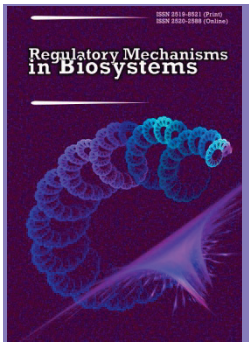

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2020, 11(2), 153–161
doi: 10.15421/022023

Effect of succinic acid on the organism of mice and their intestinal microbiota against the background of excessive fat consumption

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Article info

Received 17.04.2020
Received in revised form 21.05.2020
Accepted 24.05.2020

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Lieshchova, M. A., Bilan, M. V., Bohomaz, A. A., Tishkina, N. M., & Brygadyrenko, V. V. (2020). Effect of succinic acid on the organism of mice and their intestinal microbiota against the background of excessive fat consumption. Regulatory Mechanisms in Biosystems, 11(2), 153–161. doi:10.15421/022023

Succinic acid and its salts (succinates) positively affect the oxygenation of the internal environment, stabilize the structure and functional activity of mitochondria, and normalize the ion metabolism in the cell. Separate clinical studies and experimental surveys confirmed that having low toxicity succinic acid has well-manifested antioxidant, immunostimulating, adaptogenic properties. In this study, we determined the influence of succinic acid on the organism of laboratory animals against the backround of high-fat diet: the changes in body weight, indices of the mass of the internal organs, blood parameters and the changes in the intestinal microbiota were determined. For the experiment, we formed three experimental and three control groups of male white mice. The animals of the control group received 0.5% solution of succinic acid instead of water. In the experiment, we determined that succinic acid has no effect on the intensity of growth of weight of young mice against the background of excessive fat in their diet. Excessive consumption of fat by male mice leads to mainly disorders in the functioning of the liver, excretory and the immune systems. High-fat diet of mice is accompanied by impaired hepatic function, manifested in sharp hypoproteinemia due to globulins, increase in the activity of hepatic enzymes against the background of reduced activity of alkaline phosphatase, increase in the level of bilirubin, and decrease in glucose. Excess of fat in the diet leads to malfunctioning of the excretory system, manifested in the reduced index of kidneys' weight, high content of creatinine and reduced level of urea in the blood. Addition of succinic acid has a positive effect on the functional condition of the liver and the kidneys, especially noticeable during long-term intake. High-fat diet causes disorders in the functioning of the organs of blood circulation and immune protection, accompanied by decrease in the relative mass of the thymus and spleen, low content of hemoglobin and the number of erythrocytes, but has no significant effect on the content of other cellular elements in the blood. By the middle of the experiment, succinic acid had exacerbated these processes compared to the control, but by the end of the experiment, by contrast, these processes were alleviated. Addition of the succinic acid to high-fat diet contributed to the change in the quantitative composition of the main representatives of the obligatory microbiota (*Bifidobacterium* spp., *Lactobacillus* spp. and typical *Escherichia coli*) in the laboratory animals. Such changes in the intestinal microbiota may lead to such consequences as reproduction of the facultative microflora, and, thus, development of various diseases.

Keywords: health effects; biochemical parameters of blood; relative mass of the organ; gut microbiota; microbiome; high-fat diet.

Introduction

Development and search for effective preparations for the prevention and treatment of disorders in metabolism, correction of biochemical parameters and increase in the natural resistance of the organisms of humans and animals is a relevant problem. Metabolic correction is a direction in therapy which is based on use of preparations of natural origin which combine efficiency and safety and impose no pharmacological load due to their bioavailability (Novikov & Levchenkova, 2013). These requirements are met by natural metabolites of the Krebs cycle, particularly compounds of succinic acid (Ischeikin et al., 2012). Succinic acid is a universal intracellular metabolite with a broad range of action towards the parameters of vitality of a living organism. The system that uses succinic acid for producing energy, by its power, becomes significantly superior to the other systems of organisms' energy production. Therefore, succinic acid provides a broad range of non-specific treatment effects based on the influence on the process of tissue metabolism: cellular respiration, ionic transport, and synthesis of proteins.

In vitro experiments revealed that application of succinic acid leads to increase in consumption of oxygen by the tissues due to oxidation of the added substrates to the final products – carbon dioxide, water and heat. One molecule of succinic acid added to tissue ensures the oxidation of many endogenous substrates. Thus, transformation of succinic acid in the organism is associated with the production of energy required for the vital activity. With increase in the load on any system of organism, support of its functions is provided chiefly by the oxidation of succinic acid (Shakhmardanova et al., 2016).

The antihypoxic, disintoxication, and antioxidative effects of succinates have been determined (Novikov & Levchenkova, 2013). At the same time, biological effects of succinate are realized through activation of the production of energy of the respiratory chain of the mitochondria, during which the formation of ATP and the restoration of the equivalents are considerably enhanced, and the membrane potential stabilizes both in the mitochondria and the cells in general (Mallaisse et al., 1997).

Succinates are medical-preventive preparations of a new generation – the so called "smart drugs" – which have corrective effects on the tissues. By normalizing the metabolism in the organism, succinic acid contributes to the strengthening of the immunity. Therefore, it is recommended for clinical treatment of immune deficiencies and infectious diseases. Succinic acid affects the molecular, cellular and mediatory mechanisms of the regulation of the immune system (Kim et al., 2017). Strength and the orientation of the modification during the action of succinic acid depend on the condition of the tissues, and its final result is expressed in the optimization of the parameters of their functioning (Sakamoto et al., 1998).

Energy-synthesizing effect is especially noticeable in the conditions of hypoxy, which is the essential condition of the development of most pathological conditions. A study on the clinical efficiency of additional prescription of succinic acid to traditional therapy demonstrated positive dynamics manifested in decrease in the duration of intoxication, respiratory syndrome, and more rarely asthenia (Kitura, 2013).

In a group of children suffering from pneumonia, there was seen reliable decrease in the level of aterogenic fractions of the lipid spectrum of blood (low-density lipoprotein cholesterol (LDL-C) with low and very-low density lipoprotein cholesterol) after intake of succinic acid. At the same time, growth of the concentration of high-density lipoprotein cholesterol (HDL-C) and decrease in atherogenicity index (AI) were observed. Similar dynamics of the atherogenicity index were observed in children with bronchitides, and also children with non-complicated course of the respiratory pathology. Decrease in the level of LDL-C against the background of prescription of succinic acid along with the antiatherogenic effect indirectly positively affects also the immune status, because it suppresses the competing effect of LDL-C with antigene substrates for the receptors on the surface of the immune cells (Vahitov et al., 2014).

In rats, hypobaric hypoxia causes fibrosis in the myocardium, which inevitably leads to systolic and diastolic dysfunctions, neurohormonal activation and, finally, heart failure. At the same time, succinic acid combined with inosine functions acts as a highly-effective reserve of phosphate, which can support the level of adenosine triphosphate at the level sufficient for the support of the contractile function of the heart (Zadnipryany et al., 2019).

