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ADAPTIVE RESPONSES OF CATTLE DIGESTIVE SYSTEM AS INFLUENCED BY DIETARY ULTRAFINE IRON PARTICLES COMBINED WITH FAT DIETS

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Abstract

Fats are a concentrated source of energy; the fatty components in the diets of farm animals are economically feasible and efficient. The fatty supplements in the diet of cattle improves the palatability of the diets and reduces the rate of feed passage through the gastrointestinal tract, which increases the availability of nutrients and increases livestock productivity. However, some papers indicate a decrease in the digestibility of nutrients in the presence of dietary fat. To increase the availability of nutrients in rations, it is necessary to use additional components in the feed, in particular, ultrafine particles. They, unlike their counterparts in micro- and macro-form, have higher physical activity, chemical neutrality, and high bioavailability ensured by an increased surface area. The limited practical use of ultrafine particles (UFP) in animal husbandry is due to insufficient knowledge about their biological effects on metabolism. Here, for the first time, we evaluated the effect of an ultrafine iron preparation on pancreatic secretion, enzymatic activity of pancreatic juice, morphological and biochemical parameters of blood, and digestibility of feed enriched with sunflower and soybean oils. The aim of our research was to characterize ultrafine iron particles as modulators of metabolic activity when using vegetable fats in the diet of ruminants. The experiments were carried out on Kazakh white-headed calves aged 8 months with an average weight of 120-130 kg (a vivarium of Federal Research Centre of Biological Systems and Agrotechnologies RAS, October 2019-October 2020). A Latin square 4×4 design was applied in five replicates. Control group were fed a standard balanced basal diet (BD), group I — BD supplemented with UFP Fe, group II — BD added with sunflower oil, group III — BD added with sunflower oil + UFP Fe, group IV — BD added with soybean oil, and group V — BD added with soybean oil + UFP Fe. Oils replaced 3 % dry matter of feed concentrates. To produce UDP Fe, we used electric explosion of a conductor in an argon atmosphere (Advanced Powder Technologies, Tomsk). UFPs Fe (d = 90 nm, Z-potential 7.7±0.5 mV) are 99.8 % Fe. Before use, ultrafine iron particles were dispersed in a physiological solution using UZDN-2T (NPP Akadempribor, Russia) (35 kHz, 300 W, 10 μA, 30 min) and added at a dosage of 2.2 mg per animal. To study the exocrine function of the pancreas, a duodenal anastomosis surgery technique was performed. Pancreatic juice and chyme samples were collected over 8 hours with a 60 min interval. The activity of amylase, proteases, and lipase was measured. The blood NO metabolites and trypsin activity were measured. Feed digestibility was assessed on day 7 in balance experiments based on the amount of the consumed feed, uneaten feed and excreted feces. The digestibility coefficient (DC) was calculated as the ratio of the digested nutrients to those entered the body. Dry matter, crude protein, fat and ash contents were measured. Blood for quantitative analysis of the morphological and biochemical parameters was sampled in the morning on an empty stomach on day 7 of the experiment. The research data indicate that dietary UFP Fe with the fat diets contributed to a significant ($p \leq 0.05$) increase in the digestibility of crude fat, organic matter and nitrogen-free extractive substances, while the digestibility of crude fiber and crude protein decreased. Enrichment with UFP Fe and fatty ingredients had a stimulating effect on pancreatic secretion, leading to an increased amount of pancreatic juice. The

UFP Fe selectively changed the activity of the digestive pancreatic enzymes. UFP Fe added to BD, increased the activity of lipase by 35.7 %, intestinal proteases by 43.1 % while the amylolytic activity decreased by 28.8 %. Dietary UFP Fe combined with sunflower and soybean oils reduced the enzymatic activity of the pancreas compared to the control: in group III, the activity of lipase and intestinal proteases increased by 12.1 and 16.7%, respectively ($p \leq 0, 05$), in group V — by 133.2 and 38.4 %, respectively ($p \leq 0.05$). The BD supplementation with fatty ingredients, alone and in combination with UFP Fe, increased the level of NO-metabolites in all experimental groups compared to the control. When replacing BD with fat diets, the trypsin activity increased in group II by 106.6% ($p \leq 0.05$), in group IV by 130.9 % ($p \leq 0.05$). Added UFP Fe reduced the trypsin activity. Morphological analysis revealed a statistically significant ($p \leq 0.05$) increase in the hemoglobin content in calves of the experimental groups, in group I by 9.7 %, in group II by 31.2%, in group III by 41.9 %, in IV by 28.0 %, in V by 30.1 %. A biochemical blood test showed that all the studied parameters were within physiological norms, however, it should be noted that UFP Fe had a stimulating effect on protein, fat and carbohydrate metabolism in calves. A significant increase in the de Ritis ratio occurred in the groups that fed UFP Fe with fat diets, up to 3.98 in group III and 4.1 in group V ($p \leq 0.05$). As compared to the control, the bilirubin index (BI) increased by 17.8 % ($p \leq 0.05$) in group I and by 5.5% ($p \leq 0.05$) in group IV, in all other groups the BI values were lower than in the control.

Keywords: ultrafine particles, iron, blood morphology, blood biochemical test, pancreas, enzymes, pancreatic juice, chyme, cattle, fats, sunflower oil, soybean oil

Today, the production of high-quality beef has transformed fat from a simple additive to a valuable high-energy cereal substitute, energy source, and cellular metabolism modifier [1]. Fats as a feed agent serve as concentrated sources of energy, contain and transport fat-soluble vitamins, provide the body with essential fatty acids, and also give the feed certain aromatic, taste and structure. The addition of fat components to complete diets for farm animals is cost effective and efficient [2, 3].

