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Influence of Probiotics «Spas» And «Biosporin» At T-2 Toxication of Broiler Chickens.

Arthur I Gindullin[1#], Tatyana A Shamilova[2], Diana A Gindullina[3], Mikhail Y Tremasov[4], Arkadiy V Ivanov[4*], Alexander A Ivanov[4], Albert N Chernov[4***], Malik N Mukminov[5], and Eduard A Shuralev[5*]**

[1] Ph.D. of Biological Sciences, Scientific Researcher, Institute of Fundamental Medicine and Biology, Kazan Federal University, ul. Kremlevskaya 18, R-420008 Kazan, Russia.
[2] Ph.D. of Biological Sciences, Principal Adviser, «Ministry of Health and Care Services of Republic of Tatarstan», Kazan, Republic of Tatarstan, Russian Federation.
[3] Ph.D. of Biological Sciences, Assistant of Director, SSI «TatarSRIA of Russian agricultural academy», Kazan, Republic of Tatarstan, Russian Federation.
[4] Doctor of Biological Sciences, Professor, Head of Toxicology Department, FGBI «Federal center of toxicological, radiological and biological safety», Kazan, Republic of Tatarstan, Russian Federation.
[4*] Doctor of Biological Sciences, Professor, Director, FGBI «Federal center of toxicological, radiological and biological safety», Kazan, Republic of Tatarstan, Russian Federation.
[4**] Doctor of Biological Sciences, Deputy of Director, FGBI «Federal center of toxicological, radiological and biological safety», Kazan, Republic of Tatarstan, Russian Federation.
[4***] Doctor of Biological Sciences, Deputy of Director, FGBI «Federal center of toxicological, radiological and biological safety», Kazan, Republic of Tatarstan, Russian Federation.
[5] Doctor of Biological Sciences, Professor, Institute of Environmental Sciences, Kazan Federal University, Kazan, Republic of Tatarstan, Russian Federation.
[5*] Ph.D. of Biological Sciences, Associate Professor, Institute of Environmental Sciences, Kazan Federal University, Kazan, Republic of Tatarstan, Russian Federation.

ABSTRACT

The main «meat locomotive» in the agricultural sector for a long time has been serving the poultry industry. Advantageous characteristics of the poultry industry are low-cost of financial expenses, high pay-back period, the short period of the reproductive cycle of the organism and the acceptability of the nutritional value of meat products. These properties and criteria emphasize the poultry as one of the most outstanding and important components in the realization of the Food Security Doctrine of Russian Federation. Low quality of feed, inappropriate to zoohygienic and sanitary standards content of birds, the presence of an infectious agent are constraints, which ultimately result in the partial fulfillment of bioresource potential. The main indicator of a poor quality of feed is the presence of mycotoxins. Exposure to mycotoxin contamination of feed solved with prevention, which use probiotics. Application of probiotic preparations is an effective and promising method in the toxicosis of animals. The main aim of this article was research the influence of probiotics «Spas» and «Biosporin» in sub-chronic poisoning by T-2 toxin of broiler chickens. The application of probiotics «Spas» and «Biosporin» to broiler chickens during intoxication by T-2 toxin occur beneficial effects on clinical and hematologic, immune-biochemical parameters, thereby increasing the safety of chickens and also increased live weight gain.

Keywords: probiotic, poultry, T-2 toxin, broiler chicken, haematological and biochemical parameters.

[#]*Corresponding author*

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INTRODUCTION

In the Russian Federation has approved the Food Security Doctrine, in accordance with the guarantor of its achievement is the implementation of Food Security Plan. Key positions of increase domestic production of the country are production gain of livestock and providing the population by produced animal breeding. Poultry industry for a long time has been serving the main «meat locomotive» in agriculture and also is an important component in the realization of the Food Security Doctrine due to of qualities such as intensive breeding of poultry, the acceptability of the nutritional value of meat products, the short period of the reproductive cycle of the organism, year-round, low-cost of financial expenses, high pay-back period.

In the Russian Federation, the consumption level of poultry meat products in 2011 was 42 %, while the share of consumption of pork and beef are, respectively, 33 % and 22 %. In 2009 ratio of poultry meat in the total amount of produced meat resources was 40 %, pork – 32 %, beef – 26 %, while in the 1990s this indicator was 18; 34 and 43 %, respectively [1].

