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IRON DEFICIENCY ANEMIA IN LABORATORY RATS TO BE USED AS AN EXPERIMENTAL MODEL FOR FARMED FUR-BEARING ANIMALS

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Abstract

Iron is an essential trace element necessary for the implementation of many processes in the body (metabolism regulation, DNA and ATP synthesis, oxygen transfer, tissue respiration, erythropoiesis, immune response). In caged fur animals, iron deficiency anemia leads to significant economic losses due to a decrease in viability and fertility, and a deterioration in the fur quality. Therefore, the study of the causes of this microelementosis, the development of pharmacological agents and techniques for its prevention and treatment remain a topical issue. In our report, we present data confirming the modeling of this pathology using an atraumatic approach, i.e., the diet we proposed, which is low in cost and simple in its ingredients. As a model object, white rats were used, which, in terms of physiological parameters, are more similar to fur-bearing animals than other laboratory animals. The aim of the study was experimental modeling of iron deficiency anemia in laboratory rats in order to extrapolate the results obtained on this model to fur animals in the future. From 4-month-old white outbred laboratory rats weighing 200 g, two groups of 10 individuals were formed. Control animals received a generally accepted balanced diet which corresponded to the consumption norms for laboratory rats and was 4 g proteins, 2 g fats, 25 g carbohydrates, and 0.5-1.0 g fiber. In the experimental group a specially developed diet was applied which was 4 times less in the iron content, but corresponded to the feeding norms in terms of the nutrients, vitamins and minerals (excluding iron). After 45 days, the rats in the experimental group developed iron deficiency anemia. As compared to the control rats, receiving a diet not deficient in iron, there was a significant ($p \leq 0.05$) decrease in hemoglobin (by 37.5 g/l), hematocrit (by 18.35 %), the number of erythrocytes (by 3.57×10^{12}/l), the concentration of serum iron (by 18.44 μmol/l), the average volume of erythrocyte (by 14.02 fl), the average content of hemoglobin per erythrocyte (by 6.26 pg) and per erythrocyte mass (by 73.29 g/l). The anemia of the animals was hypochromi and macrocytic. From day 17 of the experiment, shortness of breath and increased heart rate occurred, from day 24, the body temperature decreased which indicates the development of an anemic syndrome in the rats. Up to day 14, the color of the skin and mucous membranes, as well as the general condition of the rats in both groups corresponded to the norm. After day 14, anemic skin and mucous membranes of the oral cavity were observed in rats receiving an experimental diet with a limited iron content. In addition, lethargy and general depression occurred. Our results demonstrated the ability to effectively simulate iron deficiency anemia in laboratory rats, minimizing stress and eliminating physical and mental traumatization of animals, the risk of their death, and side effects. The model has been successfully applied in evaluating the effectiveness of a complex microelement preparation based on a polymaltose complex of Fe^{3+} hydroxide. In the future, we plan to use the model of iron deficiency anemia in rats to develop methods for the prevention and correction of this pathology in farmed fur-bearing animals.

Keywords: fur farming, iron deficiency anemia, iron preparations, anemia modeling, laboratory rats

Iron is an essential micronutrient with a complex metabolism, recycling

system, and content control, which allows us to speak about Fe homeostasis in the body [1-4]. Inorganic iron supplied with food [5] is mainly in the trivalent form Fe^{3+}. It is absorbed by the duodenal mucosa with the participation of beta-3-integrin and mobilferrin, a 56 kDa protein. In the cytosol of the absorbing cell, iron binds to a complex known as paraferritin, which contains integrin, mobilferrin, and flavin monooxygenase. This complex serves as a ferrireductase and reduces iron to the divalent state Fe^{2+}, in which it is available for the formation of heme proteins [6-8]. In the cells of the intestinal mucosa, iron in the form of Fe^{3+} combines with the protein apoferritin to form ferritin, the main form of deposition of this trace element [4,] (in the bone marrow, liver, and spleen). It is believed that the amount of iron entering the blood depends on the content of apoferritin in the intestinal walls [10]. The transport of iron from the intestine to the hematopoietic organs is carried out by a complex with the blood plasma protein transferrin [8]. In the form of fumarate, the bioavailability of iron is increased [11].

