Comprehensive Biosafety Assessment of Additives based on Live Microorganisms

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***Abstract:** When developing veterinary or feed preparations, a mandatory procedure is the study of their toxicological properties in living bio-objects. The purpose of this research work was to study the toxicological effect of probiotic on the basis of autochthonous microflora of birds by identifying the parameters of acute, subacute and chronic toxicity, as well as irritating characteristics in experimental laboratory animals. In studies of subacute and chronic toxicity, the pharmacodynamics of a probiotic was studied by its effect on individual metabolic parameters, morphobiochemical composition of blood, and also the growth rate of experimental laboratory animals. A complex of toxicological studies of the developed biological product showed that the probiotic does not exhibit toxic and irritating properties, stimulates hematopoiesis, erythropoiesis, there is a slight increase in the mass of laboratory animals in connection with which it can be used for its intended purpose, regardless of the dose.*

***Keywords :** probiotic, laboratory animals, toxicity, biochemical composition of blood.*

I. INTRODUCTION

One of the main problems of veterinary medicine is the emergence of microorganisms with pathogenic properties that have become resistant to a number of antibiotic drugs. And the widespread use of these antibiotics as part of feed rations helps to reduce the own immunity of farm animals and birds. In this case, inhibition in the gastrointestinal tract of one's own microbial background is observed, which leads to the development of diseases. In this connection, for the prevention and treatment of diseases caused by imbalance in the microbial balance, the use of biological products of microbial origin is relevant [2].

Natural normoflora takes an active part in maintaining the colonization stability of the intestinal mucosa and has a large role in preventing diseases. By isolating antibiotic substances into the intestinal environment, they contribute to the inhibition of the growth of pathogenic and conditionally pathogenic microblora, which is the causative agent of various internal non-communicable diseases. The beneficial microflora in the composition of biological products has an anti-infectious, immunomodulatory effect on the body, increases protective functions, and stimulates intestinal motility and excretory functions. Biological products based on live microflora are used both for prophylaxis and therapy. However, each species of animal and bird has its own microorganisms with probiotic properties. In this connection, the use of probiotics developed on the basis of living cultures of microorganisms that were isolated from the gastrointestinal tract from one type of living organism is not always effective when used in another species [3, 4].

It follows that the development of biological products based on useful native microflora, which can replace antibiotics, is an urgent area, and the study of the properties of the new isolated useful microflora is both scientific and practical.

The aim of this research work was to study the toxicological effect of probiotican based on autochthonous microflora isolated from the blind processes of the intestine of a poultry.

II. MATERIALS AND METHODS

The object of research was a liquid probiotic supplement consisting of a composition of three types of lactobacilli, which were isolated by an independent microbiological method and identified by real-time PCR, as well as by metagenomic methods, from blind processes of the quail gastrointestinal tract.

The study of the toxicity parameters of the developed probiotic was carried out according to the recommendations [5, 6]. The study of skin-resorptive action and irritating properties on the mucous membranes of the eyes was carried out on albino 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 within 14 days. Laboratory animals were divided into groups according to the principle of pair-analogues. The number of animals in each group was 10 animals of both sexes; the content of females and males was separate. Nonlinear white mice weighing 18–20 g (age 2.5 months) and sexually mature outbred white rats weighing 180–200 g (age 2.5 months) were used as laboratory research bioobjects. A study of skin-resorptive action was carried out on 2 sexually mature albino rabbits of different sexes (body weight 2.2-2.5 kg). A study of the irritating effect of probiotican conjunctiva was performed on 2 sexually mature albino rabbits of both sexes (weight 2.9–3.1 kg).

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III. RESULTS AND DISCUSSION

The study of acute toxicity. The results of the acute toxicity of the probiotic are presented in table 1. As a result of the experiment, it was found that in the experimental groups, no deaths and diseased animals were recorded in the experimental groups, as in the intact and control groups. During a daily examination of laboratory animals, it was possible to observe that mice and rats remained mobile and active, satisfactory food intake was recorded, while maintaining all vital reflexes. In the studied range of probiotic doses, mortality in the experimental groups was not detected, which indicates the absence of a semi-lethal dose. Thus, the probiotic supplement in the studied microbial concentrations does not cause severe toxicosis in laboratory animals, and therefore it can be attributed to the 4th hazard class (low toxic substances).

