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EVALUATION OF THE IMMUNOMODULATING POTENTIAL OF FERMENTED RAW MEAT

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Abstract

Food allergy, which manifests itself as an oversensitivity of the body's immune system to certain kinds of foods, affects a significant share of the world's population and represents a serious issue. The aim of this study was to evaluate the allergenic potential of beef exposed to a probiotic microbial consortium. The developed consortium, which consisted of strains: Lactobacillus plantarum 7K, Lactobacillus paracasei k-406, Pediococcus acidilactici PDA-27 and Staphylococcus carnosus SPHC-108, exhibited high proteolytic activity against beef proteins (after three days of fermentation, the degree of hydrolysis reached up 21.5 %). The allergenic potential of fermented beef and unfermented beef was studied in the experimental animals (rats) that were exposed to simulation of artificially induced systemic anaphylactic reaction to ovalbumin. It was found that, against the background of ovalbumin sensitization, the inclusion of fermented meat into the rats' diet reduced the severity of the anaphylactic reaction by 43.5 % compared to the corresponding parameters among the rats that were fed with unfermented meat. The concentration of specific antibodies (IgG) to ovalbumin in the blood serum of the experimental animals, that were fed with fermented meat, was also significantly lower ($p \leq 0.05$). Animals that received the fermented food along with the sensitization background featured greater body weight gain at the end of the experiment. The fecal counts of lactobacteria and bifidobacteria in the animals in this group fed with fermented meat along with ovalbumin sensitization and along with anaphylactic reaction development increased fourfold in comparison with to the group of rats fed with unfermented meat. Histological studies showed that introducing fermented meat into the diet along with food sensitization did not cause significant morphofunctional changes in the mesenteric lymph node structures, whereas the animals fed with unfermented meat demonstrated the signs of decompensation and immune mechanisms stress. These results demonstrate the potential of using meat fermented with probiotic microorganisms consortium as promising approach to reducing food allergies risk.

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Introduction

Food allergies are a serious issue that affects a significant share of the world's population today. Approximately 2.5 % of the general population suffers from the food allergies: however, in industrialized countries this values reaches up to 8 % among children and 10 % among adults. The World Allergy Organization (WAO) estimates that 220–250 million people suffer from food allergies. WHO experts warn that, if current trends persist, by 2030 allergic diseases of one or another type would be able affect up to 50 % of the world's population [1,2].

Food allergy is characterized by a condition of increased body sensitivity to certain kinds of food, which develops due to immune response mechanisms. Experts note the importance of the influence of a number of factors on the food allergies onset and development, among which the condition of the intestinal microbiota is of particular importance, because interaction with a variety of intestine microorganisms plays a key role in the development of immunity mechanism [3,4]. The pathogenetic process of food allergy development is caused by an imbalance of cellular reactions of the Th1/Th2 type. Under normal conditions, the penetration of a food protein containing antigens triggers the activation of T- and B-cells of the immune system, but the absence of allergy symptoms is explained by the body's formation of natural tolerance to such substances. When a food allergy develops, this defense mechanism fails, mainly due to the hyperreactivity of Th2 cells, provoking excessive production of IgE antibodies directed specifically against food components [5]. The body's immune system needs to maintain an optimal balance of Th1/Th2-cell activity. It is the intestinal microbiota that regulates this balance by suppressing excessive reactivity of Th2 cell and this way promoting an adequate Th1 response, or by stimulating regulatory cells (Treg) [6–8]. The current scientific concepts and clinical observations confirm that changes in the composition of the intestinal microbiota under the influence of various factors significantly affect the development of the immune response and thus contribute to the development of allergic conditions.

Given the extensive data on the role of the intestinal microbiota in the pathogenesis of food allergies, some researchers propose using probiotics as a promising method for preventing and healing allergic pathology, particularly in children. Studies of correlation between microbiome structure and immunological reactions related to food allergies have shown that microbiome structure abnormalities are associated with Th1/Th2 cell responses imbalance, which is accompanied by an increase in allergic symptoms [9]. These findings support the hypothesis of a strong correlation between the characteristics of the intestinal microbiome and the functioning of key immune defense elements involved in allergic diseases development.

A number of studies demonstrate the efficiency of certain types of probiotics in the therapy and prevention of allergic disorders in children of different ages [10]. Thus, integrated therapy that involves the probiotic Bifiform Kids, combined with B vitamin complexes (vitamins B1 and B6) for 21 days, reduced the severity of gastrointestinal and dermatological manifestations of food allergies in patients with mild forms of the disease. Based on these results, the authors recommend including a combination of strains *Lactobacillus rhamnosus GG* and *Bifidobacterium animalis spp. lactis BB-12* in therapeutic protocols for the children with gastrointestinal and skin symptoms of food allergy [11].

The capability of probiotic bacteria to stimulate anti-allergic processes has also been proven in experiments *in vivo* with *Lactobacillus gasseri* (LK001), *Lactobacillus salivarius* (LK002), *Lactobacillus johnsonii* (LK003), *Lactobacillus paracasei* (LK004), *Lactobacillus reuteri* (LK005) and *Bifidobacterium animals* (LK011) [12]. Research by M. Mennini et al. (2021) was conducted to evaluate the effect of *Lactobacillus strain rhamnosus GG* on the state of intestinal microbiota and the intensity of digestive disorders in children with milk protein intolerance, this research revealed an increase in the level of tolerance to this food products, including whole cow protein, when the recipient got *Lactobacillus-based rhamnosus GG* preparations [13].

