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# Toxicological and Irritating Effects of Protein Hydrolyzate of Microbial Origin

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## Abstract

This article presents a studying the toxicological and irritating protein hydrolyzate effect for further use in industrial poultry farming. The studies' results have shown that the protein hydrolyzate used in the research work is safe in the studied dosages. During an acute experiment, it was not possible to establish the LD50. The death of the experimental mice and rats was not recorded. There were no signs of a violation of their health during the experiment. The animals remained mobile, active, and ate food well. Clinical symptoms of intoxication did not appear. In the experiments, the hydrolyzate does not cause the death of laboratory animals and loss of body weight during the experiments. On the contrary, it increases the growth of laboratory mice and rats within the range of 2.0–8.0% concerning the control group.

**Disciplinary**: Veterinary, Biotechnology.



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## 1 Introduction

The development of animal husbandry, in particular poultry farming, is directly related to solving the problem of ensuring Russia food security. One of the Russian Federation's agricultural policy's main tasks is to meet the needs of the country's population with agricultural food products of its manufacture. However, significant economic damage to industrial poultry farming is caused

by agricultural poultry diseases of bacterial etiology. They lead to large losses of young animals, high percentages of forced slaughter, reduced productivity, and product quality. The use of antibiotics to prevent and treat poultry causes the development of conditionally pathogenic and pathogenic microflora resistant to them. Therefore, in the 70s of the last century, the search for economically feasible and environmentally safe means of preventing and treating poultry diseases began, aimed at normalizing the microbial background of the digestive organs by purposefully changing the composition of the microbiota of the gastrointestinal tract [8].

Microorganisms that act as antagonists to pathogenic microflora, due to the significant content of vital biologically active components in them, as well as their ability to rapidly grow and accumulate a large biomass, the use of bacteria as raw materials for the development of new-generation biologics that can exhibit probiotic and immunostimulating properties is a cost-effective and promising direction. Therefore, in recent decades, a trend has been formed to change microorganisms' composition in the gastrointestinal tract, which led to the creation of a new class of bacterial preparations-probiotics [7].

The most important task in the design of probiotics is the selection of bacterial strains that can effectively colonize the gastrointestinal tract and remain active for a long time. To do this, they are selected from existing collections, and they are screened among wild natural and micro-organisms inhabiting the tract of animals and humans [9].

The normal microflora of the gastrointestinal tract is characterized by a qualitative and quantitative ratio in its various parts of the microorganisms populations that can maintain the metabolic and immune balance of the macroorganism. The main role in this is played by lactic acid bacteria, which are an obligatory component of the beneficial microflora of the gastrointestinal tract of animals and birds. The property of lactic acid bacteria to exhibit an immunostimulating effect is primarily due to the presence of certain components that are part of the structure of their cell wall. A special role in this is played by the glucosaminylmuramyl dipeptide (GMDP), a structural unit of the peptidoglycan of most bacteria's cell wall. The therapeutic and prophylactic effect of GMDP is that it can influence the cells of the innate immune system, thereby causing the stimulation of the effector functions of phagocyte cells and the release of cytokines that induce the proliferation, activation and differentiation of cells of the acquired immunity – T- and B-lymphocytes. Through increased production of colony-stimulating factors, GMDP induces leukopoiesis. The GMDP action result is the activation of all parts of the immune system with an increase in anti-infective immunity [6; 9; 10].

Biologics produced using lactobacilli have found extensive use in veterinary medicine. The use of drugs that can increase the natural and specific resistance of the poultry body in the Russian Federation is still quite small, but diverse; such drugs include various vitamin and mineral complexes, plant adaptogens, probiotics, etc. Experience shows that the use of lactic acid bacteria hydrolysates to correct the body's immunobiological status is promising, since they are easily absorbed and more easily involved in biochemical processes [11].

In general, it should be noted that a thorough study of the scientific and patent literature has shown the lack of effective designs of functionally adapted probiotic additives made with the use of evolutionarily fixed microorganisms of the poultry gastrointestinal tract, as well as hydrolysates based on them for use in industrial poultry farming.

This study focused on the toxicological and irritating protein hydrolysate properties of microbial origin for further use in poultry farming.

## 2 Method

Research on the effect of the biological product on the body of laboratory animals was carried out on the basis of the Veterinary Pharmacological Center, a structural division of the Kuban State Agrarian University named after I. T. Trubilin.

The study's object was a protein hydrolysate obtained by thermal acid hydrolysis of the bacterial mass of lactobacilli isolated from the gastrointestinal tract of wild birds (see Figure 1).

