ISSN 2228-9860 eISSN 1906-9642 CODEN: ITJEA8

International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies

http://TuEngr.com

BIOSAFETY OF AUTOCHTON STRAINS OF MICROORGANISMS-PROBION AND THEIR JOINT COMPOSITION

Andrei Koshchayev [1*], Albina Luneva [2], Yury Lysenko [1], Alexander Lysenko [3], Azamat Shantyz [3]

[1] *Department of Biotechnology, Biochemistry and Biophysics, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA*

[2] *Department of Parasitology, Veterinary Examination and Pet Hygiene, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA*

[3] *Department of Therapy and Pharmacology, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA.*

**Corresponding Author (Email: Koshchayev @ mail.ru).*

Paper ID: 12A1L

Volume 12 Issue 1

Received 02 September 2020
Received in revised form 02 November 2020
Accepted 06 November 2020
Available online 07 November 2020

Keywords:
Acute toxicity, Chronic toxicity, Lactobacillus, Harmlessness, Toxigenicity, Probiotic, Virulence, Probiont strains, Mice, Rats.

Abstract

The research work presents the results of studying the virulence, toxigenicity, and various types of toxicity of probiont strains isolated and identified by modern molecular genetic and microbiological methods from wild birds' gastrointestinal tract. In this work, laboratory animals were used for research, particularly nonlinear mice, rats, and albino rabbits. As a result of a complex of preclinical studies, it was revealed that experimental autochthonous strains of lactobacilli do not show signs of virulence, toxicity, toxicity, and a positive reaction to the production of hemolysin. In experiments on acute and chronic toxicity, regardless of the concentration of the studied strains introduced into the organism of laboratory animals, as well as their joint consortium in the composition of the probiotic, no death of the experimental animals was revealed, there were no signs of health disorders and body weight loss during the experiment. In general, the study results showed that the studied cultures of microorganisms and their consortium do not have a toxic effect on the organism of laboratory animals, both with a single and long-term use in the diet, which confirms their safety.

Disciplinary: Biotechnology, Bioscience.

Cite This Article:

Koshchayev, A., Luneva, A., Lysenko, Y., Lysenko, A., Shantyz, A. (2021). Biosafety of Autochton Strains of Microorganisms-Probion and Their Joint Composition. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies, 12*(1), 12A1L, 1-11. http://TUENGR.COM/V12/12A1L.pdf DOI: 10.14456/ITJEMAST.2021.12

1. Introduction

Microflora, acting as antagonists to pathogenic microflora due to the significant content of important biologically active components in them and their ability to rapidly grow and accumulate large biomass.

The use of bacteria as raw materials to develop new-generation biological products capable of exhibiting probiotic and immunostimulating properties is profitable and promising. Therefore, in recent decades, a trend has changed the composition of microorganisms in the gastrointestinal tract, which has led to the creation of a new class of bacterial drugs - probiotics [1, 7].

The most crucial task in probiotics design is selecting 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 microorganisms that inhabit the tract of animals and humans [1, 5, 6, 8].

The study of scientific and patent material showed that today there are no effective designs of probiotic supplements prepared using the autochthonous (own) microflora of the gastrointestinal tract for use in industrial poultry farming.

This research aims to study the biosafety of new strains isolated from the gastrointestinal tract of wild birds.

2. METHOD

Research work was carried out at the Department of Biotechnology, Biochemistry, and Biophysics, as well as in the laboratory of preclinical and clinical studies of veterinary medicinal products and feed additives at the FSBEI HE Kuban State Agrarian University.

Determination of virulence, toxicity, toxicity, and harmlessness (hemolysin test), as well as the study of "acute" and "chronic" toxicity of probiont strains and their combined composition, was carried out on nonlinear mice (18-20 g) and rats (180-200 g) of both sexes according to methodological recommendations [2, 3]. Before setting up the experiment, laboratory animals were quarantined (acclimatization period), the duration of which was at least 3-4 days. During the quarantine, the experimental animals were examined daily (general condition and behavior). Light mode: 12 h light, 12 h darkness. The air temperature was maintained within the range of 19-25°C, relative humidity 65-70%. Experimental animals' maintenance corresponded to the current sanitary rules for the design, equipment, and maintenance of experimental biological clinics (vivariums) [4]. The formation of groups of experimental and control animals was carried out by the method of pairs-analogs.