In the body, iron compounds are involved in oxidative reactions. Hemoglobin serves as a carrier of oxygen, myoglobin (protein of skeletal muscles and heart muscle) binds oxygen and creates a reserve to make up for its deficiency. Parenteral and enteral administration of iron salts increases the content of hemoglobin in the blood and iron in the blood serum [12-16]. Iron-containing enzymes cytochromes, cytochrome oxidase, catalase, peroxidase provide tissue respiration, iron is in the prosthetic group of ferroflavoproteins - xanthine oxidase, succinate dehydrogenase [17].

Thus, iron is necessary for the implementation of basic processes in the body (metabolism regulation, DNA and ATP synthesis, oxygen transport, tissue respiration, erythropoiesis) [17-19], it affects immunoresistance [20]. Iron deficiency can cause impaired conversion of protoporphyrin IX to heme. As a result, the content of porphyrins in erythrocytes increases (21). With iron deficiency, a hematological syndrome develops, characterized by impaired hemoglobin synthesis and manifested by erythrocytopenia and sideropenia (low iron and iron-containing enzymes) [22-24].

The variety of Fe functions determines the significant physiological abnormalities caused by iron deficiency anemia [25-27]. In animal husbandry, its etiology in most cases is associated with improper feeding and inadequate care of animals [26]. Iron deficiency anemia accounts for the majority of all diagnosed anemias [16, 28]. With this pathology, the growth and development of animals slows down [29-31], in fur-bearing animals under conditions of industrial breeding, the condition of the skin and hair integuments worsens, the quality of furs decreases [2, 32-34], including its physical and mechanical characteristics [35].

The prevalence of iron deficiency anemia in mammals, its dangerous consequences and the damage it causes determines the volume of fundamental physiological, biochemical [36-40] and genetic studies in this pathology [18, 25, 41], as well as practical developments to compensate for the iron deficiency state in humans [12, 42] and animals [13, 29-31, 43]. For these purposes, iron-deficient diets are widely used in world practice [18, 44-46], which are proposed for various animal species - rodents, dogs, cats, rabbits, guinea pigs, ferrets, pigs, sheep, goats, cows, primates and are produced in commercial scale, which allows standardization of the design of the experiments. Examples include Teklad from Envigo, USA (https://www.envigo.com/) and AIN (American Institute of Nutrition Approved Diets) [46-48], which continue to be improved. In such experiments, an adequate choice of a biological model is also important, which makes it possible to apply the results obtained on a laboratory animal to solve practical problems [49]. The most common animal models used in biomedical research are rats, usually males [18], which makes it possible to exclude the effect of hormonal changes on the

results.

In fur farms, iron deficiency anemia is a common pathology [34, 50, 51] and leads to significant economic losses due to a decrease in the viability and fertility of animals, and a deterioration in the quality of the resulting furs [26, 34, 52]. Iron deficiency anemia is most common in minks [2], in part because their diet includes marine fish [34, 53], which are rich in trimethylamine oxide (TMAO, or triox), which binds iron and converts it from divalent to non-ferrous. digestible trivalent. With regular consumption of such fish, animals, especially growing young animals, develop iron deficiency [34, 53].

Until now, in Russia, there was no publicly available information about the method of obtaining experimental models of iron deficiency anemia which would be similar to the known ones, but low-cost, accessible, atraumatic and at the same time sufficient for the selection of pharmacological prevention and treatment of this microelementosis and schemes for their use in the practice of fur farming. In our report, we present data confirming the possibility of such modeling in rats using the original diet developed by us presented in the work.

The aim of our study was to confirm the development of experimental iron deficiency anemia in laboratory rats when using a diet with a limited iron content.