Succinic acid exhibited insulinotropic effect in experimental diabetes due to significant increase in the activity of succinate dehydrogenase. At the same time, increase in the synthesis of insulin was due to the improvement of metabolic processes in the insular of the pancreas (Malaisse & Sener, 1993; Malaisse et al., 1993; Cancelas et al., 2001).

Intravenous injection of succinic acid to patients with acute purulent pyelonephritis contributed to the removal of the symptoms of toxicosis, normalized the analyses of blood compared to the use of the standard methods of treatment (Hozhnko et al., 2006). In patients with acute purulent pyelonephritis, succinic acid had a positive effect on the activity of enzymes and the content of the glycolysis products; at the earlier stages, compared to the use of the standard infusive media, there was seen achievement of normalization of the activity of the processes of peroxidation of lipids in the blood plasma and urine (Zolotykh, 2008).

Succinic acid facilitates the hormonal re-structuring of the organism during pregnancy, prevents toxicoses, supports the activity of the immune system, reduces the probability of complications (Ladriere et al., 1996). The fetus develops in optimum conditions with good provision of oxygen, the placenta barrier strengthens, thus preventing the penetration of toxins, viruses and bacteria into the organism of the child. Use of the preparations of succinic acid significantly reduces the risk of post-natal complications, and the process of birth shortens and is facilitated. Succinic acid enhances the restoration of the mother`s organism after giving birth (Lebedev et al., 2009; Evglevskij et al., 2013).

The radioprotective effect of succinic acid is explained by the influence on the metabolic processes in the cells: decrease in the oxygenation of nucleus and cytoplasm, increase in the synthesis of protein and the formation of ATP, activation of cellular respiration, inhibition of peroxidation of lipids (Ronai et al., 1987; Ivnickij & Shturm, 1990).

The adaptogenic action of succinic acid and succinates were described at numerous points in the model of mobilized stress (Westergaard et al., 1994), stress caused by the physical factors (Filatova et al., 1984). In the conditions of X-ray-driven oxidative stress in rats, succinic acid and its preparations (Reamberin) contributed to the decrease in the concentration of hydroperoxides of lipids, conjugated dienes, malondialdehyde in the blood plasma (Tihomiriva, 2005). Use of the succinate-containing preparations in the conditions of ultraviolet radiation of the organism stabilizes the peroxidation of lipids and increases the activities of different components of the antioxidative system (Simonova et al., 2018).

The anti-inflammatiry effect of succinic acid was seen with hepatitis and even liver cirrhosis. It helps in cases of gallstones, increases the production of salts and contributes to the drainage of the liver (Garnyk et al., 2012). Preparations of succinic acid together with complex therapy in the period of acute chronic cholecystitis reduce the intensity of peroxidation of lipids and increase the activity of the system of antioxidant protection regardless of the age of patients (Ryabushko, 2013). Use of succinic acid during chronic pancreatitis enhances the activity of the system of antioxidant protection, which manifests in the increase in the content of restored glutathione and increase in the catalase activity in blood, contributes to the decrease in the intensity of pain syndrome (Kitura, 2013).

Succinic acid in preparation "Reamberin 1.5% solution for infusions" is used for the treatment of intestinal infections with notable intoxications caused by shigellosis, salmonellosis, rotavirus gastroenteritis, *Klebsiella* infection and dysentery of undetermined etiology (Ferreira et al., 2000; Tihomiriva, 2005).

In veterinary practice, the succinic acid-based medical preparations have become used in the treatment and prevention of diseases in different spheres: correction of the metabolic processes, purulent-septic diseases, inflammatory processes, immune deficiencies, infectious and parasitic diseases and others (Ilnitskyi & Hierdieva, 2014; Lashin et al., 2018). Combination of succinic acid and antiparasitic preparations which usually are highly toxic significantly reduces the latter property. This was confirmed in a series of studies on agricultural animals which revealed that the use of succinic acid and levomycetin for calves causes notable stimulation of metabolic and immune processes (Evglevskij et al., 2013; Karachevceva, 2013).

Succinic acid and preparations based on it are broadly used in livestock farming as biostimulators. In broiler chickens, against the background of using the preparation with succinic acid, the activity of adenosine triphosphates in the membranes of erythrocytes reliably increased, and the morphological and biochemical blood parameters normalized (Ryzhkova et al., 2011; Lashin & Simonova, 2017).

In an experiment on cattle when using Succinic Biostimulator, the blood was found to have increase in the phagocytic activity of neutrophils, and in the post-natal period – increase in the antibacterial activity of the blood serum (Ivanov et al., 2009). During the use of Succinic Biostimulator preparation based on succinic acid and ATP-2F, the cows and their calves were observed to have a broad range of positive changes. The cows were observed to have the normalization of protein metabolism, which was expressed in the increase of concentration of the total protein of blood to the physiological norm, correction of dysproteinemia as the normalization of the content of albumins and globulins and decrease in the content of urea (Ivanov et al., 2009). Introduction of Succinic Biostimulator simultaneously with the vaccination of calves contributed to the production of virus-neutralizing antibodies to the main components of Kombovak vaccine in them (Shvets, 2011).

Succinic acid fed in the dose of 5 mg/kg first 10 days of each month starting from the age of two months and until slaughter caused positive effect on the histology of the liver of red fox (Kokorina et al., 2014).

Materials and methods

The protocol of the experimental part of the study at the stages of maintenance of animals and their withdrawal from the experiment corresponded to the principles of biological ethics, was agreed upon with the Local Ethics Committee of the Dnipro State Agrarian and Economic University (Dnipro, Ukraine). In the experiment, we used young white non-breed mice, 36 males aged 3 weeks with average body weight of 12 ± 2 g. The animals were kept in the vivarium of the Dnipro State Agrarian and Economic University. The maintenance conditions were standard. The room temperature equaled 20–22 °C. The animals were fed twice a day – in the morning and evening, had free access to food and water. To perform the experiment, three experimental (7 animals in each) and three control (5 animals in each) groups were formed (Table 1). The diet of all the mice had excessive content of fat due to adding lard (energy density of 5.24 kcal/g, fats of 60%kcal; carbohydrates accounted for 20%kcal, proteins for 20%kcal). The animals of the experimental groups received 0.5% solution of succinic acid. In the period of studies, the changes in the body weight the animals, as well as the amount of the liquid and food they consumed were recorded.

