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Adaptation mechanisms of the pancreas enzyme system of calves after the introduction of ultrafine chromium particles into the ration

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Abstract

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An important tool in regulating chromium metabolism in the body is its ability to penetrate through the intestinal wall. It accelerates with a decrease in its size, and the presence of digestive agents (vitamins, phytates, aminoacids). Chromium has an excessively low digestibility and absorption of organic (25%) and inorganic (3%) forms, while the absorption of Cr+3 occurs mainly through the kidney Cr – 80 – 95%, with small losses in the hair, through the sebaceous gland and bile (45% Cr), indicating a rapid absorption and recreation of Cr. Regardless of increase in chromium in the diet (40-240 μg/day), the level of absorption remains constant 0.4 – 2%.

Thus, the factorial dependence of chromium absorption consists of the source, size and ingredient composition of diet and a decrease in the size of Cr particles will increase the rate of absorption of chromium in the body.

The highest activity of protease and lipase was established in the first hours of selection, followed by its decrease by 3 hours. Adding chromium to the diets of bulls stimulated protease activity during all record periods, the difference with control was from 25 to 43% ($p \leq 0.05$). Characteristic is the enhancement of protease activity on the 2nd day of the experiment, followed by a decrease in its activity by 3 day. Similarly, lipase activity was formed; the difference with the control was 36% ($p \leq 0.05$). On the contrary, amylase activity decreased at hourly sampling points and increased in daily sampling.

Keywords: mineral nutrition; chromium; nanoparticles; amylase, pancreas

Introduction

The question of mineral nutrition of humans and animals is still discussed. High-energy diets, multicomponent feed mixtures and additives in the composition of modern diets require special attention optimizing the list of limited micronutrients. A priori, chromium, being an important trace element in human and animal organism, is used to correct carbohydrate, fat and lipid metabolism (Ahmed et al., 2005). Due to its low content in the components of diet, its role in the formation of the microecological status of body is poorly understood.

Deficiency and inadequate intake of chromium in organism is accompanied by a slowdown in growth and a deterioration of glucose tolerance, leading to hyperglycemia and hyperlipidemia (Ani & Moshtaghie, 1992; Egorov et al., 2001; Vertiprakhov, 2004; Ban et al., 2015). Correction of diet by sources of chromium can level the negative effects of its deficiency, and size range and shape of chromium must be taken into account, it is a key factor affecting its bioavail-

ability (Sineschekov, 1955; Boutwell, 1962; Brooks et al., 2016).

An important tool in regulating chromium metabolism in the body is its ability to penetrate through the intestinal wall. It accelerates with a decrease in its size, and the presence of digestive agents (vitamins, phytates, aminoacids). Chromium has an excessively low digestibility and absorption of organic (25%) and inorganic (3%) forms, while the absorption of Cr^{+3} occurs mainly through the kidney Cr – 80 – 95% (Fisinin et al., 2017), with small losses in the hair, through the sebaceous gland and bile (45% Cr), indicating a rapid absorption and recreation of Cr. Regardless of increase in chromium in the diet (40 – 240 μg/day), the level of absorption remains constant 0.4-2% (Samokhin, 2003; Chanda et al., 2015).

Thus, the factorial dependence of chromium absorption consists of the source, size and ingredient composition of diet, and a decrease in the size of Cr particles will increase the rate of absorption of chromium in the body (Batoyev, 1971; Fisinin et al., 2009).

In this regard, a promising direction is to study the possibility of using ultrafine chromium particles as modulators of metabolic activity, and in particular – lipid metabolism.

The aim of the research is to study the adaptation mechanisms of enzymatic system of pancreas of calves when chromium ultrafine particles (UFPs) are added to the diet.

Materials and Methods

Studies were conducted on two dairy calves with an average weight of 110 – 120 kg, at the age of 6 months.

Animal care and experimental studies were performed in accordance with the instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 on 08/12/1977 of the USSR Ministry of Health) and "The National Academy Press, Washington, DC 1996).

The studies were performed in laboratory of biological tests and assessments of the Federal Research Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (FRC BST RAS) and the experimental biological clinic of the FSBEI "Orenburg State University". Calves were kept in a specialized pen with free access to water and feed. The basic diet (BD) was balanced for basic nutrients in accordance with detailed standards of VNIIMS and included grass hay (2.5 kg), feeding molasses (0.3 kg) and mixed feed (1.5 kg) consisting of wheat, barley, fish flour and mineral additives (premix PK-60, salt, dicalcium phosphate).

To accomplish the task, the animal underwent an original operation to impose a duodenal anastomosis (Nattapon et al., 2012).