However, limiting factors in the full realization of the poultry genetic potential are low quality of feed, inappropriate to zoohygienic and sanitary standards content of birds, the presence of an infectious agent that are responsible for the changes in the functioning of the poultry organism [2].

Scientist and researchers studied and described more than 300 fungi toxins that are toxic, mutagenic and carcinogenic for both humans and animals [3, 4, 5].

The main indicator of a poor quality of feed is the presence of mycotoxins. According to estimates of the Food and Agriculture Organization of the United Nations (FAO) from 25% to 50% of the world's food crops affected by mycotoxins, which as the result leads to loss of more than 1,000 million tons of feed per year [6]. By the data of Tremasov M.Y. et al. the contamination of feed by mycotoxins is more than 30%. Effects of mycotoxins on the organism of animals, including poultry possesses immunesuppressor impact, which is characterized by allergenicity, carcinogenicity and mutagenicity.

Application of probiotic preparations is an effective and promising method in the toxicosis of animals at preventive medical approach.

Application of probiotics is environmentally safe and harmless method in the prevention of pathogenic and opportunistic microorganisms, including acute intestinal infections [8]. This is accomplished due to the ability of probiotic microorganisms to synthesize biologically active substances (BAS), to adhere toxins on the cell surface and to produce enzymes which inactivating the toxic effect.

In this paper we studied the effect of probiotics «Spas» (based on *Lactobacterium plantarum* strains) and «Biosporin» (based on *Bacillus* strains) in broiler chickens in sub-chronic poisoning by T-2 toxin.

MATERIALS AND METHODS

Application of probiotic

As the preventive means at T-2 mycotoxicosis used probiotic preparations: «Spas» based on *Lactobacterium plantarum* strains (manufacturer FGBI «Federal center of toxicological, radiological and biological safety») and «Biosporin» based on *Bacillus subtilis и Bacillus licheniformis* strains (manufacturer FGI «48 Central Research Institute of the Ministry of Defence of the Russian Federation»).

In the course of experiments we have evaluated the clinical status of chickens, haematological and biochemical parameters, changes in body weight gain at the beginning of the experiment and afterwards every 10 days after the initial injection of the toxin.

Animals treatment

Research of general toxicity effect of T-2 toxin was carried out on broiler chickens cross «Smena-7», at the age of 20 days and weighing 650-680 g, simultaneously with the application of probiotics «Spas» and

«Biosporin». Forty broiler chickens were randomly allotted to one of four groups. Each group contained 10 birds. The first (biological control) group was given normal uncontaminated feed. For modelling the mycotoxicosis second, third and fourth groups have received feed contaminated by T-2 toxin in quantity of 4 MPC (maximum permissible concentration = 50 mg/kg of feed) within 30 days. The second group was obtained only contaminated feed and which served as a model of sub-chronic T-2 mycotoxicosis. For the third and fourth groups for the prevention of mycotoxicosis were supplied orally probiotic «Spas» and «Biosporin», respectively individually 0.5 ml containing at least 10^9 CFU (colony-forming units) once a day before feeding contaminated fourage. Housing and feeding conditions of birds were according to zoohygienic and sanitary standards.

As mycotoxin producer was used the fungus *Fusarium sporotrichioides* strain 2m-15 from the collection of «Federal center of toxicological, radiological and biological safety». Qualitative and quantitative determination of T-2 toxin was carried out according to government standard (GOST 52337-2005) and using the method bioautography with confirmation of results by applying gas chromatographymass spectrometry «Hitachi-80m».

Considered parameters

In our experiment we were taken into account such parameters as average body weight gain (BWG), the amount of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP). Also we investigated the protein, glucose, erythrocytes, leukocytes, hemoglobin and erythrocyte sedimentation rate (ESR). Among other issues, it was also considered a systemic condition, the degree of feathers and chicken behavior. Through studies of the digestive system we paid attention to the food stimulation and the state of excrement. Health monitoring of chicken broiler was carried out by studying morphological parameters of blood before the experiment, at the 10-th, 20-th and 30-th day of experiment. Blood was taken from the wing vein.

Applied methods

The total amount of protein, glucose, erythrocytes, leukocytes, hemoglobin and ESR were investigated by the methodology of Antonov B.I. et al. [9] and Kondrakhin I.P. [10].