On the 15th, 30th and 45th days, the animals were withdrawn from the experiment using ketamine and xylazine intraperitoneally, putting

animals in the condition of deep sleep in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasburg, 1986; Kyiv, 2001) and the requirements of the Law of Ukraine №3447-IV from 21.02.2006 "On the protection of animals against cruel treatment". The blood from the heart was drawn for the following biochemical and morphological assays. Biochemical studies of blood included: determining the total protein using the biuret method, globulins and the protein coefficient were calculated and the albumins – according to the reaction with bromocresol green. To determine the activity of aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) we used the kinetic technique based on the Warburg optical test, alkaline phosphatase – enzymic technique with n-nitrophenyl phosphate and glucose – glucose oxidase methods. We determined the total bilirubin and the uric acid – enzymatically with uricase on Miura 200 automatic biochemical analyzer (Italy) and High Technology sets of reagents (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). The quantity of erythrocytes and leukocytes in the stabilized blood of mice was determined in Mindray BC-2800Vet automatic hematological analyzer. For the leukogram, we prepared blood smears according to Pappenheim with subsequent Romanovsky-Giemsa staining. After the autopsy, we visually evaluated the condition of the internal organs (heart, liver, lungs, thymus, spleen, stomach, small and large (colon, cecum, rectum) intestines, kidneys, testes, and the brain) for the presence of pathological changes, weighed on the scales with accuracy of up to 1 mg with the subsequent determination of the index of the weight of the organs. Also, we determined the length and body weight, roundness of the stomach.

To determine the condition of microbiota of the intestines of the experimental animals, we selected feces from the rectum, after euthanasia, observing the rules of asepsis. After dilutions in the sterile normal saline, the selected samples were examined using the technique of bacterial inoculation to special growth media. The inoculations were cultivated at the temperature of 26 and 37 °C over 24–72 h. The results were obtained by counting the grown colonies in the colony-forming units per gram of feces (CFU/g). Identification and differentiation of the isolated microorganisms were performed according to the morphological features, tinctorial, cultural, and enzymic properties (Bilan et al., 2019). Bacterioscopy of the stained smears using the generally accepted methods was used to visually confirm the studied microorganisms.

The data were analyzed in Statistica 8.0 (StatSoft Inc., USA) software. The data are presented in the tables as x ± SD (x ± standard deviation). Differences between the values in the control and experimental groups were determined using the Tukey test, where the differences were considered reliable at $P < 0.05$ (with taking into account Bonferroni correction).

Results

Relative mass of the organs throughout the experiment changed in the heart, liver, stomach, small intestine, kidney, and brain (Table 1). During the 45 days of the experiment, the constant weight remained in the lungs, spleen, large intestine and its sections (caecum, upper colon, rectum) and the testes. Succinic acid (Table 1) at the level of tendency (statistically unreliably) contributed to the increase in the relative weight of the liver at the late stages of the experiment: on the 30th day the liver was 9.9% greater than in the control group, on the 45th day – 12.6% greater. During all the stages of the experiment, we saw unreliable decrease in the relative weight of the small intestine compared with the control group (by 4.1%, 7.4% and 17.7% less compared with the control respectively on the 15th, 30th, 45th days).

During the experiment, both the surveyed groups were seen to have a tendency towards the decrease in relative weight of the kidneys, though succinic acid showed no reliable effect on the relative weight of this organ. At all the stages of the experiment, we noted a statistically unreliable increase in the relative weight of the brain of the animals that consumed succinic acid: 5.4%, 12.2% and 8.5% respectively on the 15th, 30th and 45th days. Similar changes at the level of tendency, but more manifested, were seen also for the thymus: by 56.2%, 58.8% and 36.6% correspondingly on the 15th, 30th and 45th days (Table 1). Opposite changes in the weight were characteristic for the heart (also statistically insignificant): relative mass of the heart in the groups with succinic acid in the diet decreased by 2.4%, 4.3% and 2.0% in the same stages of the experiment (Table 1).

Table 1

Change in relative mass of the organs (%), sizes and weight of body of male mice under the impact of adding succinic acid to their diet (x ± SD)

