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THE INFLUENCE OF COOKED SAUSAGE WITH INULIN ON THE PHYSIOLOGICAL INDICATORS OF LABORATORY ANIMALS

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ABSTRACT

Reducing fat content in meat products requires a complex of studies on the quality of sausage with fat substitutes and their impact on health. The article presents the study results concerning the effect of cooked sausage with inulin on the clinical and physiological parameters of laboratory animals. The experiment was performed for 26 days on 27 Wistar rats. The animals in the experimental group (Group 1) consumed cooked sausage with inulin. The control group (Group 2) was fed with cooked sausage with fat content of 20.5 ± 2.1%. Animals in the intact group (Group 3) consumed a standard diet of vivarium consisting of pearl barley porridge and compound feed. Animals in Group 1 had a minimum weight gain for 26 days of 8.6% compared to Group 2 (10.5%) and intact animals (13.0%). Group 1 rats showed a more pronounced rise in glucose levels with increase in total bilirubin and urea, decrease in creatinine, and increase in aspartate aminotransferase and alanine aminotransferase levels as compared to blood serum of animals in Group 2. Based on a decrease in total protein with increased total bilirubin, urea and glucose in Group 1 animals, the assumption was made of accelerating the catabolism of proteins and carbohydrates by the introduction of cooked sausage with reduced caloric content into the diet. It was established that the introduction of experimental meat products into the diet allows to reduce the total cholesterol, triglycerides and low-density lipoproteins in the blood and increase the level of high-density lipoproteins and lipase.

1. Introduction

In developed countries, the problem of high fat consumption is very topical. The population is concerned about the increase in mortality from cardiovascular and oncological diseases, and growing obesity. One of the measures aimed at solving this problem is to increase the production of low-calorie products. Many countries develop national programs to improve the health of the population, including measures to reduce the fat content in food. Fat has a wide range of technological properties that allow to create consumer characteristics of meat product, including texture, color, flavor, and taste. The fat content may be reduced either by replacing it by meat with a high content of muscle and connective tissue or by using non-meat ingredients with a structure different from fat (proteins, polysaccharides, and their mixtures). It should be noted that increasing the proportion of lean meat in meat product formulation may lead to unsatisfactory consumer characteristics, especially hard texture. In this regard, the selection of non-

meat ingredients that can simulate fat in the product is of particular interest. Development of fat substitutes is being carried out in many countries, including Russia. For this purpose, a diverse range of food ingredients and additives is used, i.e. vegetable and animal proteins (Sousa *et al*., 2017, Campagnol*et al*., 2013; Lee *et al*., 2016) and polysaccharides: starches, gums, fiber, etc. (De Oliveira Faria*et al*., 2015; Han M. *et al*., 2017; Alves *et al*., 2016; Henning *et al*., 2016). It should be noted that in the manufacture of meat products, protein preparations are traditionally used to replace lean meat, rather than fat. This is due to the fact that replacing the fat with vegetable or animal proteins may lead to deterioration of consumer characteristics, in view of the fact that the resulting protein gels do not able to simulate fat functions in the product. For this purpose, carbohydrates are more successfully used, among which the inulin is of special interest. A large number of works in different countries are aimed at the development of low-calorie meat products with inulin, the use of which allows to significantly reduce the fat content in meat products (Furlan*et al*., 2014, Keenan *et al*., 2014, Shoaib *et al*., 2016, Silva-Vazquez*et al*., 2018). This natural polysaccharide in a hydrated form (inulin: water ratio 1: (1 to 2)) has properties that allow to simulate fat in the product resulting in delicate soft texture, white color, and absence of foreign odor and flavor. Preliminary studies have revealed the possibility of using inulin in a hydrated form to replace the fat in meat products in the amounts up to 50% of its content in the formulation, which helps to reduce fat content by more than 40% (Semenova*et al*., 2012). Being a dietary fiber, inulin has a beneficial effect on the function of gastrointestinal tract, significantly increases the absorption of minerals, decreases cholesterol level in blood, and markedly improves the metabolism of carbohydrates and lipids (Kumar *et al*., 2016; Han K.H. *et al*., 2017). However, taking into account that meat products containing inulin are multicomponent products, studies aimed at physiological evaluation of sausage with inulin as a fat substitute in comparison with traditional sausage are needed. In this connection, the aim of the work was to study the effect of cooked sausage with inulin on the clinical and physiological parameters of laboratory rats.