Table – I: Acute toxicity of probiotic laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of administration	Probiotic dose, CFU / ml	Test result, goal.		
				got sick	died	survived
Intact	mice	–	–	0	0	10
	rats	–	–	0	0	10
Control	mice	1.0 ml saline solution, by mouth	–	0	0	10
	rats	10.0 ml saline solution, by mouth	–	0	0	10
1st experienced	mice	0.2 ml of probiotic, by mouth	$1,6 \times 10^{9}$	0	0	10
	rats	2.0 ml probiotic, by mouth	$1,5 \times 10^{10}$	0	0	10
2nd experienced	mice	0.5 ml probiotic, by mouth	$3,9 \times 10^{9}$	0	0	10
	rats	5.0 ml probiotic, by mouth	$3,9 \times 10^{10}$	0	0	10
3rd experienced	mice	1.0 ml probiotic, by mouth	$7,9 \times 10^{9}$	0	0	10
	rats	10.0 ml probiotic, by mouth	$7,9 \times 10^{10}$	0	0	10

The study of subacute toxicity. Since it was not possible to determine the semi-lethal dose of the probiotic in an acute experiment, the maximum volume of the administered biological product for mice and rats (1.0 and 10.0 ml) was taken as the main dose in the study of subacute toxicity. Experimental laboratory animals were given a probiotic supplement inside the stomach at concentrations of 1/10, 1/20 and 1/50 of the maximum administered in an acute experiment. Thus, mice received a probiotic in a volume of, respectively, 0.1; 0.05 and 0.02 ml (7.9×10^{8}; 3.9×10^{8} and 1.5×10^{8} CFU / ml), and rats 1.0; 0.5 and 0.2 ml (7.9×10^{9}; 3.9×10^{9} and 1.5×10^{9} CFU / ml). In addition to the experimental groups, groups were also formed in which similar preparations were not used: the intact group — laboratory mice and rats did not receive additional solutions, except for standard drinking and feeding; control group — instead of the biological product, laboratory mice and rats received physiological saline in the maximum volume (1.0 and 10.0 ml, respectively). The introduction of solutions was carried out daily, through a probe, orally. The results of determining the subacute toxicity of the probiotic are presented in table 2.

Table – II: Subacute toxicity of probiotic supplements in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of administration	Probiotic dose, CFU / ml	Test result, goal.		
				got sick	died	survived
Intact	mice	–	–	0	0	10
	rats	–	–	0	0	10
Control	mice	1.0 ml saline, by mouth	–	0	0	10
	rats	10.0 ml saline, by mouth	–	0	0	10
1st experienced	mice	0.1 ml probiotic, by mouth	$7,9 \times 10^{8}$	0	0	10

	rats	1.0 ml probiotic, by mouth	$7,9 \times 10^9$	0	0	10
2nd experienced	mice	0.05 ml probiotic, by mouth	$3,9 \times 10^8$	0	0	10
	rats	0.5 ml probiotic, by mouth	$3,9 \times 10^9$	0	0	10
3rd experienced	mice	0.02 ml probiotic, by mouth	$1,5 \times 10^8$	0	0	10
	rats	0.2 ml probiotic, by mouth	$1,5 \times 10^9$	0	0	10

From an experiment to monitor the general condition and behavior of animals, it was found that the probiotic in the studied dosages did not have a negative effect on the organism of the experimental biological objects. Experimental animals satisfactorily tolerated doses of the biological product. Deaths - not detected. Mice and rats during the experiment were mobile and active, and the coat was smooth with a characteristic sheen.

The effect of probiotican was studied on the increase in live weight of laboratory animals during the research period. The live weight of mice and rats was taken into account before setting up the experiment and after its completion. The results of the experiment are presented in table 3.