Figure 1: Appearance of the finished form of the hydrolyzate

The study of the toxicity parameters of a biological product of microbial origin was carried out by the requirements and recommendations [2; 3; 5]. The study of the skin-resorptive effect and irritant properties on the eyes' mucous membranes was carried out on rabbits according to GOST R ISO 10993.10-99 [1]. The study of acute, subacute, and chronic toxicity was carried out on clinically healthy laboratory animals (mice, rats), previously quarantined for 14 days. Laboratory animals were divided into groups according to the principle of pairs of analogs. The number of animals in each group was ten heads of both sexes, the content of females and males was separate. Non-linear white mice with a bodyweight of 18-20 g (age 2.5 months) and sexually mature mongrel white rats with a bodyweight of 180-200 g (age 2.5 months) were used as laboratory biological objects of research. Laboratory animals of all groups were kept under the sanitary and epidemiological requirements for the design, equipment and maintenance of experimental biological clinics (vivariums) [4].

When studying acute toxicity indicators, the biological product was administered to laboratory animals once after a 12-hour fast exposure. To obtain reliable results, the experiment was carried out in three repetitions. For oral administration, a metal intragastric probe was used. When the drug was administered, the animals were fixed in an upright position with slight tilt of head and probe was inserted into the stomach and solution was slowly injected. Feeding of the animals was carried out 3 hours after the introduction of the biological product. After applying the biological product, the laboratory animals were monitored for 2 weeks, taking into account changes in their general behavior and condition.

To select the semi-lethal value (LD50) when using hydrolysate, a dose of 10.0 ml/kg was taken as the maximum. For the experiment, five groups of animals were formed by the method of analog pairs: the intact group – laboratory mice and rats did not receive additional solutions, except for standard drinking and feeding; the control group - instead of a biological product, laboratory mice and rats received a saline solution at a maximum dose of 10.0 ml/kg; the 1st experimental group, the used hydrolysate was administered orally to laboratory animals at a dose of 2.0 ml/kg; The 2nd experimental group, mice were administered the biopreparation intragastrically at a dose of 5.0 ml/kg; the 3rd experimental group, hydrolysate was administered intragastrically to animals at a dose of 10.0 ml/kg. In some cases, due to the insignificant volumes of the injected liquid, the obtained dosages were supplemented with a saline solution to the volume that provides a comfortable introduction of the test biological product to laboratory animals.

The study of subacute and chronic toxicities of biological products makes it possible to identify the long-term effect of their main components on the functional state of individual organs, tissues and systems, as well as their ability to accumulate. Subacute toxicity lasted for 3 months, and chronic toxicity, taking into account the intended production use of the biological product and recommendations, lasted for 6 months [3]. To study subacute and chronic toxicity, hydrolysate was administered to experimental laboratory animals daily, intragastrically in dosages of 1/10, 1/20 and 1/50 of the maximum administered in the acute experiment. After dilution, the obtained doses were added to the saline solution to the volume that provides a comfortable introduction of the biological test product to the experimental animals. The laboratory animals were monitored daily, and their safety, behavior, appetite, and general condition were monitored. At the beginning and end of the experiment, individual weighing of laboratory animals was carried out. At the final stage of the experiment, laboratory animals were subjected to mortification to study the morpho-biochemical parameters of blood and an analysis of the state of their organs and tissues pathoanatomical autopsy.

The skin-resorptive effect was studied on 2 mature rabbits of different sexes (bodyweight 2.2-2.5 kg)for the biopreparation. The rabbits had their hair cut on both sides' symmetrical areas for application the day before the research. One of the clipped areas was a control. The size of the treated skin area was from 5.0 to 8.0 % of the laboratory rabbit's body surface. 1.0 ml of the biological product was applied to cotton swabs and applied to the cut area of the skin. A cotton

swab was fixed with a gauze bandage. The local irritant effect of the biological product on the skin was evaluated after 4 hours of exposure, and then after 24, 48 and 72 hours.The presence of redness, increased sensitivity, swelling, inflammation, possible manifestations of erythema and edema, as well as other general reactions of the laboratory animal to the studied biological product were taken into account. The primary irritation index was evaluated.

The study of the biological product's irritant effect on the conjunctiva was carried out on two mature albino rabbits of both sexes (weight 2.9-3.1 kg). Before the tests, the eye condition of each laboratory albino rabbit was examined. To experiment, 0.1 ml of the studied biological product was injected into the lower conjunctival sac of the right eye. After introducing the test biological product, the lower and upper eyelids were closed and held for 2-3 seconds. The control was the left eye. Examination of laboratory animals' eyes after insolation with a biological product was carried out after 1, 24, 48 and 72 hours. The following was taken into account: the condition of the conjunctiva, cornea, iris, the presence of edema, as well as other secretions from the right eye. The primary irritation index was evaluated.