Determination of total crude protein from blood plasma was carried out by the refractometric method, the quantitative ratio of protein fractions from blood serum was identified by method of Aull and McCord in a modification by Karpyuk S.A. The enzyme activity of AST, ALT, AP, the concentration of protein and lipid metabolism products, carbohydrate, total calcium, inorganic phosphorus was determined on biochemical analyzer «Microlab 300».

Definition of phagocytic activity (PA) of blood neutrophils was performed by the method of Cost S.A. et al. [11], lysozyme activity (LA) – by nephelometric method of Dorofeychuk V.G.

The number of T-lymphocytes was estimated by the method of self-generation with erythrocytes (E-ROK), B-lymphocytes – by the method of formation rosettes by Frimel Kh.

RESULTS

It has been noted that in the modeling group of mycotoxicosis the most chickens had cyanosis of comb. At the beak and oral mucosa were detected foci of necrosis. Also it was muscle tremors and diarrhea, decrease in BWG at 18.5 % (Figure 1). Sub-chronic effects of T-2 toxin on broiler chickens of this group characterized by inhibition of behavior status, loss of appetite, morphological reduction (erythrocytes at 18.3 %, leukocytes – 25.9 %, hemoglobin – 26.7 %) and biochemical (total protein – 24.6 %, albumins – 29.9 %, γ-globulins – 20.6 %) of blood parameters at the end of the experiment with the compare by the group of biological control as shown in Table 1 and 2.

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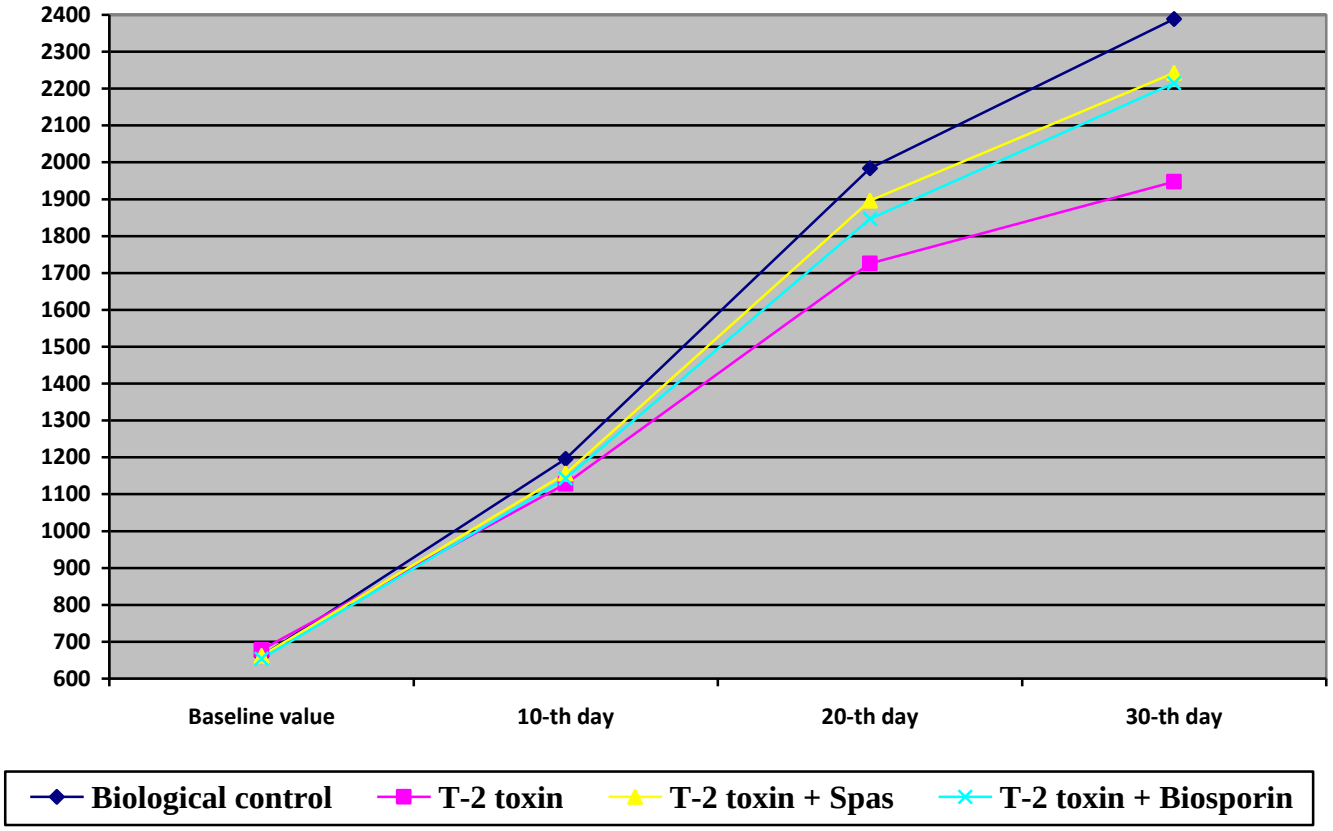