Table – III: Effect of probiotic on the growth and development of laboratory animals

Group	Body weight g		Growth per experience, g
	at the beginning of the experiment	at the end of the experiment	
Mice			
Intact	18,87±0,27	25,63 ± 0,29	6,76
Control	18,56±0,25	26,14 ± 0,25	7,58
1st experienced	18,37±0,28	31,89 ± 0,24*	13,89
2nd experienced	18,43±0,30	30,58 ± 0,27*	12,15
3rd experienced	18,69±0,27	31,43 ± 0,26*	12,74
Rats			
Intact	189,75±1,67	222,63 ± 1,85	32,88
Control	190,12±1,58	224,36 ± 1,71	34,24
1st experienced	189,65±1,72	235,93 ± 1,68*	46,28
2nd experienced	189,71±1,59	234,71 ± 1,75*	45,00
3rd experienced	190,42±1,64	234,32 ± 1,63*	43,90
* Significant difference with control (P <0.05)			

It was found that the body weight of laboratory animals, in particular, the 1-3rd experimental groups was higher than in the control group with a statistically significant difference (P <0.05). So, in the 1st experimental group the body weight of mice was higher than in the control and intact groups by 22.1 and 24.4%, in the 2nd experimental group higher by 16.9 and 19.3%, and in 3 group 20.2 and 22.6%, respectively.

A similar trend was observed in experimental groups of laboratory rats. So in the 1-3rd experimental groups, the live weight of rats was significantly higher than in the control by 5.2; 4.6 and 4.4%, respectively (P <0.05).

A study was made of the effect of probiotic supplements on the body's metabolism in experimental groups of laboratory mice and rats through their morphobiochemical blood parameters (table 4 and 5).

Table – IV: Morphological and biochemical blood parameters of laboratory mice after application of a probiotic (n = 6)

Indicator	Group				
	Intact	Control	1st experienced	2nd experienced	3rd experienced
Morphological indicators					
Erythrocytes, 10^{12} / l	8,21±0,17	8,32±0,16	8,53±0,12	8,51±0,19	8,49±0,20
Hemoglobin, g / l	125,21±2,58	122,54±2,71	131,32±2,65	133,51±2,61	128,12±2,46
Platelets, 10^9 / L	235,32±3,12	238,94±3,42	241,26±3,33	245,14±3,26	245,63±3,64
White blood cells, 10^9 / l	7,32±0,21	7,31±0,19	7,29±0,18	7,30±0,17	7,26±0,22
Biochemical parameters					
Total protein, g / l	53,99±1,25	55,04±1,36	62,72±1,01*	63,47±1,12*	63,24±1,09*
albumin, g / l	22,45±0,65	22,53±0,79	27,34±0,77*	27,26±0,71*	27,79±0,74*

globulin, g / l	31,54±0,71	32,51±0,85	35,38±0,56	36,21±0,62	35,45±0,61
A / G ratio	0,71±0,03	0,69±0,02	0,77±0,03	0,75±0,02	0,78±0,02
Cholesterol, mmol / l	1,53±0,03	1,55±0,02	1,51±0,03	1,50±0,04	1,49±0,03
Urea, mmol / l	19,16±0,43	19,21±0,51	18,87±0,39	19,12±0,40	18,97±0,39
Calcium, mmol / l	2,37±0,05	2,31±0,04	2,38±0,04	2,41±0,05	2,35±0,03
Phosphorus, mM / L	1,32±0,03	1,36±0,02	1,42±0,03	1,49±0,04	1,43±0,02
AST, Unit / L	105,86±3,45	103,53±3,73	106,52±3,51	104,75±3,49	106,48±3,62
ALT, Unit / L	85,46±2,78	88,67±2,91	84,65±2,84	85,84±2,93	86,41±2,79

* Significant difference with control (P <0.05)