Variant of the experiment	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	F ($F_{0.05}$ = 2.53)	P
Duration of the experiment, days	15	15	30	30	45	45		
n	5	7	5	7	5	7		
Heart, % of body mass	0.714 ± 0.070^{a}	0.697 ± 0.135^{ab}	0.564 ± 0.063^{ab}	0.540 ± 0.103^{ab}	0.503 ± 0.058^{b}	0.493 ± 0.053^{b}	7.03	$1.9 \cdot 10^{-4}$
Lungs, % of body mass	1.155 ± 0.097^{a}	0.980 ± 0.181^{a}	0.954 ± 0.327^{a}	0.945 ± 0.168^{a}	0.995 ± 0.166^{a}	0.991 ± 0.154^{a}	0.86	0.518
Liver, % of body mass	7.41 ± 1.14^{a}	7.10 ± 0.79^{a}	5.56 ± 0.39^{b}	6.11 ± 0.35^{ab}	5.14 ± 0.29^{b}	5.79 ± 0.51^{ab}	10.81	$5.2 \cdot 10^{-6}$
Spleen, % of body mass	0.509 ± 0.192^{a}	0.573 ± 0.206^{a}	0.594 ± 0.236^{a}	0.496 ± 0.090^{a}	0.451 ± 0.218^{a}	0.536 ± 0.139^{a}	0.46	0.803
Stomach, % of body mass	1.316 ± 0.161^{a}	1.459 ± 0.295^{a}	0.765 ± 0.104^{b}	0.958 ± 0.279^{ab}	0.828 ± 0.101^{ab}	0.791 ± 0.114^{b}	12.81	$1.0 \cdot 10^{-6}$
Small intestine, % of body mass	12.01 ± 1.72^{a}	11.52 ± 1.25^{a}	7.44 ± 0.35^{b}	6.89 ± 0.88^{b}	8.77 ± 2.56^{ab}	7.22 ± 1.05^{b}	15.72	$1.3 \cdot 10^{-7}$
Large intestine, % of body mass	2.77 ± 0.67^{a}	4.17 ± 1.72^{a}	2.38 ± 0.29^{a}	2.63 ± 0.61^{a}	2.62 ± 0.28^{a}	2.34 ± 0.35^{a}	6.82	$2.3 \cdot 10^{-4}$
– caecum, % of body mass	0.843 ± 0.119^{a}	1.146 ± 0.485^{a}	0.696 ± 0.100^{a}	0.714 ± 0.138^{a}	0.936 ± 0.168^{a}	0.734 ± 0.144^{a}	3.20	0.020
– upper colon, % of body mass	1.149 ± 0.364^{a}	2.067 ± 0.658^{a}	1.143 ± 0.053^{a}	1.568 ± 0.790^{a}	1.006 ± 0.202^{a}	0.706 ± 0.183^{a}	6.43	$3.6 \cdot 10^{-4}$
– rectum, % of body mass	0.703 ± 0.098^{a}	1.090 ± 0.508^{a}	0.554 ± 0.197^{a}	0.497 ± 0.242^{a}	0.850 ± 0.075^{a}	0.784 ± 0.144^{a}	4.06	$6.2 \cdot 10^{-3}$
Right kidney, % of body mass	0.863 ± 0.134^{a}	0.706 ± 0.102^{a}	0.635 ± 0.063^{a}	0.612 ± 0.115^{a}	0.519 ± 0.199^{ab}	0.559 ± 0.086^{ab}	5.57	$9.6 \cdot 10^{-4}$
Left kidney, % of body mass	0.804 ± 0.131^{a}	0.671 ± 0.077^{a}	0.599 ± 0.062^{a}	0.665 ± 0.145^{a}	0.540 ± 0.053^{ab}	0.634 ± 0.354^{a}	1.18	0.344
Right testis, % of body mass	0.422 ± 0.027^{a}	0.341 ± 0.140^{a}	0.435 ± 0.048^{a}	0.459 ± 0.092^{a}	0.454 ± 0.078^{a}	0.412 ± 0.088^{a}	1.46	0.230
Left testis, % of body mass	0.506 ± 0.057^{a}	0.378 ± 0.119^{a}	0.410 ± 0.040^{a}	0.459 ± 0.077^{a}	0.418 ± 0.063^{a}	0.412 ± 0.090^{a}	1.71	0.161
Thymus, % of body mass	0.089 ± 0.042^{a}	0.139 ± 0.058^{a}	0.119 ± 0.050^{a}	0.189 ± 0.073^{a}	0.191 ± 0.089^{a}	0.261 ± 0.139^{a}	3.19	0.020
Brain, % of body mass	2.40 ± 0.32^{a}	2.53 ± 0.24^{a}	2.13 ± 0.13^{a}	2.39 ± 0.67^{a}	1.88 ± 0.14^{ab}	2.04 ± 0.40^{a}	2.45	0.057
Initial body mass, g	12.40 ± 1.14^{a}	11.00 ± 0.82^{a}	11.80 ± 0.84^{a}	11.57 ± 0.79^{a}	11.80 ± 0.84^{a}	11.71 ± 0.49^{a}	1.82	0.139
Final body mass, g	13.79 ± 1.27^{a}	12.41 ± 1.07^{a}	17.11 ± 1.35^{ab}	14.70 ± 2.91^{a}	16.69 ± 0.45^{ab}	17.29 ± 3.45^{ab}	5.27	$1.4 \cdot 10^{-3}$
Body mass change, g	1.39 ± 0.29^{a}	1.41 ± 0.67^{a}	5.31 ± 1.32^{b}	3.12 ± 3.18^{ab}	4.89 ± 0.98^{b}	5.58 ± 3.08^{ab}	5.01	$1.9 \cdot 10^{-3}$
Body length, cm	6.88 ± 0.41^{a}	7.16 ± 0.66^{a}	7.62 ± 0.15^{a}	7.23 ± 0.24^{a}	7.46 ± 0.11^{a}	7.46 ± 0.46^{a}	2.22	0.078
Abdominal circumference, cm	6.50 ± 0.59^{a}	6.96 ± 0.55^{a}	6.94 ± 0.27^{a}	6.60 ± 0.35^{a}	7.06 ± 0.23^{a}	6.37 ± 0.50^{a}	2.45	0.056

Note: different letters indicate the values significantly differing one from another within a line of the Table 1 on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction.

With same initial weight in six groups of animals (Table 1), the body weight at the end of the experiment and the change in this mass in the groups which consumed this food additive and which did not significantly did not differ. Also, we have seen no reliable changes in the body length in the control and the experimental groups (Table 1). The groups of animals which consumed succinic acid in the diet were observed to have insignificant change in the abdominal circumference: increase by 7.1% on the 15th day and decrease by 4.9% and 9.8% on the 30th and 45th days.

More notable changes under the influence of succinic acid in the diet occurred at the biochemical and cytological levels. The concentration of

the total protein in the blood of animals reliably decreased by the 15th and 30th days (by 15.4% and 22.4%), and increased by the 45th day of the experiment (by 30.0%, Table 2). The concentration of albumins in the blood of animals reliably did not change. The content of globulins decreased by the 15th and 30th days (by 24.1% and 25.2%) and increased by the 45th day of the experiment (by 42.1% compared with the control groups). Protein coefficient reliably did not change during the experiment (Table 2). The concentration of the urea was also unreliably higher (by 17.4%, 16.9% and 29.5% on the 15th, 30th and 45th days) in the groups with succinic acid in the diet compared with the control groups of mice (Table 2). Increase at the level of tendency occurred in the urea nitrogen content in the blood (by 14.9%, 4.3% and 17.9% respectively).

By the 15th day the concentration of creatinine in the group with addition of succinic acid to the diet had increased by 21.7%, on the 30th – by 32.8%, and by the 45th day of the experiment it had decreased by 21.8% compared with the control groups of animals (Table 2).

The activities of the hepatic enzymes (aspartate aminotransferase and alanine aminotransferase) in different periods of the study changed in different orientations: at the beginning of the experiment the activity of both enzymes in the blood reliably increased (by 69.3% and 51.8% respectively), at the end of the experiment, the differences between the group which received succinic acid and the one which did not receive this food additive became unreliable (Table 2). De Ritis Ratio showed similar changes.

Table 2

Change in cytological and biochemical parameters of blood of male mice under the impact of addition of succinic acid to their diet (x ± SD)