In the study of Fedotova et al. [14] there is an analysis of the available experimental and clinical data on the modern methods of restoring the compositional formulation and functional activity of intestinal microflora and their potential impact on the therapy and prevention of allergic food intolerances. The authors noted that therapy with the preparations including bifidobacilli, lactobacilli and lactic acid microorganisms, used along with a basic diet and medications support, provided a positive effect on the dynamics of the disease management, contributed to the improvement of the gastrointestinal tract condition. Individual experiments demonstrated the efficiency of using the synbiotic complexes (for example, the combination of *Bifidobacterium breve M-16V, Lactobacillus rhamnosus GG* together with oligosaccharides) as a method of primary prevention of food allergy in the individuals with a high risk of developing allergic pathologies in the future [14]. However, according to the authors, there are no sufficient proofs that the inclusion of prebiotics, probiotics and synbiotics into the therapy protocols is capable of significantly increasing the efficiency of standard methods of curing food allergy as its is prescribed by the current national clinical regulations. Perhaps, the evaluation of the complex therapy results is complicated by the impossibility of determining the share of the contribution of each individual element of therapy to the overall final effect. Thus, the question of the real therapeutic value of the medications that particularly normalize the intestinal flora still remains unsolved [14]. Current international WHO recommendations for prevention of allergic diseases, based on a systematic analysis of a big array of scientific studies, declare that the available data are insufficient to recommend using prebiotics, probiotics and synbiotics for prevention and therapy of food allergies conditions [15,16]. A similar opinion is declared in the Russian clinical guidelines drawn up in accordance with the principles of proofs-based medicine [17].

Current practice shows that the recommended elimination approach, which is the only effective one, to therapy and prevention of the food allergies does not provide the necessary comfort and does not improve the quality of life for the patients. People who suffer from food allergies encounter constant anxiety and stress caused by the fear of accidental contact with the allergen, which anxiety leads to limitations in social interactions: difficulties visiting public places, restaurants, participating in family celebrations, and school events, which becomes a source of additional anxiety and thus requires special precaution measures in the patient's behavior [18]. This consideration underlines the need to develop new approaches and technologies to reduce the allergenic capacity of the food products.

One of the promising methods for reducing allergen capacity, actively used by the specialists, is enzymatic hydrolysis, which ensures the destruction of conformational and linear epitopes, ensures rupture of peptide chains, formation of short-chain peptides and free amino acids, as well as the modification of the structural features of allergens, including a decrease in their ability to bind to IgE-antibodies [19,20]. The obtained short peptides and individual amino acids lose the ability to initiate immune system responses through their interaction with antigen presenting T-cells. According to various sources, the least molecular weight of peptides, at the threshold of their reaching the allergen capacity of the product, decreases down, it ranges within the values of approximately 3.5–10 kDa [21–23]. Enzymes of animal, plant and microbial origin are widely used for enzymatic hydrolysis purposes: pepsin, trypsin, papain, alcalase, etc. [24]. However, the disadvantage of this method is, firstly, the possibility of new allergenic epitopes formation due to protein structure changes, and secondly, enzymatic processing may not affect or partially affect the target epitopes which are capable of binding to IgE

antibodies. Along with enzyme processing, a new promising and innovative direction has emerged — using the enzymatic activity of microorganisms which can reduce the allergenic potential of raw materials. One example of that is using probiotics, such as lactic acid bacteria, for the fermentation of soy milk, for example. The extract of the resulting fermented product reduced the manifestation of allergic symptoms, including hay fever caused by Japanese cedar pollen [25,26]. It is worth noting the emergence of data that non-living microorganisms and bacterial metabolites of their functioning are biologically active as postbiotic materials [27]. In particular, it was reported that introduction of soy milk extract obtained by fermentation with several strains of lactic acid bacteria caused changes in the intestinal microflora, improved the intestinal condition, etc. [28].

Using of probiotic microorganisms for fermented products production is widespread. It is well known that the allergenic potential of food varies depending on the methods of its production. The activity of probiotic bacteria results in the formation of secondary metabolic products, including short-chain fatty acids like butyrate, propionate, acetate, and others, which participate in the regulation of the human immune response and provide protection against the allergic reactions development [29]. In particular, it has been shown that butyrate stimulates the production of IL-10 and IFN-γ and reduces the rate of DNA methylation in these cytokines genes, as well as it promotes the differentiation of B-cells and increases the production of secretory IgA in the intestinal mucosa [30–32]. The main mechanism of the immune regulatory action of short-chain fatty acids, according to the researchers' data, is the increase of T-regs production and activity, which leads to the suppression of allergic inflammation [32–34]. Based on the above, it follows that postbiotics, which represent promising and potentially useful components in the therapy of allergic conditions, also provide a significant impact on the regulation and restoration of immune responses controlled by Th1/Th2 cells, peculiar for probiotic microorganisms. Furthermore, the introduction of postbiotics as functionally active ingredients in food products opens up new opportunities for expanding the range of fortified food products.

Beef and its derivatives are an important source of complete protein, essential amino acids, heme iron, and B group vitamins, all essential for a balanced diet. However, meat proteins are serum proteins: bovine serum albumin (BSA, Bos d 6) and immunoglobulins (Bos d 7), muscle proteins: actin, myosin, and tropomyosin, as well as the carbohydrate component (α - Gal) are able to cause allergic reactions. It has been noted that beef allergy or intolerance rarely occurs in isolation, and due to the similarity of proteins, cross-reactions with cow's milk, meat of other mammals, gelatin, etc. are also possible [35–38].

The purpose of our research is to evaluate the allergenic potential of beef exposed to a consortium of probiotic microorganisms effect.

Objects and methods

Methods for determining physical and chemical properties and process parameters

For the study the samples of spinal muscle tissue (*Longissimus dorsi)* were taken from beef cuts obtained from 18-month-old cattle raised at Ulekchin Agricultural Production Cooperative (Ulekchin village, Zakamensky area, Republic of Buryatia), chilled for 18–20 hours at 2–4 °C. The meat was pre-trimmed to remove fat and connective tissue. The beef muscle tissue was then minced into 120–130 g pieces by slicing the slightly chilled meat across the fibers with a sharp knife into slices approximately 2 cm thick. The test meat samples were mixed with a microbial consortium at a rate of 1 unit of enzyme activity per 100 kg of raw material. The processed meat then was massaged using a GRZK20 vacuum massager (Hualian, China) at a speed of 9 rpm, a vacuum level of –0.08 MPa, and was held still for 1–2 hours at a temperature of 18–20 °C. The processed (fermented) raw materials were packaged in film, stored at 2–4 °C for seven days, and then tested. Unprocessed (non-fermented) meat served as a control sample.

The degree of hydrolysis (*DH*) was determined with the equation 1:

$$DH = \frac{N_a}{N_g} \times 100\%, \quad (1)$$

where N_a — is amino nitrogen content, %; N_g — is total nitrogen content, %.