The inclusion of vegetable oils (coconut, palm, soybean, sunflower, flaxseed and canola) in ruminant diets reduced in vitro intestinal methane production by 40.55-48.58%. It did not affect the pH of the rumen, the amount of microbial protein, the digestibility of dry and organic matter [4]. The addition of sunflower oil to the diet of cattle (cattle) led to a decrease in the number of protozoa, a decrease in methanogenesis and the concentration of ammonia nitrogen, and an improvement in the production of microbial biomass and propionic acid in the rumen [5]. However, some researchers point to a decrease in nutrient digestibility in the presence of fat [6, 7]. There was a decrease in the digestibility of neutral detergent fibers in the rumen due to the addition of fat, while the efficiency of microbial protein synthesis increased, and the abundance of protozoa tended to decrease [8]. Fat supplements led to a statistically significant ($p \leq 0.01$) decrease in the digestibility of organic matter and neutral detergent fiber in the intestines of young fattening cattle [9].

To increase the effectiveness of feed products, such components of diets as mineral supplements, in particular metal nanopowders, are considered and studied [10-12]. Currently, the influence of ultrafine metal particles as independent additives, as well as in combination with other feed components on metabolic processes in the gastrointestinal tract, the enzymatic activity of the digestive glands, and the composition of the microbiome is being actively studied. These supplements have been shown to be effective in reducing side effects, improving nutrient bioavailability, and increasing performance [13-15].

In the presented work, we for the first time established the effect of an ultrafine iron preparation on pancreatic secretion with the additional inclusion of sunflower and soybean oils in the diet of calves. An increase in the enzymatic activity of pancreatic juice, as well as the digestibility of the nutritional components of the feed, was noted.

The purpose of the study was to evaluate the possibility of using ultrafine iron particles as modulators of the activity of metabolic processes when vegetable fats (sunflower and soybean oils) are added to the diet of calves.

Materials and methods. In vivo experiments were carried out from October 2019 to October 2020 at the Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences on calves (*Bos taurus taurus*) of the Kazakh white-headed breed. Groups of four animals aged 8 months (average live weight 120-130 kg) were formed. The experiment was performed according to the scheme of the Latin square 4×4 in five repetitions.

Animal care and research were carried out in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). Every effort has been made to minimize animal suffering and reduce the number of samples taken. Animals were kept in separate metabolic cages (1.0×2.2 m) in a room with optimal temperature and humidity (during the experiment, the ambient temperature was maintained between 23 and 25 °C), with free access to water.

The calves of the control group received a basal diet (BD) which included mixed grass hay (2 kg), a mixture of concentrates (1.5 kg), corn silage (5 kg), wheat straw (1 kg), fodder molasses (0.1 kg), table salt (0.04 kg), vitamin and mineral premix. To the BD, ultrafine particles (UFP) of Fe were added in group I, sunflower oil in group II, sunflower oil + UFP Fe in group III, soybean oil in group IV, soybean oil + UFP Fe in group V. Oils were added at the rate of 3% of the dietary dry matter by replacing the concentrated part of the diet. Animals were fed twice a day, in the morning and in the evening in equal proportions. The diets met the need for nutrients and energy but differed in the fatty acid composition of the vegetable fats [16, 17].

Ultrafine iron particles obtained by electrical explosion of a conductor in an argon atmosphere (Advanced Powder Technologies, Russia). UFP (d = 90 nm, Z-potential 7.7±0.5 mV) were 99.8% Fe. Before adding to the diet, they were dispersed in physiological saline using UZDN-2T (NPP Akadempribor, Russia) (35 kHz, 300 W, 10 μA, 30 min). Animals were fed UFP Fe after mixing with a concentrated feed mixture at a dose of 2.2 mg per snimal.

To study the exocrine function of the pancreas, duodenal anastomosis was performed [18].

The tests were carried out after 16 hours of empty stomach. Pancreatic juice and chyme were collected over 8 hours at 60 min intervals. After taking the first sample, the animals were fed, and the juice and chyme collection continued. The amount of juice and the enzymatic activity of juice and chyme were determined in cito.

Amylase activity was measured by the Smith-Roe method modified for high values of the parameter [19]. Protease activity was evaluated by Hammerstenpurified casein hydrolysis under calorimetric control (λ = 450 nm) [20], lipase and α-amylase activity, concentration of total protein, phosphorus and calcium were measured using an automatic biochemical analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with commercial veterinary biochemical kits (ZAO DIACON-DS, Russia) [21].

The concentration of blood NO metabolites was determined spectrophotometrically with the Griess reagent (an Infinite PRO F200 microplate analyzer, Tecan Austria GmbH, Austria; λ = 540 nm) [22].

Blood trypsin activity was determined using a CS-T240 automated biochemical analyzer (DIRUI Industrial Co., Ltd, China), sodium benzoyl-DL-arginine-4(p)-nitroanilide hydrochloride (BAPN) was used as a substrate.