Variant of the experiment	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	F ($F_{0.05}$ = 2.53)	P
Duration of the experiment, days	15	15	30	30	45	45		
n	5	7	5	7	5	7		
Total protein, g/L	40.2 ± 6.4^{ab}	34.0 ± 4.3^{a}	41.6 ± 5.2^{ab}	32.3 ± 5.2^{a}	40.0 ± 6.3^{ab}	52.0 ± 8.9^{b}	8.65	$3.6 \cdot 10^{-5}$.
Albumins, g/L	17.0 ± 2.5^{a}	16.6 ± 2.0^{a}	20.0 ± 2.7^{a}	16.3 ± 3.3^{a}	18.4 ± 1.7^{a}	21.3 ± 3.9^{a}	3.26	0.018
Globulins, g/L	23.2 ± 6.5^{ab}	17.6 ± 3.8^{a}	21.4 ± 6.6^{ab}	16.0 ± 5.0^{a}	21.6 ± 5.0^{ab}	30.7 ± 8.1^{b}	5.15	$1.6 \cdot 10^{-3}$
Protein coefficient, units	0.800 ± 0.255^{a}	0.986 ± 0.234^{a}	1.060 ± 0.251^{a}	1.157 ± 0.458^{a}	0.900 ± 0.200^{a}	0.771 ± 0.256^{a}	1.49	0.223
Urea, mmol/L	3.86 ± 0.82^{ab}	4.53 ± 0.95^{ab}	3.08 ± 0.22^{a}	3.60 ± 1.12^{ab}	3.96 ± 0.46^{ab}	5.13 ± 1.31^{b}	3.57	0.012
Urea nitrogen, mg%	7.32 ± 1.51^{ab}	8.41 ± 1.46^{b}	5.78 ± 0.43^{a}	6.03 ± 1.54^{ab}	7.54 ± 0.88^{ab}	8.89 ± 1.98^{b}	4.64	$2.9 \cdot 10^{-3}$
Creatinine, µmol/L	37.8 ± 5.1^{a}	46.0 ± 2.9^{ab}	32.6 ± 4.4^{a}	43.3 ± 3.0^{ab}	60.0 ± 16.2^{b}	47.0 ± 13.2^{ab}	5.57	$9.5 \cdot 10^{-4}$
Aspartate aminotransferase (AST), U/L	179 ± 16^{a}	303 ± 56^{b}	341 ± 39^{b}	291 ± 73^{ab}	159 ± 50^{a}	219 ± 31^{ab}	11.73	$2.4 \cdot 10^{-6}$
Alanine aminotransferase (ALT), U/L	55.8 ± 12.9^{a}	84.7 ± 44.1^{a}	72.6 ± 11.9^{a}	73.3 ± 23.5^{a}	93.0 ± 42.9^{a}	66.6 ± 18.1^{a}	1.09	0.384
De Ritis Ratio, U	3.36 ± 0.75^{ab}	4.11 ± 1.22^{ab}	4.72 ± 0.41^{b}	4.13 ± 0.76^{ab}	2.20 ± 1.25^{a}	3.71 ± 1.59^{ab}	3.17	0.021
Alkaline phosphatase, U/L	26.4 ± 5.8^{a}	30.2 ± 2.8^{a}	28.2 ± 2.4^{a}	25.8 ± 2.9^{a}	24.9 ± 4.5^{a}	23.2 ± 6.8^{a}	1.96	0.114
Total bilirubin, µmol/L	12.4 ± 2.3^{ab}	14.6 ± 3.1^{b}	12.2 ± 4.2^{ab}	9.7 ± 1.2^{a}	14.0 ± 2.9^{b}	14.1 ± 5.1^{ab}	1.95	0.116
Glucose, mmol/L	6.92 ± 0.87^{a}	5.81 ± 0.99^{a}	6.06 ± 1.15^{a}	6.99 ± 1.96^{a}	4.46 ± 2.02^{ab}	2.20 ± 0.40^{b}	11.94	$2.1 \cdot 10^{-6}$
Inorganic phosphorus, mmol/L	2.46 ± 0.39^{a}	2.13 ± 0.23^{a}	2.62 ± 0.18^{ab}	2.30 ± 0.21^{a}	3.28 ± 0.32^{b}	3.26 ± 0.96^{b}	6.26	$4.4 \cdot 10^{-4}$
Cholesterol, mmol/L	2.28 ± 0.42^{a}	1.51 ± 0.19^{b}	1.92 ± 0.26^{ab}	1.99 ± 0.34^{ab}	1.60 ± 0.19^{ab}	1.73 ± 0.31^{ab}	5.15	$1.6 \cdot 10^{-3}$
Hemoglobin, g/L	92.0 ± 7.8^{ab}	91.3 ± 12.7^{ab}	101.2 ± 5.8^{ab}	86.1 ± 10.5^{a}	103.6 ± 4.1^{ab}	118.6 ± 11.9^{b}	9.45	$1.7 \cdot 10^{-5}$
Hematocrit, %	28.5 ± 1.9^{a}	29.7 ± 5.1^{a}	33.5 ± 2.9^{a}	27.9 ± 4.2^{a}	32.7 ± 1.4^{a}	41.4 ± 2.5^{b}	14.32	$3.4 \cdot 10^{-7}$
Erythrocytes, 10^{12}/L	6.34 ± 0.67^{a}	5.68 ± 0.62^{a}	6.35 ± 0.57^{a}	5.43 ± 0.83^{a}	6.29 ± 0.22^{a}	7.61 ± 0.65^{b}	9.81	$1.2 \cdot 10^{-5}$
Leukocytes, 10^{9}/L	6.36 ± 3.23^{ab}	7.89 ± 2.08^{ab}	8.68 ± 4.01^{ab}	6.73 ± 0.57^{ab}	5.00 ± 0.94^{a}	8.69 ± 2.70^{b}	1.94	0.117
Thrombocytes, 10^{9}/L	257 ± 62^{a}	228 ± 76^{a}	142 ± 51^{a}	164 ± 56^{a}	196 ± 28^{a}	199 ± 74^{a}	2.47	0.055
Basophils, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–	–
Eosinophils, %	0.80 ± 0.45^{a}	1.00 ± 0.00^{a}	1.00 ± 0.71^{a}	0.57 ± 1.51^{a}	2.20 ± 2.17^{a}	1.14 ± 0.38^{a}	1.42	0.245
Myelocytes, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–	–
Neutrophils, %	22.0 ± 1.9^{ab}	19.3 ± 7.1^{a}	26.8 ± 7.7^{ab}	30.9 ± 12.3^{ab}	32.0 ± 6.8^{ab}	32.3 ± 4.1^{b}	3.32	0.017
– neutrophils segmented, %	14.6 ± 2.1^{ab}	11.9 ± 6.3^{a}	21.8 ± 7.7^{ab}	21.9 ± 9.2^{ab}	25.6 ± 7.1^{b}	23.0 ± 5.8^{b}	3.70	$9.9 \cdot 10^{-3}$
– neutrophils banded, %	7.40 ± 3.51^{a}	7.43 ± 1.72^{a}	5.00 ± 2.12^{a}	9.00 ± 4.97^{a}	6.60 ± 6.11^{a}	9.00 ± 7.62^{a}	0.55	0.739
– neutrophils juvenile, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	–	–
Lymphocytes, %	82.6 ± 3.2^{a}	77.6 ± 7.3^{a}	70.8 ± 11.5^{ab}	62.6 ± 13.6^{ab}	60.8 ± 4.5^{b}	64.1 ± 4.3^{b}	6.09	$5.2 \cdot 10^{-4}$
Monocytes, %	0.80 ± 0.45^{a}	1.86 ± 0.69^{ab}	2.80 ± 2.17^{ab}	6.00 ± 2.08^{b}	5.00 ± 6.36^{ab}	2.14 ± 1.07^{ab}	3.34	0.016

Note: see Table 1.

At the end of the experiment, the concentration of glucose in the blood of animals reliably reduced below the norm after 45 days of consuming succinic acid in the diet (Table 2). The concentration of non-organic phosphorus in the blood changed insignificantly.

