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Efficiency of Application of a Polysaccharide Enterosorbent of "Fitosorb" For Prevention of the Combined Mycotoxicoses.

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ABSTRACT

The purpose of the real researches was studying of efficiency of use of polysaccharide adsorbent Fitosorb for prevention of the combined mycotoxicoses. In experience it was modelled combined mycotoxicoses on white rats. Animals received with a diet at the same time of T-2 toxin and an aflatoxin B1 (AFB1) in a dose of 0,3 mg/kg within 30 days, and as a prophylactic adsorbent mycotoxins «Fitosorb» of was applied. Adsorbent was brought in a diet in a dose of 5 or 10 g/kg of a forage. It is established that inclusion in a diet of experimental animals of a combination of T-2 and an (AFB1) negatively affects hematologic, biochemical paramete rs of blood throughout experiment. Daily introduction of an enterosorbent «Fitosorb» to the forages struck with mold fungis and their toxins in number from 5 to 10 g/kg to a diet reduces symptoms of toxicosis, improves the general clinical condition of animals, has favorable effect on the course of physiological processes of an organism, providing correction of morphological and biochemical indicators of blood, stimulating growth of animals.

Keywords: combination of T-2 mycotoxins, B1 aflatoxin, adsorbent of mycotoxins, prevention of mycotoxicoses

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INTRODUCTION

From natural toxins – pollutants of agricultural raw materials and food, big health hazard of the population and animals is represented by microscopic mushrooms and their toxins – mycotoxins. Mycotoxins pollute food and forages at all stages of their production, transportation, storage, processings and realization [1, 2]. Mycotoxins differ in high toxicity, and many of them –immunosupressiv, mutagenic, teratogenic and cancerogenic properties [3, 4, 5]. Researches of scientists show that the animal husbandry sustains serious economic losses from decrease in efficiency and reproduction of the farm animals arising at the mycotoxicoses [4, 6, 7].

Expansion of scales of export and import of grain between the countries, observed climate change in the world, systematic use of fungicides, pesticides, protravitel of seeds, leads to increase in formation of mycotoxins in hundreds of times and to increase of a possibility of simultaneous contamination of a forage various mycotoxins [8].

From a set of the existing measures of prevention of mycotoxicoses: agrarian, the correct storage of forages, processing preparations of a chemical and biological origin, etc., recently, the increasing distribution is found by enterosorbents [9, 10, 11, 12, 13] because of set of the properties shown to preventive preparations: profitability, physiology, harmlessness, low level of inclusion in the diet, selectivity of sorption, etc.

The purpose of the real researches was studying of efficiency of application of a polysaccharide enterosorbent «Fitosorb» for prevention of the combined mycotoxicoses.

MATERIALS AND METHODS

Experiments are made on laboratory animals – the white rats of the line Wistar who have arrived from special nursery of Federal Center for Toxicological, Radiation and Biological Safety. Before statement of experiences of animals maintained on 2-week adaptation, carried out feeding according to the norms accepted in zootechnics.

Structure: cereals (wheat, barley) and products of their processing, (bran wheat), meat and offal of an animal origin (meat and bone meal), meal sunflower, vitamins and minerals (premix) (table 1).

Table 1: Diet of white rats

name	unit of measure	
Humidity, no more	%	13,50
Crude protein, not less	%	19, 00
Crude fat, no more	%	5,00
Crude cellulose, no more	%	4,00
Crude ashes, no more	%	7,00
Calcium	%	0,9-1,2
Phosphorus	%	0,6-0,9
Sodium, not less	%	0,2-0,25
Table salt, no more	%	0,20
It is in addition entered vitamins and minerals in 1 kg of compound feed		
vitamin A	thousandME	27,000
vitamin D3	thousandME	1,375
vitamin E	mg	125,00
vitamin B1	mg	30,00
vitamin B2	mg	9,50
vitamin B6	mg	15,00
vitamin B12	mg	50,00
nicotinic acid	mg	37,50
vitamin B5	mg	22,00
vitamin B9	mg	2,50

Chloride is well-cared	mg	1590,00
vitamin C	mg	70,25
vitamin K3	mg	62,50
Bétaïne	mg	365,00
Microelement		
Fe	mg	128,00
Mn	mg	18,75
Zn	mg	128,00
Cu	mg	12,50
J	mg	2,30
Se	mkg	187,50
K	g	2,60

Food is also tested for the content of mycotoxins and biosecurity indicators established in Russia - the presence of pathogenic and opportunistic pathogenic microorganisms, feed accordance with the certificate of quality security.