When analyzing the morphological and biochemical status of the blood of laboratory mice after using the probiotic, it was found that at the end of the subacute experiment, the blood counts were within the physiological norm for these biological research objects. Pathology in the metabolism was not detected. However, according to the level of individual indicators, it was possible to reveal that the use of the additive had a positive effect on the body of laboratory mice. So, in the 1st, 2nd and 3rd experimental groups of mice, the level of red blood cells and hemoglobin was more high than in the control group by 2.5 and 7.1%; 2.3 and 8.9%; 2.0 and 4.6%, respectively. The increase in the studied parameters indicates that there is an improvement in the saturation of organs and tissues of experimental animals, since hemoglobin is able to bind to oxygen, and red blood cells carry it with the blood flow through the body and, as a result, an increase in oxidation-reduction processes is observed. Also, hemoglobin increased within the normal range will provide the best release of carbon dioxide in the lungs. The leukocyte count in the experimental groups was within the physiological norm, and also was on the same level as the intact and control groups, which indicates the absence of negative effects on the body of laboratory mice from the used additives, since these blood cells are responsible for the specific and non-specific protection of the body from external and internal negative agents. A similar positive trend was found in the analysis of biochemical blood parameters of white mice. A statistically significant difference was revealed when studying the level of total protein and its individual components in the blood of the experimental groups. So, in the 1st experimental group, the content of total protein was higher than in the control by 13.9%, in the 2nd by 15.3% and the 3rd - 14.9% (P <0.05). The degree of intensity of the process of biosynthesis of protein components of the blood shows the protein coefficient (A / G), which in the first experimental group was slightly higher than in the control and intact groups by 11.6 and 8.5%, in the 2nd experimental group higher by 8 , 7 and 5.6%, and in the 3rd group by 13.0 and 9.8%, respectively. For other biochemical blood parameters, no significant difference was observed. In general, there were no negative consequences from the action of the probiotic, and in some cases its positive effect on the metabolism in the body was revealed.

Table – V: Morphological and biochemical blood parameters of laboratory rats after application of a probiotic (n = 6)

Indicator	Group				
	Intact	Control	1st experienced	2nd experienced	3rd experienced
Morphological indicators					
Erythrocytes, 10^{12} / l	5,32±0,12	5,37±0,13	5,51±0,11	5,47±0,15	5,46±0,17
Hemoglobin, g / l	102,63±3,78	105,73±3,62	110,11±3,71	109,63±3,84	112,42±3,67
Platelets, 10^9 / L	153,54±3,32	154,83±3,31	161,38±3,41	158,84±3,28	158,38±3,36
White blood cells, 10^9 / l	11,34±0,35	11,41±0,29	11,31±0,28	11,33±0,33	11,29±0,31
Biochemical parameters					
Total protein, g / l	66,13±1,34	65,13±1,21	68,80±1,36	68,51±1,31	69,60±1,27
albumin, g / l	29,64±0,82	28,98±0,78	31,12±0,76	31,02±0,79	31,78±0,81
globulin, g / l	36,49±0,74	36,15±0,69	37,68±0,54	37,49±0,59	37,82±0,61
A / G ratio	0,81±0,02	0,80±0,01	0,83±0,02	0,83±0,02	0,84±0,03
Cholesterol, mmol / l	1,87±0,03	1,83±0,04	1,79±0,03	1,81±0,03	1,81±0,03
Urea, mmol / l	5,64±0,16	5,60±0,19	5,58±0,15	5,62±0,13	5,61±0,14
Calcium, mmol / l	2,49±0,05	2,52±0,06	2,55±0,04	2,58±0,03	2,56±0,07
Phosphorus, mM / L	13,18±0,25	13,24±0,31	13,32±0,36	13,30±0,28	13,28±0,29
AST, Unit / L	153,65±3,85	155,48±3,67	158,54±3,74	156,38±3,48	156,21±3.54

ALT, Unit / L	135,17±3,45	139,24±3,21	139,49±3,67	141,82±3,42	138,15±3,33

* Significant difference with control (P <0.05)

When conducting subacute toxicity in laboratory rats, a positive effect of the studied additive on the morphological and biochemical status of animal blood was revealed. From table 5 it is seen that the content of red blood cells in the whole blood of the experimental groups of animals was slightly higher than in the control by 2.6; 1.9 and 1.7%, respectively. The level of hemoglobin in red blood cells compared with the control group in the 1st experimental group was higher by 4.1%, in the 2nd higher by 3.7%, and in the 3rd - by 6.3%. The number of other elements of whole blood was at the same physiologically normal level. In general, the morphological status of the blood of laboratory rats showed the absence of negative effects of the probiotic on the animal organism, and even vice versa, contributed to increased metabolism.