Materials and methods. Physiologically healthy female outbred laboratory rats (n = 20, aged 4 months with body weight of 200 g) were used in the experiment. According to the principle of pair-analogues, two groups of 10 rats each were formed. The conditions of the animals were in accordance with the International Guidelines for Biomedical Research Involving Animals [54]. Rats of both groups were kept in standard cages in compliance with veterinary and zootechnical requirements according to the recommendations for biological models [55]. As the main (control group I) we used a balanced diet (Delta Feeds, a feed for laboratory rats and mice P-22, AO BioPro, Russia), corresponding to the consumption norms for laboratory rats (4 g proteins, 2 g fats, 25 g carbohydrates, 0.5-1.0 g fiber), water in plenty. In group II (test group), rats received a specially designed diet (26 g individually for each animal 1 time per day), which corresponded to the age norms for animals of this species in terms of the content of nutrients, minerals and vitamins with a decrease in the content of Fe in the feed (9.12 mg/kg vs. 35.00 mg/kg in control). In both groups, access to water was not restricted. The experiment continued for 45 days.

During the entire observation period, body temperature, heart rate (HR), and respiration rate (RR) were measured every week in animals. We used a PowerLab® 8/30 recorder (ADInstruments Pty Ltd., Australia) and a piezoceramic sensor for recording respiration rate with a BNC for connecting to the recorder, an elastic cuff for fixing the sensor (different sizes depending on the type of animal). The general condition of the rats was also assessed.

At the end of the experiment (on day 45), a hematological analysis of peripheral blood of each laboratory rat collected from the tail vein with a needle into a test tube with an anticoagulant was performed. The number of erythrocytes and the amount of hemoglobin [56], hematocrit [57], the average volume of erythrocytes, the average content of hemoglobin in the erythrocyte and in the erythrocyte mass, as well as the content of iron in the blood serum [58] were determined.

From rats with iron deficiency anemia, modeled according to the method we proposed by (n = 10), two groups were formed (n = 5 each) to study the effectiveness of a new complex trace element preparation based on the Fe^{3+} hydroxide polymaltose complex (OOO A-BIO, Moscow). Animals of group I served as control (rats continued to receive the diet developed by us), in group II, it was supplemented with a complex microelement preparation at a dose of 0.1 ml for

each animal. After 30 days, hematological examination of peripheral blood was performed as described above.

Statistical analysis of the obtained data was performed using the Microsoft Excel program. The arithmetic mean of the measured parameters (*M*) and the standard error of the mean (±SEM) were calculated. To assess the significance of differences between the compared means, Student's *t*-test was used ($p \leq 0.05$).

Results. The study of the efficacy and safety of pharmacologically active compounds in experimental models is a necessary preliminary step in the search for drugs for the treatment and prevention of microelementoses in fur animals.

Various schemes for modeling iron deficiency anemia in animals are known. For example, in pigs [59] and rats [60], this microelementosis was induced by bloodletting (blood-removing induced anemia). In Russian researchers, it was proposed to administer the drug Deferoxamine subcutaneously (0.5 g/kg twice with an interval of 3 days, Patent RU 2553344 C1, publ. 10.06.2015, Bull. No. 16) [61]. A significant drawback of the proposed method is the multiple negative side effects. Another technique involves the administration of Desferal® (also a complexing compound, the dosage and frequency of administration are not indicated by the authors) [60, 61]. However, these techniques are traumatic (up to the risk of death of animals) and cause severe stress in test animals [61], which distorts the results of the experiment. In world practice, standardized iron-deficient diets are used [18, 44-46]. In Russia, the use of a diet with an iron content of 27 mg/kg for the induction of experimental iron deficiency anemia in rats was reported, but the composition of the diet was not specified by the author [61].

