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Application of retinyl acetate in rabbit aflatoxicosis

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Abstract.

The data on estimation of efficiency of co-use retinyl acetate and zeolite due to long-term exposure of aflatoxins B_1 to animal organism are presented. The experiments were carried out on 24 rabbits divided by the principle of analogues into 4 groups: biological control; priming control; experimental group (aflatoxins + retinyl acetate at a dose of 700 IU) and experimental group (aflatoxins + retinyl acetate at a dose of 1500 IU). Aflatoxin B_1 and retinyl acetate were administered in the diet of animals. The evaluation was performed using clinical, hematological and biochemical research methods. In the course of the test, experimental aflatoxicosis of animals was reproduced. The weight loss as well as a decrease in the morphological parameters of rabbits blood was noted. It indicates the toxic effect of aflatoxin B_1. At the same time a favorable effect of retinyl acetate on experimental animals is shown. It is confirmed by observations of the clinical condition and also manifests itself in less pronounced changes in hematological and biochemical parameters in the experimental groups compared with the control group of the seed.

Key words: Mycotoxin, aflatoxins, retinyl acetate, blood, rabbits.

INTRODUCTION.

Mycotoxins are secondary metabolites of microfungus belonging to one of the biogenic poisons groups that have dominated in recent years, contaminating both animal food and food products. The majority of mycotoxins are highly resistant to physicochemical factors and do not break even with prolonged heating of the substrate [1,2,4]. Microfungus affects mainly plant objects during their growing season or storage. As a result of fungal damage about 30% of the produced grain spoils during storage annually. At the same time, spoiled grain is often used to feed cattle, which can lead to their diseases and death. Researches show that animal farming has serious economic losses from production loss and farm animals reproduction that occur during mycotoxicosis [7].

According to Creepy E. [3], the most common toxigenic fungi in Europe are Aspergillus, Penicillium and Fusarium. Among them, preference is given to Aspergillus flavus and A. parasiticus, which produce aflatoxins. According to their chemical structure, aflatoxins are furocoumarins. More than 80% of the total amount of these mycotoxins from the 4 main members of the aflatoxins family (B_1, B_2, G_1 and G_2) is accounted for aflatoxin B_1, which is extremely toxic and synthesized in the largest amounts [6].

The essence of the biological effects of aflatoxins on the body is a suppression of vital functions such as protein production, nucleic acids and impaired lipid synthesis. A decrease in total and microsomal protein in the liver of rats and monkeys and a significant decrease in the concentration of protein in the serum of various animal species were also noted. Aflatoxins act directly on the cell walls and membranes of various cytoplasmic inclusions accompanied by changes in their enzymatic activity.

Aflatoxins are the strongest immunosuppressants. They are carcinogenic, mutagenic and teratogenic, cumulated in the organs and body tissue and can move along the food chain and to humans. Aspergillus parasiticus and Aspergillus flavus are produced [15]. Aflatoxin contamination causes health problems and losses in livestock production, causing mortality and morbidity [8,9]. In addition, the presence of aflatoxins can cause pathological damage in the liver and, therefore, impair the antioxidant liver functions, as mentioned by Yang F. et al. [16].

There are data on strengthening of negative effect on organism during combined contamination of animal feed [10,11,12,13,14].

Thus, the literature data indicate the relevance of the aflatoxicosis problem, the need to control the quality of animal feed and improve measures aimed at preventing animal diseases, the research of new means of treatment and prevention.

The purpose of researches was to study the effect of aflatoxin B_1 on the animal body during the use of retinol acetate at different doses.

MATERIAL AND METHODS.

The experiment with the use of 24 rabbits with a live weight of 3.5-4 kg, divided according to the principle of analogs into four groups of 6 animals each, was carried out in laboratory conditions at “Federal Centre of Toxicological and Radiation Safety of Animals (VNIVI)”. The conditions of keeping and feeding of animals corresponded to zoohygienic norms.

The first group served as a biological control and received fresh food throughout the experiment (55 days). During 25 days (from the 30th to the 55th day of the experiment) animals of the second, third and fourth groups received the main diet, contaminated with aflatoxin B_1 in concentration of 3 mac (75 μg/kg animal feed). Throughout the experiment (1-55 days) an oil solution of retinyl acetate at a dose of 700 and 1500 international units (IU) per individual were additionally introduced into the diet of the third and fourth group rabbits. We took this experimental scheme with the introduction of vitamin A 25 days before priming with a toxin in order to provide the body of rabbits with a

supply of this vitamin. We are interested in finding out the effect of different doses of retinyl acetate on the course of aflatoxicosis in animals (for example, rabbits) and how consumption of aflatoxin can affect on the vitamin content in the body.

Blood drawing for hematological and biochemical studies was carried out from the marginal ear vein on days 35, 45 and 55 of the experiment. The number of erythrocytes, leukocytes, the content of total hemoglobin in the peripheral blood was determined according to generally accepted methods on an automatic hematology analyzer Mythic 18. The total protein content was determined refractometrically. The proportion of protein fractions was nephelometric. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined on an EXPRESS PLUS analyzer. The vitamin A content was determined in blood and liver by the method described by Hosotani and Kitagawa [5]. The method is based on alkaline hydrolysis, n-hexane and ethanol were used as a solvent, and natrium ascorbat was used as an antioxidant. The results were detected on an Agilent 1260 liquid chromatograph equipped with diode array and fluorescence detector. We used the HPLC Reprosil ODS-A C18 250 * 4mm column. The autosampler was used when entering the sample.