Figure 1: Productivity index of chicken-broiler.

Table 1: Hematological parameters of chicken-broiler

Parameter	Group			
	Biological control	T-2 toxin	T-2 toxin + Spas	T-2 toxin + Biosporin
10-th day				
Erythrocytes, 10^{12}/l	2,71±0,04	2,48±0,09	2,62±0,06	2,66±0,03
Leukocytes, 10^{9}/l	27,28±0,26	25,15±1,14	26,72±0,79	26,05±0,57
Hemoglobin, g/l	61,32±0,73	52,83±1,12*	56,95±0,96*	55,53±0,74
ESR, mm/h	3,1±0,10	3,59±0,21*	3,39±0,17*	3,33±0,23
Hematocrit, %	34,6±1,50	28,2±1,85*	31,4±0,94	32,1±1,26
20-th day				
Erythrocytes, 10^{12}/l	2,76±0,32	2,39±0,16*	2,61±0,57	2,59±0,42
Leukocytes, 10^{9}/l	27,93±0,33	24,52±0,53	26,24±1,24	25,97±1,41
Hemoglobin, g/l	62,66±1,41	49,58±0,67**	54,43±1,44*	53,25±0,91*
ESR, mm/h	3,0±0,22	3,72±0,38**	3,38±0,73*	3,32±0,58
Hematocrit, %	35,9±1,50	26,3±0,94**	30,02±1,28	29,8±0,69*
30-th day				
Erythrocytes, 10^{12}/l	2,73±0,52	2,23±0,32*	2,4±0,83	2,49±0,71*
Leukocytes, 10^{9}/l	26,39±0,61	19,55±0,81**	23,93±1,32	23,72±0,82
Hemoglobin, g/l	60,53±0,85	44,35±0,74**	47,22±0,73**	48,01±1,42**
ESR, mm/h	2,87±0,32	4,18±0,63***	3,34±0,36*	3,22±0,51*
Hematocrit, %	33,93±0,76	24,31±0,795*	28,8±1,06	28,0±0,755*

* = The difference with the control group is statistically significant at P<0,05
** = The difference with the control group is statistically significant at P<0,01
*** = The difference with the control group is statistically significant at P<0,001

In groups of chicken broiler who received simultaneously with toxic food probiotics «Spas» and «Biosporin» these parameters ranged within the physiological standard or slightly fall outside this range, which

indicates that probiotic preparations possess the protective and preventive effect at the T-2 mycotoxicosis of broiler chickens. At the sub-chronic T-2 toxicosis oral administration of probiotics to experimental chickens (at the dose of 0.5 ml at least 10^9 CFU) leads to normalization of hematologic (increasing the number of erythrocytes at 7.6 and 11.7%, leukocytes – 22.4 and 21.3%, hemoglobin – 6.5 and 8.3%, consequently for the «Spas» and «Biosporin») and biochemical (increasing of total protein at 12 and 15%, albumins – 18,3 and 20% , glucose – 10.3 and 12%, respectively for the «Spas» and «Biosporin») parameters (Table 1 and 2) and also enhances BWG at 24.6 and 23%, respectively for «Spas» and «Biosporin» with comparing the modeling group of mycotoxicosis at the end of the experiment (Figure 1).

