duodenum, which was stored no longer than 12 h before the assay at 4 °C. For the analysis for 1 g of 12 duodenum, 4 ml of Ringer's solution was added and homogenate was prepared from the tissues of the organs. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected in sterile tubes and used for analysis.

The activity of pancreatic enzymes in chyme of the duodenum in vivo, in blood plasma and litter was carried out on an automatic biochemical analyzer CS-T240 ("Dirui Industrial Co., Ltd", China) using commercial biochemical sets for veterinary Diabetes Test (Russia) (pancreatic amylase, α-amylase, lipase (kit) .The determination of protease was carried out by the method of casein hydrolysis according to Anson.

Statistical processing

Statistical processing of the data was carried out using the Statistica 10.0 software package and the MS Excel 2000 software package. Data are presented as: mean (M) ± standard error of mean (m). Reliable results were considered at P≤0,05.

EXPERIMENTAL

For the period of the experiment, the feed consumption per 1 kg of gain in the control was 1.85 kg, the difference depending on the dose of injected UFP Cr was from 8 to 16% (Table 2).

Table 2: Growth of broiler chickens Arbor Aikres crosses and feed costs when included in the ration of UDP Cr (M±m, n=30, experiment in the conditions of the vivarium).

Group, µg/kg	Liveweight				Feed costs per 1 kg increment	
	Total, g		incrementperexperience (1-6 weeks)			
	7 days	42 days	kg	tothecontrol,%	kg	tothecontrol,%
0	224,7±2,4	2266±20,3	2,04±0,05		1,85	
50	230,8±4,3	2366±40,1	2,13±0,06	105	1,71	92
100	234,2±3,0	2431±36,4	2,19±0,02	108	1,70	92
200	224,5±3,2	2533±59,8	2,30±0,11	113	1,67	90
400	232,0±23,1	2536,0±78,2	2,31±0,08	113	1,55	84
Note. The increase and cost of feed in the control are taken as 100% * Differences with control are reliable at p≤0.05						

Inclusion in the diet of chromium was accompanied by change in the intensity of growth of chickens. In particular, for the period of the experiment in the III and IV experimental groups, the absolute growth of the live weight was 2308.6 and 2312.0 g, which is 3.9 and 3.1% higher than in the control. The difference with the

control in I (50 µg/kg) and II (100 µg/kg) in the experimental groups did not exceed 1.5%.

Introduction of UFP of chromium in various dosages led to unequal changes in the biochemical parameters of the blood of broiler chickens (Table 3).

Table 3: Biochemical indicators of the blood of broiler chickens

Index	Groups				
	Control	I	II	III	IV
21 days					
Totalprotein, g / l	34,93±1,2	35,93±1,2	40,89±2,9	35,18±1,5	38,26±0,3
ALaT, U/L	7,30±0,5	8,30±2,4	14,25±1,0***	7,13±3,2	13,20±1,7**
ASaT, U/L	52,50±33,0	80,20±50,1*	190,37±76,9**	188,67±33,2**	106,43±20,0**
Glucose, mmol/l	14,04±0,4	14,84±0,8	14,39±1,6	15,27±0,5	14,88±0,5
Cholesterol, mmol/l	3,08±0,1	3,44±0,2	2,99±0,6	2,73±0,3	3,22±0,3
BilirubinTotal, µmol/l	0,81±0,3	0,86±0,0	0,66±0,1	0,65±0,3	0,81±0,2
Triglycerides, mmol/l	0,65±0,2	0,62±0,2	1,69±0,2*	0,63±0,3	1,25±0,1*
Amylase, U/l	436,0±19,1	422,0±195,1	303,9±64,1	403,7±25,7	617,7±42,9*
Lipase, U/l	6,43±0,3	7,2±0,3	6,83±0,3	7,93±0,8	8,30±2,4
42 days					
Totalprotein, g / l	41,99±2,0	37,25±1,9	40,15±1,8	43,04±0,8	44,63±4,7
ALaT, U/L	23,00±2,1	17,10±1,0	26,33±1,1	24,07±2,4	15,43±1,1
ASaT, U/L	70,50±6,1	152,00±8,5***	63,83±7,8	69,43±13,7	116,6±20,9**
Glucose, mmol/l	15,45±0,4	14,34±0,6	13,48±1,1	14,06±0,5	14,21±0,2
Cholesterol, mmol/l	3,81±0,3	3,33±0,1	3,02±0,3	3,11±0,3	3,93±0,4
BilirubinTotal, µmol/l	0,65±0,1	0,76±0,2	0,76±0,2	0,76±0,1	0,55±0,2
Triglycerides, mmol/l	0,69±0,1	0,40±0,2	0,39±0,1	1,14±0,3*	2,18±0,7*
Amylase, U/l	173,3±5,6	180,3±5,6	157,03±10,4	166,3±1,2	186,3±1,2
Lipase, U/l	2,73±0,8	2,30±0,8	1,43±0,7	2,83±0,7	5,47±4,5**
* Differences with control are reliable when p≤0,05 ** Differences with control are reliable when p≤0,01 *** Differences with control are reliable when p≤0,005					

The results indicate that stimulation of protein metabolism occurred in groups with a maximum level of UFP of chromium (200-400 µg/kg). The level of protein compared to the control was 2.7-6.2% higher.