The protein content (*P*) was determined using equation 2:

$$P = N_g \times 6.25 \times 10\ (\%), \quad (2)$$

N_a and N_g were determined using the Kjeldahl method (GOST 25011-2017[1]) with the help of semi-automatic device KDN-812 (Shanghai Xianjian, China).

A consortium of microorganisms was developed based on four strains: *Lactobacillus plantarum 7K, Lactobacillus paracasei k-406, Pediococcus acidilactici PDA-27 and Staphylococcus carnosus SPHC-108,* for which a priority certificate for the invention and notification of a positive result of the formal expert examination were received[2]. The bacterial strains were obtained from the National Bioresource Center "All-Russian Collection of Industrial Microorganisms" of the National Research Center "Kurchatov Institute".

The activity of the microorganisms consortium was determined by the number of viable cells based on their content in one unit of enzyme activity (1 EA), which is equivalent to 10^9 CFU/g (cm^3)[3].

[1] GOST 25011-2017 "Meat and meat products. Protein determination methods". Retrieved from https://docs.cntd.ru/document/1200146783. Accessed January 18, 2026. (In Russian)

[2] Application No. 2025129394/10(069370) for the invention "Consortium of microorganisms with high proteolytic activity for the preparation of starters and fermented products" Dated 10.30.25. (In Russian)

[3] GOST 34272-2017 "Bacterial starter cultures for the production of dairy products. General specifications". Retrieved from https://docs.cntd.ru/document/1200157895. Accessed January 18, 2026. (In Russian)

The proteolytic activity of the microorganisms' consortium was determined according to GOST 34430-2018[4].

Methods of biological research

The allergen capacity of unfermented and fermented beef was assessed in an experiment on 21 outbred white rats (males) aged 3 months, obtained from the vivarium of Buryat State Agricultural Academy, with their initial body weight of 220–240 g.

Before the experiment, the animals underwent a week-long adaptation to laboratory conditions. Throughout the experiment, they were housed in the cages 2–3 animals per one cage under the following conditions: temperature (22 ± 3 °C), humidity (50–60 %), and a 12/12 light/darkness schedule.

After adaptation to the standard vivarium diet (SVD — holistic feed mixture from Aleysk Cereal Product JSC), the rats were divided into 3 groups (7 animals in each group).

The model of systemic anaphylaxis was reproduced according to the research [39] with some modifications. On the 1st, 3rd and 5th day of the experiment, the rats of all groups were intraperitoneally sensitized with hen egg ovalbumin (OVA) in isotonic 0.15 M NaCl solution (100 μg per rat) with Freund's complete adjuvant (1:1) in a volume of 0.2 ml each. To induce a secondary immune response, on the 21st day of the experiment, the rats were injected with another 10 μg of OVA under the same conditions. Rats in the group I (control group) received only CVD for 28 days, the rats in the group II received CVD + unfermented beef (7 g), and the rats in the group III received CVD + fermented beef (7 g). At the end of feeding (on the day 29th), 0.2 ml of blood was collected from the tail vein of the animals from all three groups to analyze the antibody level, after which a resolving dose of OVA was administered intravenously — 30 mg/kg of body weight in 0.5 ml of isotonic 0.15 M NaCl solution. Over the next 24 hours, the animals were monitored for the development of symptoms of anaphylactic shock, and their condition was scored according to the scale:

+ (1) — chills, shortness of breath;
++ (2) — weakness, ataxia, cyanosis of the extremities;
+++ (3) — convulsions, paralysis;
++++ (4) — fatal outcome.

The anaphylactic shock reaction severity was assessed in score points. The anaphylactic index (AI) was calculated as the arithmetic mean of the score points for the reaction severity in the group according to [40].

The intensity of the humoral immune response was assessed by the concentration of circulating specific IgG antibodies with the help of indirect enzyme-linked immunosorbent assay on polystyrene (ELISA) [41].

The animals' condition was monitored in general daily throughout the experiment. Behavior, coat condition, food intake, and water consumption were observed and recorded. Animal weights were also determined at the beginning and at the end of the experiment (on the 29th day).

Also on the 29th day fecal samples were collected from the rats' colons into sterile vials. The intestinal contents were re-suspended in sterile isotonic sodium chloride solution at a ratio of 1:10, followed by plating of 1 ml of the sample using serial dilutions of $1\times10^{-1}\times10^{-7}$ using the submersible method on a semi-solid thioglycollate medium in order to detect content of lactobacteria and bifidobacteria[5].

All manipulations with the animals were conducted in compliance with international moral and ethical standards in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986)[6] and in accordance with the WMA Declaration of Helsinki[7], as well as Federal Law No. 498-FZ[8]. The study protocol was approved at the Ethics committee meeting.

Methods of morphological assessment

At the end of the experiment, the animals were euthanized with chloroform, and the bean-shaped cranial mesenteric lymph nodes (CMLNs) were extracted for histological examination. After washing the samples to remove excess formalin, standard alcohol processing was performed. Paraffin blocks and sections were prepared using standard histological methods. Staining was performed with Ehrlich hematoxylin and eosin, and azure eosin. The size of the mesenteric nodules was determined in 10 fields of microscope view for the animals of each group. Measurements of the specimens' structures thickness — the relative area of the *cortex* and *medulla* to the section area — and other cellular inclusions were performed using a Micromed 3 U 3 microscope and a video eyepiece of the Toup microscope Cam 5/1 at magnification A — ×100 (PL magnification 10×/0.25); B — ×400 (PL magnification 40×/0.65).

Statistical data processing

The obtained digital histological data were subjected to statistical processing with mean values (M) calculation, calculation of standard error of the mean (m) and parametric test (Student's t-test) using the Micromed Excel software. The software Statistica and Microsoft Excel were

[4] GOST 34430-2018 "Enzyme preparations for food industry. Method for the determination of proteolitic activity". Retrieved from https://docs.cntd.ru/document/1200160228. Accessed January 18, 2026 (In Russian)

[5] GOST R 56139-2014 "Functional foods and foods for special dietary uses. Methods for detection and enumeration of probiotic microorganisms" Retrieved from https://docs.cntd.ru/document/1200115455. Accessed January 18, 2026. (In Russian)

[6] ETS No. 123, Strasbourg, 1986) (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Retrieved from https://rm.coe.int/168007a67b. Accessed January 18, 2026.