2. Materialsandmethods

2.1.Materials

2.1.1. Samples

For the experiment, cooked sausage was produced. The control sample of the cooked sausage contained 50 kg of lean pork, 28 kg of high-grade beef, 22 kg of fatback, water (25 l over the formulation), salting ingredients (sodium chloride, sodium nitrite), spices, food phosphates, and sodium ascorbate.

In the formulation of experimental products, 16 kg of fatback was replaced by 10 kg of inulin gel with inulin: water ratio of 1: 1.5, 2 kg of milk protein and 4 kg of hydrated egg protein. Cooked sausage was produced according to traditional technology (Kapovsky*et al*., 2017) with cooking at a core temperature of 72 ± 2 °C.

2.1.2.Management and stunning of animals

As a laboratory model, aged rats of the Wistar line at the age of 11 weekswith a body weight of 340-370 g were used. Animals were obtained from the Andreevka branch of the Scientific Center for Biomedical Technologies of the Russian Federal Medical and Biological Agency and passed quarantine for 14 days. Rats were randomly divideto 3 groups (n = 27): Group 1 was fed with the standard vivarium died (SVD) with addition of experimental cooked sausage; Group 2 consumed control samples

of cooked sausage; Group 3 was intact, and rats consumed SVD throughout the experiment. The standard diet of the vivarium consisted of cooked pearl barley and standard chow(Laboratorkorm, Russia). Ground samples of cooked sausage were introduced into the cooked barley. Diets of animals in all groups were balanced and contained equal amount of protein (10% of calories).

Animals were kept under similar conditions, i.e. temperature (20 ± 3 °C), humidity (48 ± 2%), illumination (light day 6.00 a.m. to 6.00 p.m.), in polysulfone cages (Tecniplast, Italy) with free access to water and feed. On every 4th day, animals were weighed on Ohaus electronic technical scales (AdventurerPro, USA), weight change was analyzed, and diet was calculated. Weighing and feeding of animals were performed daily at a strictly defined time (1.00 p.m. to 2.00 p.m.).

The experiment was conducted for 26 days. By the end of the experiment, the animals were stunned in the euthanasia chamber (VetTech, UK). From the right ventricle of the heart of stunned animals, clinical and biochemical analyses were carried out.

2.2.Methods

2.2.1.Methods for studying the physical and chemical properties of cooked sausage

The mass fraction of protein in the sausage was determined as a result of mineralization of the Kjeldahl sample and a photometric measurement of color intensity of indophenol blue, which is proportional to the amount of ammonia in the mineralized sample. Fat content was determined by the method based on the extraction of total fat with hexane or petroleum ether with a boiling point of 50 to 60 °C in the Soxhlet extraction apparatus. The mass fraction of carbohydrates was determined by the calculation method, subtracting the values of moisture, fat, protein and ash mass fractions from the 100 g of the product.

Determination of the meat product color characteristics in the CIELab system was carried out using a spectrocolorimeter (Spectroton, Russia) while simultaneously measuring reflection coefficients of samples at 24 fixed wavelengths in increments of 13 nm in the visible spectral range from 380 to 720 nm, followed by mathematical processing of the measurement results by microprocessor controller integrated in the measuring unit.

To determine color stability during storage, the color stability criterion (U, %) was used (Semenova*et al*., 2007).

The shear force was determined using Instron-3342 universal testing machine, USA, with subsequent recording and export of measurement results to an Excel file.

The pH value was registered by a potentiometric method using Zamer-1 portable pH-meter (Russia).

The water activity was determined by the cryoscopy method using AWK-20 instrument (Germany).

2.2.2.Methods for studying the physiological parameters of laboratory rats

2.2.2.1.Clinical and biochemical blood analyses

Clinical analysis of blood samples was performed on Abacus Junior Vet 2.7 automatic veterinary hematological analyzer (DiatronMesstechnik GmbH, Austria) using Diatron reagent kits. Blood serum studies were performed on BioChemSA biochemical analyzer (HTI, USA) using reagent kits (HTI, USA).

2.2.2.2 Postmortem examination

Postmortem examination was carried out by visual inspection of internal organs. The absolute weight of the liver, kidneys, spleen, and heart was determined by weighing on electronic scale with an accuracy of

± 0.001 g (AcculabVicon, USA), and the relative weight of organs (spleen, kidney, liver, heart) was calculated (Dzhimak*et al*., 2015).

2.3. Statistical analysis

Statistical processing of the data was carried out by the analysis of variance using Microsoft Excel 2010 and STATISTIKA software packages; the difference of the compared indicators was considered significant with a probability of difference more than 95% ($p < 0.05$).