Table 2: Ratio of total protein and it's fractions in blood serum of chicken-broiler

Parameter	Group			
	Biological control	T-2 toxin	T-2 toxin + Spas	T-2 toxin + Biosporin
10-th day				
Total protein, g/l	39,7±0,46	33,6±0,33*	38,2±0,26	37,7±0,34
Albumins, %	51,45±0,79	44,7±1,89*	47,2±0,67	48,85±5,31
α-globulins,%	9,35±1,47	10,87±1,03*	10,37±2,41	10,55±1,62*
β-globulins, %	9,5±0,98	11,2±1,73*	10,7±0,97*	10,86±1,16*
γ-globulins, %	29,7±1,43	26,2±0,83	27,68±1,27	28,75±0,94
20-th day				
Total protein, g/l	36,9±0,38	29,8±0,33*	32,7±0,28	32,9±0,26
Albumins, %	53,1±1,27	42,3±0,99**	46,93±1,82	47,97±2,42
α-globulins,%	9,63±1,62	12,01±1,81**	10,91±1,83*	11,03±1,72*
β-globulins, %	9,23±1,23	12,2±2,16***	11,14±1,37**	11,29±2,01**
γ-globulins, %	28,04±2,14	23,44±2,91*	25,06±1,32	26,38±1,86
30-th day				
Total protein, g/l	31,3±1,24	23,6±1,18**	26,43±1,65*	27,11±0,88*
Albumins, %	49,54±0,63	34,7±1,49**	41,05±1,29*	41,64±1,38*
α-globulins,%	9,21±0,69	12,73±1,23***	11,26±1,52**	11,48±1,26**
β-globulins, %	8,89±0,91	13,62±1,62***	11,42±0,82**	11,52±1,34**
γ-globulins, %	32,36±1,28	25,69±1,55**	28,44±1,86*	29,35±1,43

* = The difference with the control group is statistically significant at P<0,05
** = The difference with the control group is statistically significant at P<0,01
*** = The difference with the control group is statistically significant at P<0,001

According to the data from table 2, the effect of probiotic preparations «Spas» and «Biosporin» testifies about positive dynamics of changes in total protein and its fractions.

De-emphasis of the protein value in the modeling group of mycotoxicosis is characterized that in the organism occurs insufficient its digestibility and therefore unsatisfactory functioning of gastrointestinal tract (GIT)-inhabiting bacteria.

Data about the biochemical indicators of the experiment are shown in table 3, which indicates that the simulating group of T-2 mycotoxicosis is characterized by a decrease of glucose at 20.5%, whereas in the preventive groups by «Spas» and «Biosporin» it were 12.3 and 11.0 %, respectively, by comparison with the biological control.

Biochemical parameters of the blood at the end of experiment have showed enzyme activity increase of ALT at 53.1 % and AST – 36.9 % in the group of broiler chickens, which were supplied only contaminated feed by T-2 toxin. The enhancement of enzyme activity in the groups which were received «Spas» and «Biosporin» with feed contaminated by T-2 toxin, was 30.5 and 39.7 % for ALT; 16.4 and 20.9 % for AST, respectively with the comparison by biological control.

Raising activity of the enzymes ALT and AST in the blood of modeling group by comparison with preventive groups by probiotic preparations «Spas» and «Biosporin» is testimony to the fact that injury of liver, heart and GIT were more intensive.

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Table 3: Biochemical parameters in blood serum of chicken-broiler