[7] WMA Declaration of Helsinki — ethical principles for medical research involving human subjects Retrieved from https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ Accessed January 18, 2026.

[8] Federal Law of the Russian Federation dated December 27, 2018 No. 498FZ "On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation." Retrieved from https://docs.cntd.ru/document/552045936. Accessed May 17, 2025. (In Russian).

used to analyze biochemical parameters and biological test results. The significance of biological test parameters differences was determined using the nonparametric Mann–Whitney rank test. A probability of 0.05 was chosen as the level of significance.

Results and discussion

To produce fermented meat raw materials with reduced allergenic potential, the selection of effective probiotic cultures is critically important. Therefore, when developing multi-strain probiotic consortia (for fermentation starters and functional products with certain predetermined properties), it is necessary to use biocompatible strains with both probiotic properties and high synergistic proteolytic activity [42].

We have developed a consortium of the microorganisms that feature high biochemical and proteolytic activity, suitable for the production of fermented food products. The above-specified microorganism strains *(Lactobacillus plantarum 7K, Lactobacillus paracasei κ-406, Pediococcus acidilactici PDA-27 u Staphylococcus carnosus SPHC-108)* were cultured in a milk medium at 37 °C in the optimal quantitative ratio of 3:2:2:1, respectively. Analysis of the degree of proteolysis demonstrated a significant superiority of the microbial consortium over its monoculture (monostrain) analogues. In particular, in terms of the ability to peptonize protein, the bacterial consortium showed higher proteolysis rates (the clearing zone was equal to 27–29 mm). Monocultures of the studied strains demonstrated proteolytic activity with a clearing zone diameter in the range of 15–25 mm. The maximum level of protein hydrolysis (78 %) was registered using this consortium after 12 hours of fermentation, which fact proves a synergistic effect of the proteolytic action of the consortium compared to the relevant parameters of monocultures.

Further, the use of the selected consortium as sources of proteases for the hydrolytic cleavage of beef muscle proteins was the subject of interest. Figure 1 shows the dynamics of beef protein hydrolysis (control sample — unfermented beef; experimental sample — fermented beef).

As the data presented shows, the proteolytic process intensified during the post-slaughter period: in the control sample the degree of hydrolysis in the initial period was low, accounting to 6.23 %. During further studies, the degree of protein hydrolysis increased, reaching 8.85 % on the third day.

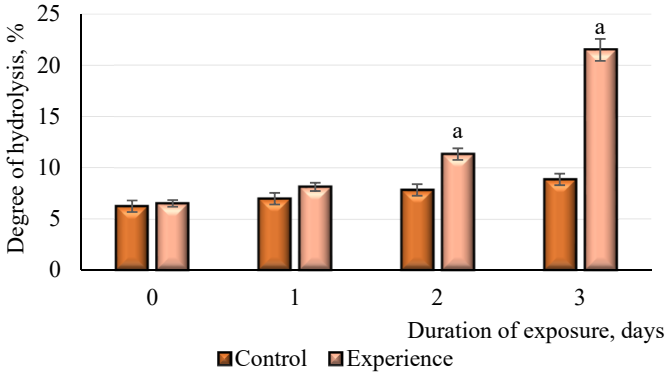

Figure 1. Dynamics of hydrolysis of beef proteins exposed to the effect of the developed consortium of microorganisms (a-p ≤ 0.01 in comparison with the control sample, according to Student's t-test)

Protein proteolysis processes were more active in the experimental sample. After two days, the degree of hydrolysis was 11.32 %. The significant increase in the analyzed parameter was noted after three days, reaching up to 21.5 %, which is 3.3 times higher than that value of the original raw material ($p \leq 0.01$).

Similar data were obtained by the group of authors Bazhenova et al. in the study [43], where a consortium of probiotic microorganisms (including *Lactobacillus paracasei)* was used for horse meat fermentation. Its notable proteolytic activity was observed compared to the monoculture effect: the degree of hydrolysis after three days increased fourfold compared to the original raw material. The authors came to the conclusion that proteolytic modification of proteins affected with the consortium led to a decrease in the antigenic epitopes content level and decreasing the sensitizing activity of the meat raw material.

Yang et al. [44] showed that co-fermentation with the combination of *Staphylococcus cohnii, S. saprophyticus* and *Lactobacillus plantarum* enhanced proteolytic processes in a meat product — Chinese bacon — and provided a positive effect on the quality of the food product.

This way the developed consortium of microorganisms exhibited its proteolytic activity against beef muscle tissue proteins, which opens up the opportunities for its using in the production of a wide assortment of food products.

As it has been noted above in the analysis of references, the studies including the most recent, demonstrate that enzymatic hydrolysis of food proteins is an effective method for reducing the allergen capacity of the food product [19,20,41]. To define the presence or absence of these anti-allergenic properties, the severity of the systemic anaphylaxis reaction and the level of the humoral immune response were assessed in rats sensitized with a model allergen (OVA) against the background of getting a standard vivarium diet and a diet supplement including unfermented and fermented beef.

At the beginning of the experiment, the rats were kept in normal physiological condition. They were active and alert; their muscles were toned; their fur was tightly attached to their bodies, smooth, clean, and shiny; their skin was elastic; visible mucous membranes were pale pink; no inflammatory reactions were observed. Their eyes were bright red. Defecation and urination were within norm.

During the experiment, no significant changes in the animals' clinical condition were observed. The rats actively consumed the food, including unfermented and fermented beef.

The results of changes in the rats' weight at the beginning and end of the experiment are presented in the Table 1.

Table 1. Body weight of the rats in a model experiment sensitized with OVA ($M \pm m$)

Groups of animals sensitized with OVA on the model of anaphylactic reaction of the body, which received	Body weight at the beginning of the experiment, g	Body weight at the end of the experiment, g	Body weight gain, %
I. SVD (control group)	228.0 ± 12.0	243.3 ± 11.8	6.7
II. SVD+ unfermented beef		249.9 ± 12.0	9.6
III. SVD+ fermented beef		271.8 ± 13.6[a, b]	19.2

Note: [a, b] — indicators are reliable in regards to the groups I and II ($p \leq 0.05$) according to the Mann-Whitney test.