There was no contamination of a laying, a forage and the water capable to affect results of research. Periodic water analysis, laying and a stern on microbiological contamination is carried out to laboratories.

Researches were conducted on 72 males of the white rats divided by the principle of analogs into 4 groups. The first group of intact animals served as biological control and during 30 days of experience received the "pure" compound feed which isn't containing mycotoxins, to rats of the second group set "a toxic forage", contaminated combined T-2 toxin and an AFB1 (in doses of 0,3 mg/kg of a forage that the 3rd are multiply exceeded by the most admissible level); the third group received "a toxic forage" and an enterosorbent of "Fitosorb" in number of 5 g/kg of a diet; the fourth group received "a toxic forage" and an enterosorbent of "Fitosorb" in doses of 10 g/kg of a diet.

For pilot studies toxin and an AFB1 produced by Fermentek ltd (Israel) used crystallic T-2, purity of toxin made not less than 97%. Toxins an animal entered inclusion in their diet by consecutive and careful hashing. As a prophylactic used organic polysaccharide adsorbent of mycotoxins the «Federal center of toxicological, radiation and biological safety" developed by together with the Kazan state technological university (Kazan) received by way of special processing from a cover of cereals.

During experiments studied a clinical condition of animals, consumption of a forage, hematologic, biochemical indicators, change of body weight, registered life expectancy. At skilled and control animals capture of blood was carried out a dekapitation.

The quantity of erythrocytes, leukocytes, hemoglobin determined by the hematologic analyzer "Mythic 18", the speed of subsidence of erythrocytes (SOE) – on Panchenkov's device, the content of the general protein, bilirubin, glucose, activity of enzymes of alaninaminotransferase (ALT), aspartataminotransferase (AST), the alkaline phosphatase (AP) carried out to serums of blood of animals by means of the biochemical analyzer "Microlab 300".

The processing of digital material was performed by variational statistics using Student's criterion for authenticity. Student's t-test was used for all statistical analyses. Differences were considered significant at a significance level of $P<0.05$.

RESULTS OF RESEARCHES

Research of hematologic parameters of white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent «Fitosorb» is conducted.

At all rats receiving toxin without sorbent the expressed clinical symptoms of toxicosis already on 10 days of experiment in the form of loss of appetite, oppression, anorexia and diarrhea while, less expressed clinical picture 60% had animal 3 groups, and 36% - 4 groups were observed.

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The mass of the white rats receiving a toxic forage to 20 days of experience was for 10,4% (p <0,01) lower in comparison with control group, and to 30 days decrease has made 21,5% (p<0,001). Inclusion in "a toxic forage" of an enterosorbent "Fitosorb" promoted reduction of negative effect of mycotoxins by a gain of live mass of rats. So, in the third and fourth groups decrease in body weight to 20 days has made 5,5 (p <0,05) and 3,1% respectively, to 30 days - 15,0 (p <0,001) and 6% (p <0,05) respectively in comparison with control group.

Results of hematologic blood tests of white rats, at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent "Fitosorb", are presented in table 2 from which it is visible that decrease in indicators in group of the rats receiving "a toxic forage" without application of a sorbent for all the time of research, was below basic data.

Table 2: Hematologic indicators of white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent "Fitosorb"

Term of research, days	Group	Indicator			
		Erythrocyte, $x10^{12}$/l	Leukocyte, $x10^{9}$/l	Hemoglobin, g/l	SOE, mm/ hour
10	1	8,46±0,19	13,2±0,26	180,0±5,73	1,07±0,07
	2	8,72±0,18	11,0±0,77*	174,0±4,11	0,78±0,07*
	3	8,76±0,23	12,6±0,84	182,0±2,49	0,40±0,05***
	4	8,74±0,24	12,8±0,92	182,8±2,07	0,63±0,11*
20	1	8,50±0,21	14,0±0,29	176,5±3,82	1,15±0,12
	2	7,60±0,17*	11,6±0,79*	147,0±5,12**	2,23±0,52
	3	7,81±0,28	12,2±0,57	165,0±4,69	1,78±0,14*
	4	8,00±0,25	12,7±0,33*	169,5±5,02	1,33±0,17
30	1	8,33±0,31	13,8±0,43	173,5±4,65	1,15±0,12
	2	6,30±0,21**	10,0±0,53**	135,3±4,46**	2,83±0,48*
	3	7,26±0,19*	11,2±0,87*	144,0±3,40**	1,45±0,12
	4	7,54±0,23	11,8±0,42	154,8±3,63*	1,30±0,12