A similar positive dynamics was revealed in the analysis of biochemical blood parameters of experimental rats. Blood counts were located at the level of physiological norm. The total protein in the blood serum of the 1st, 2nd and 3rd experimental groups was higher than in the control group by 5.2 and 6.9%. There was no negative effect on the biochemical parameters of blood serum of laboratory rats.

Thus, the results of the subacute toxicity of the probiotic showed that the biological product did not have a negative (toxic) effect on the body of experimental laboratory mice and rats.

The study of chronic toxicity. The effect of probiotic on the body The results of a study of the effect of the biological product on the body weight of experimental laboratory animals, as well as the growth are presented in table 7. From the data of table 7 it was found that the introduction of probiotic in the diet of experimental groups had a statistically significant increase in the body weight of laboratory animals. In the first, in the second and third experimental groups of mice, the mass of animals at the end of the experiment was higher than in the control group by 3.85 g (10.9%), 3.56 g (10.1%) and 3.25 g (9.2%), respectively (P <0.05). The increase in bioobjects was similar to their body weight, more in the experimental groups than in the control group by 3.92 g (23.3%), 3.54 g (21.0%) and 3.3 g (19.6%) . In the 1st experimental group of laboratory rats, there was a tendency to increase the mass of animals relative to rats in the control group by 10.19 g or 4.1%. In the 2nd and 3rd experimental groups, the animal body weight was significantly higher than in the control group by 11.75 g (4.8%) and 10.3 g (4.2%), respectively (P <0, 05). The increase in the 1-3rd experimental groups of rats was higher than in the control group by 10.0 g or 19.0%. Laboratory animals in the chronic experiment were carried out similarly to the subacute experiment, however, the test period was 6 months. An additional pathological dissection of experimental animals was also performed to study the effect of the biological product on the organs and tissues of mice and rats.

Studies have shown that laboratory animals satisfactorily tolerated the studied doses of probiotic. Deaths of animals - not detected. There are no violations in the physiological picture of laboratory animals. Mice and rats during the chronic experiment remained viable, movements were active. Coat is normal (table 6).

Table – VI: Chronic toxicity of probiotic in laboratory animals (n = 10)

Group	Kind of animal	The volume of injected fluid, method of administration	Probiotic dose, CFU / ml	Test result, goal.		
				got sick	died	survived
Intact	mice	–	–	0	0	10
	rats	–	–	0	0	10
Control	mouse	1.0 ml saline, by mouth	–	0	0	10
	rats	10.0 ml saline, by mouth	–	0	0	10
1st experienced	mice	0.1 ml probiotic, by mouth	$7,9 \times 10^8$	0	0	10
	rats	1.0 ml probiotic, by mouth	$7,9 \times 10^9$	0	0	10
2nd experienced	mice	0.05 ml of probiotic, oral	$3,9 \times 10^8$	0	0	10
	rats	0.5 ml probiotic, by mouth	$3,9 \times 10^9$	0	0	10
3rd experienced	mice	0.02 ml probiotic, by mouth	$1,5 \times 10^8$	0	0	10
	rats	0.2 ml probiotic, by mouth	$1,5 \times 10^9$	0	0	10