As shown in Table 1, the animals in the study groups showed normal growth rate. Rats in the experimental group III demonstrated greater weight gain at the end of the experiment compared to the groups I and II. The average weight gain in the group that received fermented meat was 2.9 and 2.0 times greater respectively, than in the groups I and II ($p \leq 0.05$). The significant weight gain in the group III may be related to its higher bioavailability and digestibility; however, further additional studies of direct digestibility parameters are required to make a final conclusion.

Table 2 presents data on the assessment of anaphylactic reaction severity in the animals. The data in Table 2 show that the anaphylaxis reaction severity in the groups I and II did not differ significantly from each other, although the AI in the group II was 4.5 % higher than in the group I of the animals ($p > 0.05$). In the group III, the anaphylaxis reaction severity accounted to 40.9 % and 43.5 % lower, respectively, in comparison with the groups I and II. In the group III no severe reactions (+++ and +++++) were observed. There were no fatal cases in any groups.

Table 2. Results of assessing the systemic anaphylaxis reaction severity in the rats during the experiment (M)

Groups of animals sensitized with OVA on the model of anaphylactic reaction of the body, which group received	Anaphylactic index (AI)	Severe reactions, %	Mortality rate, %
I. SVD (control group)	2.2	28.6	0.0
II. SVD+ unfermented beef meat	2.3	28.6	0.0
III. SVD+ fermented beef meat	1.3[a, b]	0.0	0.0

Note: [a, b] — indicators are reliable in regards to the groups I and II ($p \leq 0.05$) according to the Mann-Whitney test.

Figure 2 presents the data on the assessment of the severity of the anaphylactic shock reaction in three groups of experimental rats. As it follows from the Figure 2, adding of unfermented beef into the rats' diet (group II) caused a redistribution of the anaphylactic shock reaction severity: there was no mild manifestation (+), and the severity of the reaction (++) was 1.3 times higher than in Group I. The degree of the reaction (+++) was similar to that in the group I. When fermented meat was introduced into the rats' diet (group III), the severity of the anaphylactic shock reaction became the least, since 71.4 % and 28.6 % accounted for the lowest reaction score points (+ and ++), while the score points (+++) were absent.

Figure 3 represents the serum antibody concentrations to ovalbumin in three experimental groups of rats. As can be seen from this figure, humoral immunity in the group II,

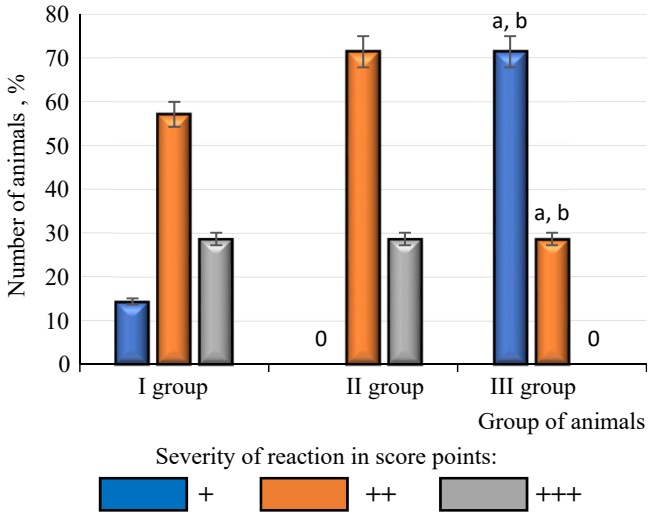

Figure 2. Distribution of anaphylactic shock reaction severity in the rats experimental groups ([a, b] — indicators are reliable in regards to the groups I and II, $p \leq 0.05$) according to the Mann-Whitney test

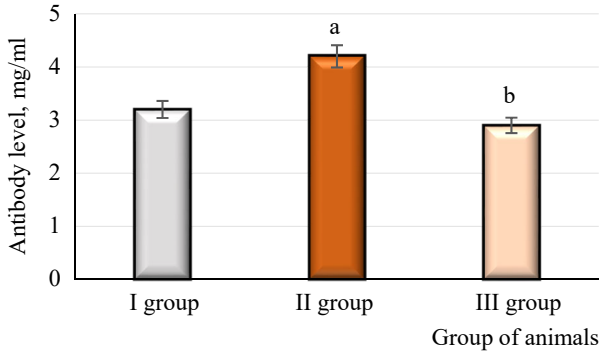

Figure 3. The intensity of the humoral immune response in rats of different experimental groups ([a, b]– indicators are reliable in regards, respectively, to the group I or II, $p \leq 0.05$) according to the Mann-Whitney test.

which consumed unfermented beef, was significantly higher than in the group I, which received SVD only. Humoral immunity in the group III, which received fermented beef, was significantly lower than in the group II (by 1.4 times) and even lower than in the group I ($p > 0.05$). This proves a significant increase in the level of sensitization of animals to the model allergen (OVA) upon consumption of unfermented beef and a decrease in sensitization as a result of fermented beef consumption.

The similar studies were implemented by Simonenko et al. [45] conducted a study on enzymatic hydrolysates of mare's milk, which demonstrated their effect on anaphylaxis in OVA sensitized rats. The parameters like anaphylactic shock severity and the level of specific IgG antibodies were recorded. The authors found that consumption of

hydrolysates did not affect the reaction severity, but led to a 1.9-fold decrease in the level of IgG antibodies to OVA ($p < 0.001$).

Thus, studies conducted on a model of systemic anaphylaxis to OVA revealed that rats that consumed fermented beef in their diet showed higher weight gain at the end of the experiment, a less severe anaphylactic shock reaction, and lower levels of antibodies to OVA compared to the rats fed with standard diet and the rats fed with unfermented beef after sensitization. Apparently, the degree of consortium-fermented beef hydrolysis achieved in the experiment reduced the immunomodulatory effect after ovalbumin sensitization.

The obtained results are consistent with the chosen approach and the efficiency of hypoallergenic meat products for children produced by domestic manufacturers (trademarks "Tema", "Agusha", etc.) and foreign (companies "Nestle", "Gerber", etc.), which use raw materials with low sensitizing activity and which use hydrolysis as a method of biological transformation of allergenic proteins [46,47].

