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DIFFERENT CHROME SOURCES INFLUENCE ON MORPHO-BIOCHEMICAL INDICATORS AND ACTIVITY OF DIGESTIVE ENZYMES IN Wistar RATS

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Abstract

Nowadays, issues of mineral nutrition of humans and animals are quite relevant. High-energy rations, multicomponent feed mixtures and additives in the diets require special attention when optimizing limited microelements. A priori, chromium, being an important trace element in animals, is used to correct carbohydrate, fat and lipid metabolism. Due to its low content in the components of diets, its role in the formation of the microecological status of the body is poorly understood. At the same time, its biological availability in the body depends on the source of chromium. In the present work, using a model object, the Wistar rats, we for the first time compared the biological effects of various chromium sources, i.e. picolinate (CrPic), nanoform (NP Cr_2O_3) and chloride ($CrCl_3$) at doses of 300 and 500 μg/kg feed, according to a set of indicators (feed digestibility, hematological parameters, activity of digestive enzymes, composition of intestinal microflora) and established greater bioavailability and more pronounced positive effect of picolinate and chromium nanoparticles on body weight and hematological parameters and ambiguous influence of the studied forms on the activity of digestive enzymes and intestinal microflora. The purpose of this work was to study the biological effect of chromium in various forms and dosages on Wistar rats. The studies were carried out on 105 white male rats weighing 70-80 g under standard vivarium conditions (Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences). Animals were divided into seven groups (n = 15 each). The control group was fed a common diet. The group I diet included Cr_2O_3 NPs at a dose of 300 μg/kg feed (NP 300), group II — $CrCl_3$ at a dose of 300 mg/kg ($CrCl_3$ 300), group III — chromium picolinate (CrPic) at a dose of 300 mg/kg (CrPic 300), group IV — Cr_2O_3 NPs at a dose of 500 μg/kg of feed (NP 500), group V — $CrCl_3$ at a dose of 500 mg/kg ($CrCl_3$ 500), and group VI — CrPic at a dose of 500 mg/kg (CrPic 500). Nanoparticles were introduced into the feed by mixing. The introduction of chromium in the form of CrPic and Cr_2O_3 NPs at a dose of 500 μg/kg, under the same feed consumption, was accompanied by an increase in the body weight of rats by 22.6 and 22.2 % ($p \leq 0.05$). The effect of Cr_2O_3 NPs expressed as the absence of the reaction of lymphocytes, monocytes and granulocytes, in other cases their level exceeded control values by 14 to 45 %. Synthesis of hemoglobin was adequate to stimulation of erythropoiesis, but an increase in the number of platelets resulted in blood sludging, an increase in viscosity and difficulty in perfusion through vessels. This symptom was typical for NP Cr_2O_3 300, $CrCl_3$ 300 and $CrCl_3$ 500 (the difference with the control is from 70 to 90 %, $p \leq 0.05$). High digestibility of CrPic and NPs Cr_2O_3 500 (from 20.2 to 34.0 %), accompanied by manifestation of hepato- and nephrotoxicity, with signs of oxidative stress, decreased activity of amylase and lipase in the blood plasma, indicating a depressant effect of high doses of chromium on enteropancreatic circulation of the digestive enzymes and metabolic disorders of Mg and Fe in the blood. Triglycerides, like true fats, decreased at maximum doses of chromium in the form of chloride and picolinate, confirming their participation in lipid metabolism, causing splitting of excess fat in the body, and reducing the ratio of fat to body weight ratio from 2 (control group) to 0.82 (NP Cr_2O_3 500). The indicators of bilirubin and creatinine clearly demonstrate the absence of toxicity at low doses, i.e. Cr_2O_3 NPs 300 and CrPic 300. Amylase activity in the pancreas is increased at a dose of 300 μg/kg