Parameter	Group			
	Biological control	T-2 toxin	T-2 toxin + Spas	T-2 toxin + Biosporin
10-th day				
Glucose, mmol/l	10,28±0,32	9,07±0,96	9,53±0,69	9,71±0,62
Cholesterin, mmol/l	3,17±0,28	3,34±0,23	3,26±0,19	3,29±0,21
Creatinine, mmol/l	14,1±0,33	12,9±0,23	13,4±0,41	13,6±0,36
ALT, U/l	4,29±0,31	5,03±0,28*	4,78±0,41	4,83±0,36**
AST, U/l	37,19±0,67	43,88±0,39*	41,03±0,72	42,14±0,29*
Alkaline phosphatase, U/l	202±2,01	234±3,16*	216±2,97	211±3,28
20-th day				
Glucose, mmol/l	9,9±0,16	8,24±0,52*	8,81±0,34	9,02±0,47
Cholesterin, mmol/l	3,09±0,11	3,87±0,39	3,63±0,44	3,72±0,56
Creatinine, mmol/l	14,3±0,28	11,7±0,11*	12,8±0,26	12,9±0,18
ALT, U/l	4,12±0,46	5,61±0,43***	5,08±0,37	5,19±0,51***
AST, U/l	37,01±0,48	47,56±0,67**	43,17±0,39*	43,96±0,55**
Alkaline phosphatase, U/l	191±3,15	267±5,31***	228±3,32**	230±2,94**
30-th day				
Glucose, mmol/l	9,0±0,28	7,15±0,41**	7,89±0,46*	8,01±0,53*
Cholesterin, mmol/l	3,09±0,11	3,87±0,39	3,63±0,44	3,72±0,56
Creatinine, mmol/l	14,0±0,19	10,9±0,23**	11,9±0,24*	12,2±0,31*
ALT, U/l	4,03±0,56	6,17±0,49***	5,26±0,53**	5,63±0,48***
AST, U/l	36,62±0,52	50,1±0,58***	43,99±0,76*	44,31±0,41**
Alkaline phosphatase, U/l	177±2,86	284±7,95***	235±4,83***	241±5,17***

* = The difference with the control group is statistically significant at P<0,05
** = The difference with the control group is statistically significant at P<0,01
*** = The difference with the control group is statistically significant at P<0,001

Table 4: Indicator of nonspecific resistance in blood serum of chicken-broiler

Parameter	Group			
	Biological control	T-2 toxin	T-2 toxin + Spas	T-2 toxin + Biosporin
10-th day				
T-lymphocytes, %	51,7±0,36	48,1±0,43	49,9±0,24	50,1±0,33
B-lymphocytes, %	22,6±0,28	20,3±0,53	21,5±0,14	21,83±0,25
PA, %	28,6±0,31	24,77±0,23*	25,82±0,28	26,14±0,41
LA, %	17,22±0,17	16,1±0,23	16,48±0,26	16,57±0,34
20-th day				
T-lymphocytes, %	50,56±0,21	45,28±0,28	47,89±0,36	48,12±0,25
B-lymphocytes, %	21,22±0,16	18,15±0,42*	19,41±0,31	19,83±0,19
PA, %	27,44±0,20	22,54±0,43*	24,47±0,23	24,76±0,33
LA, %	16,97±0,10	15,02±0,13*	15,53±0,22	15,81±0,27
30-th day				
T-lymphocytes, %	49,79±0,33	42,16±0,36*	46,16±0,23	46,37±0,17
B-lymphocytes, %	20,12±0,66	16,27±0,31*	17,91±0,31	18,23±0,19
PA, %	26,23±0,35	19,67±0,26**	22,13±0,51*	23,12±0,24*
LA, %	16,14±0,22	12,79±0,28**	13,86±0,37*	14,26±0,21*

* = The difference with the control group is statistically significant at P<0,05
** = The difference with the control group is statistically significant at P<0,01

Immunological parameters of broiler chickens in the simulating group of T-2 mycotoxicosis indicates about immunosuppression. Reduction of fractions T- and B-lymphocytes in this group vis-à-vis biological control were 15.3 and 19.1 % finally of the experiment, respectively (Table 4).

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Calculation of economic efficiency (Table 5) of probiotics in the industrial conditions at poultry LLC «Ak Bars» showed that the more economic effect was obtained with using the probitic «Spas» (2.9 rub per 1 spent rub) compared with the «Biosporin» (1.1 rub per 1 spent rub).

Table 5: Economic efficiency of probiotics application at T-2 toxicosis at LLC «Ak Bars»

Parameter	Group			
	1	2	3	4
Quantity of chicken broiler	25	25	25	25
Mortality	0	7	2	3
Daily average weight gain, g	57,6	42,3	52,7	52,0
Average price of 1 kg product, rub	90	90	90	90
Average price of broiler chicken, rub	50	50	50	50
Economic loss				
– mortality of chicken broiler, rub		606,84	239,72	237,3
– decrease of body weight gain, rub		1652,4	529,2	601,56
Total, rub		2259,24	768,92	838,86
Expenses				
Price of probiotic, rub/l			550	672
Volume of probiotic usage, ml			149,5	149,5
Expenses involved in probiotic , rub			82,22	401,85
Salary to specialists and workers, rub			299,88	299,88
Total, rub			382,1	701,73
Economic effect of probiotic's application, rub			1108,21	768,64
Economic efficiency per 1 spent rub			2,9	1,1

The above-mentioned indicates about possible dysfunction of lymphoid organs in the modeling group of mycotoxicosis, whereas in groups where used probiotics «Spas» and «Biosporin» the lesion of these organs were less.