3.Results anddiscussions

3.1. Physical and chemical properties of sausage

The replacement of fatback by inulin gel had no significant effect on pH, water activity and color indices (Table 1). The data obtained were consistent with the results of Furlan*et al.* (2014), who found that using of inulin makes it possible to simulate fat in minced semi-finished products while reducing fat content by 20-35% without changing the appearance and color of the minced meat and sensory properties of the finished product.

According to the results of studies, an increase of color stability in experimental sausage was found to be 4.1%, which is obviously explained by a decrease in ultraviolet-induced oxidation processes due to the introduction of inulin instead of fatback. The use of inulin contributed to an increase in shear force of experimental cooked sausage by 17.1% ($p < 0.05$) compared to control. The data obtained are consistent with the results of Derek *et al*. (2012), who found that with an increase in the proportion of fat replacement with inulin, the density of sausage increases, which may be due to the formation of interacting microcrystal groups forming a gel network.Studies of the chemical composition showed that the introduction of inulin reduced the fat content by 47.3% ($p < 0.05$), while the caloric content decreased by 28.8% ($p < 0.05$) compared to the control (Table 2).

Table 1. The results of physical and chemical studies of cooked sausage

Parameters	Sample	
	Control	**Experimental**
pH	6.24±0.04	6.28 ± 0.03^{ns}
Shear force, Pa	21.7±1.2	25.4 ± 0.5^{s}
Water activity, units	0.9742±0.006	0.9744 ± 0.0003^{ns}
Lightness, units	69.7±2.3	67.6 ± 3.1^{ns}
Redness, units	9.4±0.6	9.7 ± 0.2^{ns}
Yellowness, units	14.0±0.7	14.6 ± 0.3^{ns}
Color stability, %	71.3	75.4^{ns}

^{ns}not significant $p \geq 0.05$; ^{s} significant at $p < 0.05$.

Table 2. Chemical composition of cooked sausage

Sample	Mass fraction in the product, %			Caloric content, kcal
	proteins	**fat**	**carbohydrates**	
Control	12.3±0.5	20.5±2.1	-	233.7
Experimental	13.7 ± 0.8^{ns}	10.8 ± 0.9^{s}	3.6 ± 0.4^{s}	166.4^{s}

^{ns}not significant $p \geq 0.05$; ^{s} significant at $p < 0.05$.

3.2.Clinical and physiological parameters of laboratory rats

3.2.1.Changes in weight of laboratory rats

Observations of rats in Groups 1 and 2, in which the experimental sausage was added to the diet, as well as for the intact.

Group 3 animals consuming the standard diet, did not reveal any abnormalities in the physiological state. The sausages in the diet was completely eaten. Weight dynamics of the animals showed that the rats in Groups 1 and 2 gained weight more rapidly in the first day of the experiment (Figure 1). Group 1 animals intensively gained weight on the first 4 days (on average, up to 7 g/day), and in the period from 4th to 26th day, weight stabilization was noted, when the rats gained on average not more than 4 g/day. Group 2 rats also gained weight quite intensively during the first 4 days. Further observation showed an increase in animal weight on average of 5 g/day. Intact animals in Group 3 consistently gained weight throughout the experiment on average of 4-6 g/day. Weight gain by the end of the experiment was as follows: animals in Group 1 consuming experimental products - 13.1 ± 0.6%, rats in Group 2 consuming control samples – 15.0 ± 2.8%, intact animals in Group 3 – 15.4 ± 2.3%.