of Cr_2O_3 NPs. Dietary CrPic in a similar dosage stimulated the activity of lipase and protease, whereas in the 12 duodenal ulcer it led to a decrease in the activity of amylase and lipase. $CrCl_3$ and CrPic at a dosage of 500 μg/kg reduced the activity of lipase in the duodenum. The specific effect of Cr NPs 500 μg/kg on the microecological status of the organism was manifested in a decrease in the number of lactic acid bacteria by 55.9 %. The number of bifidobacteria was significantly higher, by 48.6 % ($p \leq 0.05$), in the PicCr 500-fed group. The number of enterobacteria in the NP 300-fed group was 34.8 % lower than the control, while in the other groups their number increased 24.0-33.7 times ($p \leq 0.05$). From the totality of the estimated parameters of the intestinal microflora, the use of nanoparticles in the composition of the diet is promising due to the lack of resistance. Thus, chromium of CrPic 500, NP Cr_2O_3 300 does not show a toxic effect on the body, and has a stimulating effect on growth, development, digestibility of chromium, the activity of the digestive enzymes and microecological status of the organism, which puts these forms in the category of promising sources of chromium for the correction of metabolism and the microbial composition of the gastrointestinal tract of animals.

Keywords: rats, chromium concentration, productivity, blood biochemical parameters, digestive enzymes, intestinal microflora

Chromium (Cr) is a necessary element for animals and humans, but the mechanism of its biological action is not fully understood. It is known that chromium enhances the function of insulin, as well as stimulates the rate of insulin-induced swelling of isolated mitochondria [1], increases the respiratory factor of epididymal fat pad [2], interacts with the thyroid gland [3], and plays an important role in the metabolism of proteins and nucleic acids by significantly increasing the stimulation of amino acids in liver protein in vitro [4]. Okada et al. [5] found that the direct interaction of Cr with DNA leads to a significant stimulation of RNA synthesis in vitro, and also identified a unique protein containing 5-6 Cr atoms, which is characterized by anabolic functions. A new Cr-binder [6] was found that potentiates the effect of insulin in the conversion of glucose into lipid and carbon dioxide in isolated adipocytes [6].

Although chromium exists in nature in oxidation states from Cr^{2-} to Cr^{6+}, relatively inert complexes of Cr^{3+} may function as structural components that bind ligands with the proper dimensional orientation, contributing to the enzymatic catalysis, and also support the tertiary structure of proteins or nucleic acids [7]. In chickens, the inclusion of additional chromium in the diet improved the physiological status and productivity under cold and heat stress [8, 9], and in pigs, the addition of 200 rg of chromium picolinate (CrPic) in the diet increased the rate of nitrogen absorption [10].

The modern livestock breeding has a tendency to replace traditional mineral sources of trace elements with new organo-mineral or nanoforms, due to their better digestibility, bioavailability, and prolonged action [11, 12]. It is shown that the metabolic response of the body to chromium depends on its chemical form: organic sources have higher bioavailability (10-25%) than inorganic sources (3%) [13]. Reduction of the size of chromium particles may increase the rate of digestion and absorption. Lien et al. [14] found that CrPic nanoparticles were significantly better absorbed than typical CrPic, which led to an increase in the content of chromium in the blood serum of rats. In the case of the inclusion of chromium nanoparticles in the diet of pigs, the area of the longissimus muscle and the chromium content in tissues increased, while the fat ratio and the thickness of back fat decreased [15, 16]. In the studies in rats, nanosized chromium significantly increased the mean increment of body weight, the feed efficiency, the ratio of fat deposits, the insulin concentration in the blood serum, and the content of chromium in the organs [17].

It may be assumed that the change in feed conversion, meat productivity, and biochemical parameters in animals is based on the mechanisms of chromium participation in digestion and metabolism through the stimulation of digestive enzymes. The study of alternative forms of trace elements in the diet of

animals is a necessary tool for the management of digestion, the formation of productivity, and the nutritional value of animal products.

In the present paper, the biological effect of various forms of chromium (picolinate, nanoforms, and chloride) at doses of 300 and 500 rg/kg of feed on a set of indicators (digestibility, the hematological parameters of blood, the activity of digestive enzymes, and the composition of intestinal microflora) was compared for the first time on the model object (Wistar rats); the study showed high bioavailability and a more significant positive effect of picolinate and chromium nanoparticles on body weight and hematological parameters, as well as the ambiguous effect of the studied forms on the activity of digestive enzymes and intestinal microflora.