The results of studying the effect of the biological product on the body weight of experimental laboratory animals, as well as the growth are presented in table 7. From the data of table 7 it was found that the introduction of a probiotic in the diet of experimental groups had a statistically significant increase in the body weight of laboratory animals. In the first, in the second and third experimental groups of mice, the mass of animals at the end of the experiment was higher than in the control group by 3.85 g (10.9%), 3.56 g (10.1%) and 3.25 g (9.2%), respectively (P <0.05). The increase in bioobjects was similar to their body weight, more in the experimental groups than in the control group by 3.92 g (23.3%), 3.54 g (21.0%) and 3.3 g (19.6%) . In the 1st experimental group of laboratory rats, there was a tendency to increase the mass of animals relative to rats in the control group by 10.19 g or 4.1%. In the 2nd and 3rd experimental groups, the animal body weight was significantly higher than in the control group by 11.75 g (4.8%) and 10.3 g (4.2%), respectively (P <0, 05). The increase in the 1-3rd experimental groups of rats was higher than in the control group by 10.0 g or 19.0%.

Table – VII: Effect of probiotic on the growth and development of laboratory animals

Group	Body weight g		Growth per experience, g
	at the beginning of the experiment	at the end of the experiment	
Mice			
Intact	18,45±0,42	34,64 ± 0,47	16,19
Control	18,54±0,44	35,37 ± 0,44	16,83
1st experienced	18,47±0,38	39,22 ± 0,42*	20,75
2nd experienced	18,56±0,39	38,93 ± 0,49*	20,37
3rd experienced	18,49±0,43	38,62 ± 0,44*	20,13
Rats			
Intact	195,32±2,87	245,56 ± 1,13	50,24
Control	194,67±2,64	246,48 ± 1,11	51,81
1st experienced	195,14±2,83	256,67 ± 1,16	61,53
2nd experienced	196,34±2,72	258,23 ± 1,22*	61,89
3rd experienced	194,89±2,79	256,78 ± 1,07*	61,89
* Significant difference with control (P <0.05)			

Similarly to the subacute experiment in chronic experience, we also studied the effect of probiotic on the organism of laboratory animals, through their morphological and biochemical parameters of whole blood and serum. The research results are presented in table 8 and 9.

Table - VIII: Morphological and biochemical blood parameters of laboratory mice after application of a probiotic (n = 6)

Indicator	Group				
	Intact	Control	1st experienced	2nd experienced	3rd experienced
Morphological indicators					
Erythrocytes, 10^{12} / l	8,03±0,12	8,10±0,14	8,34±0,14	8,39±0,13	8,38±0,12
Hemoglobin, g / l	102,76±2,87	105,38±2,94	110,34±2,89	108,36±2,77	109,83±2,92
Platelets, 10^9 / L	201,53±3,73	203,17±3,56	208,42±3,68	210,11±3,73	206,82±3,84
White blood cells, 10^9 / l	7,21±0,22	7,20±0,18	7,19±0,19	7,22±0,16	7,22±0,18
Biochemical parameters					
Total protein, g / l	51,25±0,76	52,73±0,67	59,62±0,81*	60,57±0,80*	59,65±0,75*
albumin, g / l	21,13±0,79	21,26±0,75	25,14±0,81	25,75±0,79	24,96±0,83
globulin, g / l	30,12±0,71	31,47±0,69	34,48±0,68	34,82±0,71	34,69±0,75
A / G ratio	0,70±0,02	0,67±0,03	0,72±0,03	0,74±0,02	0,72±0,03
Cholesterol, mmol / l	1,67±0,03	1,62±0,04	1,53±0,03	1,55±0,04	1,54±0,03
Urea, mmol / l	21,46±0,52	20,89±0,49	21,64±0,45	20,84±0,46	20,17±0,43
Calcium, mmol / l	2,14±0,03	2,11±0,03	2,22±0,02	2,24±0,03	2,19±0,03
Phosphorus, mM / L	1,14±0,03	1,17±0,02	1,20±0,03	1,19±0,03	1,17±0,04
AST, Unit / L	125,27±3,56	122,02±3,73	126,28±3,84	124,27±3,63	123,87±3,81
ALT, Unit / L	91,63±1,76	90,46±1,85	91,47±1,68	90,97±1,73	89,68±1,95