Determination of the virulence of the tested strains. The 2nd passage culture, grown on a solid nutrient medium, was washed off with a 0.9% sodium chloride solution. In the resulting suspension, the concentration of microbial cells was determined using an optical turbidity standard. A series of tenfold dilutions were made from the resulting suspension. The resulting suspension of various concentrations (doses) of microbes was administered orally. The experiment used at least 10 animals per group. The administered dose was contained in a volume of 1.0 ml. The

test suspension of probiont strains was administered to mice fractionally during the day. The observation period was 14 days. The animals were observed daily, noting the number of live and dead animals in the experiment protocol. At the end of the observation period, the LD_{50} was calculated.

Determination of the toxigenicity of the tested strains. The same principle studied the toxicity of microbes as virulence. For toxigenicity determining, the test strain culture was sown on a liquid nutrient medium, kept in a thermostat at an optimum temperature for growth for ten days to accumulate the toxin in it if the strain produces it. Then it was filtered through a bacterial filter. The resulting clear filtrate was introduced undiluted. Each dose of the filtrate was tested simultaneously on 10 laboratory animals when administered intraperitoneally.

Determination of the toxicity of the tested strains. The toxicity of the tested strains was checked by intraperitoneal administration of a suspension of the test strain (at the maximum concentration of microbial cells) killed by heating at a temperature of 100°C for 30 min. The heated culture, native, was injected into 10 animals in a volume of 1.0 ml and determined the LD_{50} or maximum tolerated dose. The animals were observed for 14 days.

Hemolysin test. 5% defibrinated ram blood was added to the MRS nutrient medium melted and cooled to 48-50°C. After pouring the medium into Petri dishes in a thin layer (1.5-2 mm), an 18-hour culture was inoculated on the solidified and dried surface by streaking to obtain isolated colonies. The dishes were incubated at a temperature of (37±1)±C for 24-48 h, after which the results were recorded.

Determination of the dermonecrotic properties of the tested strains. For this purpose, white-skinned rabbits of the chinchilla breed weighing 1.5-2.5 kg were used in 3 heads per group. Different microbial suspension concentrations in a volume of 0.1-0.2 ml were injected intradermally into the back. The skin at the suspension injection site was preliminarily freed from wool and treated with 70% ethanol. At the injection site, the skin was stretched with the left hand's fingers, and the needle was inserted with the right hand at an acute angle. The daily result was recorded for 3-4 days. The appearance of swelling, redness, and the presence of necrosis were noted.

Study of "acute" toxicity. In the study of "acute" toxicity, three different doses of the tested cultures of strains and a probiotic were administered once orally to determine the LD50 or the maximum tolerated dose. The control group of animals was injected with a 0.9% sodium chloride solution in the same way. Intact animals were formed from the same group (control group 2). The total duration of observations of the animals was 7 days. The animals' death, body weight, and the presence or absence of possible clinical symptoms of intoxication, including impaired coordination of movement, presence of seizures, their nature, and scalp condition, the color of mucous membranes, and tail position were recorded daily.

Determination of "chronic" toxicity. The strains and their composition as part of the probiotic were administered daily, the duration of administration was 30 days. "Chronic toxicity" was

determined in nonlinear mice and rats. Observation of the animals was carried out during the introduction of strains and biological products and the next 7 days. The death of animals, body weight, as well as presence or absence of possible clinical intoxication symptoms, including impaired coordination of movement, presence of seizures, their nature, and scalp condition, the color of mucous membranes, tail position, were recorded daily. At the end of the experiment, blood was taken to analyze its morpho-biochemical parameters, and pathomorphological studies were carried out, taking material from the internal organs of surviving animals. Before the collection of internal organs, they were visually examined, and macroscopic changes were recorded.

The results obtained in the course of research experiments were processed by the method of variation statistics. The difference was regarded as significant at $P < 0.05$.

