ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Efficacy of The Adsorbent "Fytosorb" In Case of Combined Mycotoxicosis In Young Weaned Pigs Against the Background of Infection Load.

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ABSTRACT

2 groups of 3 young pigs (females) each were made. Group 1 served as toxic control and received a ration contaminated with T-2 toxin at a dose of 70 µg/kg of feed, zearalenone at a dose of 50 µg/kg of feed and deoxynivalenolat a dose of 1000 µg/kg of feed. Group 2 received a ration contaminated with T-2 toxin at a dose of 70 µg/kg of feed, zearalenone at a dose of 50 µg/kg of feed and deoxynivalenolat a dose of 1000 µg/kg of feed; Fytosorbwas also added to their ration at a dose of 0.5% of the ration. All the young pigs were infected with the Clostridium pathogenic culture from the strain collection of the FGBNU "FTSTRB-VNIVI". All the animals were vaccinated with the vaccine against escherichiosis. The experiment lasted 30 days. Joint intake of fusariotoxins into the organism of young pigs with feed in permitted doses during 30 days - T-2 toxin at a dose of 70 µg/kg of feed, zearalenone at a dose of 50 µg/kg of feed and deoxynivalenol at a dose of 1000 µg/kg of feed against the background of infection load by Clostridium microorganisms causes clinically pronounced mycotoxicosis accompanied by the activation of lipid peroxidation, a decline in immunological parameters: a decrease in the number of T- and B-lymphocytes, the titer of specific protective antibodies and the development of pathological processes in the tissues and organs of young pigs. A daily addition of enter sorbent "Fytosorb" to the feed at a dose of 5 g/kg of feed reduces the symptoms of toxicosis, improves the general condition of animals, has a beneficial effect on physiological processes of the body providing correction of morphological and biochemical parameters of blood and stimulating the growth and development of animals.

Keywords: a combination of mycotoxins T-2, zearalenone, deoxynivalenol and Clostridium microorganisms, immunity, mycotoxicosis prophylaxis, adsorbent "Fitosorb".

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ISSN: 0975-8585

INTRODUCTION

The problem of obtaining biologically complete and harmless livestock products, including in ecologically polluted regions, remains unsolved both in scientific and theoretical and in practical terms. A significant part of feed contains mycotoxins, heavy metals and pollutants. This causes latent forms of intoxication of livestock, a decrease in liveability, inefficient use of feed, a decrease in consumer properties of livestock and poultry products [1].

Mycotoxins are thermally stable poisonous substances that are products of the metabolism of microscopic molds; affecting plant raw materials, they affect feed causing intoxication in farm animals (mycotoxicosis) [2-4].

Studies of scientists show that animal husbandry suffers serious economic losses because of a decrease in the productivity and reproduction of farm animals that are caused bymycotoxicosis [2-6]. Researchers are particularly interested in immunosuppressive properties of mycotoxins [7-18].

The toxins produced by the fungus Fusarium sp. - T-2 toxin, zearalenone and trichothecene mycotoxins have a negative effect. Most often, these mycotoxins are produced by fungi simultaneously, that additionally creates a synergistic effect [19-29]. Previously, we had studied the combined effects of fusariotoxins T-2, zearalenone, deoxynivalenol and infectious agents on the young weaned pigs' organism [18]. Their negative influence in small doses was described by other authors [15, 16, 31-34].

The search for prophylactic drugs is an urgent task for veterinary medicine. Due to the combination of the properties applicable to prophylactic drugs: efficiency, physiology, harmlessness, low percentage of introduction into the ration, selectivity of sorption, etc. enter sorbents are widely used [27,34]. Previously, the adsorption properties of lignins and polysaccharides had been studied [35-40]. The purpose of the present research was to study the preventive efficacy of the organic adsorption "Fytosorb" in case of combined impact of such fusariotoxinsas T-2 toxin, zearalenone, deoxynivalenol and infectious agents on the organism of young weaned pigs.