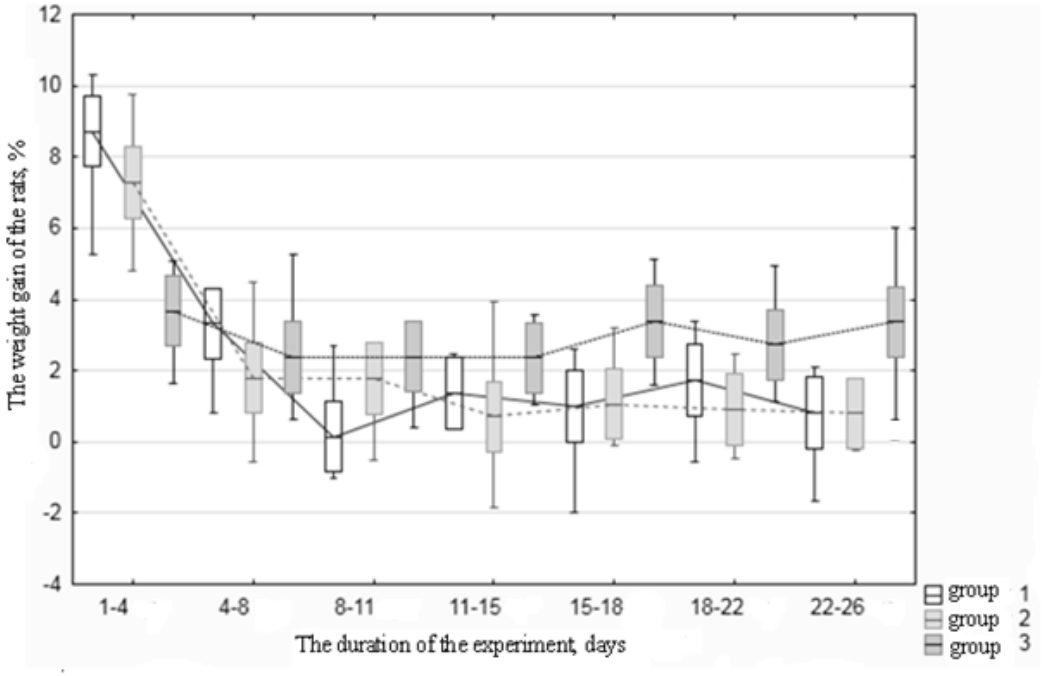

Figure 1. Body weightdynamics of experimental rats during the experiment

3.2.2 Clinical blood analysis

The results of clinical blood analysis showed some changes in the hematological indices of experimental rats compared to intact animals.

Redistribution of white blood cells (WBC) was most pronounced in animals fed with experimental and control samples of cooked sausages, which was characterized by a significant decrease in lymphocyte content and an increase in mixture of monocyte, eosinophil, basophil and immature cells by up to 40% (Figure 2). Group 1 rats also showed an increase in granulocytes by 35%.

With respect to hematological parameters characterizing the functional activity of red blood cells of Group 1 rats, a statistically significant decrease in the volume of erythrocytes compensated by an increase in hemoglobin was revealed with a red blood cell content (erythrocytes and hematocrit) not significantly different from intact rat values (Table 3). Other parameters characterizing the functional activity of platelets of the rats in Groups 1 and 2 were not significantly different from intact animals.

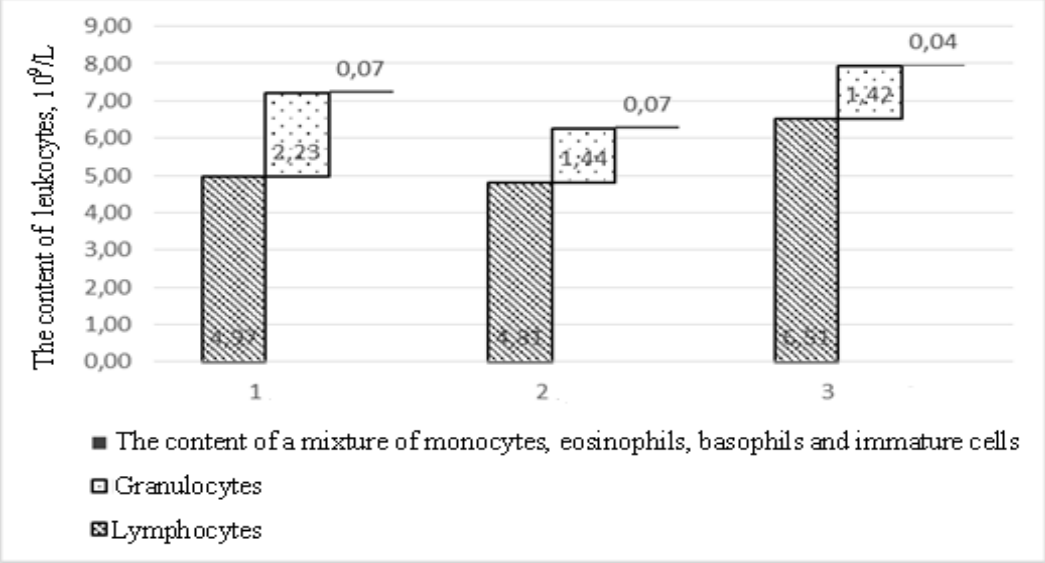

Figure 2. Differential blood count of experimental rats

Table 3. Morphological indicators of animal blood characterizing the functional state of erythrocytes