In the study of subacute and chronic toxicities, the pharmacodynamics of hydrolysate was studied by its effect on individual metabolic parameters, the morphobiochemical composition of blood, as well as the growth rate of laboratory animals.

The morphological status of the blood of laboratory animals was studied. The number of cells (red blood cells, white blood cells, and platelets) in whole blood was counted in a chamber with a Goryaev grid. The content of hemoglobin in red blood cells was measured on the HemoCueHb analyzer. Biochemical parameters of blood serum of experimental animals were studied on a semi-automatic desktop analyzer Statfax4500.

The data obtained in the experiments were subjected to biometric processing, considered significant at $P < 0.05$.

# 3 Result and Discussion

## 3.1 Study of Acute Toxicity

The parameters of acute toxicity of microbial hydrolysate were determined on non-linear mice and sexually mature white mongrel rats. The results of the acute toxicity of the hydrolysate are presented in Table 1.

**Table 1**: Acute toxicity of hydrolyzate in laboratory animals (n = 10)

| Group | Kind of animal | Liquid dose, injection method | Test result, heads | | |
|---|---|---|---|---|---|
| | | | got sick | died | survived |
| Intact | mouse | – | 0 | 0 | 10 |
| | rats | – | 0 | 0 | 10 |
| Control | mouse | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| | rats | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| 1st experimental | mouse | 2.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 2.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 2nd experimental | mouse | 5.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 5.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 3rd experienced | mouse | 10.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 10.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |

Since in the 2nd experimental group, the volume of the administered liquid for rats and mice was insignificant, it was necessary to add a saline solution to the received doses to a level that provides a comfortable introduction of the test biological product to the experimental animals. As a result, it was revealed that in the experimental groups, regardless of the concentration of the bacterial hydrolysate used, there were no obvious changes in the general condition of the animals, they remained viable and active. During the daily examination of mice and rats, observing that the laboratory animals remained mobile and active, and satisfactory feed consumption was recorded, while maintaining all vital reflexes. In the studied range of doses of bacterial hydrolysate, mortality in the experimental groups was not detected. Thus, the developed hydrolysate of lactic acid bacteria does not cause pronounced toxicosis in rats and mice. Therefore it can be attributed to the 4th hazard class (low-toxic substances) according to GOST 12.1.007-76.

## 3.2 Study of Subacute Toxicity

Because the LD 50 of bacterial hydrolysate was not determined in the acute experiment, the maximum dose administered to laboratory animals in the acute experiment, namely 10.0 ml/kg, was adopted as the main dose for subacute toxicity. According to the requirements, experimental white mice and rats were injected with bacterial hydrolysate inside the stomach in dosages equal to 1/10, 1/20 and 1/50 of the maximum used in the acute experiment. When studying hydrolysate's subacute toxicity, laboratory animals received the following doses of the drug-1.0, 0.5 and 0.2 ml/kg, respectively. For the convenience of administering small doses of the drug to animals, they were mixed with saline solution. Groups were also formed: intact-laboratory mice and rats did not receive additional solutions, except for standard drinking and feeding; control – instead of a biological product, laboratory mice and rats received a saline solution at a maximum dose (10.0 ml/kg). The administration of the studied solutions was carried out daily, through a probe, orally. The results of the study of the subacute toxicity of bacterial hydrolysate are presented in Table 2.

**Table 2**: Subacute toxicity of lactobacillus hydrolyzate on laboratory animals (n = 10)

| Group | Kind of animal | Liquid dose, injection method | Test result, heads | | |
|---|---|---|---|---|---|
| | | | got sick | died | survived |
| Intact | mouse | – | 0 | 0 | 10 |
| | Rats | – | 0 | 0 | 10 |
| Control | mouse | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| | Rats | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| 1st experimental | mouse | 1.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | Rats | 1.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 2nd experimental | mouse | 0.5 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | Rats | 0.5 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 3rd experienced | mouse | 0.2 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | Rats | 0.2 ml / kg hydrolyzate, oral | 0 | 0 | 10 |

The conducted studies showed that no negative consequences were detected in the experimental animals when using the biological product. The safety of the animals is 100 %. Mice and rats during the testing period were mobile and active, the coat was smooth with a characteristic shine.

During the testing period, the effect of hydrolysate on the live weight gain of laboratory animals was studied. The live weight of mice and rats was taken into account at the beginning and end of the experiment. The results of the studies are in Table 3.