At the beginning of the experiment (on the 15th day) the addition of succinic acid contributed to decrease in the cholesterol concentration in the blood of animals (by 33.8%), and by the 30th and 45th days it had unreliably increased (by 3.6% and 8.1%) compared with the control groups of animals. Concentration of hemoglobin and hematocrit showed a reliable increase by the 45th day of the experiment (by 14.5% and 26.6% respectively). Cellular composition of blood showed no reliable differences between the control and experimental groups (Table 2).

During the study on microbiota of the intestine in mice kept 45 days on a high-fat diet (Table 3), we observed decrease in the number of representatives of the genera *Bifidobacterium*, *Lactobacillus* and typical *Escherichia coli* (lac+). Decrease in the quantity of microorganisms of *Bifidobacterium* genus was seen only on the 45th day of the studies, but in the animals to whose diet succinic acid was added, the number of the representatives of this genus had the tendency towards decrease only from the 30th day. By the end of the experiment, the difference in the quantity of bacteria of this genus between the control and experimental groups reached more than 10 times. Reliable decrease of *Lactobacillus* spp. was seen from the 30th day of the research in both the control and experimental groups, but in the diet with addition of succinic acid, the number of these microorganisms decreased more significantly.

In mice of the experimental group, we observed a slight increase in the quantity of lactose-positive *E. coli* on the 30th day of the study and its decrease by the 45th day, similarly to the control group. Among other representatives of the intestinal microbiota, no reliable differences were seen.

Discussion

The high-fat diet on which the experimental animals were maintained did not lead to significant and excessive increase in the body weight by the 15th, 30th and even 45th days of the experiment. Addition of succinic acid to such diet also had no effect on the intensity of weight gain of animals compared to the control, as well as the normal parameters for this species and age of animal. Increase in the body weight of animals was seen during excess of carbohydrates (fructose) in their diet, while high-fat diet had no effect on the weight gain in the animals (Ozkan & Yakan, 2019; Brygadyrenko et al., 2019). An important indicator of the influence of different substances on the organisms of human and animals, especially against the background of the metabolic processes, is the absolute and relative mass of the organs. Therefore, in our study, the relative weight of the organs changed throughout the experiment. Excessive content of fat in the diet led

to decrease in this parameter in the kidneys, thymus, spleen, and the testes. At the same time, the relative weight of the heart, liver and the brain after 15 days of intake of high-fat diet was higher than the norm for their age category (Abrashova et al., 2013).

Table 3

The number of microorganisms (lg KOE/g of feces) in group of mice on the high-fat diet with addition of succinic acid (x ± SD, n = 7, the 45 days duration of the experiment)

Variant of the experiment	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	Without succinic acid	With succinic acid	Norm*
Duration of the experiment, days	15	15	30	30	45	45	
n	5	7	5	7	5	7	
Bifidobacterium spp.	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	10.0 ± 0.0^{a}	9.0 ± 1.1^{ab}	9.3 ± 0.9^{ab}	7.8 ± 1.2^{b}	8–10
Lactobacillus spp.	12.2 ± 0.8^{a}	12.2 ± 0.8^{a}	10.6 ± 1.4^{ab}	9.2 ± 2.1^{ab}	8.0 ± 1.6^{b}	7.2 ± 2.6^{b}	5–11
Escherichia coli (lac +)	7.3 ± 0.5^{ab}	7.0 ± 3.5^{ab}	7.7 ± 0.5^{a}	7.4 ± 1.4^{ab}	5.3 ± 1.2^{b}	5.1 ± 2.6^{ab}	7–8
E. coli (lac-)	2.1 ± 0.1^{a}	2.2 ± 0.3^{a}	2.5 ± 0.7^{a}	2.2 ± 1.3^{a}	1.3 ± 0.9^{a}	1.8 ± 1.6^{a}	2
Enterococcus spp.	7.3 ± 0.5^{a}	4.5 ± 2.9^{a}	9.1 ± 0.2^{b}	6.4 ± 3.2^{a}	5.4 ± 3.9^{a}	4.2 ± 3.5^{a}	7–8
Proteus spp.	2.2 ± 0.2^{a}	2.6 ± 0.8^{a}	1.7 ± 1.3^{a}	1.8 ± 0.9^{a}	1.5 ± 1.1^{a}	2.7 ± 1.5^{a}	2
Enterobacter spp.	2.4 ± 0.2^{a}	2.9 ± 0.2^{a}	1.8 ± 1.3^{a}	1.9 ± 1.5^{a}	2.1 ± 1.5^{a}	1.2 ± 1.9^{a}	2
Citrobacter spp.	1.9 ± 1.3^{a}	1.9 ± 1.7^{a}	3.0 ± 0.7^{a}	2.7 ± 0.4^{a}	2.4 ± 1.7^{a}	2.5 ± 1.9^{a}	2
Clostridium spp.	0.0 ± 0.0^{a}	0.5 ± 1.2^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.5 ± 1.2^{a}	4
Staphylococcus spp.	3.4 ± 0.3^{a}	2.3 ± 1.8^{a}	3.8 ± 0.4^{a}	3.5 ± 0.7^{a}	3.5 ± 0.3^{a}	3.8 ± 0.2^{a}	2–3
Candida spp.	2.3 ± 0.5^{a}	2.8 ± 0.4^{a}	3.6 ± 0.1^{a}	2.8 ± 1.4^{a}	3.5 ± 0.2^{a}	3.0 ± 0.6^{a}	2

Notes: for each genus and species of microorganism (in one line) the selections significantly not differing between each other are indicated in same letters ($P < 0.05$) according to the results of Tukey test taking into account Bonferroni correction; the norm – according to Makarova et al. (2016).

In the conditions of high-fat diet, the relative weight of the liver gradually decreased by the 30th and especially 45th days, and in the group with the addition of succinic acid, this parameter was nonnreliably higher and practically reached the norm for this age category of the animals. Also, the excess of fat in the diet contributed to the impairment of the protein-synthetic function of the liver. The total amount of protein in the blood following the excess of fat in the diet was lower than the reference values at all stages of the experiment. Addition of succinic acid exacerbated this process by the 15th and 30th days, but caused rapid increase and the normalization of the total protein in the blood by the 45th day. This was chiefly due to the globulin fraction, where these processes were similar. High content of fat in the diet contributed to insignificant increase of the level of albumins in blood, and succinic acid somewhat slowed this process at the beginning of the experiment and stimulated them at the end, driving this parameter closer to the control group and the reference values by the 45th day (Abrashova et al., 2013).