Feed digestibility was assessed for 7 days in balance experiments, based on the amount of feed consumed by animals, uneaten leftovers, and the amount of excreted feces. The digestibility coefficient (DC) was calculated as the ratio of

digested nutrients to those taken. After freezing, drying, and homogenization, the content of dry matter, organic matter, crude protein, crude fat, nitrogen-free extractives (NFE), and ash in feces and feed was analyzed according to the recommendations of the Association of Official Agricultural Chemists [23]. Digestibility was assessed as described by S. Hashemi et al. [24].

Blood for morphological and biochemical tests was taken from the jugular vein into vacuum tubes with a coagulation activator (thrombin) in the morning on an empty stomach on day 7 of the experiment. A CS-T240 automatic analyzer (DIRUI Industrial Co., Ltd, China) and commercial kits for veterinary medicine (DiaVetTest, Russia) were used.

Statistical analysis was performed using ANOVA methods (Statistica 10.0 software package, StatSoft, Inc., USA) and Microsoft Excel. Means (*M*) and standard errors of the means (±SEM) are sunmitted. The significance of differences between the compared indicators was determined by Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

Results. Table 1 shows the composition and quality indicators of the diets fed to the experimental calves. The difference in the content of crude fat, crude protein and metabolic energy was quite significant, 45.5, 8.3 and 12.9%, respectively.

1. Composition and quality parameters of the diets fed to Kazakh white-headed calves (*Bos taurus taurus*) in the experiment (FSC of biological systems and agricultural technologies RAS, 2019-2020)

Parameter	Diet		
	basal	added with sunflower oil with soybean oil	added with soybean oil
Composition of the diet			
Mixed grass hay, kg	7.0	7.0	7.0
Concentrates, kg	2.0	2.0	2.0
Sunflower oil, kg		0.3	
Soybean oil, kg			0.3
Molasses fodder, kg	0.6	0.6	0.6
Premix PK-60, kg	0.06	0.06	0.06
Salt, kg	0.02	0.02	0.2
UFP Fe, mg		2.2	2.2
Nutritional value of the diet			
Dry matter, kg	8.42	8.42	8.42
Crude fiber, kg	2.56	2.56	2.56
Crude fat, kg	0.244	0.355	0.355
Crude protein, kg	0.72	0.66	0.66
NFES, kg	5.4	5.0	5.0
Calcium, g	42.2	42.6	43.2
Phosphorus, g	30.0	29.8	30.4
ME, MJ	63.0	71.1	71.1

N o t e. NFES — nitrogen-free extractive substances, ME — metabolic energy. The vitamin-mineral premix contains Mn — 48 mg, Zn — 36 mg, Fe — 60 mg, Cu — 10 mg, — - 0.24 mg, Co — 0.12 mg; vitamin A — 2640 IU, vitamin D — 302 IU; vitamin E — 17 mg (per 1 kg of concentrate).

The main challenge in the production of high quality beef is to provide animals with the necessary nutrients to meet metabolic needs and increase productivity. However, traditional grains in cattle diets adversely affect dry matter content and inhibit fiber digestion [25, 26]. The use of fats in diets is essential in the feeding of farm animals. Lack of fat leads to growth retardation, disruption of reproductive function, reduced productivity and poor product quality. The presence of a large amount of fat in the diet creates a load on the digestive system as a whole, especially in cattle. When diets are saturated with fats, the activity of digestive enzymes changes, as a result, complex food components are not broken down well enough and are poorly absorbed [27]. Dietary fat that is not biolyzed and biohydrogenated by rumen microorganisms but is digested in the lower digestive tract is known as bypass fat or rumen protected fat (inert fat) [28]. The introduction of fats into the diet of cattle grazing on pastures increases the production of meat and dairy products. However, increasing the amount of fats and fatty acids

inhibits the digestion of fiber in the rumen and reduces the digestion of organic matter in the anterior part of the stomach [29].

In our work, with the introduction of sunflower and soybean oil into the diets, the digestibility of crude fat decreased by 38.2 and 10.9% ($p \leq 0.05$) vs. control, respectively (Table 2). UFP Fe added to the basal diet increased the digestibility of organic matter by 9.6% ($p \leq 0.05$), crude fat by 2.2% ($p \leq 0.05$), and nitrogen-free extractive substances by 9% ($p \leq 0.05$). Z. Khan et al. [30] revealed that feeding calves with a diet high in iron reduced average daily body weight gain, dry matter intake, and feed nutrient digestibility.

2. Nutrient digestibility coefficients (%) **in Kazakh white-headed calves (*Bos taurus taurus*) fed diets supplemented with vegetable oils and ultrafine Fe particles** (n = 4, M±SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020)

Parameter	Group					
	control;	I	II	III	IV	V
Dry matter	74.3±0.04	70.8±0.03*	76.1±0.01*	72.1±0.02*	60.9±1.05	68.4±0.03*
Organic matter	87.8±0.30	96.2±0.40*	83.83±0.21*	98.7±0.42	59.4±0.03*	95.8±0.05*
Crude protein	76.3±3.60	71.0±4.10	81.4±2.30*	74.5±2.82	72.8±1.02	70.4±2.4
Crude fat	72.7±1.23	74.3±1.40*	44.9±1.88*	62.7±1.54*	64.8±0.75*	76.4±1.23*
Crude fiber	37.4±0.18	36.6±0.20	45.5±0.08*	37.5±0.12	43.7±0.45*	36.2±1.12
NFES	80.3±0.90	88.2±0.70*	82.3±1.10	91.8±0.93*	75.6±0.38*	84.3±0.24*

N o t e. NFES — nitrogen-free extractive substances. For a description of the groups, see the Materials and methods section.