1. The diet for modeling iron deficiency anemia in white laboratory rats

Ingredient	Daily dose per animal, g
Sodium chloride	0.3
Magnesium sulfate · 7H20	0.01
Sodium phosphate	0.4
Calcium gluconate	0.55
Potassium chloride	0.02
Microcrystalline cellulose	0.25
Tea powder	0.08
Vitamin blend	0.003
Sunflower oil	2.0
Abiopeptide dry	2.0
Semolina	20.0
Corn starch	0.67

N o t e. The composition of the vitamin blend is specially selected and contains the following components (one daily dose): α-tocopherol acetate (0.24 mg), ascorbic acid (1.8 mg), calcium pantothenate (0.072 mg), nicotinamide (0.48 mg), pyridoxine hydrochloride (0.072 mg), retinol palmitate (0.044 mg), riboflavin (0.048 mg), rutoside (0.24 mg), thiamine hydrochloride (0.048 mg), folic acid (1.68 mcg), cyanocobalamin (0.048 μg). Each rat received individually 26 g of the mixture per day.

For the induction of iron deficiency anemia, we have proposed and tested a diet, the composition of which is presented in Table 1.

When developing an experimental model, an adequate choice of animal is important [44, 49]. The most common biological models are mice [43, 62] and rats [15, 18, 25, 39, 44]. In our experiment, rats were model animals which are more similar to fur-bearing animals, and in particular to minks, in terms of physiological and biochemical features. An important advantage of rats as laboratory animals is that they are quite resistant to infectious diseases. Males are usually used [18], as well as females at the age of 3-5 months (up to 6 months of age, only about 1% of individuals start reproduction) [54, 55]. We used 4-month-old female white outbred laboratory rats based on the fact that, in particular, in minks, it is the iron deficiency anemia of females that poses a serious problem. Anemia-prone adult male breeding males are mostly sterile, while females have reduced body weight, a high percentage of infertility, cannibalism, and loss of maternal

instinct [52]. Their puppies are underweight at birth, often suffer from maldigestion, grow poorly, often die at an early age, and the survivors remain small and even dwarf [52].

In our experiment, when rats received the experimental diet, the blood hemoglobin significantly ($p \leq 0.05$) decreased, hematocrit, erythrocytes and serum iron concentration also decreased compared to control by 37.5 g/l, 21.35%, 3.57×10^{12}/l and 18.44 μmol/l), respectively, hypochromia and microcytosis were recorded (Table 2) which are characteristic signs of iron deficiency anemia.

2. Hematological parameters in white laboratory rats characterizing the state of iron deficiency anemia ($M \pm$SEM)

Parameter	Group I (control) ($n = 10$)	Group II ($n = 10$)
Erythrocytes, $\times 10^{12}$/l	7.82±0.43	4.25±0.37*
Hemoglobin, g/l	121.1±6.2	83.6±0.4*
Hematocrit, %	44.58±3.55	23.23±2.18*
Average erythrocyte volume, fl	62.26±6.17	51.24±3.16*
Average hemoglobin content per erythrocyte, pg	22.64±2.32	16.38±1.13*
Average hemoglobin content in erythrocyte mass, g/l	348.45±20.11	275.16±28.12*
Serum iron concentration, μmol/l	45.68±2.74	27.24±3.82*

N o t e. The groups formed from 4-month-old rats were used in a 45-day test.
* Differences from control are statistically significant at $p \leq 0.05$.

The significant hematological changes we identified are similar to those described in animals of other species where these indicators, i.e., a decrease in hemoglobin [40], a decrease in hematocrit [63], erythrocytopenia, a decrease in blood iron concentration, and hypochromia and microcytosis [64] which are characteristic of iron deficiency anemia.

3. Dynamics of physiological parameters of white laboratory rats with experimental iron deficiency anemia ($M \pm$SEM)

Days	Group ($n = 10$)	Parameter		
		RR	HR	T
One day prior to the experiment	I	99±7	398±20	37.3±0.2
	II	98±10	402±15	37.4±0.3
Day 3	I	101±11	411±21	37.5±0.3
	II	106±9	409±19	37.3±0.2
Day 10	I	102±8	387±18	37.4±0.3
	II	112±11	406±21	37.5±0.4
Day 17	I	109±8	395±12	37.6±0.4
	II	129±9*	428±10*	37.4±0.4
Day 24	I	112±11	403±16	37.4±0.3
	II	132±8*	429±17	36.4±0.4*
Day 31	I	99±7	409±15	37.3±0.3
	II	133±8*	439±14*	36.5±0.4*
Day 38	I	110±9	413±16	37.5±0.2
	II	138±10*	448±15*	36.8±0.4*
Day 45	I	104±6	404±18	37.4±0.3
	II	137±8*	453±21*	36.9±0.4

N o t e. The groups formed from 4-month-old rats. RR — respiration rate, HR — heart rate, T— body temperature.
* Differences from control are statistically significant at $p \leq 0.05$.