* - p<0,05 ** p<0,01 *** p<0,001

Follows from table 2 that the quantity of erythrocytes at the rats of the 2nd group receiving "a toxic forage" to 10 days of experiment has slightly increased, rather biological control, by 3,1%, to 20 days there was a decrease by 10,5% and to 30 days - for 26,5% (p<0,01). The maintenance of leukocytes in blood of rats of the second skilled group constantly decreased: to 10 days - for 16,7%, to 20 days – for 17,1% and to 30 days – for 27,5%. To 10 days the amount of hemoglobin decreased on 3,3, in 20 days - on 16,7 (p<0,01) and in 30 days - by 22,1% (p<0,01). Speed of subsidence of erythrocytes has decreased by 10 days of experiment in the second group on 27,1 (p<0,05), to 20 days has increased on 94, to 30 days - by 146,1% (p<0,05).

In the third group of animals prevented from mycotoxins by introduction of an enterosorbent in dose of 0,5% of a diet, change of hematologic indicators was the following: the quantity of erythrocytes, rather control data, on 10 days slightly increased - by 3,6%, then to 20 and 30 days has decreased on 8,1 (p<0,05) and 11,5% (p<0,01), respectively; decrease in quantity of leukocytes was noted throughout all experiment which has made to 10 days - 5; to 20 days - 12,9 (p<0,05) and to 30 days of-19,2% (p<0,05); on 10 days have registered slight increase of content of hemoglobin in blood of skilled animals in comparison with data of the first group for 1,1% which then to 20 and 30 days has decreased by 6,5 and 17% (p<0,01); SOE has decreased to 10 days on 62,6 (p<0,001), then has increased to 20 days – on 54,8 (p<0,05) and to 30 days - by 26,1%.

The fourth group of animals prevented by introduction of an enterosorbent in dose of 1% of a diet observed the following dynamics of hematologic indicators: the quantity of erythrocytes, rather control data, on 10 days slightly increased - by 3,4%, then to 20 and 30 days has decreased by 6 and 9,4% (p<0,05), respectively; decrease in quantity of leukocytes was registered throughout all experiment and it has made to 10sut-3,4; to 20 days – 9,6 (p<0,05) and to 30 days of-14,8% (p<0,05); on 10 days have registered slight increase of content of hemoglobin in blood of skilled animals in comparison with data of the first group for 1,53% which then to 20 and 30 days has decreased by 4 and 10,8% (p<0,05); SOE has gone down to 10 days on 41,4 (p<0,05), then has increased to 20 days – on 15,7 and to 30 days - by 13,04%.

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During experiment determination of content of glucose, general protein and its fractions (table 3) have conducted researches of some biochemical indicators of blood, in particular.

Table 3: Biochemical indicators of blood of white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent "Fitosorb"