* Differences from control are statistically significant at $p \leq 0.05$.

UFP Fe in the diets statistically significant increased the digestibility of crude fat, in group III vs. group II by 39.6% ($p \leq 0.05$), in group V vs. group IV by 17.9% ($p \leq 0.05$). A similar trend occurred in the digestibility of organic matter and NFES. It should also be noted that dietary UFP Fe reduced the digestibility of crude fiber and crude protein in groups I, III and V.

Additional enrichment of diets with mineral components and changes in the quantitative and qualitative composition of the feed significantly affect pancreatic secretion and the activity of digestive enzymes [31-34]. The structure and composition of the diet, as well as the volume and frequency of feeding, have a regulatory effect on digestive functions due to reflexive and humoral mechanisms.

In the first hour before feeding the animals, pancreatic secretion in all groups was significantly lower than after feeding. Secretion increased in the reflex and gastric phases, and then decreased in the intestinal phase, in the period 360-480 min after the start of measurements.

3. The amount of pancreatic juice (ml) **excreted in Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles** (n = 4, M±SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020)

Time, min	Group					
	control	I	II	III	IV	V
0-60	32.0±2.81	48.0±3.24	28.0±1.72	109.0±19.02	18.0±2.43	88.0±12.23
60-120	66.0±3.42	76.0±3.61*	48.0±3.51	138.0±6.52	78.0±6.72*	141.0±5.64*
120-180	67.0±5.32	80.0±5.12	58.0±4.62*	228.0±4.11*	72.0±7.12	218.5±14.64
180-240	59.0±4.71	84.0±6.04	50.0±5.03	156.5±1.93*	62.0±5.83	184.5±3.83
240-300	55.5±5.20	88.0±4.22	48.0±3.72	142.5±9.92	5.0±4.31	168.5±11.31*
300-360	59.5±4.11	91.0±2.81	47.0±3.30*	77.0±5.02	48.0±5.31	81.5±18.04
360-420	67.0±6.31	90.0±4.52	38.0±5.43	23.0±6.23	46.0±3.82	39.0±6.40
420-480	51.5±4.70	84.0±3.02*	50.0±5.22	40.0±2.90	42.0±3.22	28.0±3.91
0-480	457.5±37.83	641.0±32.40*	367.0±55.42*	914.0±55.53*	417.0±45.22*	949.0±75.81*

N o t e. During the first hour of the experiment, the indicators were recorded on an empty stomach. For a description of the groups, see the Materials and methods section.

* Differences from control are statistically significant at $p \leq 0.05$.* Differences from control are statistically significant at $p \leq 0.05$.

When replacing the control compound feed with experimental samples with UFP Fe, the amount of pancreatic juice produced increased, which indicates an increase in the load on the pancreas with such diets (Table 3). Thus, when using the basal diet with UFP Fe, the amount of pancreatic juice increased by 40.1% over the entire time of the experiment ($p \leq 0.05$). With sunflower oil added to the diet, the production of pancreatic juice decreased by 19.8% ($p \leq 0.05$), with soybean oil by 8.8% ($p \leq 0.05$) vs. control. Dietary UFP Fe stimulated the secretion of pancreatic juice by 149.0% in group III vs. group II, and by 127.6% in group V vs. group IV ($p \leq 0.05$).

4. Activity of pancreatic juice enzymes in Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles ($n = 4$, $M \pm$SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020)

Parameter	Group					
	control	I	II	III	IV	V
Lipase, U/l	90.9±18.2	123.4±19.4*	773.0±14.8*	101.9±12.7*	667.0±37.0*	212.0±11.3*
Amylase, $mg \cdot ml^{-1} \cdot min^{-1}$	5137.5±450.0	3337.5±330.0	2537.0±400.0	1698.4±330.0	1931.0±69.0	1456.0±34.0
Proteases, $mg \cdot ml^{-1} \cdot min^{-1}$	133.5±24.3	191.0±22.6	249.0±21.1*	155.8±14.6*	200.0±12.6*	184.8±13.5*
Total protein, g/l	0.46±0.12	0.48±0.16	0.18±0.01	0.38±0.01*	0.33±0.01	0.41±0.020
Phosphorus, mol/l	0.14±0.02	0.12±0.03*	0.03±0.00	0.10±0.00	0.08±0.01	0.10±0.010*
Calcium, mol/l	2.33±0.12	2.46±0.15*	2.43±0.22*	2.26±0.18	2.39±0.10*	2.41±0.12
α-Amylase, U/l	416.0±4.8	536.0±6.2	578.0±11.5	559.1±6.7	767.0±13.8	758.0±16.8

N o t e. For a description of the groups, see the Materials and methods section.
* Differences from control are statistically significant at $p \leq 0.05$.

The introduction of additional ingredients and a change in the qualitative composition of the diet lead to a selective change in the activity of digestive enzymes [35-38]. UHF Fe added to the BD led to a significant increase in the activity of lipase (by 35.7%, $p \leq 0.05$), intestinal proteases (by 43.1%) vs. a decrease in amylolytic activity (by 35.0%, $p \leq 0.05$). In group I, the content of phosphorus in the pancreatic juice decreased by 14.3% ($p \leq 0.05$) with an increase in the amount of Ca by 5.6% ($p \leq 0.05$) vs. control (Table 4).