The immunomodulatory effect observed in the group II of the rats that were fed with unfermented meat is likely related to the presence of common allergenic epitopes that give cross-reactions with ovalbumin sensitization. Meat allergies are primarily associated with beef consumption and are more common among the people who suffer from milk and egg allergies. However, other cases are also possible. Research of Revyakina [48] provides examples of anaphylaxis in patients with an allergy to cats when those patients consumed pork (cat-pork syndrome), when consuming meat with an allergy to antibiotics and to other medications.

An important condition for reducing the allergen capacity of the meat products with the help of fermentation starter cultures is their viability during their passing through the gastrointestinal tract (GIT), which directly determines the proteolytic activity and probiotic properties of microorganisms.

Figure 4 shows the results of the bifidobacilli and lactobacilli content analysis of the gastrointestinal tract, determined in the feces of the three studied groups of the rats, on which the model of systemic anaphylaxis to the model allergen OVA was simulated.

As it is shown in Figure 4, adding unfermented beef into the diet during OVA sensitization increased the levels of lacto- and bifidobacteria content by an order of magnitude. Furthermore, introducing fermented meat into the diet during the experimental model of systemic anaphylaxis increased this indicator by 57.1 and 4.0 times, respectively, compared to the groups I and II of the experimental rats ($p \leq 0.01$), thus reaching an average value of 4×10^6 CFU/g. This may indicate that during sensitization of the experimental animals, introducing into their diet the meat fermented by microorganisms consortium increases the amount of beneficial intestinal microflora, which is likely responsible for the attenuated allergenic potential of

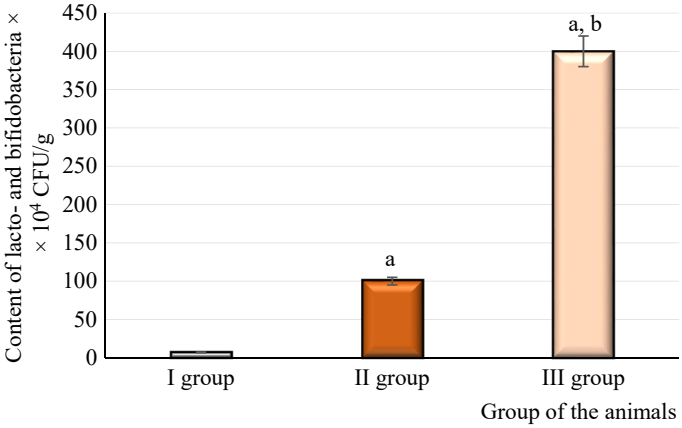

Figure 4. The content of lacto- and bifidobacteria in the excrement of experimental groups of rats ([a, b] — indicators are reliable in regards to, respectively, the group I or II, $p \leq 0.01$) according to the Mann-Whitney test

fermented beef compared to unfermented beef. Whether this is due to the replenishment of beneficial microorganisms that restore the balance of Th1/Th2 cells activity, or due to the biological activity of probiotic metabolites — it still remains unsolved. Further research is needed to assess the impact of the studied consortium on the microbiota composition.

For manifestation of probiotic effect it is important that beneficial microorganisms reach the intestine and colonize it in sufficient quantities. Research shows that for probiotics to adhere to intestinal cells, their numbers in the human body must reach at least 10^6–10^7 CFU/g (CFU/ml). At these concentrations, beneficial microorganisms exert a probiotic effect by modulating the activity of the intestinal microbiota, inhibiting pathogens, alleviating gastrointestinal disorders, and promoting the activation of the immune response [49].

According to modern scientific data, the survival of probiotics in the gastrointestinal tract depends on the type of carrier product (food matrix), the biological characteristics of the strains, and the use of protective technologies (in particular, encapsulation). The review [50] summarizes the main process approaches that ensure the safety of probiotics from production and storage to passage through the gastrointestinal tract. According to Rubio et al. [51], *Lactobacillus strain rhamnosus CTC1679,* used as a starter culture for fermented meat products, successfully survived the harsh gastrointestinal environment and maintained viability in a human clinical trial.

Research results confirm the efficiency of probiotic therapy in experimental models. For example, in the work of Kim et al. al. [52] mice with OVA-induced allergy were given *Lactobacillus acidophilus* and *Bifidobacterium lactis.* Mice given probiotics had suppressed production of OVA-specific antibodies (IgE, IgG1, IgA) in serum and feces, indicating their antiallergenic potential.

Authors Lu et al. [53] studied the effect of three probiotic strains (*L. plantarum, L. reuteri, B. longum)* on mice allergic to OVA. Administration of probiotics not

only alleviated allergy symptoms and reduced the level of OVA-specific IgE, but also changed the composition of the intestinal microbiota. For example, the *B. longum strain* increased the content of bifidobacteria and bacteria producing short-chain fatty acids.

The data obtained are also consistent with modern studies on the efficiency of postbiotics. Unlike probiotics, postbiotics are naturally formed in fermented foods (yogurts, tea fungus kombucha, kefir, sour cabbage, etc.), they are stable over a wide range of temperatures and pH, and their adequate amount can be controlled under production and storage conditions where survival is not the major determining factor [54]. The efficiency of postbiotics in the management of clinical manifestations of food allergies has been noted in a number of studies; in particular, oral administration of bacterial lysate is indicated by the group of authors Bodemer et al. as the best outcome in the therapy of atopic dermatitis in children [55], way of respiratory diseases prevention and therapy [56].

Histological studies of the cranial lymph nodes of three studied groups of rats were conducted. The mucosa is the site of initial contact with the allergen, Peyer's patches provide the primary inductive response, and the mesenteric lymph nodes act as a "decision center" for determining whether to induce food tolerance or initiate food allergic response [57,58].

In a model of OVA systemic anaphylaxis in experimental animals, adding of fermented and non-fermented meat to the rats' diet contributed to antigenic stimulation and structural and functional rearrangement of the mesenteric lymph nodes.

Morphometric parameters of the mesenteric lymph nodes of the studied groups of animals are presented below in the Table 3.

In experimental animals, the cranial mesenteric lymph nodes, which collect lymph from the small intestine and the initial part of the large intestine, are small in dimensions and feature variable shape. They are arranged in a chain of 5–9 nodules along the cranial mesenteric artery and its branches. The mesenteric lymph node is covered by a convoluted connective tissue capsule with an average dimensions of 7.5 to 14.5 μm. In the mesenteric lymph nodes, the border between the cortex and medulla is well differentiated. Figure 5 shows the lymph nodes of the rats in the group I, which contained both primary lymphoid nodules and secondary ones with formed germinal centers that show distinct dark and light zones (Figure 5a and Figure 5b), which indicates the reaction of the body's immune system to OVA sensitization [59].