A syringe for collecting the 12 duodenal chyme was attached to a fistula from an isolated segment using a special rubber adapter. Physiological experiment started in the morning. Chyme was selected in the morning before feeding on an empty stomach after a 12-hour fast and 1 and 3 hours after feeding. The experiment was repeated within 15 days for each type of feed.

Calves of the control group received the basic diet, and chromium nanoparticles in a dose of 200 mg/kg were additionally introduced to the diet of the experimental animals.

UDPs of chromium were obtained by plasma-chemical synthesis (LLC "Platina", Moscow). Before being included in the diet, nanoparticles were dispersed in physiological solution using UZDN-2T (Academpribor Scientific Production Enterprise, Russia) (35 kHz, 300 W, 10 μA, 30 min). They were introduced to the feed by the method of step mixing.

Laboratory studies were carried out in the laboratory of "Agroecology of Technogenic Nanomaterials" and the Testing Center (Federal Scientific Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, accreditation certificate of RA.RU.21PF59 as of 02.12.15).

Blood was collected into vacuum tubes from the jugular vein with the addition of an anticoagulant, for biochemical parameters – into vacuum tubes with a clotting activator (thrombin). Morphological blood analysis was performed on an automatic hematology analyzer URIT-2900 Vet Plus (URIT Medical Electronic Group Co., Ltd., China), biochemical analysis of blood serum was performed on an automatic analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with commercial kits for veterinary (CJSC "DIAKON-DS", Russia).

The activity of pancreatic enzymes was measured by the following methods. Amylase was determined according to Smith-Roy in a modification to determine high activity of enzyme (Sizova et al., 2015) proteases were determined by the hydrolysis of casein purified according to Hammerstein with calorimetric control (wavelength 450 nm) and lipase on an automatic biochemical analyzer CS-T240 ("Dirui Industrial Co., Ltd", China) using commercial biochemical veterinary kits DiaVetTest (Russia).

Experiments on calves were performed in 15-fold repetition. Statistical analysis was performed using ANOVA methods (Statistica 10.0 software package, StatSoft Inc., USA) and Microsoft Excel. Statistical processing included the calculation of the mean (M) and standard error of the mean (± SEM). The reliability of differences in the compared indicators was determined by Student's t-test. The level of significant difference was set at $p \leq 0.05$.

Results and Discussion

Considering the biochemical parameters (Table 1), the effect of Cr on body of calves was manifested in the established hyperglycemic effect, expressed in an increase in glucose by 10.6% ($p \leq 0.05$) in the experimental group compared with control values. Triglycerides, like true fats, increased by 14.3%, it attests to the effect of chromium on lipid metabolism, causing splitting of excess fat in body, it is confirmed by studies (Zhitkovich et al., 1996; Dzagurov et al., 2013), but there is also a reverse effect, which is expressed in increasing cholesterol and lowering the triglyceride concentration after 12 weeks of supplementation with 200 μg of chromium.

Table 1. Biochemical blood parameters of calves

Parameters	Group	
	Control	Experimental
Glucose, mol/l	5.08±3.2	5.68±2.8
Total protein g/l	105.5±11.2	95.6±9.3*
ALT, units/ l	19.4±6.2	17.4±8.2
AST, u/l	64.1±9.4	59.1±8.1*
Cholesterol, mol/l	1.54±0.8	1.28±0.6*
Triglycerides, mmol/l	0.18±0.009	0.21±0.018*
Alkalinephosphatase, u/l	83±18.4	82±21.4
Iron, μmol/l	17.1±3.1	14.6±6.2
Magnesium, mol/l	0.75±0.06	0.72±0.08

Note: * – the results are statistically significant ($p \leq 0.05$)

At the same time, the additional inclusion of chromium NPs in the compound feed at a dose of 200 μg/kg of feed led to a depression of protein metabolism, expressed in a decrease in its level in the experimental group by 9.6% ($p \leq 0.05$).

Data on the content of Fe in blood are interesting. If chromium and iron at low levels of concentration mainly occupy different binding sites (Lien et al., 2009, Xueting, 2018), then at higher levels they compete for these sites, it manifested in a decrease in iron metabolism in the experimental group.

Analysis of the reaction of aminotransferases, as an indicator of cell damage, established a decrease in the activity of AST and ALT in the experimental group of 10.4 and 7.9%, respectively. Indicators of alkaline phosphatase and magnesium in the groups were almost at the same level, against the background of iron decrease by 14.7% compared with the control values.

Given the complex mechanisms of digestion in cattle, it is impossible to exclude the participation of chromium in metabolic processes, it was demonstrated with dynamics of activity of digestive enzymes in chyme (Table 2).