Fatty diets stimulated lipase and intestinal proteases, in group II 8.5-fold ($p \leq 0.05$) and 1.9-fold ($p \leq 0.05$), respectively, in group IV 7.3-fold and 1.5-fold ($p \leq 0.05$) vs. control. Due to fat components in diets, the activity of the amylase decreased.

UFP Fe addede to the diets containing sunflower and soybean oils reduced the enzymatic activity of the pancreas, that is, the load on the pancreas decreased. In group III compared to control, the activity of lipase and proteases significantly increased, by 12.1 and 16.7%, respectively ($p \leq 0.05$), in group V by 133.2 and 38.4% ($p \leq 0.05$). Fat components in the diet increased the secretion of lipase, but not amylase. Obviously, an increase in the amount of any nutrient leads to an increase in the production of digestive enzymes in the pancreas.

A downward trend also occurred in the acticity of intestinal proteases. Proteolytic activity decreased by 52.0% for BD + UFP Fe vs. BD, but the differences were not significant. A statistically significant decrease in protease activity occurred, by 28.8% ($p \leq 0.05$) in group II, by 62.9% ($p \leq 0.05$) in group III, by 50.0% ($p \leq 0.05$) in group V, by 3.4% in group IV. The activity of lipase in the duodenal chyme increased in animals receiving fat diets, however, the introduction of UFP Fe led to a decrease in the parameter value. Thus, , lipolytic activity decreased 4.3-fold ($p \leq 0.05$) in group I, 4.6-fold ($p \leq 0.05$) in group III, and 4.5-fold ($p \leq 0$ .05) in group V.

Dietary UFP Fe significantly reduced the activity of pancreatic amylase in the duodenal chime, by 16.0% with dietary sunflower oil and by 66.0% with

dietary soybean oil ($p \leq 0.05$) (Fig. 1).

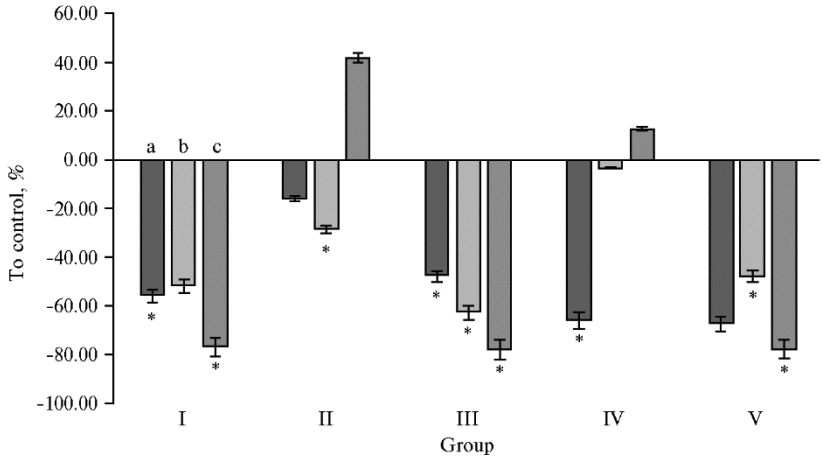

Fig. 1. Changes in the activity of digestive enzymes of pancreatic juice in duodenal chyme of Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles vs. control: a — amylase, b — protease, c — lipase ($n = 4$, $M \pm$SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020). For a description of the groups, see the Materials and methods section.

The activity of nitric oxide metabolites in the blood serum mediates a whole cascade of physiological processes, including the regulation of vascular tone, plasma and platelet hemostasis, neurotransmission and the formation of an immune response, inhibition of the proliferation of smooth muscle cells and has a significant effect on metabolic processes in the digestive tract [39]. In our study, an increase in the content of NO metabolites was observed in all experimental groups relative to the control values (Fig. 2).

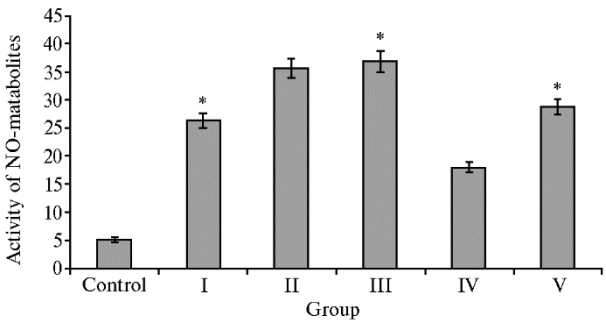

Fig. 2. The content of NO metabolites in the blood serum of Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles ($n = 4$, $M \pm$SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020). For a description of the groups, see the Materials and methods section.

* Differences from control are statistically significant at $p \leq 0.05$.

Additional administration of UFP Fe statistically significantly increased the content of nitric oxide metabolites 5.1-fold ($p \leq 0.05$) in group I, 7.2-fold ($p \leq 0.05$) in group III, 5.6-fold ($p \leq 0.05$) in group V. Insufficient production of NO in animals from the control group is associated with the development of disorders in the cardiovascular and other body systems. S.V. Rama Rao et al. [40] found that excessive production of NO, which provides an antimicrobial effect in inflammation, could turn from an adaptation link into a link in pathogenesis and become no less dangerous damaging factor for the body than NO deficiency. An increase for NO-metabolites in calves in group III fed UFP Fe indicated a compensatory reaction of the body to the changing lipid profile of the diet.