The standard markers of the normal function of the liver were also the parameters of the activity of blood enzymes: AST, ALT, alkaline phosphatase (Nazarenko & Kishkun, 2000; Yu et al., 2012). High-fat diet led to increase in the activity of AST in blood, especially against the background of succinic acid, by the 15th day (practically twice), significant heightening of it by the 30th day, and decrease, less notable during consumption of succinic acid, by the 45th day of the experiment. Also, high activity of ALT was mostly observed in the middle of the experiment (15th day): in the conditions of excessive fat in the diet it gradually increased. Addition of succinic acid caused increase in the activity of the enzyme at the beginning of the experiment (15th day) and gradually caused its decrease by the end of the experiment; but all the same, this indicator remained higher than the normal parameters for healthy animals. As with the alkaline phosphatase, its activity in all the groups of animals was significantly lower than the parameters of the norm (Abrashova et al., 2013). Liver dysfunction also suggests significant decrease in the level of urea and increase in the level of bilirubin against the background of fat excess in the diet. At the same time, in the groups of animals which additionally received succinic acid, the decrease in the level of urea was much less manifested compared with the control.

The level of the glucose in blood of mice on a high-fat diet was significantly reduced, and the addition of succinic acid led to aggravation of this process, especially by the beginning (by the 15th day) and at the end of the experiment (by the 45th day). In an experiment inducing experimental diabetes, the insulinotropic effect of succinic acid due to increase in the activity of succinate dehydrogenase and improvement of metabolism in the insular of the pancreas were confirmed (Malaisse & Sener, 1993; Malaisse et al., 1993; Ladriere et al., 1999; Ladriere & Malaisse, 2000a, 2000b; Cancelas et al., 2001). Both high-fat diet and the use of succinic acid led to disorders in the functioning of the excretory system. In the process of the experiment, the relative weight of the kidneys was below the norm and such biochemical parameters as creatinine and urea in the blood, with which the function of the kidneys is monitored, significantly differed from the values of the healthy animals (Jia et al., 2014). The level of creatinine in animals which consumed succinic acid increased by the 15th and 30th days of the experiment, but by the end of the experiment (on the 45th day) rapidly decreased compared with the control. The level of urea throughout the experiment was below the norm in all the groups, but addition of succinic acid to the diet alleviated this deviation from the norm: the concentration of urea in the blood was higher in the groups with succinic acid compared with the animals that received only the high-fat diet.

Both high content of fat and addition of succinic acid to the diet provoked changes in the organs of the system of blood circulation and the organs of immune protection. At the organ level, we observed reduced indices of the thymus mass during the first stage of the experiment (until the 30th day) and spleen until the end of the experiment (on the 45th day) against the background of excess of fat in the diet. Succinic acid contributed to the normalization of the relative mass of thymus, but only by the end of the experiment (by the 45th day). The relative weight of spleen was not affected by succinic acid, remaining below the normative values for this particular age group of animals. Througout the period of the study, we observed low content of blood hemoglobin in the conditions of reduced number of erythrocytes. At the same time, by the middle of the experiment, succinic acid worsened these processes compared to the control, but, by contrast, relieved them by the end of the experiment.

Addition of succinic acid in the dose of 5 mg/kg of body weight to the feed reduces the time of the formation of the immunity, and durations of its humoral and cellular phases increase. Immune-stimulating properties were seen in chitosan succinate during daily subcutaneous injection in the dose of 2.6 mg/kg of live weight 3 days before vaccination of cows against leptospirosis.

The level of globulins in the experimental groups was reduced and only by the end of the experiment did it increase, reaching the reference values at addition of succinic acid to the diet. Total number of leukocytes in all the groups of animals significantly did no change, indicating absence of inflammatory process in the organism. Succinic acid did not significantly affect this parameter at the beginning of the experiment, but led to significant increase in the quantity of blood leukocytes in the blood of mice at the end of the experiment compared with the control group of animals. The total number of lymphocytes did not change either in the animals receiving a high-fat diet, or succinic acid.

Intestinal microbiota is composed of hundreds of bacteria, fungi and protists and is essential for numerous biological processes, such as digestion of nutrients, production of vitamins and resistance to colonization by bacterial pathogens (Ferreira et al., 2011). Around 10^{14} microorganisms live in the lower part of the human intestine, and many of these microorganisms have developed mutualistic and commensal associations with the

host, actively participate in many of its physiological processes. Nonetheless, dysbiosis (altered microbial composition of the intestine) with other predisposing genetic factors and the environmental factors may contribute to the metabolic disorders in the host, leading to a number of diseases (Haraken et al., 2016). Gut microbiota play an important role in the improvement of digestion, especially of indigestible substances, and also energy conversion (Dethlefsen et al., 2006, 2007; Turnbaugh et al., 2006; Round & Mazmanian, 2009; Foster & McVey Neufeld, 2013). The diversity and composition of intestinal microbiota is affected by the diet of host, life style and the factors of the environment (Maslowski & Mackay, 2011; Graf et al., 2015).

Currently, the role of the gut microbiota in the development of obesity and disorders related to it, such as metabolic synfrom, has been determined (Backhed et al., 2004; Cani et al., 2008; De La Serre et al., 2010; Mosso et al., 2010; Cani, 2013; Neves et al., 2013; Chistiakov et al., 2015; Kostina et al., 2015; Murphy et al., 2015; Kravchuk et al., 2016; Sun et al., 2018). Development of obesity is a complex process which includes genetic susceptibility and environmental factors which remain only partly understood. In such cases, intestinal microbiota is becoming more and more recognized as an important factor which connects genes, environment and the immune system (Musso et al., 2010). Over the past three decades, obesity has emerged as an endemic disease which became broadly distributed in the developed countries, rapidly turning into a serious problem in some developing countries (Bouchard, 2000; Haraken et al., 2016).

The results of the studies conducted on people and laboratory animals indicate decrease in the number of microorganisms of Bacteroidetes type and increase in the number of the representatives of Firmicutes (due to microorganisms of *Lactobacillus* genus) in patients with obesity, compared with thin patients in the control and patients with anorexia (Armougom et al., 2009). The data of Koliada et al. (2017) demonstrate that adults suffering from obesity had a higher level of Firmicutes and lower level of Bacteroidetes compared with the people with normal weight and thin people. Santacruz et al. (2010) report that pregnant patients with obesity (24th week) were determined to have reduced number of *Bifidobacterium* and Bacteroidetes, but increase in the number of identified representatives of Firmicutes phylotype (for example, *Staphylococcus* spp.) and Proteobacteria (for example, family Enterobacteriaceae, such as *Escherichia coli*) compared with pregnant patients with normal weight.

Cox & Blaser (2013), in the studies on mice without microbes, while injecting gut microbiota of pregnant women in their third semester, observed increase in the weight and the development of resistance to insulin, by contrast with the experiment on mice injected with microbiota of pregnant patients in their first trimester.

Bäckhed et al. (2007) determined that unlike mice with intestinal microbiota, the animals without microbes are protected against obesity which develops after consuming food with high content of fats and rich in sugar.

During the studies on cecum microbiota of mice with obesity, Ley et al. (2005) determined a 50% decrease in the number of Bacteroidetes and respectively a higher amount of Firmicutes. At the same time, the number of separate representatives of these bacterial phylotypes inside the groups of animals did not differ significantly. Similar results, Ley et al. (2006) were obtained during the study of obese people compared to thin people. They determined that the relative share of microorganisms of Bacteroidetes phylotype decreased in obese people and that this proportion increased with losing weight on two types of low-calorie diet (limitation of fat and carbohydrates).