The work objective was to study the biological effect of chromium in various forms and dosages on Wistar rats.

Techniques. The studies were carried out on 105 white male Wistar rats weighing 70-80 g under standard vivarium conditions (Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences). The diet of animals (GOST R 50258-92) corresponded to the rules of laboratory practice during preclinical studies in the Russian Federation (GOST R 51000.3-96 and R 51000.4-96). 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 and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)." Rats were kept in separate cells with free access to water and feed.

Based on the results of previous tests [16, 17], the doses of chromium in the experiments were 300 and 500 rg/kg of feed. After the preparatory period (7 days), the animals were divided into seven groups (n = 15 each). The control group was fed a common diet. The diet of group I included Cr_2O_3 NPs at a dose of 300 μg/kg feed (NP 300), group II received $CrCl_3$ at a dose of 300 mg/kg ($CrCl_3$ 300), group III received chromium picolinate (CrPic) at a dose of 300 mg/kg (CrPic 300), group IV received Cr_2O_3 NPs at a dose of 500 rg/kg of feed (NP 500), group V received $CrCl_3$ at a dose of 500 mg/kg ($CrCl_3$ 500), and group VI received CrPic at a dose of 500 mg/kg (CrPic 500). Nanoparticles were introduced into the feed by step-type mixing.

Ultrafine particles (UFPs) of chromium were obtained by the method of plasma-chemical synthesis (OOO Platina, Moscow; d = 91 nm, specific surface of 9 m^2/g, Z-potential 93±0.52 mV, the Cr content of 99.8%). Nanoparticle preparations were dispersed in a saline solution using UZDN-2T (NPP Akadempribor, Russia; 35 kHz, 300 W, 10 μa, 30 min). The inorganic form of Cr in the form of chromium chloride $CrCl_3 \cdot 6H_2O$ a.r.g. (19.5% Cr) (AO Reakhim, Russia) and chromium picolinate (contains 10% organic chromium, ZAO Evalar, Russia) were also used.

At the beginning and end of the experiment (day 21), growth rates and feed consumption were taken into account. Blood was taken from the tail vein into vacutainer tubes with the addition of anticoagulant, for biochemical parameters – into vacutainer tubes with a clot activator (thrombin). Blood morphological analysis was carried out on the automatic hematology analyzer URIT-2900 Vet Plus (URIT Medical Electronic Group Co., Ltd, China), biochemical analysis of blood serum on the automatic analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with commercial veterinary kits (ZAO DIACON-DS, Russia).

After decapitation of rats on day 21 under Nembutal anesthesia (5 individuals from each group), bio-substrates were selected for analysis. The concentration of chromium was determined at the beginning and end of the experiment in milled samples of biomaterial (all tissues and systems of the body), feed and

feces, followed by ignition in the microwave decomposition system Multiwave 3000 (Anton Paar, Austria). The elemental composition was studied by the atomic emission spectrometry (Optima 2000 V, Perkin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA) methods.

The digestibility of chromium was evaluated on the basis of the general collection of biological substrates (feces, urine) that took place during the digestion trial (5 days) in individual metabolic cages (http://цтт-ягпу.рф/dxl-d). The digestibility of Cr (D, %) was calculated by the formula: D = {[(individual feed intake × % Cr) – (amount of feces and urine per period of 5 days in grams × % Cr)] × (individual feed intake for rats × % Cr)} × 100.

The duodenum and pancreas were removed through the abdominal wall cut from the animals after slaughter to assess their enzymatic system. To prepare tissue samples of the studied organs, the sub-samples (1 g) were rubbed in cooled Ringer's solution (4 ml), the homogenate was centrifuged at 3000 rpm for 10 min (CM-12, Fabrika NV-group, Russia). Amylase activity was determined according to the method by Coles [18], lipase activity by Boutwell [19], protease with the use of the technique by Batoev [20]. The activity of the studied enzymes was expressed in conventional units (con. un., the difference between the indications of the sample with the substrate and the blank sample per 1 g of wet sub-sample of intestinal mucosa tissue for 1 min).