The growth-stimulating effect of UFP was determined by the growth of NO metabolites throughout the entire experiment in group III (200 µg/kg), the difference with the control group (59.8 µmol/l) was 20.0% (p≤0.05). In other groups, the indices compared with the control group did not exceed 5%.

The marker of energy and lipid metabolism is the level of triglycerides in the blood. On day 21 in broilers who received chromium at a dose of 100 and 400 µg/kg as part of the ration, compared with the control, the triglyceride level increased by 61.6% and 48.5%, respectively. With the prolongation of the effect towards the end of the accounting period, where the III and IV groups were characterized by the maximum values.

The effect of UFP was expressed in stimulating the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (ASaT). At day 21, the level of ALT in the II and IV groups was almost 2 times higher than in the control (p≤0,05), against the background of an equal level of bilirubin. In the case of ASaT, the differences were comparable to the level of UFP of chromium in the diet, significant differences of 3.5 times (p≤0.05) were characteristic for dose of 200 µg/kg.

By the end of the experiment on day 42, the level of ALT in the blood serum of broiler chickens was increased. The level of ALT decreased in the groups with the minimum and maximum dose of UFP of chromium, while the activity of ASaT, on the contrary, increased by 53.7% and 39.6% (p≤0.05) at the given dosages. The level of bilirubin in the groups did not differ significantly.

ISSN: 0975-8585

The functional significance of UFP chromium was expressed in the activity of amylolytic enzymes in the blood plasma (Table 3). On the 21st day the maximum level of amylase was established in the IV test group, the difference with the control values was 29.5% ($p \leq 0.05$). In other groups, no significant differences were found. A similar dynamics was characteristic of lipase. At a concentration of 200 and 400 mg/kg UFP Cr, lipase activity on days 21 and 42 was 19 to 30% higher than the reference values. By the end of the experiment, lipolytic activity decreased.

Transformation of substances in the body depends on the effective work of the digestive glands and their ability to adapt to changing feeding conditions (Figure 1).