3. RESULT AND DISCUSSION

3.1 Virulence of the Tested Strains

Virulence is a biological property of microorganisms that characterizes the degree of their pathogenicity. In contrast to pathogenicity, virulence is not specific, but an individual feature of a microbe, which can be intensified or weakened up to complete disappearance under the influence of various factors. The virulence results of the studied cultures are in Tables 1 and 2.

Table 1: Results of studying the virulence of *Lactobacillus sp. 1* in mice and rats (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	Strain dose, CFU/ml	The test result, heads		
				got sick	dead	survived
1st experimental	mice	1.0 ml Lactobacillus sp.1, orally	5.4×10^{10}	0	0	10
	rats					10
2nd experimental	mice		5.4×10^{9}	0	0	10
	rats					10
3rd experienced	mice		5.4×10^{8}	0	0	10
	rats					10

Table 2: Results of studying the virulence of *Lactobacillus sp. 2* in mice and rats (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	Strain dose, CFU/ml	The test result, heads		
				got sick	dead	survived
1st experimental	mice	1,0 ml Lactobacillus sp.2, orally	3.5×10^{10}	0	0	10
	rats					10
2nd experimental	mice		3.5×10^{9}	0	0	10
	rats					10
3rd experienced	mice		3.5×10^{8}	0	0	10
	rats					10

The results of the virulence of probiont strains used showed that, regardless of the concentration of laboratory animal cultures introduced into the body, no death of experimental animals was detected, there were no signs of health disorders and body weight loss by the end of the observation period (on the 14th day). It was not possible to determine LD_{50}, and the initial concentration of the tested strains was taken as the maximum tolerated dose, which for *Lactobacillus sp. 1* was 5.4 x 10^{10} CFU/ml, and for *Lactobacillus sp.2* 3.5 x 10^{10} CFU/ml, which will then be used as starting points in the assessment of "acute toxicity". Thus, the studied cultures of lactobacilli do not show virulence.

3.2 Toxicity of the Tested Strains

The results of studying the toxigenicity of the probiont strains under study are in Table 3. The absence of death of animals, signs of health disorders, and loss of body weight in mice and rats by the end of the experiment was established, which indicates the absence of a toxic agent in the filtrate of the tested cultures. Thus, Lactobacillus sp. 1 and sp. 2 are not toxigenic.

Table 3: Results of studying the toxigenicity of the studied cultures in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	The test result, heads		
			got sick	dead	survived
1st experimental	mice	1.0 ml filtrate *Lactobacillus sp.1*, intraperitoneally	0	0	10
	rats				10
2nd experimental	mice	1.0 ml filtrate *Lactobacillus sp.2*, intraperitoneally	0	0	10
	rats				10

3.3 Toxicity of the Tested Strains

The results of the study of the toxicity of the studied lactobacilli show in Table 4. As a result of studying the toxicity of the strains in the experimental groups, all experimental animals survived. No signs of health disorders and loss of body weight in mice and rats were recorded by the end of the experiment. Thus, the probiont strains under study are not toxic.

Table 4: Results of studying the toxicity of the studied cultures in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	The test result, heads		
			got sick	dead	survived
1st experimental	mice	1.0 ml dead weight *Lactobacillus sp.1*, intraperitoneally	0	0	10
	rats				10
2nd experimental	mice	1.0 ml dead weight *Lactobacillus sp.2*, intraperitoneally	0	0	10
	rats				10

3.4 *Hemolysin Test*

An additional indicator of the safety of the tested strains is the hemolysin production test. Some bacteria produce hemolysins (substances that destroy red blood cells), which are pathogenic factors. In this regard, the production of hemolysin in many cases is a marker of virulence. On blood agar, the grown colonies of such microorganisms surround the zones of enlightenment, see the results in Figure 1.