**Table 3**: Influence of microbial hydrolyzate on the growth and development of laboratory animals

| Group | Body weight, g | | Experience gain, g |
|---|---|---|---|
| | at the beginning of the experiment | at the end of the experiment | |
| Mouse | | | |
| Intact | 19.45±0.28 | 24.76 ± 0.45 | 5.31 |
| Control | 19.13±0.31 | 24.43 ± 0.41 | 5.30 |
| 1st experimental | 19.63±0.30 | 27.84 ± 0.51 | 8.21 |
| 2nd experimental | 19.23±0.35 | 26.69 ± 0.47 | 7.46 |
| 3rd experienced | 19.41±0.25 | 26.37 ± 0.39 | 6.96 |
| Rats | | | |
| Intact | 195.38±1.67 | 219.49 ± 2.86 | 24.11 |
| Control | 194.03±1.74 | 221.62 ± 2.64 | 27.11 |
| 1st experimental | 195.11±1.69 | 224.58 ± 2.79 | 29.47 |
| 2nd experimental | 196.21±1.78 | 223.51 ± 2.68 | 27.46 |
| 3rd experienced | 195.79±1.73 | 223.38 ± 1.81 | 27.59 |

Table 3, it can be seen that when conducting a subacute experiment in experimental groups of laboratory animals, there was a slight tendency to increase the live weight of mice and rats. So it was found that in 1-3 experimental groups of mice at the end of the study, the live weight was higher than in control group by 13.9, 9.3 and 7.9 %. The increase in the 1st experimental group was 8.21 g, in the 2nd group was 7.46 g and in the 3rd-6.96 g, which is more than in the control group by 2.91, 2.16 and 1.66 g, respectively. A similar situation was observed among the experimental groups of laboratory rats. The results of weighing laboratory rats showed that in the 1st experimental group, the animals' weight was higher than in the control group by 1.3 %. The 2nd experimental group was higher than in the control group by 0.9 % and in the 3rd group-by 0.8 %. The increase in the 1-3 experimental groups of rats was slightly greater than in the control group by 8.7; 1.3 and 1.8%, respectively.

**Table 4**: Morphological and biochemical parameters of the blood of laboratory mice after the use of hydrolyzate (n = 6)

| Index | Group | | | | |
|---|---|---|---|---|---|
| | Intact | Control | 1st experimental | 2nd experimental | 3rd experienced |
| *Morphological indicators* | | | | | |
| Hemoglobin, g / l | 128.14±3.54 | 129.64±3.67 | 133.26±3.48 | 131.72±3.49 | 134.63±3.58 |
| Leukocytes, $10^9$ / l | 7.17±0.14 | 7.19±0.17 | 7.20±0.14 | 7.19±0.15 | 7.16±0.17 |
| Erythrocytes, $10^{12}$ / l | 8.67±0.12 | 8.62±0.11 | 8.85±0.11 | 8.73±0.10 | 8.71±0.12 |
| Platelets, $10^9$ / l | 223.83±3.97 | 222.56±3.87 | 223.12±3.84 | 222.64±3.93 | 224.37±3.91 |
| *Biochemical indicators* | | | | | |
| Total protein, g / l | 55.13±2.21 | 56.21±2.04 | 56.32±2.17 | 58.27±2.08 | 57.37±2.10 |
| albumin, g / l | 23.54±0.56 | 22.96±0.59 | 23.76±0.61 | 23.11±0.57 | 23.87±0.58 |
| globulin, g / l | 31.59±0.87 | 33.25±0.93 | 32.56±0.89 | 35.16±0.91 | 33.50±0.93 |
| A / H ratio | 0.71±0.03 | 0.69±0.01 | 0.73±0.02 | 0.72±0.02 | 0.72±0.03 |
| Urea, mM / l | 6.61±0.23 | 6.63±0.29 | 6.81±0.27 | 6.58±0.28 | 6.52±0.25 |
| Cholesterol, mM / L | 2.61±0.03 | 2.57±0.03 | 2.58±0.03 | 2.53±0.04 | 2.59±0.03 |
| Calcium, mM / l | 2.24±0.06 | 2.26±0.05 | 2.28±0.04 | 2.31±0.05 | 2.27±0.06 |
| Phosphorus, mM / l | 1.35±0.03 | 1.37±0.04 | 1.41±0.02 | 1.39±0.03 | 1.38±0.03 |
| AsAT, U / l | 101.34±3.67 | 103.76±3.53 | 104.76±3.76 | 102.76±3.86 | 103.12±3.72 |
| ALAT, U / l | 88.69±1.87 | 87.45±1.93 | 86.73±1.85 | 88.82±1.94 | 87.79±1.79 |

The effect of hydrolysate on the morpho-biochemical status of the blood of laboratory animals was studied. Table 4 presents the results of morphological and biochemical parameters of the blood of laboratory mice.