Indicators of amylase and lipase activity in blood serum may not always indicate the physiological stress of pancreatic function when changing diets, since there is an extrapancreatic production of these enzymes. Trypsin is the optimal marker for detecting changes in the physiological state of the pancreas, since it is

specific to this organ. It has been established that the entry of trypsin into the blood reduces the release of enzymes with pancreatic juice, while the administration of a trypsin inhibitor, on the contrary, is accompanied by an increase in the secretion of enzymes [41].

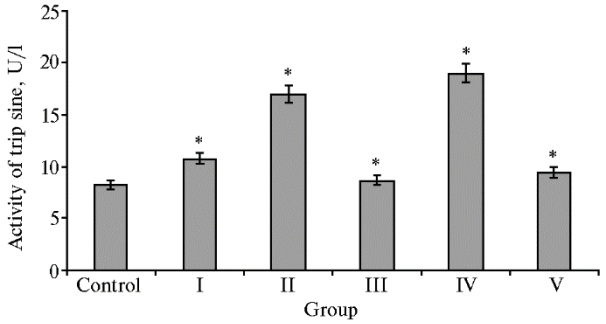

Fig. 3. Trypsin activity in the blood serum of Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles (n = 4, M±SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020). For a description of the groups, see the Materials and methods section.

* Differences from control are statistically significant at $p \leq 0.05$.

In our experiments, the introduction of UFP Fe into the diet in all experimental groups increased the activity of trypsin in the blood serum, which plays a critical role in initiating the cascade of activation of digestive enzymes in the intestine (Fig. 3). With the introduction of UFP Fe, trypsin activity increased by 31.2% ($p \leq 0.05$) vs. control. When the basal diet was replaced with fat diet, trypsin activity increased in group II by 106.6% ($p \leq 0.05$), in group IV by 130.9% ($p \leq 0.05$) vs. control. When UFP Fe was addaed to fat diets, trypsin activity decreased by 48.8% ($p \leq 0.05$) in group III vs. group II and by 50.5% ($p \leq 0.05$) in group V vs. group IV.

Trypsin activation by proteolytic breakdown of trypsinogen in the pancreas can lead to a series of events that induce pancreatic self-perception. One of the consequences of the autosomal recessive disease cystic fibrosis is insufficient transport of trypsin and other digestive enzymes from the pancreas [42].

5. Blood morphology in Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles (n = 4, M±SEM, FSC of biological systems and agricultural technologies RAS, 2019-2020)

Parameter	Group					
	control	I	II	III	IV	V
Leukocytes, $\times 10^9$/l	7.5±1.32	7.1±1.20	7.2±1.63	6.9±1.32	11.8±2.22*	8.2±1.72*
Lymphocytes, $\times 10^9$/l	2.9±0.83	2.6±0.63	4.1±1.21	3.2±1.13	5.5±1.21	4.3±1.32
Monocytes, $\times 10^9$/l	1.3±0.32	1.2±0.25	1.5±0.31	1.2±0.22	2.5±0.42	1.6±0.33
LMR	2.21	2.13	2.72	2.61	2.22	2.50
Granulocytes, $\times 10^9$/l	1.90±0.63	2.10±0.52	5.30±0.82*	4.30±0.62*	4.80±0.61	4.36±0.71
Erythrocytes, $\times 10^{12}$/l	5.08±2.91	4.98±2.22	5.37±3.13	4.88±2.50	7.62±1.81*	4.86±1.61*
Hemoglobin, g/l	93.0±11.12	102.0±9.81*	122.0±13.80*	132.0±11.51*	119.0±14.70*	121.0±12.22*
Hematocrit, %	20.1±4.34	22.6±3.63	21.6±4.91	22.0±2.82	24.2±3.94	20.8±2.61
MCH, g/l	349±26.92	324±28.63	440±26.42	388±36.53	424±27.72	368±34.23
Platelets, $\times 10^9$/l	201±19.81	212±14.32	224±17.71	216±18.22	220±21.70	206±14.63

N o t e. LMR is the lymphocytes-to-monocytes ratio, MCH quantifies the amount of hemoglobin per red blood cell. For a description of the groups, see the Materials and methods section.

* Differences with the control group are statistically significant at $p \leq 0.05$.

Blood morphology in calves changed when the basal diet was replaced with the experimental diet supplemented with soybean oil. The number of leukocytes increased statistically significantly by 57.3% ($p \leq 0.05$) vs. control, but the added UFP Fe led to a 30.5% decrease in leukocytes vs. group IV ($p \leq 0.05$) (Table 5).

The addition of UFP Fe led to increased absorption of iron from the gastrointestinal tract, improved synthesis of iron-containing metabolites (including hemoglobin), and stimulation of erythropoiesis [32]. In experiments on 4-month-old heifers treated with iron nanopowder, the number of erythrocytes increased by 19.6%, and the amount of hemoglobin by 17.1% compared to control.

We found that the UHF Fe added to both control and fat diets reduced the number of lymphocytes and monocytes. The number of erythrocytes decreased in group I by 2.0% vs. control, in group III by 9.0% vs. group II, in group V by 36.2% ($p \leq 0.05$) vs. group IV.