* Significant difference with control (P <0.05)

Table – IX: Morphological and biochemical blood parameters of laboratory rats after application of a probiotic (n = 6)

Indicator	Group				
	Intact	Control	1st experienced	2nd experienced	3rd experienced
Morphological indicators					
Erythrocytes, 10^{12} / l	5,24±0,15	5,28±0,16	5,35±0,15	5,33±0,16	5,36±0,17
Hemoglobin, g / l	111,64±2,34	109,38±2,46	118,65±2,41	117,73±2,47	115,27±2,40
Platelets, 10^9 / L	143,65±2,45	147,62±2,32	145,38±2,53	148,23±2,52	145,32±2,62
White blood cells, 10^9 / l	11,01±0,19	11,24±0,19	10,57±0,18	11,03±0,18	10,96±0,21
Biochemical parameters					
Total protein, g / l	62,58±0,75	63,24±0,72	65,22±0,75*	66,32±0,76*	66,29±0,61*
albumin, g / l	28,32±0,74	28,23±0,68	29,85±0,61	30,11±0,71	30,32±0,65
globulin, g / l	34,26±0,62	35,01±0,58	35,37±0,56	36,21±0,59	35,97±0,60

A / G ratio	0,82±0,02	0,80±0,02	0,84±0,02	0,83±0,02	0,84±0,03
Cholesterol, mmol / l	2,12±0,05	2,07±0,03	1,98±0,03	1,99±0,04	2,05±0,03
Urea, mmol / l	5,43±0,12	5,51±0,09	5,41±0,11	5,45±0,12	5,48±0,10
Calcium, mmol / l	2,23±0,04	2,27±0,03	2,31±0,03	2,28±0,04	2,27±0,03
Phosphorus, mM / L	11,26±0,22	11,35±0,25	11,38±0,19	11,33±0,23	11,29±0,22
AST, Unit / L	167,87±3,35	165,97±3,21	170,12±3,33	166,75±3,26	168,64±3,19
ALT, Unit / L	139,64±3,11	143,37±3,06	141,68±3,09	144,85±3,18	140,39±3,02

* Significant difference with control (P <0.05)

Blood counts of all the studied experimental laboratory animals were normal for these types of biological objects. Pathology in the studied indicators, similar to the experiments in the subacute experiment, was not detected. Both in the subacute and in chronic experience when using a probiotic for the analyzed blood parameters, it was possible to draw a conclusion about the positive effect of the supplement on the body of mice and rats.

During an autopsy of laboratory experimental biological objects, as well as intact and control groups of animals, changes and pathologies in the location and structure of internal organs were not detected. The organs in the thoracic and abdominal cavities were positioned anatomically correctly.

Thus, the prolonged use of probiotic supplements on nonlinear white mice and sexually mature mongrel white rats did not have a toxic effect on their body.

The study of skin-resorptive and irritating properties. The use of a probiotic suggests its possible contact with the skin or mucous membranes of the eyes, and therefore, in order to exclude any negative effect in such cases, it was important to study the irritating characteristics of the probiotic on the surface of the skin and the conjunctiva of the eyes of laboratory animals. Bioobjects of research were albino rabbits.

The results of cutaneous applications showed that during the experiment the laboratory albino rabbits did not have: local redness, increased sensitivity, swelling, inflammation, erythema and edema. The primary irritation index was recorded as zero.

The conjunctival test showed a slight reddening of the mucous membrane with the simultaneous release of the lacrimal glands immediately after the administration of the biological product, which took 2 minutes. In the future, no negative impact was recorded. The overall behavior and condition of the animals at the analyzed time intervals (1; 24; 48 and 72 hours) remained positive. The primary irritation index according to GOST R ISO 10993-10-2009 was equal to zero.

IV. CONCLUSION

The complex of pharmaco-toxicological studies of probiotics proved that the biological product used, based on the autochthonous microflora of poultry, does not exhibit toxic and irritating properties, can improve metabolism, and therefore can be used in industrial poultry farming regardless of the dose.

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