As a result of the conducted studies, there was no statistically significant difference in the use of lactobacillus hydrolysate in the experimental groups. All indicators were at the level of the physiological norm. Simultaneously, in the experimental groups of laboratory mice, there was a slight tendency to improve the studied parameters compared to the intact and control groups of animals. Slight acceleration of metabolic processes in the body of mice of the 1st-3rd experimental groups was found due to increased blood content of red blood cells, hemoglobin, total protein, and electrolytes. Thus, Table 4 shows that in the 1st, 2nd and 3rd experimental groups, the number of red blood cells was higher than in the control group by 2.7, 1.3 and 1.0%, respectively. The value of hemoglobin in red blood cells in the 1st experimental group was overestimated than in the control group by 2.8 %, in the 2nd experimental group-by 1.6% and in the 3rd by 3.8 %. The content of platelets and white blood cells in the context of the studied groups was at the same level. From the biochemical characteristics of blood serum, we would like to note a slight increase in total protein in the 2nd and 3rd experimental groups compared to the control, by 3.7 and 2.1 %.

The results of morphological and biochemical parameters of the blood of laboratory rats are in Table 5.

**Table 5**: Morphological and biochemical parameters of the blood of laboratory rats after the use of the hydrolyzate (n = 6)

| Index | Group | | | | |
|---|---|---|---|---|---|
| | Intact | Control | 1st experimental | 2nd experimental | 3rd experienced |
| *Morphological indicators* | | | | | |
| Platelets, $10^9$ / l | 141.94±3.85 | 148.62±3.79 | 151.29±3.92 | 148.34±3.82 | 149.21±3.86 |
| Erythrocytes, $10^{12}$ / l | 5.45±0.14 | 5.39±0.17 | 5.52±0.15 | 5.48±0.19 | 5.49±0.20 |
| Leukocytes, $10^9$ / l | 10.21±0.32 | 10.15±0.27 | 10.45±0.29 | 10.74±0.34 | 10.04±0.31 |
| Hemoglobin, g / l | 110.21±3.08 | 108.32±2.89 | 108.56±3.07 | 105.83±3.15 | 107.48±2.98 |
| *Biochemical indicators* | | | | | |
| Total protein, g / l | 63.45±1.69 | 64.35±1.82 | 65.57±1.76 | 64.78±1.73 | 65.21±1.67 |
| albumin, g / l | 28.32±0.81 | 28.45±0.79 | 29.74±0.74 | 29.43±0.85 | 29.74±0.76 |
| globulin, g / l | 35.13±0.73 | 35.90±0.68 | 35.83±0.74 | 35.35±0.73 | 35.47±0.81 |
| A / H ratio | 0.80±0.02 | 0.79±0.03 | 0.83±0.02 | 0.83±0.02 | 0.83±0.03 |
| Urea, mM / l | 5.21±0.14 | 5.32±0.17 | 5.31±0.13 | 5.27±0.15 | 5.33±0.16 |
| Cholesterol, mM / L | 1.73±0.03 | 1.75±0.04 | 1.71±0.03 | 1.78±0.04 | 1.76±0.03 |
| Calcium, mM / l | 2.78±0.06 | 2.64±0.05 | 2.69±0.07 | 2.71±0.06 | 2.75±0.05 |
| Phosphorus, mM / l | 12.35±0.32 | 12.21±0.31 | 12.32±0.37 | 12.37±0.41 | 12.25±0.37 |
| AsAT, U / l | 56.35±1.35 | 58.48±1.46 | 58.12±1.28 | 57.61±1.32 | 59.19±1.37 |
| ALAT, U / l | 61.64±1.27 | 60.85±1.47 | 61.72±1.26 | 62.11±1.28 | 59.54±1.34 |

* The difference with the control is reliable (P < 0,05)

The analysis of whole and serum blood of laboratory rats showed similar results after hydrolysate as in mice. Pathologies in the context of the studied groups could not be identified.

In general, the results of subacute toxicity of microbial hydrolysate showed that the biological product did not harm the body of experimental laboratory mice and rats.

## 3.3 Study of Chronic Toxicity

According to the materials and research methods, the study of the chronic toxicity of hydrolysate was carried out in a similar way to the podstrom experiment, but the test period lasted 6 months. Additionally, the biological product's effect on the organs and tissues of laboratory animals was studied by their pathoanatomical autopsy after killing.