Samples of intestinal contents were collected in sterile Eppendorf tubes. For the complex study of microflora, 0.1 ml of each of the 10-fold dilutions was plated on nutrient media according to the standard technique [21]. Endo agar, meat-and-peptone agar (MPA), yolk-salt agar (YSA) (OOO NIFTS, Russia), Rogosa agar, Bifidobacterium Agar, BCA (HiMedia Laboratories Pvt. Ltd, India) were used for the studies. The final result of the quantitative content of bacteria in a gram of feces was expressed as CFU/g.

All experiments were carried out in 3-fold repetitions. Statistical processing of the obtained results included the calculation of the mean value (*M*) and standard errors of the mean (±SEM). The significance of differences between compared indicators was defined according to Student's *t*-criterion. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using ANOVA (Statistica 10.0 software package, StatSoft Inc., USA) and Microsoft Excel.

Results. Against the background of almost the same feed consumption during the experiment period (830±17 g/animal), the addition of 500 μg/kg CrPic and 500 NP to the diet was accompanied by an increase in rat weight by 22.6 and 22.2% ($p \leq 0.05$) and a decrease in the ratio of abdominal fat to body weight. The weight of the liver and kidneys did not differ significantly from the control values. Similarly, in the studies by Lien et al. [14], the addition of Cr to the diet (0.2 mg) increased the average daily growth of the body weight without an increase in feed intake and reduced fat deposits. A similar effect of organic and nanoforms of chromium in comparison with its mineral form has been reported earlier [13].

The positive balance of chromium accumulation with the use of 300 and 500 μg/kg CrPic in the diet was observed; this result may be compared with the results of the investigation by Wang et al. [15]. The bioavailability of Cr NP reached the highest positive balance at a dose of 500 μg/kg and was 81.6% higher than the control. In other groups, chromium digestibility was in the range of 4.3-7.5% and was the lowest when using $CrCl_3$ (Table 1).

Previously in the investigations of Wang et al. [15], the ability of chromium nanoparticles to stimulate growth indicators has been described while increasing metal deposition in muscles, heart, liver, and kidneys at the same time.

1. Morphometric parameters and digestibility of various forms of chromium in Wistar rats (M±SEM, vivarium experiment)

Indicator	Group						
	control	I	II	III	IV	V	VI
Initial body weight, g	69.30±2.95	114.20±2.80	75.50±2.88	87.90±1.19	101.80±7.63	81.10±3.13	100.30±5.50
Live weight at the end of the experiment, g	118.60±3.50	149.30±0.10	131.20±9.86	146.70±7.01	152.30±6.30*	123.30±4.17	151.50±6.50*
Liver weight, g	5.55±0.49	5.03±0.43	6.88±0.43	7.90±0.45*	6.50±0.32	6.23±0.67	6.70±0.45
Kidney weight, g	1.20±0.08	1.13±0.06	1.15±0.16	1.43±0.27	1.23±0.19	1.43±0.23	1.53±0.22*
Internal fat, g/weight ratio	1.33±0.21 2	1.21±0.10 1.06	1.80±0.37* 1.38	1.63±0.21 1.12	1.25±0.09 0.82	1.37±0.79 1.12	2.10±0.30* 1.39
Growth, g	49.30±1.10	35.10±0.90*	55.70±1.20	58.80±2.30*	50.50±1.35	42.20±0.95	51.20±1.32
Cr content in the diet, mg/kg	0.13	0.43	0.43	0.43	0.63	0.63	0.63
Cr content in feces, mg/kg	0.97±0.01	3.18±0.12***	3.27±0.18***	2.75±0.05***	3.30±0.13***	4.83±0.24***	4.02±0.74***
Cr content in the body, mg/kg	0.18±0.05	0.05±0.00*	0.05±0.00*	0.06±0.00	0.21±0.04	0.06±0.00*	0.09±0.00*
Cr digestibility, %	6.7±0.3	7.6±0.4*	4.9±0.6*	20.2±1.1**	34.5±1.3*	4.3±0.9*	20.2±1.6