Parameters	Group of animals		
	1	2	3
RBC, 10^{12}/L	8.19±0.18^{a}	7.98±0.24^{a}	8.12±0.10^{a}
Hemoglobin, g/L	147.22±2.23^{ab}	144.05±3.74^{b}	150.75±1.26^{a}
Hematocrit, %	41.25±0.80^{a}	42.64±1.14^{a}	44.31±0.56^{a}
Mean corpuscular volume, µm^3	49.44±1.32^{b}	53.67±1.21^{a}	54.51±0.58^{a}
Mean corpuscular hemoglobin concentration, g/L	353.13±3.14^{a}	334.01±4.20^{b}	337.80±2.05^{b}
Red cell distribution width, %	16.34±0.43^{a}	15.84±0.32^{a}	15.44±0.34^{a}

Means in the same column with different superscript letters are significantly different ($p<0.05$).

3.2.3. Biochemical blood analysis

The results obtained in the biochemical analysis of serum from experimental animals are presented in Table 4. Evaluation of protein metabolism indices revealed non-significant increase in total protein content in Group 1 animals consuming experimental products, mainly due to the albumin fraction, with a statistically significant increase of urea level. In Group 2 rats consuming control samples, a slight but significant decrease in total protein content was observed by up to 10%, as compared to the intact Group 3. An increase in glucose

level by more than 20% in serum of 33% of experimental rats in Group 1 was observed, but the averaged index was not statistically different from the values of comparison groups. With regard to bilirubin, one of the key indicators of pigment metabolism, a significant increase in the concentration of the total fraction of this compound in the blood of the rats in Groups 1 and 2 was observed by 38.3% and 27.2%, but these values did not exceed the physiological normal rates for rats of this age.

Compared to intact rats in Group 3, study of a number of serum enzymes in experimental rats revealed in Group 1 a significant increase in the activity of cytoplasmic enzyme ALT (by up to 40%), with a slight non-significant change in the activity of AST (by more than 10%), which has mitochondrial cytoplasmic localization. In group 1, the De Ritis coefficient reflecting the ratio of AST/ALT activities was 3.28 ± 0.77, which is more than 1.5 times lower than the value of this coefficient in the group of intact rats.

The noted tendency to increase the total protein content, including albumin, glucose, and total bilirubin with the increase in the activity of intracellular enzymes of aminotransferase group may indicate an enhancement in the functional activity of the liver and, as a result, acceleration of protein and carbohydrate catabolism in animals, in which diet experimental meat products were added.

When analyzing the biochemical parameters characterizing the lipid metabolism of experimental animals, a statistically significant decrease in the total cholesterol content (Figure 3) and triglycerides (Figure 4) by up to 25% was found, with non-significant decrease in high-density lipoproteins (HDL) (Figure 5) and low density lipoproteins (LDL) (Figure 6) (by not more than 10%) compared to the values of intact animals. In Group 1 consuming experimental sausage, there was a slight decrease in cholesterol, triglycerides and LDL level ($p > 0.05$). It should be noted that a significant increase in lipase content in the serum of animals in Group 1 detectable at 68.72 ± 3.42 U/L was observed versus 36.27 ± 3.04 U/L and 55.13 ± 5.74 U/L in control rats of Group 2 and intact rats of Group 3, respectively. The data obtained are consistent with the results of Kumar *et al.* (2016), who established that when consuming soluble fiber, there is an increase in the metabolism of carbohydrates and lipids.

Table 4. General biochemical indicators of animal blood

Parameters	**Normal**	**Group of animals**		
		1	**2**	**3**
Total protein, g/L	50-80	76.40±1.46^{a}	70.96±0.70^{b}	75.76±2.07^{a}
Albumin, g/L	30-50	42.22±1.64^{a}	39.58±1.06^{a}	42.69±1.72^{a}
Creatinine, µmol/L	9-70	57.84±1.17^{a}	58.34±1.71^{a}	59.25±0.64^{a}
Urea, mmol/L	4.3-8.6	7.20±0.59^{a}	5.47±1.06^{b}	5.42±0.63^{b}
Glucose, mmol/L	7.7-12.2	11.42±2.98^{a}	8.87±1.72^{a}	9.02±2.35^{a}
Bilirubin (total), µmol/L	0-8.5	3.13±0.25^{a}	2.65±0.53^{ab}	1.93±0.49^{b}
Aspartate aminotransferase (AST), U/L	20-180	158.42±14.65^{a}	151.12±11.75^{a}	178.71±26.79^{a}
Alanine aminotransferase(ALT), U/L	10-80	46.73±6.75^{a}	27.63±8.63^{b}	28.68±6.43^{b}
Activity Ratio (AST/ALT)	-	3.28±0.77^{a}	5.04±0.66^{ab}	5.36±0.89^{b}

Means in the same column with different superscript letters are significantly different ($p<0.05$).