MATERIALS AND METHODS

Experiments were carried out in young weaned pigs of the Large White breed. Before the experiments, the animals were kept in 2-week quarantine, the feeding was carried out according to the norms adopted in zootechnics. Experimental and control animals were divided into groups according to the principle of analogues. 2 groups of 3 young pigs (females) each were made. Group 1 served as toxic control and received a ration contaminated with T-2 toxin at a dose of 70 μg/kg of feed, zearalenone at a dose of 50 μg/kg of feed and deoxynivalenol at a dose of 1000 μg/kg of feed. Group 2 received a ration contaminated with T-2 toxin at a dose of 70 μg/kg of feed, zearalenone at a dose of 50 μg/kg of feed and deoxynivalenol at a dose of 1000 μg/kg of feed; Fytosorb was also added to their ration at a dose of 0.5% of the ration. All the young pigs were infected with the Clostridium pathogenic culture from the strain collection of the FGBNU "FTSTRB-VNIVI" which previously had been isolated from the pathological material of the dead young pigs when studying the mass death of young pigs on one of the farms in the Republic of Tatars tan. Infection was performed orally in all the groups of animals. Each young pig was poured into a suspension at a dose of 2 ml that contained $1x10^6$Clostridium cells. The experiment lasted 30 days.

All the animals were vaccinated with the vaccine against escherichiosisthat was made in FGBNU "FTSTRB-VNIVI". The vaccine was injected intramuscularly into the posterior surface of the thigh at a dose of 1 ml on the 15th day of the experiment.

For experimental studies, crystalline T-2 toxin, deoxynivalenol, zearalenonefrom the laboratory of mycotoxins in FGBNU "FTSTRB-VNIVI" with the mycotoxin purity of 99.8%, 96.7% and 98.3%, respectively, were used. As a producer of T-2 toxin and zearalenone, Fusarium sporotrichioidesfungus of 2M15 strain was used, kindly provided by Dr. Sc. A.N. Kotik; as a producer of deoxynivalenol, Fusarium graminearumof W32 strain from the collection of microscopic fungi ofFGBNU "FTSTRB-VNIVI" was used. Fytosorb is an organic adsorbent based on acid detergent fiber and lignin of the grain shell [27].

Toxins were added to the feed of the animals and stirred thoroughly. Doseswere taken at the maximum permissible concentration level permitted in Russia. The feed was also pre-tested for the content of mycotoxins permitted in Russia and biological safety - the existence of pathogenic and opportunistic pathogenic micro flora, the feed corresponded to the certificate of quality.

During the experiment, the general clinical condition of animals, feed intake, hematological, biochemical and immunological parameters, changes in the body weight were studied, life expectancy and pathologic anatomical picture were registered. The blood was taken from the tail vein in experimental and control animals.

The number of red blood cells, white blood cells, monocytes, lymphocytes, platelets, hemoglobin concentration were determined with the Mythic 18 hematology analyzer, the levels of total protein, bilirubin, glucose, activity of Alanine aminotransferase, Aspartate aminotransferase, alkaline Phosphatase enzymes in blood serum of animals were determined with theMicrolab 300 chemistry analyzer.

The levels of T-and B-lymphocytes in peripheral blood were determined by spontaneous erythrocyte rosette assay. Titres of antibodies to the vaccine against colibacillosis of pigs were determined in the serum agglutination test. The degree of intensity of the process of lipid peroxidation (LPO) was estimated by the accumulation of secondary products of LPO -malonic dialdehyde (MDA) in reaction with 2-thiobarbituric acid.

The processing of the digital material was carried out by the method of variation statistics using the Student's t- test.

RESULTS AND DISCUSSION

The results of hematological blood tests in case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent “Fytosorb” are presented in Table 1.