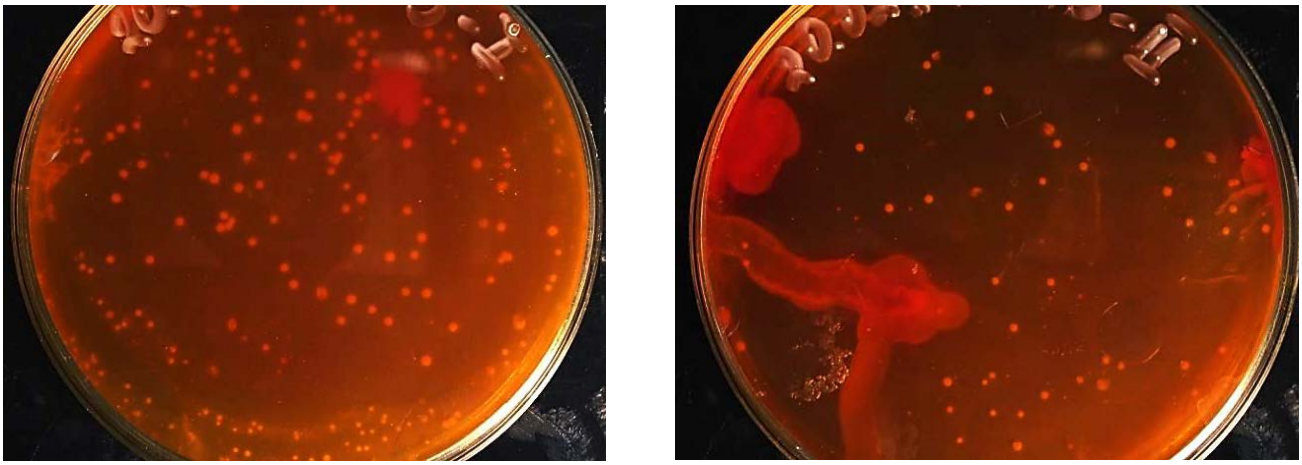

Figure 1: Hemolysin production by lactobacilli (no clearing zones)

The hemolytic properties of microbes were studied on an MRS medium with defibrinated ram blood. As a result of the experiment, during the cultivation of the studied strains on 5% blood agar, the hemolysis (clearing) zone around the colonies was not revealed, the result of hemolytic properties was negative. Thus, the cultures of lactic acid bacteria used are safe.

3.5 Dermonecrotic Properties of Strains

Three groups of rabbits were formed. The control group, which were injected subcutaneously with saline in a volume of 0.1 ml, and two experimental ones, which were injected with the corresponding microbial suspension in a volume of 0.1 ml. The result was taken into account daily for 4 days. The injection place of suspension is shown in Figure 2.

As a result of the experiment, there was no swelling, redness, areas of necrosis, and other pathologies at the injection site of solutions both in the control and in the experimental groups. Thus, the probiont strains under study do not have dermonecrotic activity. The volume of injected fluid, the method of medication.