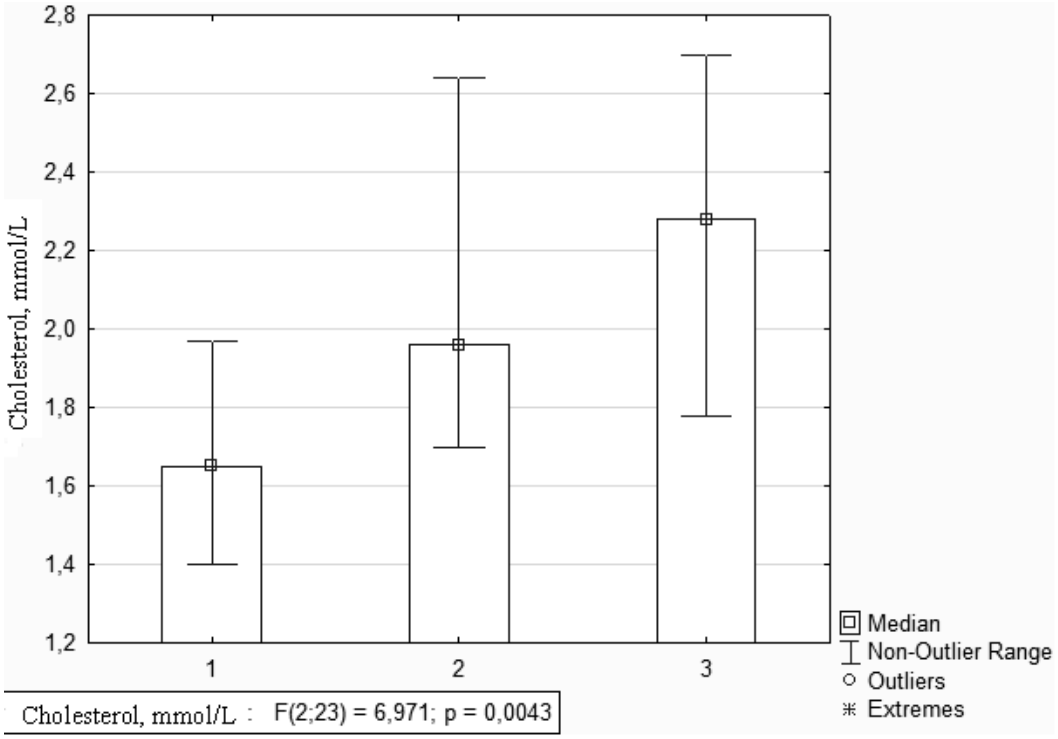

Figure 3. Cholesterol content in the animal blood

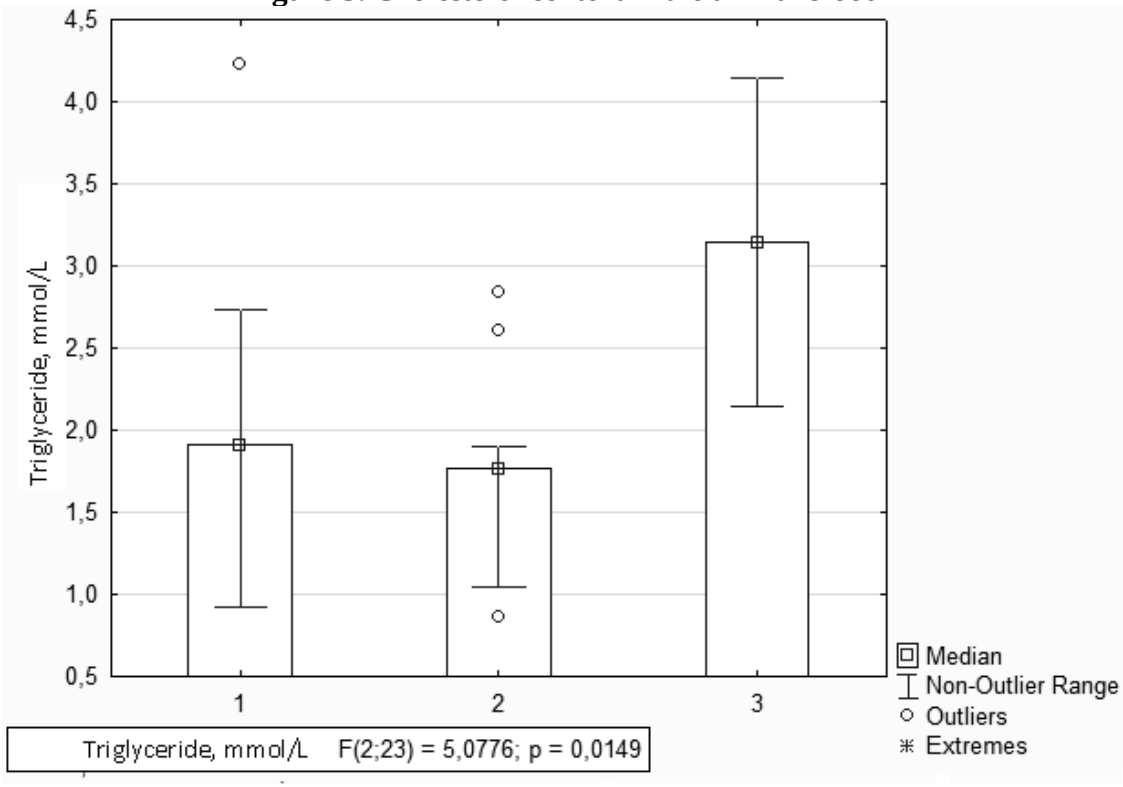

Figure 4. Triglyceride content in the animal blood

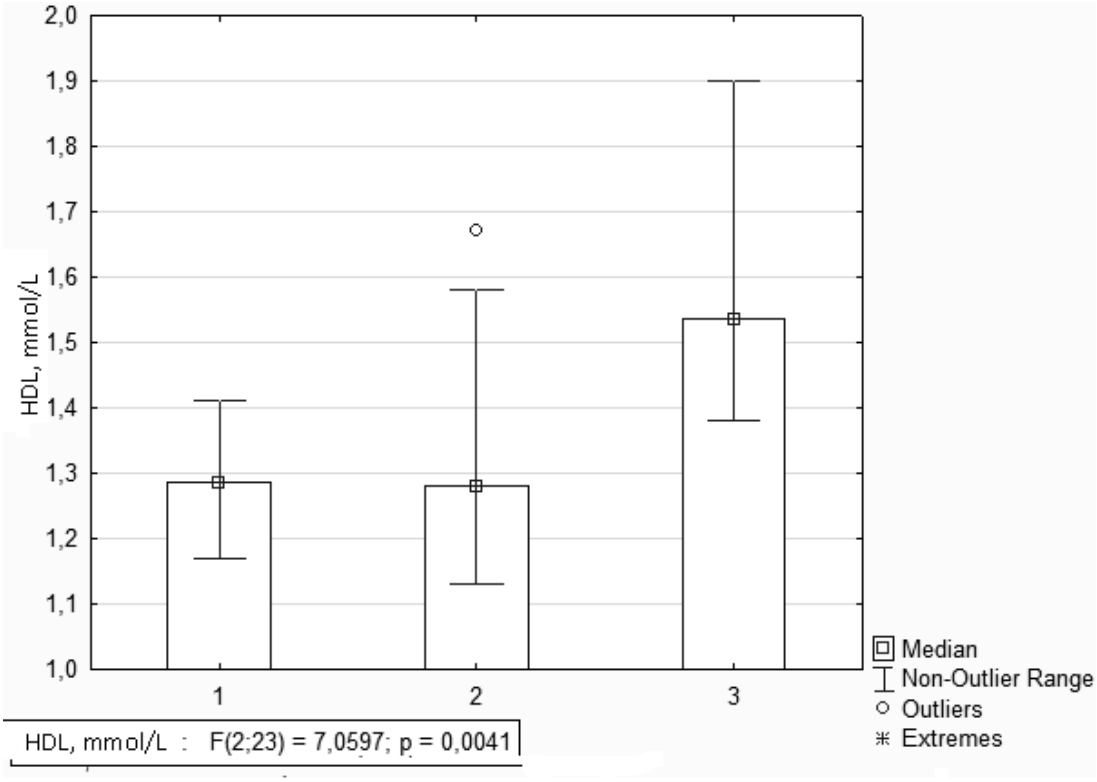

Figure 5.HDL content in the animal blood

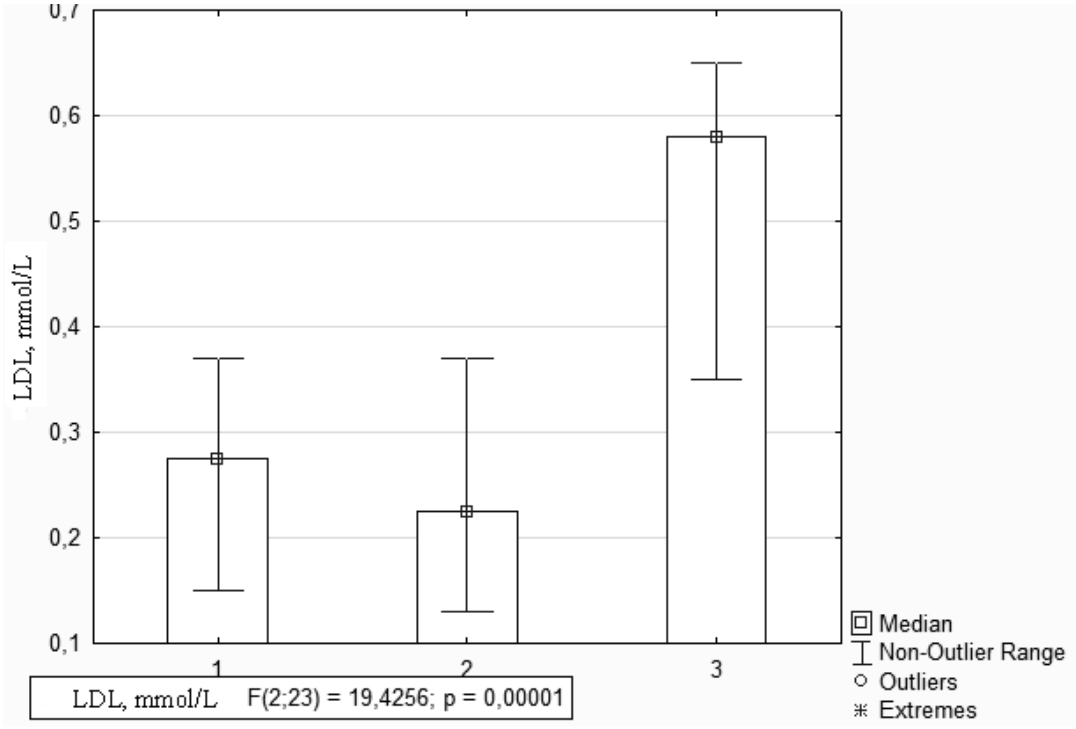

Figure 6.LDL content in the animal blood

3.2.4 Postmortem examination

Postmortem examination of animals did not reveal any significant abnormalities in the state of the internal organs (digestive tract, respiratory system, circulatory and hematopoiesis organs, urinary system, and internal secretion organs); there were no significant changes in the relative weight of the organs analyzed.

4. Conclusions