Table 1: Hematologicalparametersin case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent “Fytosorb” (n = 3)

Parameter	Group of the animals / day of the experiment							
	1				2			
	Basic data	10	20	30	Basic data	10	20	30
Red blood cells, $x10^{12}/L$	5.37 ±0.15	6.1 ±0.13 *	5.8 ±0.11	4.06 ±0.18 ***	5.38 ±0.14	5.16 ±0.17	5.23 ±0.15	5.18 ±0.18 **
Total number of white blood cells, $x10^{9}/L$	15.63 ±0.42	19.3 ±0.51*	26.9 ±0.44** *	19.1 ±0.49	16.32 ±0.34*	17.5 ±0.41	16.4 ±0.50	16.9 ±0.47
Hemoglobin, g/L	92.0 ±2.0	95.0 ±1.4	91.0 ±1.3	79.0 ±2.2 **	92.0 ±1.3	89.0 ±1.6	91.0 ±1.6	88.0 ±1.4 **
Platelets, $x10^{9}/L$	236.0 ±6.72	393.0 ±7.93 ***	639.6 ±8.85 ***	62.0 ±11.4 ***	241.0 ±8.45	281.4 ±5.64 **	304.1 ±9.95 **	255.0 ±8.31

* - $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

As can be seen from the data presented in Table 1, there were fluctuations in the white blood cell counts. Thus, on the 10th day of the experiment, a significant increase in the total number of leukocytes of 23.5% was noted in Group 1 of animals that received mycotoxins with feed, on the 20th daythe increase was 72.1%, at the end of the experiment, the increase in the number of leukocytes was only 22.2% in comparison

with biological control. In Group 2, the changes in the number of leukocytes were not significant and at the end of the experiment, an increase in the number of leukocytes was 3.6%.

Synergistic effect of mycotoxins on erythrocytes, the inhibition of erythropoietin, the development of anemia and tissue oxygenation is indicated by hematological parameters presented in Table 1. In the young pigs of Group 1, there was a decrease of 24.4%, 14, 1% and 73.7% in the levels of erythrocytes, hemoglobin and platelets, respectively, in comparison with the basic data, whereas in Group 2,a decrease in the levels of erythrocytes and hemoglobin was 3.7% and 4.3%, respectively. Thus, the data obtained indicate the negative effect of mycotoxins on hematological parameters of animals in case of combined chronic addition to feed in concentrations close to the maximum permissible ones in the feed and against the background of infection load.

During the experiment, some biochemical parameters of blood serum of young pigs against the background of chronic combined intake of mycotoxins were tested. The results of the study are presented in Table 2.

Table 2: Biochemical parameters in case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent "Fytosorb" (n = 3)

Parameter	Group of the animals / day of the experiment							
	1				2			
	basic	10	20	30	basic	10	20	30
Total protein, g/l	62.3 ±0.92	62.6 ±0.90	58.1 ±0.82*	55.5 ±0.94**	60.4 ±0.89	62.8 ±0.94	63.1 ±0.92	61.8 ±0.93
Total bilirubin, mcmol/L	1.5 ±0.1	4.2 ±0.15***	4.9 ±0.22**	12.7 ±0.57***	1.5 ±0.27	2.2 ±0.18 *	4.5 ±0.14 **	5.1 ±0.11 ***
Glucose, mmol/L	3.2 ±0.11	2.9 ±0.14*	2.5 ±0.13***	1.9 ±0.17***	3.4 ±0.15	2.9 ±0.11 *	4.0 ±0.17	4.1 ±0.16
ALT, U/L	27.4 ±1.85	46.3 ±1.92**	80.6 ±1.63***	59.2 ±1.91	32.7 ±1.89	32.0 ±1.91	38.4 ±1.50 *	43.1 ±1.77 *
AST, U/L	34.6 ±2.42	60.2 ±2.23**	75.2 ±2.48***	56.4 ±2.47	41.3 ±2.45	40.5 ±2.46	49.2 ±2.54 *	52.5 ±2.52

* - $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Against the background of the development of mycotoxicosis, there was a decrease of 10.9% in the level of total protein in the blood serum of animals on 30th day compared to the basic data, which is probably due to the weakening of synthetic processes in the liver. This pathological disorder is successfully corrected by the addition of a sorbent `to the ration.