Iron deficiency anemia induced in rats from group II affected the physiological state of the animals, the respiration rate, heart rate, and body temperature (Table 3). Consequently, the body of laboratory rats from the experimental group as a whole reacted to the development of the disease, while from day 17 significant changes began, when shortness of breath and palpitations were noted in the animals. From day 24, body temperature decreased which indicates the development of anemic syndrome.

During the experiment, we also assessed the color of the skin and mucous membranes and the general condition of the animals. Until day 14, these indicators in laboratory rats from the test and control groups corresponded to the norm. After 2 weeks, in rats fed an experimental diet with a limited iron content, we

observed anemia of the skin and mucous membranes of the oral cavity, as well as lethargy and depression of the general condition.

Laboratory rats with iron deficiency anemia, modeled by our method, were further used to study the effectiveness of a new complex trace element preparation based on the Fe3+ hydroxide polymaltose complex (Table 4). The studied drug in 1 ml contains 50 mg Fe(III) as active substances, Cu 0.1 mg, Co 0.2 mg, Se 0.07 mg, Mn 0.6-0.7 mg, Zn 0.6-0.7 mg. As excipients, the preparation is 1.5 g methylhydroxybenzoate, 0.15 g propylhydroxybenzoate, 100 g sucrose, 140 g sorbitol (drinking water up to 1.0 l). In appearance, the drug is an odorless, opaque, reddish-brown liquid.

4. Hematological parameters in white laboratory rats with experimental iron deficiency anemia in an experiment to study the effect of a complex trace element preparation based on a polymaltose hydroxide Fe^{3+} complex ($M \pm$SEM)

Parameter	Group I (control) (n = 5)	Group II (n = 5)
At the beginning of the test		
Erythrocytes, $\times 10^{12}$/l	4,42±1,63	4,35±0,31
Hemoglobin, g/l	81,8±0,4	82,3±0,8
Hematocrit, %	23,34±1,82	22,27±2,32
Average erythrocyte volume, fl	61,21±1,19	58,45±3,63*
Average hemoglobin content per erythrocyte, pg	16,45±3,56	15,91±2,17
Average hemoglobin content in erythrocyte mass, g/l	278,65±15,41	271,92±21,78
Serum iron concentration, μmol/l	24,91±3,93	26,85±3,14*
At the end of the test		
Erythrocytes, $\times 10^{12}$/l	5,14±0,24*	7,71±0,39*
Hemoglobin, g/l	85,3±5,2*	118,7±4,8*
Hematocrit, %	36,41±3,82*	44,12±2,74*
Average erythrocyte volume, fl	58,92±7,46	57,44±5,31
Average hemoglobin content per erythrocyte, pg	15,35±3,19*	23,54±2,71*
Average hemoglobin content in erythrocyte mass, g/l	288,56±19,53*	353,27±16,25*
Serum iron concentration, μmol/l	27,65±2,71*	46,32±1,68*

Note. A 30-day experiment was performed. The manufacturer of the drug is A-BIO LLC, Moscow.
* Differences from control are statistically significant at $p \leq 0.05$.