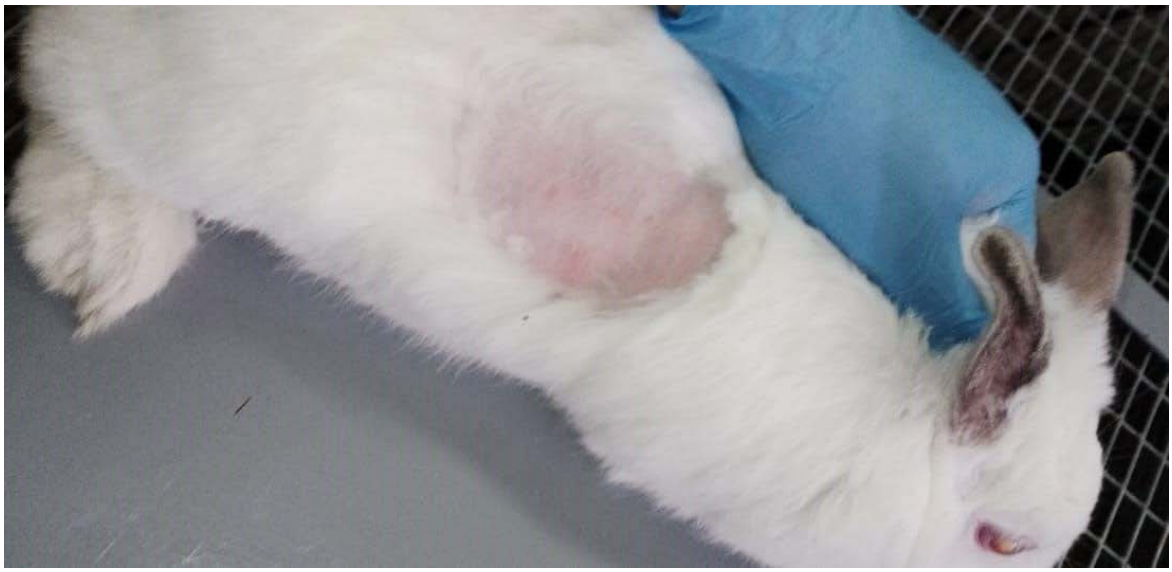

Figure 2: Injection area of research objects

3.5.1 Acute Toxicity

The study of the parameters of "acute" toxicity of the studied cultures and their consortium in the composition of the probiotic was carried out on nonlinear white mice and rats. The maximum tolerated concentrations studied in probiont strains' virulence study, namely, for Lactobacillus sp.1 5.4 x 1010 CFU/ml and Lactobacillus sp.*2* 3.5 x 10^{10} CFU/ml. Three doses were studied for their joint composition. As the maximum allowable dose, the concentration of microorganism cells contained in 1.0 ml of the probiotic was used, and additionally, doses contained in volumes of 0.2 and 0.5 ml were used. The acute toxicity results are in Table 5.

The results of a 7-day study of "acute" toxicity showed that in the 1st control group (intact), in the 2nd control group, and all experimental groups (1st to 5th) with a daily observation of laboratory animals, it was revealed that they remained mobile, active, ate food mixtures well, deaths of mice and rats were not registered, clinical symptoms of intoxication did not appear, the

condition of the scalp, as well as the color of the visible mucous membranes, were normal. Thus, the studied probiont strains and their combined composition (probiotic) do not cause severe toxicosis in laboratory animals.

Table 5: Results of the study of acute toxicity of the studied cultures and probiotic in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	Strain dose, CFU/ml	The test result, heads		
				got sick	dead	survived
1st control (intact)	mice	–	–	0	0	10
	rats					10
2nd control	mice	1.0 ml physical solution, orally	–	0	0	10
	rats					10
1st experimental	mice	1.0 ml Lactobacillus sp. 1, orally	5.4×10^{10}	0	0	10
	rats					10
2nd experimental	mice	1.0 ml Lactobacillus sp. 2, orally	3.5×10^{10}	0	0	10
	rats					10
3rd experienced	mice	0.2 ml probiotic, orally	1.8×10^{9}	0	0	10
	rats					10
4th experimental	mice	0.5 ml probiotic, orally	4.5×10^{9}	0	0	10
	rats					10
5th experienced	mice	1.0 ml probiotic, orally	8.9×10^{9}	0	0	10
	rats					10

3.5.2 Chronic Toxicity

The chronic toxicological experiments are to characterize the degree of the damaging effect of the studied strains and probiotic supplements during their long-term administration (30 days), to identify the most sensitive organs and systems of the body, as well as to study the degree of reversibility of the damage caused by them. As the studied doses for the test cultures and their joint composition in the probiotic composition, the maximum tolerated concentrations studied in the study of "acute" toxicity were used. Chronic toxicity results are in Table 6.

Table 6: Results of studying the "chronic" toxicity of the studied cultures and probiotic in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of medication	Strain dose, CFU/ml	The test result, heads		
				got sick	dead	survived
1st control (intact)	mice	–	–	0	0	10
	rats					10
2nd control	mice	1.0 ml physical solution. orally	–	0	0	10
	rats					10
1st experimental	mice	1.0 ml Lactobacillus sp. 1. orally	5.4×10^{10}	0	0	10
	rats					10
2nd experimental	mice	1.0 ml Lactobacillus sp. 2. orally	3.5×10^{10}	0	0	10
	rats					10
3rd experienced	mice	1.0 ml probiotic. orally	8.9×10^{9}	0	0	10
	rats					10

The results of daily visual observation of the behavior of laboratory mice and rats for 30 days and the next 7 days showed that the experimental animals in all groups remained active and mobile, satisfactorily ate food and consumed water, clinical signs of intoxication and mortality of biological objects from the action of the studied strains and probiotic not found. The coat was smooth with a characteristic luster. The skin and the color of the visible mucous membranes were pale pinks, typical of healthy animals.