In the animals of the group II, against the background of OVA systemic anaphylaxis and the unfermented beef inclusion into the diet, the significant structural and morpho-functional transformations were detected in the cranial mesenteric lymph nodes (Table 3). In the lymph node preparations of this group of animals, the formation of a finely looped cellular structure in the parenchyma of the cortex and a large-loop cellular structure in the medullary zone was noted (refer to the Figure 6b). Microscopy of the preparations revealed a slight increase in the dimensions of the primary and secondary lymphoid nodules in the cortex against the background of thinning of their capsule down to 7.5 μm. In contrast to the group I, large number of swollen sinuses of the node filled with lymphoid cells were observed (Figure 6a and Figure 6b). In the preparations obtained from this group of animals, a significant reduction in capsule thickness was achieved by increasing the area of marginal sinus by 2.2 times. A decrease in the quantity of primary and secondary lymphoid nodules, which are the lymph nodes dynamic structures,

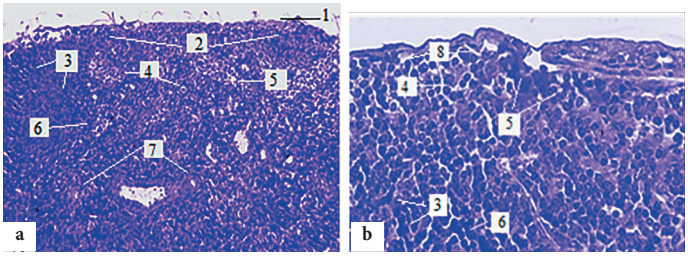

Figure 5. Micrographs of the mesenteric lymph node in the group I: a) cortex and medulla: 1 — capsule; 2 — cortex; 3 — primary lymphoid nodules clusters; 4 — secondary lymphoid nodules with a dark center; 5 — secondary lymphoid nodules with a light center; 6 — paracortical zone; 7 — medulla; 8 — cortical sinus. b) cortex. Stained with hematoxylin and eosin. A — zoom (PL 10×/0.25); B — zoom (PL 40×/0.65)

Table 3. Morphometric parameters of the cranial mesenteric lymph nodes structures in rats in the experiment ($M \pm m$)

Structure	Group I	Group II	Group III
Capsule thickness, μm	13.5 ± 1.5	7.5 ± 2.0[a]	14.5 ± 2.5[b]
Marginal sinus area, μm^2	670.5 ± 25.5	1505.0 ± 105.0[a]	755.5 ± 25.0[b]
Area of the cerebral sinus, μm^2	3475.0 ± 350.0	1300.0 ± 250.0[a]	4252.5 ± 450.0[b]
The number of primary lymphoid nodules on the section	12.5 ± 1.0	5.0 ± 1.5[a]	13.5 ± 2.0[b]
The number of lymphoid nodules with germinal centers on the section	9.0 ± 2.5	6.5 ± 3.0	12.5 ± 2.0[b]
Thickness of a lymphoid nodule with a light germinal center, μm	13.0 ± 4.5	11.4 ± 5.0	14.0 ± 6.5
Thickness of a lymphoid nodule with a dark germinal center, μm	9.0 ± 4.0	11.5 ± 4.0	8.0 ± 3.5
Paracortical zone thickness, μm	155.0 ± 25.0	95.0 ± 30.0	165.0 ± 35.0
Paracortical zone, % of the slice area	18.0 ± 4.0	14.0 ± 2.0	19.0 ± 3.5
Medulla, % of section area	30.0 ± 4.0	43.0 ± 5.5	28.5 ± 4.5
Cortex, % of section area	51.5 ± 10.5	42.5 ± 3.5	52.5 ± 6.0

Note: [a, b] indicators are reliable in regards to, respectively, the group I or the group II ($p \leq 0.05$) according to Student's t-test.

and form and disappear under the effect of various factors, was observed. A slight decrease in the area (as a percentage of the lymph node cross-sectional area) of the paracortical and cortical zones was noted, while an increase in the area of the medullary zone relative to the lymph node cross-sectional area was observed.

In some sections with expanded medulla, compression and displacement of the cortex to the periphery of the node were observed, with a significant reduction in the paracortical zone to 95 μm (Table 3 and Figure 6a). The table shows that, together with a slight increase in the primary and secondary nodules dimensions, there was a decrease in the thickness and quantity of lymphoid nodules with a light proliferation center, compared to the corresponding values in the group I (Table 3). According to references data, a significant increase in the sinuses of the mesenteric lymph nodes indicates increasing of drainage function of lymph through them [60].

Based on the obtained results, it can be assumed that the inclusion of beef meat provided a negative effect on the anaphylaxis reaction course, as evidenced by structural and functional changes associated with a decrease in barrier function, the development of hyperplasia and suppression of the lymphoid structure.

In most of the CMLN preparations of group III animals (inclusion of fermented beef meat in the diet against the background of the anaphylactic shock model), all the studied quantitative and dimensional parameters and the morphological picture practically corresponded to those in the group of animals on SVD and sensitized with OVA (group I), or changed insignificantly and insignificantly ($p > 0.05$) (Table 3 and Figure 7).

The experimental data obtained above in the systemic anaphylaxis model to OVA allow us to draw the general conclusion that introducing unfermented beef into the diet of sensitized animals (the group II) resulted in significant structural changes in the mesenteric lymph nodes, manifested by functional decompensation of the lymph nodes in response to dietary changes. Capsule thinning and sinus swelling indicate signs of strain on the filtration mechanisms and immune control of lymph flowing from the intestine. This condition can be classified as adaptive hyperplasia of lymphoid tissue with a predominant sinus component, demonstrating the active participation of lymph nodes in the immune response and lymph filtration during dietary changes.

The morphological pattern of CMLN in a model of OVA systemic anaphylaxis with the fermented beef inclusion into the diet (the group III) did not negatively affect the sensitization process compared to unfermented meat. The correspondence of most measured parameters to values close to those in control animals (the group I) indicates that fermented meat did not cause significant changes in the anaphylaxis reactions course or in lymph node structure that would indicate the allergenic effect severity.