Term of research, days	Group	Indicator					
		Glucose, mmol/l	General protein, g/l	Fractions of protein, %			
				albumin	α- globulin	β- globulin	γ- globulin
10	1	6,62±0,38	66,74±3,23	41,4±1,49	15,6±0,50	14,5±0,74	28,5±1,29
	2	6,37±0,37	60,33±1,03	37,6±1,89*	17,8±0,66*	19,4±0,47*	25,2±2,39
	3	6,84±0,50	62,53±2,17	39,3±1,42*	16,7±0,82	18,3±1,05*	25,7±1,64
	4	6,88±0,24	62,56±1,79	41,0±0,71	16,3±0,52	16,9±1,65	25,9±1,84
20	1	6,37±0,18	64,38±1,07	39,7±1,23	14,5±0,78	16,8±1,09	29,0±1,63
	2	5,51±0,14	54,46±4,38	32,5±1,80	17,7±0,43*	26,5±1,36**	23,3±0,83*
	3	6,25±0,33	62,14±2,77	34,2±1,41*	16,9±0,76	22,7±1,07**	26,2±0,38
	4	6,13±0,20	63,15±1,96	36,7±2,14*	16,1±0,73	20,4±0,75*	26,8±2,23
30	1	6,62±0,16	68,35±1,09	40,5±2,29	15,2±0,72	16,7±1,0	27,6±1,55
	2	4,97±0,44	50,35±6,2*	30,8±2,12*	21,3±1,17*	28,6±1,4***	19,3±1,55*
	3	7,25±0,27	60,56±1,6**	34,9±1,71	17,1±0,71	24,5±1,64**	23,5±1,58
	4	7,17±0,24	63,40±2,46	38,6±0,85	16,3±0,94	21,5±1,14*	23,6±1,66

* p<0,05 ** p<0,01 *** p<0,001

From table 3 it is visible, the white rats receiving "toxic forage" have more expressed decrease in level of glucose in blood serum to 10 days of experiment - on 3,78; to 20 days - on 15,7 and to 30 days - for 25% that indicates violation of a carbohydrate exchange.

In the prevented third and fourth groups at animals on 10 days of experiment registered increase of concentration of glucose in blood for 3,32 and 15,1%, respectively; on 20 days for 1,9 and 3,8%, respectively; to 30 days glucose level in blood has increased by 9,5 and 8,3%, respectively again.

Proceeding from data of table 3, it is possible to note that at rats of the second group the content of the general protein in blood serum in comparison with control data has decreased on 30 days - by 26,3% (p<0,05). The group of the rats prevented by introduction to a diet of 0,5% of an enterosorbent, decrease in the general protein observed on 30 days - for 11,4% (p<0,01); in the fourth group to the same term for 7,2%.

Decrease in percentage of albumine in serum of blood of rats concerning group of biological control happened in all groups throughout all experiment. In the second group of rats decrease on 10 days has made 9,1 (p<0,05), on 20 days – 18 and on 30 days – 23% (p<0,05). In the third group – on 5,1 (p<0,05), - on 13,8 (p<0,05), - for 13,8%; in the fourth group - on 1,1, - on 7,8 (p<0,01), - 4,5% in the same terms of research.

Content of α-globulins at rats of the second group in comparison with control to 10, 20 and 30 days of experiment has authentically increased na14,1, 21,9 and 39,9% respectively. The maintenance of fraction of α-globulins at rats of the third group in comparison with control to 10 days of experiment has increased on 7,1, to 20 days – 4,5, to 30 days – by 12,6%. Content of α-globulins in the fourth group of rats in comparison with control to 10 days of experiment has increased on 4,5, to 20 days – 11,2, to 30 days – by 7,0%.

Throughout all experiment registered increase of maintenance of fraction of β-globulins in serum of blood of experimental animal all groups, rather this biological control. So, at rats of the second group the content of β-globulins to 10 days has increased on 33,4 (p<0,01), to 20 days – on 57,7 (p<0,01) and to 30 days – 70,8% (p<0,001); content of β-globulins at rats of the third group to 10 days of experiment has increased on 26,2 (p<0,05), to 20 days – 35,1 (p<0,01), to 30 days – by 46,4% (p<0,01); content of β-globulins at rats of the fourth group to 10 days of experiment has increased on 16,2, to 20 days – 21,4 (p<0,05), to 30 days – by 28% (p<0,05).

The maintenance of γ-globulins fraction in protein of blood of rats of the second group in comparison with data of group of biological control to 10 days of experiment has decreased on 11,5, to 20 days – on 19,7 (p<0,05), to 30 days – by 30% (p<0,05).

In the γ-globulins prevented in comparison with control contents has gone down to 30 days – in the third for 14,8% and in the fourth groups for 14,5%.

During research have carried out determination of activity of enzymes, contents of cholesterol and urea in serum of blood of white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent of "Fitosorb" whose results are yielded in table 4.