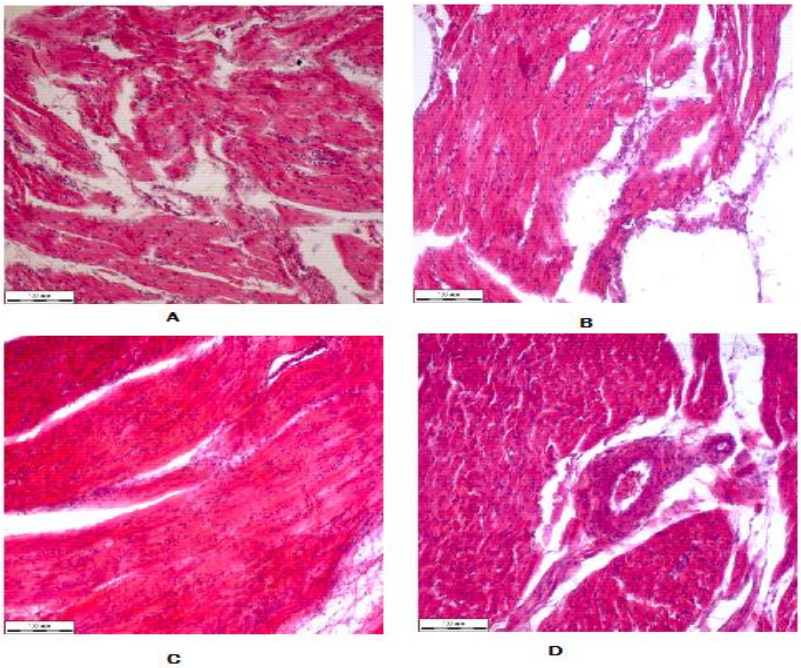

Figure 2: Cordis of broiler chicken: A = Biological control; B = T-2 toxin; C = T-2 toxin + Spas; D = T-2 toxin + Biosporin (dyeing by haematoxylin Garris, aqueous solution of eosin and carboltoluol, microscope objective ×420).

Less economic effect of probiotic «Biosporin» can be explained by the high commercial cost of the product.

In the group of simulated T-2 toxicosis in the preparation of cordis were detected swelling vascular wall, interstitial focal infiltration by leukocytes and lymphocytes (Figure 2).

DISCUSSION

Toxigenic microscopic fungi produce metabolites which affecting agricultural goods: cereal crops and fodder that subsequently causes of various etiologies.

For the prevention of mycotoxicosis and regeneration microflora in veterinary medicine use different sorbents, antioxidants, detoxifying solutions and also probiotics [8].

There are a lot of experimental data that T-2 toxin promotes the oxygen starvation and destructive changes in the hematopoietic and immune organs [12].

Results of hematological studies in groups with «Spas» and «Biosporin» have shown that probiotics significantly improve hematopoiesis, which is confirmed by researchers [13].

Increase of ALT in the blood due to structural damage of hepatocytes and growth of the permeability of the cell membranes, therefore this indicates about affection of the functional properties of the liver. AST is an enzyme that involved in the metabolism of amino acids and contained in many organs (cordis, kidney, liver and others.). Its increasing signals about injury of the cordis, liver and GIT. Similar results were obtained by Fekete S. et al. [14] and Garies M. et al. [15].

Thus, we can conclude that the application of probiotics «Spas» and «Biosporin» to broiler chickens during intoxication by T-2 toxin occur beneficial effects on clinical and hematologic, immune-biochemical parameters, thereby increasing the safety of chickens.

CONCLUSION

In the modeling group of mycotoxicosis the most chickens characterized by inhibition of behavior status, loss of appetite, morphological and biochemical reduction of blood parameters and decrease in body weight.

It was found that the usage of probiotics based on *Lactobacterium* and *Bacillus* improved the general physiological state of broilers, improved biochemical parameters of blood and also increased live weight gain.

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