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

2. Blood morphology of Wistar rats on day 21 after addition of dietary chromium in various forms and dosages (M±SEM, vivarium experiment)

Indicator	Group						
	control	I	II	III	IV	V	VI
Leucocytes, $\times 10^9$/l	7.50±3.50	11.00±1.60*	10.70±1.10	12.20±2.60*	7.10±1.70	8.80±2.10	9.00±2.10
Lymphocytes, $\times 10^9$/l	3.80±1.80	6.50±2.10*	5.80±0.70*	5.10±1.40*	3.80±1.10	4.60±0.90*	5.20±1.20*
Monocytes, $\times 10^9$/l	1.60±0.80	2.10±0.80	1.70±0.10	2.30±0.90	1.60±0.80	1.70±0.01	1.70±0.30
Granulocytes, $\times 10^9$/l	2.10±0.78	2.40±0.90	3.00±0.30	4.80±1.10*	1.70±1.07	2.50±0.08	2.40±0.20
Erythrocytes, $\times 10^{12}$/l	4.69±0.63	5.79±1.10	5.70±0.70	5.64±1.80	5.67±1.70	5.21±0.80	5.36±1.10
Hemoglobin, g/l	103.00±15.60	128.00±22.10*	125.00±3.60*	125.00±23.10*	119.00±12.50	115.00±12.60	119.00±23.60
Hematocrit, %	26.40±7.80	31.40±11.20	30.70±11.50	32.00±9.90*	28.00±9.68	28.10±3.60	29.10±2.40
Thrombocytes, $\times 10^9$/l	120.00±25.30	233.00±35.20*	237.00±23.60*	169.00±25.30	110.00±15.60	204.00±9.80*	186.00±12.50

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

3. Blood biochemical parameters of Wistar rats on day 21 after addition of dietary chromium in various forms and dosages (*M*±SEM, vivarium experiment)

Indicator	Group						
	control	I	II	III	IV	V	VI
Glucose, mmol/l	3.11±1.40	1.99±0.90	2.44±1.10	3.78±1.80	7.00±2.10*	7.74±2.10*	2.32±1.60
Total protein, g/l	63.60±9.80	68.30±10.30	57.90±16.30	63.30±9.70	64.90±11.60	62.50±12.10	61.60±9.90
Total bilirubin, μmol/l	8.90±0.40	8.30±2.10	6.80±1.10*	7.10±0.90	11.60±1.50	10.90±2.10	9.60±1.90
Cholesterol, mmol/l	1.85±1.90	1.59±0.68	1.48±0.78	2.27±0.68	1.85±1.30	1.80±0.80	1.45±0.35
Triglycerides, mmol/l	9.63±1.80	10.80±3.60	8.61±2.80	7.69±2.10	6.84±2.10	5.88±1.20*	5.69±1.60*
Urea, mmol/l	7.20±1.10	7.60±2.40	5.90±2.10	5.30±1.90	4.00±1.40*	6.40±1.30	6.10±1.80
Creatinine, μmol/l	29.70±8.90	27.60±11.30	33.90±9.60	30.80±11.10	19.70±4.30	33.90±2.10	36.10±3.10
Calcium, mmol/l	1.80±0.40	2.00±0.11	1.88±0.01	2.27±0.20	1.98±0.60	1.94±0.27	2.10±0.21
Iron, μmol/l	41.00±7.60	41.10±11.4	27.30±8.70*	29.70±7.70*	49.50±8.10	35.80±4.10	32.40±5.60
Amylase, IU/l	1266.00±42.30	1112.90±45.30	1130.60±39.60	1578.00±23.60	1414.00±19.60	643.80±122.30*	648.00±214.30*
Lipase, IU/l	2.40±0.70	4.80±2.40	0.80±1.10**	2.10±1.10	3.10±0.90	1.10±0.01*	1.60±0.03*
Magnesium, mmol/l	0.90±0.04	0.68±0.04	0.78±0.01	0.45±0.01	1.17±0.07	0.63±0.01	0.52±0.02
Phosphorus, mmol/l	3.65±0.80	3.82±1.40	3.59±1.40	4.04±2.10	1.87±1.30**	5.18±1.20*	3.86±1.60