When carrying out "chronic" toxicity, we studied the effect of the test objects on the weight gain of laboratory mice and rats during the period of experiments. The weights of laboratory animals were taken into account at the beginning experiment and the 30th day after its completion. The results of the live weight of laboratory animals are in Table 7.

Table 7: Results of the influence of the studied cultures and probiotic on the live weight of laboratory animals

Group	Body weight, g		Experience gain, g
	at the beginning of the experiment	at the end of the experiment	
Mice			
1st control (intact)	18.5 ± 0.3	22.4 ± 0.3	3.9
2nd control	18.3 ± 0.4	22.5 ± 0.4	4.2
1st experimental	18.5 ± 0.3	24.2 ± 0.4	5.7
2nd experimental	18.7 ± 0.4	24.5 ± 0.4	5.8
3rd experienced	18.6 ± 0.3	26.8 ± 0.3*	8.2
Rats			
1st control (intact)	193.5 ± 2.1	210.8 ± 1.8	17.3
2nd control	192.7 ± 2.3	211.3 ± 1.8	18.6
1st experimental	193.3 ± 1.9	217.7 ± 2.1	24.4
2nd experimental	191.9 ± 2.0	218.3 ± 2.0	26.4
3rd experienced	192.2 ± 2.1	222.6 ± 1.8*	30.4

* The difference with the 2nd control group is significant ($P < 0.05$)

When studying the influence of the studied cultures of lactic acid bacteria and their compositional form of probiotic on the live weight of laboratory animals, it was found that in the 1st and 2nd experimental groups of mice, compared with the same in the 2nd control group, there was a positive dynamics in live weight, which was higher by 7.6 and 8.9%, respectively, but the difference was not significant. A similar situation was recorded in the groups of laboratory rats. In the 1st and 2nd experimental groups, there was an increase in animals' live weight compared to the 2nd control by 3.0 and 3.3%. Statistically significant differences in live weight were found in the 3rd experimental group of mice and rats concerning the 2nd control group. So in the 3rd experimental group of mice at the end of the experiment, the live weight exceeded the 2nd control group of mice by 19.1% ($P <0.05$), and in rats by 5.3% ($P <0.05$) with significant differences.

The influence of the probiont strains and probiotic additives on the morphological and biochemical parameters of the blood of laboratory animals was studied. The research results are in Tables 8 and 9.

Table 8: Data on morphological and biochemical parameters of the blood of mice (n = 6)

Index	Group				
	1st control (intact)	2nd control	1st experimental	2nd experimental	3rd experienced
Leukocytes, 10^9 / l	7.5±0.2	7.4±0.2	7.5±0.3	7.6±0.3	7.7±0.2
Erythrocytes, 10^{12} / l	8.3±0.2	8.4±0.2	8.5±0.3	8.5±0.2	8.7±0.3
Platelets, 10^9 / l	241.2±3.1	240.4±3.3	242.8±3.1	242.2±3.3	243.6±3.4
Hemoglobin, g / l	122.3±2.3	123.4±2.5	125.4±2.5	125.8±2.3	126.6±2.4
Total protein, g / l	52.5±1.2	52.8±1.1	54.3±1.0	53.8±1.1	56.3±1.3
Glucose, mM / l	4.5±0.2	4.6±0.1	4.6±0.1	4.7±0.2	4.6±0.1
Urea, mM / l	6.2±0.2	6.4±0.1	6.4±0.2	6.3±0.2	6.4±0.2
Cholesterol, mM / L	2.5±0.1	2.5±0.1	2.4±0.1	2.5±0.1	2.3±0.1
Calcium, mM / l	2.3±0.1	2.4±0.1	2.3±0.1	2.4±0.1	2.5±0.1
Phosphorus, mM / l	1.3±0.02	1.3±0.03	1.4±0.01	1.4±0.02	1.5±0.02
AsAT, U / l	115.6±3.5	113.8±3.6	116.5±3.5	115.7±3.4	116.4±3.6
ALAT, U / l	55.4±2.7	56.7±2.9	54.7±2.6	55.84±2.9	56.4±2.7