Table 4: Activity of enzymes, content of cholesterol and urea in serum of blood of white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent "Fitosorb"

Term of research, days	Group	Alkaline phosphatase, E/l	ALT, E/l	AST, E/k	Cholesterol, mmol/l	Urea, E/l
10	1	137,00±6,07	62,60±2,51	122,88±4,26	1,61±0,08	6,50±0,42
	2	171,28±7,51*	76,22±2,56**	142,30±6,49*	1,53±0,09*	6,24±0,35
	3	152,25±5,86	63,85±2,65	131,50±5,09	1,53±0,09	4,91±0,25*
	4	154,30±8,22	64,20±2,80	126,75±4,30	1,81±0,05	6,15±0,76
20	1	142,40±10,35	73,64±3,68	118,63±4,57	1,16±0,20	6,23±0,34**
	2	195,68±9,26**	99,85±4,88**	158,50±6,56**	1,55±0,04	3,18±0,20***
	3	158,93±8,30	91,53±3,48**	146,00±3,30**	1,28±0,06	3,78±0,20**
	4	160,23±10,22	83,10±3,51	135,35±5,81	1,47±0,17	4,20±0,19**
30	1	141,33±8,98	64,56±2,72	133,50±7,19	1,29±0,11	4,67±0,22
	2	212,23±10,58**	112,87±4,00***	226,75±6,03***	1,38±0,08	7,85±0,58
	3	190,70±6,65**	101,40±3,60***	200,13±7,45**	1,27±0,06	6,53±0,45
	4	168,40±5,37*	84,80±3,90***	178,30±4,77**	1,29±0,08	4,42±0,17

* - p<0,05 ** p<0,01 *** p<0,001

Analyzing these tables 4, increase of activity of alkaline phosphatase in blood of animals of the second group, in comparison with data of biological control to 10 days - on 25 (p<0,05), to 20 days - on 37 (p<0,01), to 30 days - for 50,2% has been revealed (p<0,01). Increase of activity of alkaline phosphatase in the third group of rats of rather control data has made 11,1; 11,6 and 35% (p<0,01) to similarly previous terms of research. Rats of the fourth group had an increase of activity of this indicator to 30 days - for 19,2% (p<0,05).

In the second group of animals activity of ALT in blood to 30 days of research, in comparison with control data, has increased by 73,3% (p<0,001). At a blood test of rats of the third group to 30 days, in comparison with control data, registered increase in activity of ALT by 57,1% (p<0,001), ALT in serum of blood of white rats of the fourth group has increased to 30 days by 31,3% (p<0,001).

In blood of animals of the second group activity of AST, in comparison with biological control, on 10 days of researches was 15,8 higher (p<0,05), on 20 days – on 33,6 (p<0,01), on 30 days – for 70% (p<0,001). At rats of the third group on 10, 20 and 30 days, in comparison with control data, registered increase in activity of AST on 7,1; 23,1 (p<0,01) and 50% (p<0,001), respectively. Activity of AST in serum of blood of white rats of the fourth group has increased on 10, 20 and 30 days on 3,1; 14,1 and 33,6% (p<0,001), respectively.

In blood of rats of the second group observed reliable decrease in level of cholesterol in comparison with data of group of biological control on 10 days for 17,4% (p<0,05), on 20 and 30 days of research have registered increase for 33,6 and 7% respectively. The group of the rats prevented by introduction to a diet of 0,5% of an enterosorbent observed decrease in level of cholesterol on 10 days for 5%, to 20 days registered his increase for 26,7%, to 30 days there was a decrease in level of cholesterol to 1,6%. In blood of rats of the fourth group on 10 and 20 days of experiment observed increase of level of cholesterol for 12,4 and 10,3% respectively, and to 30 days this indicator didn't differ from control data.

Concentration of urea in blood of rats of the second group on 20 days of researches was below control for 48,9% (p<0,001), and on 30 days - is 68% higher. In the third group of animals the content of urea, in comparison with control data, has decreased by 10 and 20 days on 24,5 (p<0,05) and 39,3% (p<0,01) respectively, and to 30 days it was 39,8% higher. In the fourth group of animals the content of urea, in comparison with control data, on 10 days has increased - by 5,4%, and on 20 and 30 days has decreased - on 32,6 (p<0,01) and 5,3% respectively.