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

4. Activity (IU/ml) **of digestive enzymes in the pancreas and duodenum of Wistar rats on day 21 after addition of dietary chromium in various forms and dosages** (*M*±SEM, vivarium experiment)

Group	Duodenum			Pancreas		
	amylase	protease	lipase	amylase	protease	lipase
Contro;	81.8±6.7	2.0±0.4	0.9±0.2	31.5±0.5	2.0±0.2	4.1±0.4
I	90.7±13.2	4.6±1.2	0.3±0.1	88.8±4.7*	1.9±0.1	4.4±0.7
II	58.4±10.8	3.6±0.2	2.3±0.6	32.8±10.2	2.6±0.1	4.3±0.2
III	37.4±5.1*	3.1±0.2	2.7±0.2*	5.8±2.3*	4.1±0.4*	13.7±0.5*
IV	80.7±9.8	2.5±0.3	1.3±0.3	32.3±1.1	2.5±0.4	5.9±0.7
V	72.8±13.3	2.2±0.2	2.8±0.4*	54.4±7.8	2.6±0.4	3.7±0.6
VI	85.7±7.5	2.9±0.7	4.1±0.5*	55.4±9.4	1.7±0.1	5.2±0.3

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

It happens due to better absorption of Cr nanoform in the intestine and the high penetration of Cr particles through the blood capillaries [23]. The absorbed chromium binds mainly to transferrin, distributes in tissues depending on the chemical state in which the element entered the body [24], and is excreted in the urine and feces in the form of acetate and citrate complexes for 4 days in an amount of 60-90% of the received chromium [25].

To avoid distortion in the interpretation of the data when assessing the effects of different forms of chromium on the body, especially in concentrations exceeding biotic, it is necessary to use markers. They should exclude hidden toxic effects that are associated with metabolic disorders and expressed in the accumulation of fat, changes in hematological and biochemical parameters of blood [27] (Table 2).

In this experiment, the peculiarity of the dose of NP 500 was the absence of reaction of lymphocytes, monocytes, and granulocytes, while at a dose of NP 300, these parameters exceeded the control values by 14-45% ($p \leq 0.05$). The observed effects of chromium on the group of formed elements are similar to those of copper and iron NP addition [28]. The addition of chromium to the rat diet stimulated erythropoiesis; the greatest effect was achieved at a concentration of CrPic 300: the hematocrit index in animals in group III was by 17.5% ($p \leq 0.05$) higher than in the control group. Hemoglobin synthesis corresponded to the stimulation of erythropoiesis, but an increase in the number of thrombocytes led to blood sludge, increased viscosity, and difficulty in perfusion through the vessels. These symptoms were typical for NP 300, $CrCl_3$ 300 and $CrCl_3$ 500 (difference with the control group from 70 to 90%, $p \leq 0.05$). The reason for this effect could be the thrombogenicity of nanoparticles, and for $CrCl_3$ this could be a manifestation of the toxic properties of this compound.

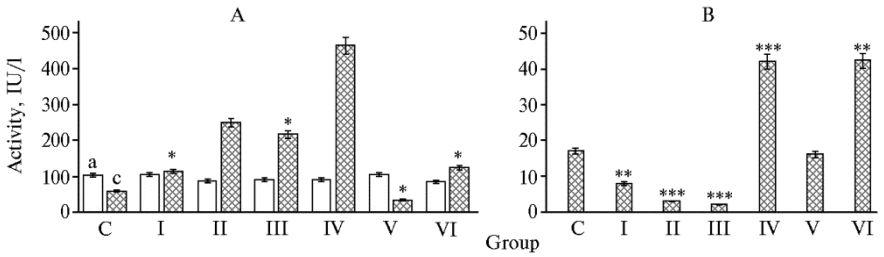
Two aspects of the biocompatibility of nanomaterials, the thrombogenicity and hemolytic activity require special attention, because after penetration through the barrier structures of the body, nanoparticles are in the lymph and blood flow, which implies their contact with both macromolecules of blood plasma and lymph and with shaped elements. It is known that particles with a negative surface charge can trigger the formation of a blood clot by contact activation of the clotting cascade, leading to the formation of fibrin; in other words, they activate the external pathway of blood clotting.