Studying the morphological and biochemical data of the blood of laboratory mice showed positive dynamics in the experimental groups in terms of the studied parameters. However, in the groups' context, no significant difference was found. Moreover, all indicators were within the physiological norm for a given type of laboratory animal. The positive dynamics in erythrocytes content should be noted, which level in the 1st, 2nd, and 3rd experimental groups was higher than in the 2nd control group by 1.2 and 3.6%, platelets 1.0; 0.7 and 1.3% and hemoglobin by 1.6; 1.9 and 2.6%. The positive dynamics in the studied indicators indicate a better saturation of organs and tissues of mice with oxygen since hemoglobin is responsible for binding with oxygen, and red blood cells (erythrocytes) carry it with the bloodstream. As a result, an acceleration of redox reactions in the body is observed. The amount of total protein in the mice's blood in the experimental groups also slightly exceeded the control groups. In contrast, the 1st experimental group's total protein was higher than in the 2nd control by 2.8%, in the 2nd experimental group by 1.9%. The highest value was observed in the 3rd experimental group, which surpassed the similar control group by 6.6%. No particular difference was found for the rest of the biochemical parameters, and they were at the normal level.

Table 9: Data on morphological and biochemical parameters of the blood of rats (n = 6)

Index	Group				
	1st control (intact)	2nd control	1st experimental	2nd experimental	3rd experienced
Leukocytes, 10^9 / l	11.3±0.3	11.5±0.4	11.3±0.4	11.4±0.3	11.5±0.4
Hemoglobin, g / l	103.6±3.7	104.7±3.6	106.1±3.7	106.4±3.8	107.4±3.6
Platelets, 10^9 / l	152.5±3.6	153.3±3.5	154.3±3.4	154.8±3.2	155.8±3.4
Erythrocytes, 10^{12} / l	5.3±0.2	5.3±0.1	5.5±0.2	5.4±0.2	5.5±0.1
Total protein, g / l	66.1±1.5	65.1±1.3	68.6±1.6	68.5±1.8	69.3±1.5
Glucose, mM / l	4.1±0.2	4.2±0.1	4.2±0.2	4.2±0.1	4.2±0.2
Urea, mM / l	5.6±0.1	5.6±0.2	5.5±0.2	5.6±0.1	5.5±0.2
Cholesterol, mM / L	1.8±0.04	1.8±0.03	1.7±0.03	1.8±0.05	1.8±0.04
Calcium, mM / l	2.4±0.1	2.5±0.1	2.6±0.1	2.6±0.1	2.6±0.1
Phosphorus, mM / l	13.1±0.3	13.2±0.3	13.3±0.4	13.4±0.2	13.5±0.3
AsAT, U / l	53.6±2.1	54.5±1.8	55.4±2.2	54.3±1.8	55.1±1.9
ALAT, U / l	65.3±2.4	65.6±2.3	65.4±1.9	66.3±2.3	66.7±2.1

The analysis of the blood of laboratory rats' morphological and biochemical parameters also revealed a positive trend in the studied parameters in the experimental groups, in the diet of which the studied cultures and the probiotic were used. Similarly to mice, erythrocytes, hemoglobin, platelets, total protein, calcium, and phosphorus levels were also slightly increased in the blood of rats of the experimental groups, within the physiological norm, which confirms the positive effect of the objects of research on the organism of laboratory animals.

During the postmortem examination of mice and rats of all the studied groups, changes in the location and structure of internal organs were not established; from the perspective of anatomy, the organs in the cavities were located correctly. Deviations and changes in the macroscopic structure of organs were not established, fluid in the cavities was not detected, the presence of fat was noted in the retroperitoneal tissue, and in the perirenal state, the lumen of the trachea and bronchi of mucus, catarrhal and purulent exudate was not found, the lung tissue was pink, without foci of necrosis.

The submandibular lymph nodes and salivary glands were rounded, uniform pinkish or yellowish, and moderately dense. The thyroid gland was tightly attached to the larynx, had a standard size and density, pinkish-reddish in color.

The stomach is not enlarged, the lumen is not filled with anything. The mucous membrane of the stomach is pale pink, shiny, folded. The mucous membranes of the small and large intestines are unchanged.