Results of research of a mineral exchange at white rats at the combined chronic T-2 and an aflatoksikoza against application of an enterosorbent "Fitosorb" are presented in table 5.

Table 5: Indicators of a mineral exchange at white rats at the combined chronic T-2 and an aflatoxicoses against application of an enterosorbent "Fitosorb"

Term of research, days	Group	Indicator	
		General calcium, mmol/l	phosphorus inorganic, mmol/l
10	1	2,39±0,07	1,43±0,02
	2	2,01±0,10*	1,36±0,03
	3	2,08±0,11	1,39±0,09
	4	2,14±0,04*	1,42±0,02
20	1	2,39±0,03	1,38±0,09
	2	1,73±0,06***	1,06±0,10
	3	2,16±0,06*	1,50±0,15
	4	2,07±0,03**	1,14±0,04*
30	1	2,46±0,16	1,46±0,10
	2	1,66±0,19*	2,15±0,29
	3	1,71±0,09**	2,04±0,11*
	4	1,80±0,10*	1,60±0,06

Proceeding from data of table 5, it is possible to note that over all experiment in blood of rats of all groups there was a decrease in content of the general calcium. So in the second group of rats the content of the general calcium on 10,20 and 30 days has decreased, concerning control, on 15,9 (p<0,05), 27,6 (p<0,001) and 32,5% (p<0,05) respectively. In the third group the maintenance of this indicator in blood of rats to similar terms of researches, in comparison with control has decreased on 13; 9,6 (p<0,05) and 30,5% (p<0,01) respectively. At a blood test of rats of the fourth group reduction of content of the general calcium by 10, 20 and 30 days of research on 10,5 (p<0,05), 13,4 (p<0,01) and 26,8% (p<0,01) respectively was noted.

In the second group of rats the content of phosphorus inorganic in blood of rats, on 10 and 20 days of experiment, in comparison with control indicators, was 5 lower also than 22,8%, and to 30 days above - for 46,1%. In the third prevented group the content of phosphorus of research, inorganic on 10 days, was insignificant below data of control - for 2,5%, and already on 20 and 30 days there was an increase by 8,7 and 38,6% (p<0,05) respectively.

In the fourth prevented group the content of phosphorus of experiment, inorganic on 10 days, was insignificant below data of control – on 0,7, and already to 20 days has gone down on 18 (p<0,05); to 30 days of experiment observed increase of this indicator for 8,9%.

CONCLUSION

Inclusion in a diet of experimental animals of a combination of T-2 and aflatoxin of B1 negatively affects hematologic parameters of blood throughout experiment that is expressed in progressing erythrocyto-leykopenia and anemias that is one their characteristic symptoms of mycotoxicoses and indicates serious destructive changes in the haematogenic and immunocompetent bodies [14].

Destructive action of a combination of T-2 of toxin and B1 aflatoxin on hepatocytes and a liver are demonstrated by results of the researches of a proteinaceous and enzymatic profile of blood of experimental animals conducted by us which will be coordinated with literary data. Toxic action of combinations of T-2 toxin and an AFB1 and other toxins is shown by a great number of authors [15, 16]. About the inhibitory effect of the

combination of T-2 toxin and AFB1 on the growth and manifestation of a similar clinical picture it was reported both earlier and is probably explained by development of syndromes of refusal of a forage and bad absorption (or malabsorption). T-2 toxin inhibits synthesis of proteins of a liver [17] that leads to a giperaminoatsidemiya – excess of free amino acids, increase in concentration in a tryptophane brain, and finally, serotonin in midbrain tissues, brain bark that influences perception of saturation [18].

Leads apoptosis of cells of a digestive tract of a trichothecenes [19], and decrease in amount of enzymes of a pancreas and the bilious acids necessary for emulsification and digestion of fats to development of the second syndrome an aflatoxin of B1 [20].

Daily introduction of an enterosorbent "Fitosorb" to forages, contaminated T-2 toxins and an AFB1 in concentration of 0,3 mg/kg, in a dose from 5 to 10 g/kg to a diet reduces symptoms of toxicosis, improves the general clinical condition of animals, has favorable effect on the course of physiological processes of an organism, providing correction of morphological and biochemical indicators of blood, stimulating growth and development of animals.

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