The loss of cell mass in the liver caused intractable hypoglycemia in the blood of experimental animals receiving toxic food, since the synthesis of glucose from amino acids and glycerin occurs in the liver. The glucose level decreased by 21.9% and 40.6% on the 20th and 30th days, respectively, in the animals of Group 1compared to the basic data, whereas in Group 2,a decrease in glucose concentration was 17.6% on 20th day with a subsequent increase of 20.6% on 30th day.

The massive damage to the integrity of hepatocytes membranes is indicated by an increase in the serum of such hepatobiliary enzymes as Aspartate aminotransferase and Alanine aminotransferase. In the blood serum of the young pigs affected by mycotoxins, the activity of Alanine aminotransferase and Aspartate aminotransferase increased by 116.1% and 56.4%, respectively, thatcorrespondsto the literature sources. Against the background of the protective effect of the adsorbent "Fytosorb", an increase in the activity of Alanine aminotransferase and Aspartate aminotransferase was 31.8% and 27.1%, respectively.

ISSN: 0975-8585

In case ofmycotoxicosis, excessive and uncontrolled activation of lipid peroxidation occurs that ultimately can lead to a pathological condition that is accompanied by the imbalance between enzymatic and non-enzymatic components of the antioxidant defense system.

One of the main quantitative markers most often used to assess the level of the antioxidant system is the end product of lipid peroxidation - malonic dialdehyde, an increase in which carries information about the depth and degree of the pathological process.

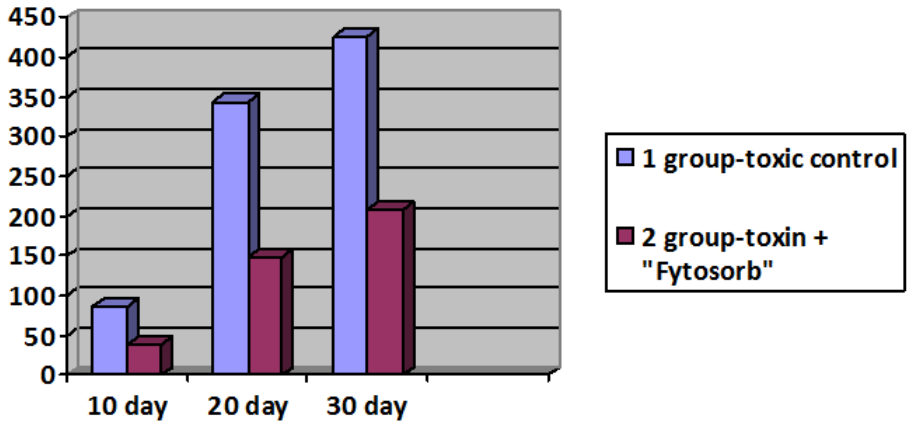

Figure: MDA concentration in the blood in case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent "Fytosorb"

According to the data presented in Figure 1, a significant increase in MDA in the blood was recorded in the toxic control group as compared to the basic data: 86.1% on 10th day of the experiment,343.6% on 20th day, 426.7% (4 times)on 30th day. The addition of the enterosorbent"Fytosorb"to the ration contributed to the repair of disorders of the antioxidant system, ason 30th day of the experiment, the MDA level in Group 2 was 2 times higher compared to the basic data.