The direct participation of iron in the hemoglobin synthesis contributed to an increase in the content of hemoglobin in the experimental groups treated with UFP Fe. The blood hemoglobin level in animals of the experimental groups increased statistically significantly ($p \leq 0.05$) in group I by 9.7%, in group II by 31.2%, in group III by 41.9%, in group IV by 28.0%, in group V by 30.1% vs. control. The high iron content of heme makes hemoglobin an ideal molecule for targeted iron extraction during endogenous exposure to UFP. Significant levels of blood erythrocytes and hemoglobin indicates more intense redox processes in the animsl body and corresponds to higher productivity parameters [43].

The highest number of platelets was noted in the experimental group II (224×10^9/l), which was 11.4% higher than the control value ($p \leq 0.05$). Competition for heme iron between blood cells and a pool of exogenous bacteria together with an intense erythropoiesis, low absorption of iron by the endothelium and increased motility of the gastrointestinal tract could cause some increase in the parameter.

The number of formed elements of the blood leukocytes in control and test groups was within the physiological norm. In the leukogram which reflects the percentage ratio of different populations of leukocytes, no deviations from the norm were recorded. The lymphocytes-tomonocytes ratio (LMR) which characterizes the relationship between the affector and effector parts of the immune response, showed that LMR prevailed in group II and then decreased in the series group III > group V > group IV > control > group I (see Table 5).

The revealed blood morphological parameters in calves from the test groups corresponded to a higher metabolic activity.

6. Blood biochemical parameters in Kazakh white-headed calves (*Bos taurus taurus*) fed diets with vegetable fats and ultrafine Fe particles (n = 4, M±SEM, Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, 2019-2020)

Parameter	Group					
	control	I	II	III	IV	V
Total protein, g/l	72.05±3.98	76.05±2.82*	86.85±4.51*	144.70±4.81*	99.43±6.98	154.50±4.54*
Albumin, g/l	29.00±6.12	38.00±5.41	36.00±5.80*	38.00±4.72	42.00±5.33*	44.20±3.82
Glucose, mmol/l	3.41±0.87	4.94±0.55*	3.52±0.63	4.94±0.46*	4.21±0.74*	5.22±0.41*
Triglycerides, mmol/l	0.29±0.07	0.34±0.06*	0.37±0.03	0.09±0.01*	0.45±0.09*	0.07±0.01*
Cholesterol, mmol/l	2.67±0.19	1.08±0.05*	3.63±0.31*	0.92±0.03	4.99±0.81*	1.06±0.07
AlAT, U/l	23.80±4.31	22.50±3.81*	31.80±5.12	26.60±2.71*	28.80±5.11	26.30±2.91
AsAT, U/l	44.20±5.93	42.20±2.92*	52.30±6.33	105.80±6.12*	54.90±5.82	108.60±4.82
De Ritis coefficient (AsAT/AlAT ratio)	1,86	1,87*	1,64	3,98*	1,91*	4,12*
Bilirubin total, rmol/l	2.43±0.07	2.61±0.08*	3.25±0.08*	2.16±0.12	3.67±0.09*	1.94±0.22
Bilirubin direct, rmol/l	1.11±0.13	1.01±0.11	1.72±0.18	1.05±0.02	1.59±0.16	1.84±0.06**
Billyrubin index	2.19	2.58*	1.91*	2.05*	2.31*	1.05
LDH, U/l	3049±56.05	3856±62.21*	5272±64.31	3659±51.12*	4098±63.70*	3426±42.01*
α-Amylase, U/l	415.00±23.11	712.00±30.22	471.00±63.12*	358.00±16.21	423.00±21.91	346.00±11.62
Lipase, U/l	17.30±3.42	18.00±2.20	16.80±1.21	8.00±0.63	28.40±3.91	8.60±0.52
Urea, mmol/l	3.20±0.72	4.20±0.63	5.10±0.91*	5.00±0.91	4.60±0.92	4.40±0.55
Creatinine, rmol/l	74.50±6.31	81.20±5.11	88.70±7.23*	93.10±5.31	89.60±7.21	92.80±4.83
γ-GT, U/l	18.30±2.60	23.20±3.21	32.40±4.11	24.00±2.12	23.60±3.13	21.00±2.32
Uric acid, rmol/l	15.50±3.22	16.00±2.82	18.90±4.32	21.20±3.82	16.10±3.92	19.80±3.61
Iron, rmol/l	19.20±3.81	22.70±4.61*	33.40±5.12*	49.30±4.41*	34.60±4.31	36.80±3.62*
Magnesium, mmol/l	1.22±0.21	1.08±0.08	1.73±0.31	0.84±0.02	1.68±0.91	0.78±0.06
Calcium, mmol/l	2.45±1.12	2.68±1.21	3.01±1.22	2.60±0.58	2.71±0.83	2.32±0.12
Phosphorus, mmol/l	1.54±0.04	2.04±0.08	2.03±0.06	1.97±0.09	1.68±0.62	1.44±0.23

N o t e. AlAT — alanine aminotransferase, AsAT — aspartate aminotransferase, LDH — lactate dehydrogenase, γ-GT — glutamyl transpeptidase. For a description of the groups, see the "Materials and methods" section.
*, ** Differences with the control group are statistically significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Changes in biochemical parameters reflect the adaptation of all body systems, including digestion and general homeostasis, to changing feeding conditions [43]. In our experiments, the introduction of UFP Fe into the diet stimulated protein metabolism in calves (Table 6). An increase in the amount of total blood protein indicated a better assimilation of feed nitrogen, which was also facilitated by an increase in enzymatic activity [29]. When the control diet was replaced with fat, this indicator increased by 20.6% in group II ($p \leq 0.05$), and by 38.0% in group IV. Additional introduction of UFP Fe increased the content of total protein in group I by 5.6%, in group III by 100.8%, and in group V by 114.6% vs. control.