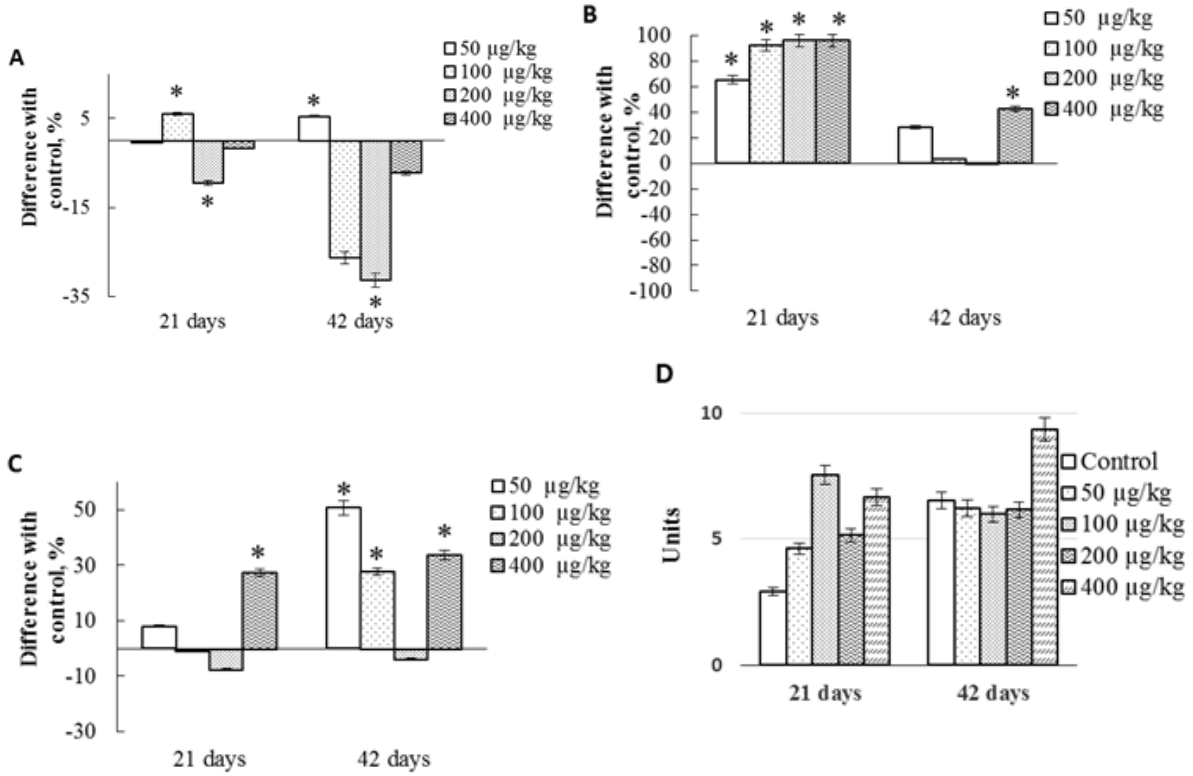

Figure 1: Activity of the digestive enzymes of the duodenum of broiler chickens: (A) amylase, (B) lipase and (C) protease by the administration of UFP of Cr_2O_3 at various dosages. (D) pH of intestinal contents of birds. Note: * Differences with the control are reliable at $p \leq 0.05$

On day 21, the amylase activity was significantly higher than the control at dose of 100 µg/kg (5.95%), and below the control at 200 µg/kg (9.45%). The activity of lipase on day 21 significantly increased in all experimental groups with an increase in the concentration of UFP (correlation level 0.82) to 96%. The protease activity was significantly higher than the control (+37.66%) at a concentration of 400 µg/kg of the UFP Cr_2O_3. At the same time, pH values of intestinal contents were in the range of values <7, which is known to be the optimal conditions for the manifestation of enzyme activity and shows that changes in the activity of enzymes were mediated by other mechanisms.

On the 42 day we observed a slightly different picture of changes in the activity of digestive enzymes. The activity of amylase in all experimental groups was below the control values (by 7.29-31.21%), except for the concentration of 50 µg/kg of feed, where the activity of amylase was significantly higher than the control by 5.45%. The lipase activity was significantly higher at a chromium concentration of 400 µg/kg of feed (by 42.26%, respectively). Protease activity was significantly higher than control in all experimental groups, except for a dose of 200 µg/kg. The pH activity differed from the control values only at 400 µg/kg and was in the alkaline range (>7 units). Thus, the preservation and increase in lipase and protease activity at a given pH can be an example of adaptation of enzymes, including the consequence of the stabilization of enzymes of UFP of chromium, which has been shown in a number of studies. A decrease in the activity of amylase at a

concentration of 400 µg/kg may indicate a sensitivity of this enzyme to a change in the acid-base balance of the medium.

DISCUSSION

In our study, stimulation of growth and the coefficient of productive use of feed corresponded to a dose range from 100 to 200 mg/kg of feed. A similar growth-stimulating effect was obtained by the researchers when tested as an additive for UFP Fe, Cu, Zn [22-23].

However, [24] reported that a dose of 300 µg/kg of NanoCrPic does not affect feed intake and body weight in rats. At the same time, [25] indicated that broilers receiving 1200 ppb CrPic had increased body weight, feed intake and improved feed efficiency. [26] reported that the broiler diet, supplemented with inorganic trivalent chromium and organic acid, improved the conversion rate of food. In pigs supplemented with CrPic, the weight increased at a dose of 50 and 200 ppb, and decreased at 100 ppm, but the efficiency of feed utilization did not influence [27].

The results of the studies [28-29] confirmed that the additional Cr (200 ppb) increased the average daily weight gain without increasing the efficiency of the feed. In contrast, Cr, added at 0-800 ppb, significantly reduced feed intake and weight gain with increasing Cr dose, but this did not affect feed efficiency [27]. In other studies [29-30], it was found that CrPic at a dose of less than 250 ppb does not affect the weight gain of pigs in the active phase of growth.

The specific mechanism of action of UDP was accompanied by stimulation of protein metabolism. The pronounced effect is established in groups with additional introduction into the diet of UDP chromium (200-400 µg/kg), and is confirmed by the growth of NO-metabolites throughout the entire experiment. In our study, there is no difference in glucose and cholesterol levels, which is consistent with the data [31]. Researchers [24] reported that the addition of chromium in the form of NanoCrPic (300 µg/kg) in the rat diet did not change the HDL LDL + VLDL profile, but lowered the LDL-C level.