During the entire observation period, all laboratory animals were healthy. The survival rate in all groups was 100 %. When giving different doses of the biological product, no external signs of toxicosis were revealed, mice and rats ate food well, were mobile, and the coat was smooth with a characteristic gloss (Table 6).

**Table 6**: Chronic toxicity of the hydrolyzate in laboratory animals (n = 10)

| Group | Kind of animal | Liquid dose, injection method | Test result, heads | | |
|---|---|---|---|---|---|
| | | | got sick | died | survived |
| Intact | mouse | – | 0 | 0 | 10 |
| | rats | – | 0 | 0 | 10 |
| Control | mouse | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| | rats | 10.0 ml / kg physical solution, orally | 0 | 0 | 10 |
| 1st experimental | mouse | 1.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 1.0 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 2nd experimental | mouse | 0.5 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 0.5 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| 3rd experienced | mouse | 0.2 ml / kg hydrolyzate, oral | 0 | 0 | 10 |
| | rats | 0.2 ml / kg hydrolyzate, oral | 0 | 0 | 10 |

The results of the biological product's effect on the change in body weight of laboratory animals are presented in Table 7.

**Table 7**: The effect of hydrolyzate on the growth and development of laboratory animals

| Group | Body weight, g | | Body weight, g |
|---|---|---|---|
| | at the beginning of the experiment | at the beginning of the experiment | |
| Mouse | | | |
| Intact | 18.96±0.42 | 32.13 ± 0.64 | 13.17 |
| Control | 18.71±0.39 | 33.73 ± 0.72 | 15.01 |
| 1st experimental | 19.07±0.40 | 35.59 ± 0.78 | 16.52 |
| 2nd experimental | 19.14±0.37 | 34.37 ± 0.76 | 15.23 |
| 3rd experienced | 18.83±0.38 | 34.61 ± 0.69 | 15.78 |
| Rats | | | |
| Intact | 192.12±2.49 | 237.17 ± 3.47 | 45.05 |
| Control | 191.42±2.59 | 237.76 ± 3.62 | 46.34 |
| 1st experimental | 192.59±2.62 | 241.89 ± 3.59 | 49.30 |
| 2nd experimental | 191.58±2.54 | 239.82 ± 3.51 | 48.24 |
| 3rd experienced | 192.81±2.57 | 240.53 ± 3.49 | 47.72 |

When using a biological product for a long time, a slight superiority in the live weight of laboratory animals was established compared to the group where similar biological products were not used. Thus, Table 7 shows the absolute live weight of laboratory mice in the 1-3 experimental groups was higher at the end of the experiment than in the intact and control groups by 10.8 and 5.5%, 6.9 and 1.9%, 7.7 and 2.6%, respectively. The increase in the experimental groups was also

slightly higher than in the control group by 1.51 g (10.1 %), 0.22 g (1.5%) and 0.77 g (5.1%), respectively.

In the 1st experimental group of laboratory rats, the animals' weight was higher than in the control group by 1.7 %, in the 2nd by 0.9 % and in the 3rd-1.1 %. The increase in rats for the entire experiment in the 1st, 2nd and 3rd experimental groups was also slightly higher than in the control group by 2.96 g (6.4 %), 1.9 g (4.1%) and 1.38 g (2.9%), respectively.

The results of the developed biological product's effect on the morphological and biochemical parameters of the blood of laboratory animals are in Tables 8 and 9.

**Table 8**: Morphological and biochemical parameters of the blood of laboratory mice after the use of hydrolyzate (n = 6)