Liver - not enlarged, dark cherry color, with a smooth surface and moderately dense texture. The liver capsule is thin, transparent, smooth, shiny, without foci of necrosis and degenerative changes.

Heart - no pathological changes.

The lungs are pale pink, without pathologies.

The kidneys are not enlarged in volume. The tissue is elastic, the kidneys' capsule is easily separable, and the cortex and medulla layers are visible on the cut. Hemorrhages and macrostructural changes in the renal tissue were not identified.

The spleen is dark cherry, with a smooth surface, dense texture.

In general, the results of comprehensive studies indicate that there is no additional load on the organs and systems of the body with prolonged oral administration of a probiotic supplement.

4. CONCLUSION

The probiont strains used in this work, which have been isolated and identified from the gastrointestinal tract of wild birds by modern molecular genetic and microbiological methods, are safe. As a result of a complex of preclinical studies, it was revealed that these lactobacilli cultures do not show signs of virulence, toxicity, toxicity, and a positive reaction to the production of hemolysin. In experiments on acute and chronic toxicity, regardless of the concentration of the studied strains introduced into the organism of laboratory animals, as well as their joint consortium in the composition of the probiotic, no death of experimental animals was revealed, there were no signs of health disorders and loss of body weight during the experiment, animals remained mobile, active, ate feed mixtures well, no clinical symptoms of intoxication appeared, the condition of the scalp, as well as the color of the visible mucous membranes, remained normal. In general, the study results showed that the studied cultures of microorganisms and their probiotic form do not have a toxic effect on the organism of laboratory animals, both with a single and long-term use in the diet, which confirms their safety.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

6. ACKNOWLEDGEMENT

The research was carried out with the Kuban Science Foundation's financial support in the framework of the scientific project No. MFI-20.1/80.

7. REFERENCES

[1] Lysenko Yu.A. (2020). Development and pharmacological substantiation of the use of new feed additives

of microbial origin in poultry meat farming. Dissertation of the doctor of biological sciences, 476p.

[2] Guidelines for the sanitary and epidemiological assessment of the safety and functional potential of probiotic microorganisms used for food production (MU 2.3.2.2789-10), 104p.

[3] System for pre-registration preclinical study of drug safety. Selection, verification and storage of industrial strains used in the production of probiotics. Methodical instructions (MUK 4.2.2602-10), 60p.

[4] Sanitary and Epidemiological Rules. Sanitary and Epidemiological Requirements for the Design, Equipment and Maintenance of Experimental Biological Clinics (Vivariums). SP 2.2.1.3218-14, 7p.

[5] Radchenko V.V., Ilnitskaya E.V., Shuvaeva T.M., Alexandrova M.V., Luneva A.V., Lysenko Yu.A., Basova N.Yu., Nekrasov A.N., Koschaev A.G. (2020). Comparative analysis and probiotic potential of new strains of the genus Lactobacillus from evolutionarily fixed associations of the gastrointestinal tract of wild birds. *Biopharmaceutical Journal*, *12*(1), 44-49.

[6] 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. DOI: 10.14456/ITJEMAST.2020.236

[7] Luneva A., Lysenko Y., Koshchaev A., Nesterenko A., Guzenko V. (2019). Comprehensive biosafety assessment of additives based on live microorganisms. *International Journal of Engineering and Advanced Technology*, 9(1), 2477-2483.

[8] 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. DOI: 10.14456/ITJEMAST.2020.230

Professor Dr.Andrei Koshchayev is at the Department of Biotechnology, Biochemistry and Biophysics, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA. He holds a Doctor of Biological Sciences

Albina Luneva is an Associate Professor of the Department Parasitology, Veterinary Examination and Pet Hygiene, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA. She is a Candidate of Biological Sciences.

Yury Lysenko is an Associate Professor of the Department of Biotechnology, Biochemistry and Biophysics, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA. He is a Candidate of Biological Sciences

Professor Dr.Alexander Lysenko is at the Department of Therapy and Pharmacology, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA. He holds a Doctor of Veterinary Sciences

Professor Dr.Azamat Shantyz is at the Department of Therapy and Pharmacology, Kuban State Agrarian University named after I.T. Trubilin, RUSSIA. He holds a Doctor of Veterinary Sciences.