There were the hyper- and hypoglycemic effects of chromium in various forms and dosages on the body of rats (Table 3). Since 65-70% of circulating glucose in the blood is utilized by the central nervous system, this determines the danger of hypoglycemic conditions that significantly change the metabolism of the brain, which ultimately leads to the death of neurons and the disruption of its function [29]. Thus, in experimental groups IV and V, there was a significant increase in the glucose content (by 2.49 and 2.25 times, $p \leq 0.05$). The concentration of total protein between the groups did not differ significantly. The maximum decrease in the creatinine content was observed in the blood of rats from group IV (by 33.7%), which, along with a decrease in the urea content by 44.4% ($p \leq 0.05$), was one of the criteria for disorders in the excretory apparatus [17].

The amount of triglycerides as true fats decreased at the maximum doses of chromium in the form of chloride and picolinate, which indicates the effect of chromium on lipid metabolism, the splitting of excess fat in the body and reducing the ratio of fat to weight from 2.00 (control) to 0.82 (NP 500). The influence of chromium on lipid metabolism is also mediated by its regulatory action on the functioning of insulin.

The activity of pancreatic enzymes of amylase and lipase in blood serum decreased with an increase in the content of $CrCl_3$ and CrPic in the feed, which

indicates the depressing effect of the used forms of chromium on the entero-pancreatic circulation of digestive enzymes. When NP 500 was added, the content of phosphorus decreased by 48.8% ($p \le 0.05$). The data on the blood content of iron serum are interesting. At a low concentration, chromium and iron mainly occupy different binding sites [30, 31], while at a higher level, they compete for the sites, which was manifested in a decrease in iron metabolism in the experimental groups receiving a diet with chloride and chromium picolinate. The analysis of the aminotransferase reaction (Fig.) as an indicator of the presence of damages in the cells indicated a decrease in the activity of AspAT by 42.7% at the dose of NP 500 ($p \le 0.01$) and the increase in NP 300, CrPic 300, and CrPic 500 by 1.97 ($p \le 0.001$); by 3.75 ($p \le 0.001$); and by 2.14 times ($p \le 0.01$) in comparison with the control against the background of a stably high content of bilirubin. This fact can be explained by the massive release of enzymes into the bloodstream after the destruction of cells caused by the onset of oxidative stress and various pathological processes. A significant decrease in the activity of ALT by 10.7% ($p \le 0.05$) under the influence of NP 500 may be indirect evidence of the disruption of glomerular filtration, as evidenced by a decrease in the content of creatinine by 33.7% ($p \le 0.05$) relative to the control group (Fig., A).



The activity of blood transaminases (A) **and γ-glutamyltransferase** (B) **in Wistar rats on day 21 after addition of dietary ultrafine chromium particles of different forms and dosages:** a — alanine aminotransferase, b — aspartate aminotransferase; C — control, I — NP Cr_2O_3 (300 μg/kg feed), II — $CrCl_3$ (300 mg/kg), III — CrPic (300 mg/kg), IV — NP Cr_2O_3 (500 μg/kg), V — $CrCl_3$ (500 mg/kg), VI — CrPic (500 mg/kg) (M±SEM, n = 15, vivarium experiment).
*, **, and ** Differences with control are statistically significant at $p \le 0.05$, $p \le 0.01$, and $p \le 0.001$, respectively.

The analysis of the blood activity of γ-glutamyltransferase (γ-GT) revealed a significant increase in the case of adding NP 500 and CrPic 500 to the diet, which could be due to cell death, stagnation within the ducts or toxic effects against the background of intoxication [32]. The introduction of NP Cr_2O_3, $CrCl_3$, and CrPic at a dose of 300 mg/kg led to a decrease in this indicator. NP Cr_2O_3 at a dose of 500 mg/kg had the greatest impact on hematological parameters. The ambiguous manifestation of the activity of transamination enzymes, on the one hand, may indicate the destruction of cell membranes, on the other − a weak induction of microsomal oxidation under the influence of mixed valence NP [33]. The other forms of chromium in the used dosages did not lead to critical changes in the hemostatic system.