| Index | Group | | | | |
|---|---|---|---|---|---|
| | Intact | Control | 1st experimental | 2nd experimental | 3rd experimental |
| *Morphological indicators* | | | | | |
| Platelets, $10^9$ / l | 197.69±3.79 | 201.06±3.59 | 198.27±3.65 | 197.59±3.62 | 199.85±3.71 |
| Erythrocytes, $10^{12}$ / l | 8.53±0.32 | 8.47±0.28 | 8.56±0.27 | 8.49±0.29 | 8.55±0.30 |
| Leukocytes, $10^9$ / l | 7.34±0.18 | 7.31±0.20 | 7.29±0.17 | 7.27±0.16 | 7.30±0.17 |
| Hemoglobin, g / l | 107.48±2.86 | 108.12±2.68 | 107.75±2.74 | 108.86±2.74 | 109.15±2.69 |
| *Biochemical indicators* | | | | | |
| Total protein, g / l | 55.34±1.76 | 54.79±1.63 | 56.87±0.73 | 55.87±1.62 | 56.76±1.69 |
| albumin, g / l | 22.56±0.65 | 21.98±0.66 | 22.59±0.71 | 22.63±0.68 | 22.51±0.70 |
| globulin, g / l | 32.78±0.72 | 32.81±0.78 | 34.28±0.79 | 33.24±0.76* | 34.25±0.78 |
| A / H ratio | 0.68±0.02 | 0.67±0.03 | 0.67±0.02 | 0.68±0.02 | 0.67±0.03 |
| Urea, mM / l | 2.85±0.05 | 2.86±0.04 | 2.79±0.04 | 2.81±0.04 | 2.82±0.03 |
| Cholesterol, mM / L | 6.54±0.24 | 6.32±0.22 | 6.28±0.27 | 6.11±0.20 | 6.48±0.24 |
| Calcium, mM / l | 2.04±0.04 | 2.01±0.03 | 2.08±0.03 | 2.11±0.04 | 2.07±0.03 |
| Phosphorus, mM / l | 1.04±0.03 | 1.07±0.02 | 1.11±0.03 | 1.08±0.03 | 1.09±0.03 |
| AsAT, U / l | 117.96±3.25 | 119.62±3.52 | 119.72±3.43 | 118.94±3.41 | 118.78±3.32 |
| ALAT, U / l | 85.21±1.83 | 84.94±1.93 | 86.51±1.92 | 85.38±1.85 | 86.91±1.91 |

**Table 9**: Morphological and biochemical parameters of the blood of laboratory rats after the use of hydrolyzate (n = 6)

| Index | Group | | | | |
|---|---|---|---|---|---|
| | Intact | Control | 1st experimental | 2nd experimental | 3rd experienced |
| *Morphological indicators* | | | | | |
| Erythrocytes, $10^{12}$ / l | 5.32±0.11 | 5.31±0.09 | 5.38±0.12 | 5.32±0.08 | 5.34±0.11 |
| Leukocytes, $10^9$ / l | 10.58±0.32 | 10.37±0.31 | 10.47±0.27 | 10.51±0.29 | 10.42±0.31 |
| Platelets, $10^9$ / l | 131.54±2.87 | 137.82±3.02 | 133.32±3.11 | 138.94±2.98 | 135.52±2.96 |
| Hemoglobin, g / l | 119.29±3.43 | 117.52±3.52 | 118.98±3.59 | 118.38±3.45 | 119.63±3.51 |
| *Biochemical indicators* | | | | | |
| Total protein, g / l | 63.61±1.69 | 63.89±1.62 | 64.79±1.59 | 65.07±1.64 | 64.68±1.66 |
| albumin, g / l | 27.83±0.56 | 28.11±0.53 | 29.15±0.51 | 28.57±0.49 | 29.42±0.55 |
| globulin, g / l | 35.78±0.76 | 35.78±0.81 | 35.64±0.73 | 36.50±0.70 | 35.26±0.77 |
| A / H ratio | 0.77±0.02 | 0.78±0.02 | 0.81±0.01 | 0.78±0.02 | 0.83±0.02 |
| Urea, mM / l | 5.13±0.15 | 5.18±0.19 | 5.21±0.14 | 5.25±0.16 | 5.19±0.17 |
| Cholesterol, mM / L | 2.25±0.05 | 2.27±0.04 | 2.14±0.04 | 2.11±0.04 | 2.09±0.03 |
| Calcium, mM / l | 2.15±0.06 | 2.17±0.05 | 2.19±0.04 | 2.20±0.04 | 2.14±0.06 |
| Phosphorus, mM / l | 11.02±0.33 | 11.23±0.32 | 11.18±0.29 | 11.05±0.34 | 11.09±0.32 |
| AsAT, U / l | 56.67±1.45 | 55.27±1.54 | 56.54±1.48 | 58.14±1.52 | 56.83±1.56 |
| ALAT, U / l | 66.38±1.32 | 63.11±1.12 | 61.93±1.09 | 62.13±1.21 | 60.73±1.17 |

From the data in Tables 8 and 9, it can be seen that, as in the case of subacute toxicity, all laboratory animals' indicators were within the physiological norm. A slight tendency to improve the studied parameters in the animals of the experimental groups was revealed. Deviations and pathologies through the morpho-biochemical status of the blood of mice and rats were not established.

When conducting a pathoanatomical autopsy of laboratory animals of all experimental groups, changes in the cavities and the structure of the internal organs of the studied biological objects, were not recorded.

Thus, the long-term use of lactic acid bacteria hydrolysate did not have a toxic effect on laboratory animals' body.