The increase in triglycerides against the background of the growth-stimulating effect does not agree with the data of [32-33], where high cholesterol and triglycerides are characteristic for chromodeficiency states. Such an ambiguous reaction of the organism is probably connected with the rearrangement of the enzymatic system, as well as an additional load on the mitochondrial apparatus of the liver cells [34], through the participation of chromium in the stimulation of the protein velocity [35] and lipid metabolism in the liver [28], against the background of high-energy rations characteristic of modern bird crosses.

Ultrafine chrome nanoparticles altered the homeostatic blood indices, promoting an increase in the activity of alanine aminotransferase (ALaT) and aspartateaminotransferase (ASaT). High activity of endogenous transferases on the one hand may be signs of impaired liver, kidney and pancreas function. But since there is no reaction of markers of inflammation (creatinine, bilirubin, urea), it can be assumed that the metabolic function of chromium can be associated with subtle mechanisms of its participation in stimulating the production of chromodulin (LMWCCr). Chromodulin accepts chromium molecules from biological molecules, including transferrin, and stimulates hepatoprotective activity [5]. The role of chromodulin in protein synthesis and anticholestatic action is not excluded. The reason for the increase in serum enzyme activity may be the result of the synthesis or resynthesis of micronutrients, the increase in the permeability of cell membranes, the activity of digestive enzymes.

The functional significance of UFP chromium was expressed in stimulating the activity of digestive enzymes in the blood plasma. In groups with the highest doses of UFP Cr (200 and 400 µg/kg), the activity of lipase and amylase was greatest. This fact, possibly, indicates the presence of stress in the adaptive mechanisms of the translocation of digestive enzymes through the bloodstream, and also confirms the hypothesis of enteropancreatic circulation of digestive enzymes [36] in different age periods.

The high amylolytic and proteolytic activity in the chicken broth chicken of the broiler chickens is consistent with the data obtained by [37], where a complex of chymotrypsin with UFP of selenium contributed to a shift in the pH of the hydrolytic activity to the alkaline region with simultaneous increase in the maximum enzymatic activity in comparison with free enzyme.

It should be noted that the peculiarity of intestinal digestion in birds in comparison with mammals is a higher concentration of hydrogen ions in the chyme, i.e. lower pH values in all parts of the small intestine. It was also shown that the addition of 0.1% organic acid positively influenced the morphology of the intestines of broilers [38]. At the same time, the hydrolytic activity of enzymes is affected by the pH of the medium and can vary depending on its magnitude [39].

As is known, the presence of hydrolytic enzymes in other media of the body, except pancreatic juice, is due to their recycling. In this case, the principle of recycling of enzymes refers to the activity of all exocrine digestive glands, as they and their glandulocytes are duacrinous, that is, they transport the enzymes both exo- and endocrine. The first way of the appearance of pancreatic enzymes in the blood and lymph is the increment of enzymes by the glandulocyte glands. A number of authors [40-41] postulate that the circulating enzymes are a depot for their subsequent pancreas recovery. The second mechanism is a resorption from the excretory ducts of the gland, the so-called "enzyme evasion". The third mechanism is the resorption of enzymes from the small intestine [41, 42].

Preservation and increase in the activity of lipase and protease at a given pH can be an example of adaptation of enzymes, including the consequence of stabilization of enzymes of UFP of chromium, which has been shown in a number of studies. Decrease in the activity of amylase at a concentration of 400 µg/kg may indicate a sensitivity of this enzyme to a change in the acid-base balance of the medium.

Stimulation of digestive enzyme production in our experiment at low and high doses in the first case did not lead to visible growth changes. But at high doses, against the background of unstable high endogenous transferase rates, there was stress in the redistribution of digestive enzyme activity in duodenal chyme.

In general, the promise of using nanoscale fertilizing is due to the fact that one of the main factors determining the physical characteristics of nanoscale objects is the developed surface, which contributes to the predominance of surface phenomena. Due to its size, comparable to the size of cells, viruses, proteins, DNA, UDF can approach the bioobject, interact and communicate with it [43].

CONCLUSION

Thus, the use of UFP of Cr_2O_3 in the composition of the ration in the range of doses from 100 to 200 µg/kg had a more stable position in terms of the parameters to be evaluated in terms of the aggregated parameters (weight gain, feed costs, biochemical parameters of blood serum, activity of digestive enzymes in dodenal chyme). These doses will have a beneficial effect on the morpho-biochemical parameters of the body and can be used as a source of chromium for agricultural poultry.

ACKNOWLEDGMENTS

The study was supported by the Program of Basic Scientific Research of the Russian Academy of Sciences (No. 0761-2018-0031)

Conflict of Interest: Authors declares that they has no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

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