Experimental data indicate that the highest activity of protease and lipase was established within the first hours of selection, followed by its decrease by 3 hour. Addition of chromium to the diets of bulls stimulated protease activity during all record periods, the difference with control was from 25 to 43% ($p \leq 0.05$). Characteristic is the enhancement of protease activity on the 2nd day of the experiment, followed by a decrease in its activity by 3 days. Similarly, lipase activity was formed, the difference with the control was 36% ($p \leq 0.05$). On the contrary, amylase activity decreased at hourly sampling points and increased in daily sampling in the experimental group.

Depending on the quality of protein in the diet, the amino acid composition of calf chyme changed (Table 3). The addition of chromium depressed the release of all amino acids with chyme in larger quantities than in the first period.

Thus, adding Cr to calves diets caused ambiguous reactions in hematological parameters of blood, it was expressed in the established hyperglycemic effect, reducing cholesterol concentration, reducing iron metabolism, it is characteristic of high doses of chromium [16-18].

A decrease in the activity of aminotransferases, on the one hand, may indicate no toxic effect of chromium nanopar-

Table 2. The activity of enzymes of duodenal chyme in calves (n = 15, M ± m)

Parameters	Sampling time		
	Before feeding	1 hour after	3 hours after
Amylase, U/l			
1 day	4.8±0.61 5.9±0.52*	3.3±0.32 3.76±0.21	0.9±0.07 2.1±0.06*
2 day	6.9±0.56 7.3±0.73	4.8±0.64 4.66±0.33	2.1±0.04 1.3±0.08
3 day	9.21±0.8 14.28±1.2*	4.3±0.28 5.2±0.01	1.4±0.07 3.9±0.07
Protease, U/l			
1 day	2.2±0.3 2.91±0.3	2.6±0.3 3.02±0.86	1.4±0.05 1.9±0.04
2 day	3.2±0.42 5.1±0.4*	3.1±0.3 5.42±0.72*	1.8±0.3 3.1±0.2
3 day	2.4±0.3 2.27±0.2	2.1±0.21 2.35±0.17	1.1±0.08 1.37±0.09
Lipase, U/l			
1 day	0.17±0.04 0.18±0.02	0.32±0.03 0.57±0.08	0.12±0.01 0.14±0.02
2 day	0.21±0.02 0.32±0.05	0.43±0.05 0.72±0.03*	0.21±0.02 0.17±0.03
3 day	0.67±0.09 1.15±0.04*	0.23±0.04 0.16±0.004	0.18±0.03 0.28±0.05*

Note: indicators of the control feed are in the numerator, indicators of the experimental feed are in the denominator, * – the results are statistically significant ($p \leq 0.05$)

Table 3. Amino acid content in chyme on the background of inclusion of chromium nanoparticles in the diet, %

Name of indicators (amino acid composition)	1 period	2 period
Arginine	0.8±0.09	0.6±0.04
Lysin	1.23±0.15	1±0.3
Tyrosine	0.76±0.05	0.66±0.02
Phenylalanine	0.85±0.07	0.8±0.09
Gistidine	0.35±0.024	0.27±0.02
Leycin-isoleucine	2.15±0.5	2±0.5
Methionine	0.74±0.06	0.33±0.04
Valin	0.95±0.05	0.87±0.09
Prolin	0.83±0.06	0.81±0.04
Treonin	1.17±0.3	0.98±0.1
Serin	0.94±0.08	0.85±0.05
Alanine	1.31±0.2	1.11±0.2
Glycine	2±0.3	1.42±0.3
Cistin	1.9±0.2	1.62±0.13

ticles on the body, on the other, a violation of the protein-synthesizing function of the liver, as evidenced by a decrease in the protein level in the blood plasma of calves from the experimental group. At the same time, the activity of protease and lipase in duodenal chyme increased due to the possible inclusion of adaptation mechanisms to maintain homeostasis of lipid and protein metabolism (Samanta, 2008).

Conclusions

Chromium nanoparticles are non-toxic, have a diverse effect on hematological parameters and metabolism in the body of calves. This is expressed in the stimulation of carbohydrate and lipid metabolism, against the background of a decrease in protein, which is confirmed by a decrease in amino acids in chyme.

Adding Cr to calves rations is accompanied by an increase in glucose concentration by 10.6% ($p \leq 0.05$), triglycerides by 14.3%, while cholesterol is reduced by 16.9%.

The highest activity of protease and lipase was established in the first hours of selection, followed by its decrease by 3 hours. Adding chromium to the diets of bulls stimulated protease activity during all record periods, the difference with control was from 25 to 43% ($p \leq 0.05$). Characteristic is the enhancement of protease activity on the 2nd day of the experiment, followed by a decrease in its activity by 3 day. Similarly, lipase activity was formed, the difference with the control was 36% ($p \leq 0.05$). On the contrary, amylase activity decreased at hourly sampling points and increased in daily sampling.

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