## 3.4 Study of Skin-Resorptive and Irritant Properties

As an additional study of the possible negative properties of hydrolysate on the animal body, a study of the skin-resorptive and irritating effects of the biological product on laboratory rabbits was conducted (see Figure 2).

**Figure 2**: Skin resorptive action studying of the hydrolyzate.

As a result of the conducted studies, it was found that according to GOST R ISO 10993-10-2009, the index of primary irritation in all cases was equal to zero in terms of the level of manifestation of reactions. The biological product did not have irritating properties on the skin and conjunctiva of the eyes.

Thus, the hydrolysate of lactic acid bacteria because in the context of tests it did not show a toxic and irritating effect on laboratory animals, can be used in industrial poultry farming regardless of the dose of application.

# 4 Conclusion

The complex of pharmacotoxicological studies has shown that the protein lysate of microbial origin used in the research work, obtained by thermal acid hydrolysis of the bacterial mass of lactobacilli isolated from the gastrointestinal tract of wild birds, is safe in the studied dosages. In the study of biologic in acute experiment set-lethal dose failed, had not observed death of experimental mice and rats, there were no signs of a breach of their health during the experiment. The animals remained mobile, active, and well ate poop, clinical symptoms of intoxication appear. Results of long-term experiments (subacute and chronic toxicity) They also confirmed the absence

of toxicological effects of the studied hydrolysate. Found that the biological product does not cause death of laboratory animals, there has been no loss of body weight during the experiment, but on the contrary, the growth of mice and rats slightly increased in the range of 2.0–8.0% of the animals remained mobile, active, condition of the hair and skin, as well as the coloration of the visible mucous membranes remained normal, indicators of morphological and biochemical blood composition were within the physiological norm. The results of cutaneous applications and conjunctival tests on laboratory rabbits demonstrated the absence of the studied protein hydrolysate's irritating effect, since there were no signs of edema, erythemas, erosions, purulent effusions.

## 5 Availability of Data And Material

Data can be made available by contacting the corresponding authors.

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## 7 References

GOST. (2000). Medical products. Assessment of the biological effect of medical devices. Part 10. Investigation of irritant and sensitizing effects, GOST R ISO 10993.10-99, 1999-12-29, Gosstandart of Russia, 38 p.

Tishkov, A. I., Argunov, M. N., Lyashko, N. I. (1987). Guidelines for the toxicological assessment of new drugs for the treatment and prevention of non-communicable animal diseases. Voronezh, 23 p.

Smirnov, A. M., Dorozhin, V. I. (2008). Scientific and methodological aspects of the study of toxic properties of pharmacological drugs for animals. Russian Agricultural Academy, Moscow, 120 p.

SP 2.2.1.3218-14 (2014). Sanitary and epidemiological requirements for the device, equipment and maintenance of experimental biological clinics (vivariums), 7 p.

Khabriev, R.U. (2005). Guidelines for the experimental (preclinical) study of new pharmacological substances: publishing «Medicine», 832 p.

Lysenko, Yu.A., Koshchaev, A.G., Lysenko, A.A., Omarov, R.S., Shlykov, S.N. (2020). Biological properties of microorganisms isolated from drone milk of honeybees. *International Transaction Journal of Engineering, Management and Applied Sciences and Technologies*.11(12), 11A12K, 1-10.

Luneva, A.V., Lysenko, Yu.A., Koshchaev, A.G., Nesterenko, A.A., Guzenko, V.I. (2019). Comprehensive biosafety assessment of additives based on live microorganisms. *International Journal of Engineering & Advanced Technology*. 9(1), 2477-2483.

Lysenko, Yu.A., Koshchaev, A.G., Luneva, A.V., Omarov, R.S., Shlykov, S.N. (2021). Organic meat production of broiler chickens hubbard Redbro cross. *International Journal of Veterinary Science, 10*(1), 25-30.

Luneva, A., Koshchayev, A., Nesterenko, A., Volobueva, E., Boyko, A. (2020). Probiotic potential of microorganisms obtained from the intestines of wild birds. *International Transaction Journal of Engineering, Management and Applied Sciences and Technologies, 11*(12), 11A12E, 1-9.

Lysenko, Y., Luneva, A., Koshchaev, A., Lifentsova, M., Gorpinchenko, E. (2019). Quality assessment of biological product of microbial origin. *International Journal of Engineering & Advanced Technology*. 9(1), 2484-2488.

Koshchayev, A., Luneva, A., Murtazaev, K., Lysenko, Yu., Omarov, R. (2020). The study of the effectiveness of the use of a new feed supplement albit-bio in the diet of quail. *Advances in Animal & Veterinary Sciences*. 8(12), 1333-1339.

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