Zhang et al. (2009) determined that Firmicutes phylotype dominated in people suffering from obesity, but quantitatively decreased in three patients who underwent Roux-en-Y gastric bypass surgery, which led to changes in food consumption (without limitation of the dietary components), digestion and proportional increase in the class Gammaproteobacteria.

Microorganisms of *Lactobacillus* and *Clostridium* genera are attributed to insulin-resistance, and *Lactobacillus* positively correlates with the levels of glucose and HbA1c on an empty stomach, whereas *Clostridium* had negative correlation with these parameters (Karlsson et al., 2013). Therefore, the search for methods for solving the issues related to the regulation of changes in the proportions and localization of bacterial flora of the intestine is being conducted all around the globe, as well as development of various strategies which would contribute to the domination of physiologically positive microorganisms. Therefore, Cani et al. (2007) found a positive effect of prebiotic of oligofructose in mice, while applying a high-fat diet, on restoration of the amount of *Bifidobacterium* bacteria, which are able to reduce the level of endotoxin, improve the barrier function of the mucous membrane of the intestine and prevent the development of diabetes.

Liu et al. (2019) determined that adding 1% aqueous extracts of green tea, oolong and black tea to the high-fat diet of C57BL/6J mice for 28 weeks to the same extent improved the tolerance to glucose and decreased consumption of fats, and thus weight gain, increase in hepatic lipids and weight of the white fat tissue. This was accompanied by significant decrease in the level of polysaccharides in the plasma and significant stimulation of the production of short-chain fatty acids. Tea extracts changed the total composition of the intestinal microbiota and decreased the relative quantity of Rikenellaceae and Desulfovibrionaceae families. Furthermore, the tea aqueous extracts also changed the quantity of the essential operational taxonomic units (OUT), including OTU473 (Alistipes), OTU229 (Rikenella), OTU179 (Ruminiclostridium) and OTU264 (Acetatifactor), and at the same time the ratio of Firmicutes/Bacteroidetes did not change.

In turn, Chen et al. (2018), during similar studies but using Kudingcha (KDC) from *Ilex kudingcha* Fuzhuan brick-tea (FBT), detected a weakening of the metabolic syndrome in mice and improvement of the diversity of intestinal microbiota. KDC decreased the relative number of Erysipelotrichacea, while FBT decreased the ratio of Firmicutes/Bacteroidetes and increased the relative number of Bifidobacteriaceae. Gong et al. (2020) determined the ability of theabrownins of Liupao tea, during the diet with high content of fat, to improve the structure and quantity of intestinal flora of *Bacteroides*. Seo et al. (2015) determined that enzymic extract of green tea changed the composition of intestinal microbiota (for example, ratio of Firmicutes/Bacteroidetes and Bacteroides/Prevotella), and this was related to the decrease in the fat mass, reduction of inflammation and weakening of non-resistance to glucose. Anhe et al. (2014) report that polyphenol-rich extract of cranberry protects mice against diet-caused obesity and metabolic disorders, which is attributed to the proportional increase in the number of *Akkermansia* spp. In the *in vitro* study on microbial ecosystem of the intestine, Kemperman et al. (2013) surveyed the effect of mixture of polyphenols which the tea contained and the ones in the extract of red wine (RWGE). The research revealed that in the context of model system these complex polyphenols can modulate separate representatives of gut microbiota of humans. The most noticeable changes were the inhibition of the representative of Firmicutes type and increase in the quantity of representatives of proteobacteria. Black tea stimulated the reproduction of *Klebsiella*, *Enterococcus* and *Akkermansia* and decreased the synthesis of *Bifidobacterium*, *B. coccoides*, *Anaeroglobus* and *Victivallis*. Red wine extract contributed to growth of *Klebsiella*, *Alistipes*, *Cloacibacillus*, *Victivallis* and *Akkermansia*, while the amounts of *Bifidobacterium*, *B. coccoides*, *Anaeroglobus*, *Subdoligranulum* and *Bacteroides* were decreased.

Succinic acid and its derivatives have a broad potential for application to feeds for animals. On the basis of the described studies on the development of compositions and methods of use of succinic acid in fodders, we determined improvement in the conversion of the feed and modulation of intestinal microbiota. In the alternative variants, it was determined that succinic acid improves the digestion of fodders and supports the animals' health, contributing to correct digestion and the supporting the immune system (Broz et al., 2008). In the *in vitro* experiment, succinic acid exerted higher activity towards the pathogenic and conditionally-pathogenic bacteria (commensal *Escherichia coli*, pathogenic *Escherichia coli* K88, *Salmonella enterica* subsp. *enterica*, serotype *enteritidis* and *typhimurium*, *Enterococcus faecalis* and *Clostridium perfringens*) isolated from the intestinal contents of piglets than towards the useful bacteria (*Lactobacillus acidophilus* and *Lactobacillus fermentum*). Minimum inhibiting concentration needed to inhibit growth of 90% of the harmful microbiota accounted for 31,250 mM compared to 125,000 mM – for the useful one.

Conclusions

Succinic acid has no effect on the intensity of body weight gain in young mice against the background of excessive content of fat in the diet.

Excessive fat feeding leads to malfunctioning of the parenchymal organs, which is accompanied by decrease in the relative weight of the liver, kidneys, testes and the intestine and increase in the relative mass of the heart, lungs and brain. High-fat diet of mice is accompanied by mostly disorders in the liver, followed by acute hypoproteinemia, chiefly due to globulins, rapid increase in the activity of the liver enzymes against the background of fall in the activity of alkaline phosphatese, increase in the level of bilirubin, decrease in glucose. Also, the excess of fat in the diet leads to impairment in the function of the excretory system, manifested in decreased index of weight of the kidneys, high level of creatinine and reduced level of urea in the blood. Addition of succinic acid has a positive effect on the functional condition of the liver and kidneys, which is especially manifested during its long term intake. Against the backround of high-fat diet, succinic acid impairs the functioning of the organs of blood circulation and immune protection, accompanied by decrease in the relative mass of the thymus and spleen, low content of hemoglobin and number of erythrocytes, but does not significantly affect the content of other cellular elements of blood. At the same time, by the middle of the experiment, succinic acid exacerbated these processes compared with the control, and by the end of the experiment, by contrast, molified them.

Adding succinic acid to high-fat diet contributed to the change in the quantitative composition of the main representatives of the obligate microbiota (*Bifidobacterium* spp., *Lactobacillus* spp. and typical *Escherichia coli*) in the laboratory animals. Such changes in gut microbiota may lead to reproduction of the representatives of facultative microbiota and, therefore, the development of various diseases.

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