An upward trend was also observed for albumin (see Table 6). Its amount was statistically significant ($p \leq 0.05$) increased by 24.1% in group II and by 44.8% in group IV. In the test groups, the exceeding of the urea content vs. control ranged from 31.2% in group I up to 59.4% ($p \leq 0.05$) in group II.

Unbound iron also is an inducer of lipid peroxidation and protein peroxidation. Metals in microparticles have a low degree of release and assimilation rate, thereby eliminating toxic effects on the body and intestinal microflora [12]. The change in fat metabolism was assessed by the content of triglycerides and cholesterol in the blood serum. When UFP Fe was added, the amount of triglycerides increased by 17.2% ($p \leq 0.05$) (see Table 6).

Vegetable oils in animal diets have a significant effect on blood lipid profile [45]. In our experiment, when replacing the control diet with fat, the content of triglycerides increased in group II by 27.6%, in group IV by 55.2% ($p \leq 0.05$). UFP Fe added to fat diets contributed to a significant decrease in the amount of triglycerides in the blood serum of calves, in group III by 68.9% ($p \leq 0.05$), in group V by 75.9% ($p \leq 0.05$) vs. control. A similar trend occurred for the content of cholesterol (see Table 6).

The impact of UFP Fe on carbohydrate metabolism in animals was assessed by the glucose content in blood serum. The parameter value increased statistically significantly ($p \leq 0.05$), by 44.9% in groups I and III, by 53.1% in group V vs. control (see Table 6).

Dietary UFP Fe contributed to an increase in the content of iron ($p \leq 0.05$), however, the amount of magnesium, calcium and phosphorus decreased insignificantly, with the exception of group III. When the control diet was replaced with fat diet, the content of P increased in group II by 31.8%, in group IV by 9.1%, the content of Ca increased in group II by 22.9%, in group IV by 10.6%, the content of Mg increased in group II by 41.8%, in group IV by 37.7% vs. control.

UFP Fe added to the control and fat diets contributed to both stimulation and inhibition of certain processes. An increase in the AsAT/AlAT ratio (de Ritis coefficient) could indicate chronic processes associated with parenchymal liver damage due to heavy metal intoxication. AsAT in the de Ritis coefficient reflects the activity of a central metabolic link. Its regulate the use of substrates in the Krebs cycle with their subsequent aerobic oxidation, performs ammonia detoxification, involving ammonia into the urea synthesis cycle, and also provides recovery of the aspartate level in tissues which decreases with an imbalance of amino acids and hypoxia [43, 45]. The higher the AlAT level, the lower the de Ritis coefficient.

The content of AlAT increased in the groups which received fat diets. i.e., in group II by 33.6%, in group IV by 21.0%, however, these changes were not statistically significant. Additional administration of UFP Fe led to a decrease for AlAT by 5.5% ($p \leq 0.05$) in group I vs. control and by 16.4% ($p \leq 0.05$) in group III vs. group II that received the same diet except the addition of iron. The de

Ritis coefficient was significantly ($p \leq 0.05$) higher in the groups receiving UFP Fe with fat diets (see Table 6).

The blood bilirubin index (BI) was also calculated, which characterizes the excretory function of the liver and shows the degree of toxicity of UFP Fe. BI increased by 17.8% ($p \leq 0.05$) in group I and by 5.5% ($p \leq 0.05$) in group IV vs. control, in all other test groups, the BI values were lower than in the control.

Thus, the ultrafine iron (UFP Fe) when added to the fat diets of Kazakh white-headed calves contributes to a significant increase in the digestibility of crude fat, organic matter, and nitrogen-free extractive feed substances, while the digestibility of crude fiber and crude protein decreased. Enrichment of rations with UFP Fe and fatty components has a stimulating effect on pancreatic secretion, leading to an increase for pancreatic juice production. UFP Fe selectively changes the activity of the digestive enzymes of the pancreas. Due to UFP Fe added to the control (basal) diet, the lipase activity increases by 35.7%, intestinal proteases by 43.1% while the amylolytic activity decreases by 28.8%. The use of UFP Fe with fat diets reduced the enzymatic activity of the pancreas vs. the control group. With sunflower oil, the activity of lipase and intestinal proteases increased by 12.1 and 16.7%, respectively ($p \leq 0.05$), with soybean oil by 133.2 and by 38.4% ($p \leq 0.05$). Supplementation of the diet with fatty components, separately and in combination with UFP Fe, led to an increase in the of NO-metabolite content in all test groups compared to control. With sunflower and soybean oil, an increase occurres in the trypsin content by 106.6% ($p \leq 0.05$) and 130.9%, respectively. UFP Fe, when aadded, decreases the trypsin content. Morphological analysis revealed a statistically significantly higher blood hemoglobin in animals of the test groups, by 9.7-41.9% ($p \leq 0.05$). All the studied biochemical parameters were within acceptable physiological norms. However, UFP Fe had a stimulating effect on protein, fat and carbohydrate metabolism. An increase in the de Ritis coefficient occurred in the groups receiving UFP Fe with sunflower and soybean oil in the diets. In all other test groups, the values of the bilirubin index were lower than in the control, which indicates a rather low toxicity of the preparation of ultrafine iron particles.

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