Table 3: Results of the evaluation of cellular immunity in case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent "Fytosorb" (n = 3)

Group of the animals	Day of the research	Parameter	
		T- lymphocytes	B- lymphocytes
1	Basic data	52.8±2.36	27.4±1.12
	10	53.7±2.83	28.6±1.49
	20	48.3±2.14	26.1±0.38
	30	32.1±2.97*	23.2±1.81
2	Basic data	53.4±2.54	29.2±0.96
	10	53.9±2.52	28.4±0.65
	20	55.7±2.74	29.9±1.04*
	30	63.2±3.01*	32.5±1.67

* - p ≤0.05; ** p ≤0.01; *** p ≤0.001

As can be seen from Table 3, during the study of simulatedmycotoxicosis in the toxic control group, an imbalance in the functioning of the body's defense systems was observed, which was characterized by a decline in T-cells of the immune system, a decrease in the number of B-lymphocytes. So in comparison with the basic data, at the end of the experiment the animals of Group 1 had a statistically significant decrease of

39.1% in the number of T-lymphocytes and 15.3% in the number of B-lymphocytes, whereas in Group 2 there was an increase of 18.4% and 11.3%in T- and B-lymphocytes, respectively.

Against the background of mycotoxicosis, there was a decrease in the production of specific antibodies againstclostridiosis in animals that was also influenced by the acquired T-deficiency. The addition of the sorbent to the ration of animals against the background of mycotoxicosiscontributed to an increase in B-lymphocyte differentiation, the induction of antibodies againstclostridiosis. So, the antibody titer in Group 2 was 1:80 compared to 1:40 in Group 1 of toxic control.

During the experiment, the following aspects were observed. In the first half of the experiment (first 2 weeks), the general condition of animals in both groups was satisfactory.

In animals of Group 1that received toxic feed containing mycotoxins, feed intake was lower than in Group 2. 6-8 days later, a partial refusal of feed was observed, which lasted 3-4 days, then the animals consumed feed again, but in smaller quantity, the young pigs were also much less active. In the second half of the experiment, the animals were sluggish, heaped up, pressed their stomachs, there was a gastrointestinal upset. On 21st-23d days, the young pigs in the toxic control group were worse, had a high temperature, 1 young pig died. While studying its internal organs, pathogenic microorganisms - agents of infection Clostridium were identified. In Group 2,the animals didn't suffer from ill health.

The results of registration and calculation of zootechnicalparameters of animals are presented in Table 4.

Table 4: Zootechnicalparametersin case of mycotoxicosis in the young pigs against the background of infection load and without the use of the sorbent "Fytosorb" (n = 3)

Parameter	Group of the animals	
	1	2
Weight of the body at the beginning of the experiment, kg	14.65 ±0.22	14.86 ±0.23
Weight of the body at the end of the experiment, kg	19.1 ±0.64*	21.79 ±0.37
Weight gainin30 days, kg	4.45	6.93
Average daily gain, g	148.3	231.0
Feed consumed per 1 heading 30 days, kg	22.6	26.8
Feed conversion	5.07	3.86
Liveability of animals, %	66.6	100

* - p ≤0.05; ** p ≤0.01; *** p ≤0.001

The data presented in Table 4 indicate that the highest weight gain was in Group 2 - 231 g, the lowest gain was in Group 1 - 148.3 g (39.2%). Feed conversion also differed in the groups: 5.07 - in Group 1 and 3.86 - in Group 2. The liveability of animals in Group 2 was 100%, in the toxic control group - 66.6%.

CONCLUSION

Joint intake of fusariotoxins into the organism of young pigs with feed in permitted doses during 30 days - T-2 toxin at a dose of 70 µg/kg of feed, zearalenone at a dose of 50 µg/kg of feed and deoxynivalenol at a dose of 1000 µg/kg of feed against the background of infection load by Clostridium microorganisms causes clinically pronounced mycotoxicosis accompanied by the activation of lipid peroxidation, a decline in immunological parameters: a decrease in the number of T- and B-lymphocytes, the titer of specific protective antibodies and the development of pathological processes in the tissues and organs of young pigs.

A daily addition of enter sorbent "Fytosorb" to the feed at a dose of 5 g/kg of feed reduces the symptoms of toxicosis, improves the general condition of animals, has a beneficial effect on physiological processes of the body providing correction of morphological and biochemical parameters of blood and stimulating the growth and development of animals.

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