At the end of the experiment (after 30 days), in rats from group I (control), hematological parameters (erythrocytes, hemoglobin, hematocrit, average hemoglobin content in erythrocytes, average hemoglobin content in erythrocyte mass and serum iron concentration) were still reduced - by 2.57×10^{12}/l, 33.4 g/l, 7.71%, 8.19 pg, 64.71 g/l and 14.67 μmol/l, respectively. In rats from group II (test), hematological parameters returned to normal, which indicated the elimination of iron deficiency anemia. Thus, a positive experience has been obtained in using the developed model of experimental iron deficiency anemia in assessing the effectiveness of its correction schemes. It was shown that the use of a complex microelement preparation of a microelement preparation based on the Fe^{3+} hydroxide polymaltose complex allowed normalization of hematological parameters (erythrocytes, hemoglobin, hematocrit, average hemoglobin content in an erythrocyte, average hemoglobin content in an erythrocyte mass and serum iron concentration) in laboratory rats after modeling iron deficiency anemia.

Based on the results of the experiments, it can be argued that we have proposed a model of iron deficiency anemia in laboratory rats. The model is effective, atraumatic, low-cost, accessible and sufficient for solving the problems of practical fur farming in assessing the effectiveness of the developed techniques for correcting this pathology [13, 15, 32, 42] and the safety of the agents used, given the pathophysiological effects of excess iron [65-68].

R.M. Kaufman and P. Simeon [44] studied the effect of an iron-deficient diet on iron absorption in rats weighing 200-350 g. The total iron content of the diet used in the study was 3.90 μg/g (daily feed intake 10 g); in a standard commercial diet, the iron content was 186 μg/g (daily feed intake of 10-15 g). The authors concluded that iron absorption is controlled by the depletion of its reserves

from a different pool than in the liver and erythrocytes. The basis was the data that with a lack of iron in the diet, its absorption in rats increased (after at least 5 days of limited iron intake), the lack of iron in the diet did not affect erythropoiesis, and up to 14 days the content of serum iron in animals in the experimental group did not differ from normal [44]. The observation time, depending on the variant of the experiment, ranged from 2 to 30 days [44]. These and other available data indicate that body adaptive responses and iron homeostasis [4, 36, 44, 67] need to be considered when developing a model of iron deficiency anemia. In our model, the daily feed intake was 26 g, the iron content in the proposed diet was 9.12 mg/kg vs. 35.00 mg/kg in the control, that is, the difference was approximately 4-fold vs. 48-fold in the report by R.M. Kaufman and P. Simeon [44]. Our experiment lasted 45 days and resulted in the significantly decreased amount of hemoglobin, hematocrit, erythrocyte count, serum iron concentration, average erythrocyte volume, average hemoglobin content in erythrocytes and average hemoglobin content in erythrocyte mass in the laboratory rats. Additional observations confirmed that from day 17 the general condition and physiological parameters of the animals changed and anemic syndrome developed.

Thus, our results demonstrate that the provided simple and available diet can effectively simulate iron deficiency anemia in laboratory rats, minimizing stress and eliminating the physical and mental traumatization of animals, the risk of their death and the unreliability of the results. The iron deficiency of the animals was characterized by a decrease ($p \leq 0.05$) in hemoglobin concentration by 37.5 g/l, in hematocrit by 21.35%, in erythrocytes by 3.57×10^{12}/l, in the blood iron concentration by 18.44 μmol/l, in mean erythrocyte volume by 14.02 fl, in mean hemoglobin content per erythrocyte by 6.26 pg, and in erythrocyte mass by 73.29 g/l compared to the control (iron-free diet). There were shortness of breath and palpitations from day 17 of the experiment and a decrease in body temperature from day 24, which indicates the development of an anemic syndrome in animals. Up to day 14, the skin and mucous membrane color, as well as the general condition of the animals in both groups corresponded to the norm, but after day 14, in rats fed feed with a limited iron content, we observed anemia of the skin and mucous membranes of the oral cavity, lethargy and oppression of the general state. The proposed model was successfully applied to study the effect of a complex microelement preparation based on the Fe^{3+} hydroxide polymaltose complex. Further, we plan to use the model of iron deficiency anemia in rats to develop methods for the prevention and correction of iron deficiency anemia in other animals, including fur animals, which, by their biological features and physiology are similar to rats.

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