The ability of chromium to penetrate into the intestine quickly (1 h after feeding) and form stable difficult to absorb hydrates in it suggests its participation in the development of digestive enzymes [34]. In this study, the stimulation of amylase activity was observed in the pancreas after addition of NP 300 and the decrease in this indicator with the use of CrPic 300. At the same time, CrPic has a stimulating effect on the activity of lipase and protease. In the duodenum, the reduction of amylase activity by 54.3% ($p \le 0.05$) and increased activity of lipase by 67.7% ($p \le 0.05$) was typical for CrPic 300. Adding of NP CrCl3 and CrPic to the diet of rats at a dosage of 500 mg/kg stimulated the lipase activity (Table 4).

Despite the absence of data in the international scientific literature that may explain the mechanism of NP chromium action on the digestive enzymes, the option to modulate their activity through induction seems to be promising. Adsorption of biological macromolecules on the surface of nanoparticles may change their spatial structure and some functional properties. Thus, the enzyme α-chymotrypsin adsorbed on single-layer carbon nanotubes loses 99% of its activity due to a violation of the secondary structure [35]. When adsorbing proteins on larger nanoparticles, significant changes in the structure and function of proteins are observed due to the formation of additional contacts [36].

Taking into account the multiple effect of chromium on the activity of digestive enzymes, it should be emphasized that nanoparticles and picolinate have a similar effect due to possible direct contact with the active center of the enzyme [37].

5. The number of microorganisms ($\times 10^6$ CFU/g) **in the large intestine of Wistar rats on day 21 after addition of dietary chromium in various forms and dosages** (M±SEM, vivarium experiment)

Микроорганизмы	Group						
	control	I	II	III	IV	V	VI
Lactobacilli	14.3±2.1	9.3±1	10±1.1	12.6±2.1	6.3±0.8*	8.0±1.1	8.6±0.4
Bifidobacteria	14.9±4.1	11.7±0.9	12.3±1.5	13.1±0.9	6.5±0.4	22.0±2.2	29.0±2.5*
Enterobacteria	0.8±0.1	0.5±0.1*	25.0±2.3**	29.0±3.1**	23.0±3.7**	20.6±2.4*	26.0±2.9**

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

The number of lactobacilli in group IV was 55.9% ($p \leq 0.05$) lower than in the control group; in other groups, it did not change significantly. The number of bifidobacteria in group VI was 48.6% higher than in the control group ($p \leq 0.01$). The number of enterobacteria in the group of NP 300 was lower than in the control group by 34.8% ($p \leq 0.05$), while in the other groups their number increased 24.0-33.7 times (Table 5).

Thus, chromium in the form of nanoparticles and picolinate at a dose of up to 300 μg/kg can be used as a bacteriostatic agent to correct the content of bifidobacteria in the intestine. The content of bilirubin and creatinine, and an increase in the activity of amylase in the pancreas, lipase and protease in the duodenum have demonstrated a lack of toxicity of NP 300 and CrPic 300 for Wistar rats. The use of chromium at a dose of 500 rg/kg, regardless of the form of the element, was accompanied by a manifestation of hepatotoxicity and nephrotoxicity with signs of oxidative stress. High digestibility of CrPic and NP 500 (from 20.2 to 34.0%) had a depressing effect on energy metabolism and enteropancreatic circulation of digestive enzymes, reduced the triglyceride content and the ratio between the amount of fat and body weight. Depression of lipid metabolism was confirmed by decreased activity of amylase in the duodenum. In general, the prospects for the use of nanoscale diets are explained by the predominance of surface interactions. Due to their size, comparable to the size of cells, viruses, proteins, and DNA, nanoparticles can approach the biological object and bind to it, being involved in biochemical processes in the body.

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