Thus, during this work, it has been established that the use of inulin gel instead of fatback allowedto produce cooked sausage with a reduced fat content without impairing sensory properties.

As a result of the biological experiment, it can be stated that 26-day introduction of low-calorie meat products with inulin into the diet of aged laboratory rats promotes a decrease in the average weight gain of animals from 7 g to 4 g per day, while the body weight of animals at the end of the experiment exceeded the initial mass of experimental rats by 13%, while in control and in intact groups this indicator was 15%.

Changes in hematological and biochemical blood indices of experimental animals were identified that were characterized by redistribution of white blood cells: a significant decrease in lymphocyte count by more than 20% with an increase in monocyte, eosinophil, basophil, immature cell and granulocyte count by up to 40%. The increase in total protein, albumin, glucose, total bilirubin content and aminotransferase activity indicates an enhancement in the functional activity of the liver and, as a result, acceleration of protein and carbohydrate catabolism, as well as intensification of active proliferation of lymphoid tissue cells.

The study of key lipid metabolism indices, i.e. total cholesterol, triglycerides, high-density lipoproteins (HDL), and low density lipoproteins (LDL), revealed a significant decrease in these parameters in rats consuming cooked sausage with inulin: total cholesterol and triglycerides by up to 25%, HDL and LDL by 10 %. Lipase increase in serum of animals in the experimental group by more than 20% was also revealed.

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