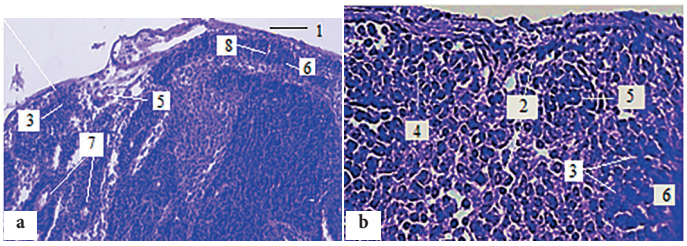

Figure 6. Micrographs of the mesenteric lymph node in group II: a) cortex and medulla: 1 — capsule; 2 — cortex; 3 — primary lymphoid nodules clusters; 4 — secondary lymphoid nodules with a dark center; 5 — secondary lymphoid nodules with a light center; 6 — paracortical zone; 7 — medulla; 8 — cortical sinus. b) — cortex. Stained with hematoxylin and eosin. A — zoom (PL 10×/0.25); B — zoom (PL 40×/0.65)

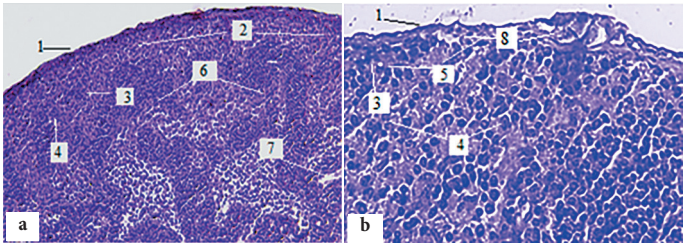

Figure 7. Micrographs of the mesenteric lymph node in group III: a) cortex and medulla: 1 — capsule; 2 — cortex; 3 — clusters of primary lymphoid nodules; 4 — secondary lymphoid nodules with a dark center; 5 — secondary lymphoid nodules with a light center; 6 — paracortical zone; 7 — medulla; 8 — cortical sinus; b) — cortex. Stained with hematoxylin and eosin. A — zoom (PL 10×/0.25); B — zoom (PL 40×/0.65)

Limitations of the study

When interpreting the obtained results, several methodological limitations should be considered. First, the model of ovalbumin-induced systemic anaphylaxis used does not fully reproduce the physiology of food allergy in humans, which limits the direct extrapolation of the findings. Second, although IgG was chosen as a marker of the humoral response in this study, the lack of a parallel determination of IgE serves as methodological limitation, because this isotype is considered to be the main mediator of class I allergy. The obtained data on IgG only indirectly proves an immunomodulatory effect but do not allow differentiation between IgE- and non- IgE -mediated pathways. Third, the relatively small quantity of the rats in each group requires confirmation of the identified patterns in more representative samples array. Further studies with a larger quantity of the animals, measurement of IgE content level, and using allergen-specific test systems are required to verify the obtained data.

Conclusion

The immunomodulatory potential of beef meat exposed to an effect of probiotic microorganisms consortium was evaluated.

The consortium was represented by four strains: *Lactobacillus plantarum 7K, Lactobacillus paracasei κ-406, Pediococcus acidilactici PDA-27 u Staphylococcus carnosus SPHC-108* exhibited high proteolytic activity during beef

muscle proteins hydrolysis. After two days of meat fermentation, the degree of hydrolysis was 11.32 %, and after three days it was 21.5 %, which is 3.3 times higher than the hydrolysis rate of the control sample.

The allergenic potential of fermented and unfermented beef was studied using a model of systemic anaphylactic reaction in the experimental animals. Fermented beef was found capable to reduce the severity of the systemic anaphylactic reaction to a model allergen (OVA) in the sensitized animals. Sensitized animals that received fermented meat featured a significantly lower anaphylactic index (1.3 points) compared to the animals in the control group (2.2 points) who consumed SVD, and the rats that consumed SVD and unfermented meat (2.3 points). The anaphylaxis reaction severity decreased by 40.9 % and 43.5 %, respectively. The concentration of specific antibodies (IgG) to ovalbumin in the blood serum of the rats that consumed fermented meat was significantly lower ($p \leq 0.05$), than in the group of the rats that consumed non-fermented meat, and which level approached to the level in the control group.

The animals that were fed with fermented meat during sensitization showed greater weight gain at the end of the experiment. The average weight gain in the group of rats that ate fermented meat was 19.2 %, which was 2.9 times higher than in the control group and 2.0 times higher than in the group of rats that consumed unfermented meat. These results indicate the potential for improved digestibility and absorption of fermented meat, but further studies of direct metabolic parameters are required to draw final conclusion.

Changes in the intestinal microbiota of sensitized animals were also noted. The levels of lacto- and bifidobacteria in the feces of the animals that were fed with fermented meat increased by 57.1 and 4.0 times, respectively, in comparison with the control group and the group of rats that consumed unfermented meat. The average value was approximately 4×10^6 CFU/g. Thus, a correlation was observed between increased levels of lacto- and bifidobacteria and less severe anaphylaxis, which requires further study of the mechanisms of their protective effect.

Histological studies of mesenteric lymph nodes of the rats showed that introducing fermented beef into the diet during OVA sensitization did not cause significant changes in lymphoid tissue, and the nodes were virtually identical to those in the rats' control group, which were fed with standard vivarium diet. On contrary the rats fed with unfermented meat during sensitization showed significant changes, thus evidencing increased antigen load and stressed immune mechanisms.

Thus, data obtained in the systemic anaphylaxis model showed that using fermented meat in the diet after its exposure to the developed consortium of microorganisms, reduced the severity of the systemic anaphylactic reaction to the model allergen in the sensitized animals, contributed to greater weight gain and increased the population of beneficial intestinal microflora.

The results confirm that using the probiotic microorganisms for meat fermentation is a promising approach for reducing food allergies risk and developing hypoallergenic food products. The mechanisms of interaction between intestines microbiota, allergy, and fermentation process technologies are currently being intensively studied, and this study contributes to this field, opening up the opportunities for development of effective allergy prevention and therapy strategies, as well as improving the quality of life of people who suffer from food allergies.

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