The processing of digital material was performed by the method of variation statistics using the Student's plausibility criterion on a personal computer using Excel programs. The difference between the compared values was considered reliable at $P \leq 0.05$ levels.

RESULTS AND DISCUSSION.

The results of daily observations of the clinical status of rabbits showed that all animals receiving a diet contaminated with a toxin without superinduction of retinyl acetate expressed clinical signs of toxicosis in the form of depression, reduction of feed intake, gastrointestinal upset in the form of diarrhea, decrease in hematological and biochemical values. Rabbits of the third and fourth experimental groups showed similar signs of a clinical condition 3-5 days later.

From 30 to 55 days of research there was a decrease in body weight of rabbits by 10.3% of the initial data in the group of animals treated with mycotoxin without retinyl acetate. In the experimental groups there was also a decrease of the test items during this period. Thus, the body weight of animals of the third and fourth groups from 30 to 55 days of experience decreased by 7.1 and 4.9%, respectively, at the same time exceeded the data of the control group of seed by 2.2 and 5.9%, respectively. The body weight of the rabbits group of biological control increased by the end of the experiment by 39.8% relative to the initial data and was higher than the weight of the second group rabbits by 27.8%.

Hematological blood values of the second group rabbits presented in table 1 indicate the negative effect of aflatoxin B_1 on these parameters, indicating serious destructive changes in the blood-forming organs.

At the same time, the study of hematological parameters of the third and fourth groups rabbits in the diet that was administered retinyl acetate prevented the manifestation of these changes. The decrease of this group indicators were less pronounced, but the changes were natural.

Compared with the control, animals receiving “toxic food” had the total protein content decrease of blood by 5.4; 18 and 26%, respectively at 35, 45 and 55 days of the study decreased; the amount of albumin - by 2.4; 7.4 and 11.8%, respectively; indicators of the third group rabbits - by 5.2; 14.3 and 23.7% and 1.3; 5.5 and 9.2%, respectively; indicators of the fourth group rabbits - by 3.7; 12.0 and 20.8% and 3.0; 5.3 and 9.0%, respectively.

Table 1 - Morphological blood value of rabbits with aflatoxicosis on the background of the use of retinyl acetate

Animals group	Index		
	Leucocytes, 10^9 /L	Erythrocytes, 10^{12} /L	Haemoglobin, GM/DL
1	2	3	4
35 days			
The first	9.39±0.22	5.50±0.17	123.56±2.07
The second	9.25±0.2	5.14±0.18	116.28±1.79*
The third	9.21±0.18*	5.32±0.15	125.57±2.03
The fourth	9,15±0.2*	5.20±0.20	126.63±1.54
45 days			
The first	9.48±0.21	5.39±0.17	124.87±1.90
The second	8.53±0.18**	4.72±0.19	112.11±2.01*
The third	8.73±0.19*	4.93±0.19	118.74±1.69*
The fourth	8.80±0.17*	4.91±0.20	119.16±1.64
55 days			
The first	9.41±0.17	5.44±0.18	123.92±1.67
The second	7.76±0.16**	4.12±0.16**	102.57±1.81**
The third	7.97±0.19**	4.41±0.18*	111.08±1.92**
The fourth	8.03±0.18**	4.43±0.17*	112.22±1.83**

Important: * - P<0.05; ** - P<0.01

Table 2 - Biochemical blood value of rabbits with aflatoxicosis on the background of the use of retinyl acetate

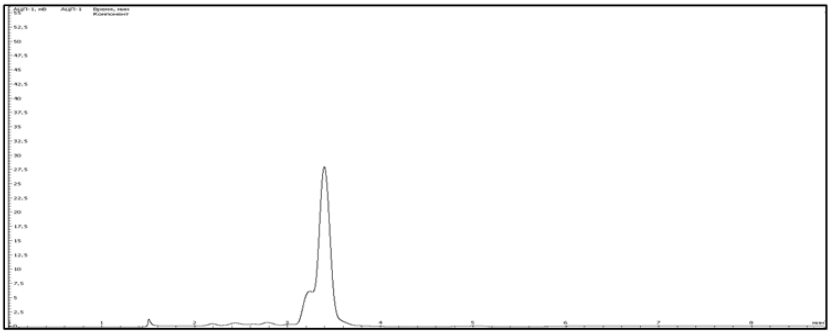
Index	Animal group / research period			
	the first	the second	the third	the fourth
1	2	3	4	5
	35 days			
Total protein, g/l	71.68±0.56	67.81±0.62	67.98±0.67	69.04±0.42
Albumins, %	55.81±1.1	54.47±1.22	55.07±1.26	54.12±1.32
AST	14.97±0.82	16.48±0.85	15.37±0.76	14.80±0.79
ALT	11.20±0.52	11.94±0.49	11.43±0.56	11.22±0.43
	45 days			
Total protein, g/l	72.13±0.75	59.14±0.62**	61.82±0.68**	63.42±0.53**
Albumins, %	55.93±0.97	51.78±1.06*	52.84±0.84	52.94±0.91
AST	14.76±0.72	17.94±0.83	16.00±0.76	15.31±0.88
ALT	11.26±0.45	12.50±0.51	11.66±0.62	11.39±0.41
	55 days			
Total protein, g/l	71.5±0.68	53.11±0.74**	54.72±0.77**	56.83±0.69**
Albumins, %	55.78±0.96	49.22±0.93**	50.65±1.03*	50.71±0.86*
AST	14.82±0.75	19.38±0.79*	17.00±0.94*	16.32±0.85*
ALT	11.19±0.49	13.29±0.54*	12.22±0.42*	11.87±0.58*

Important: * - P<0,05; ** - P<0,01

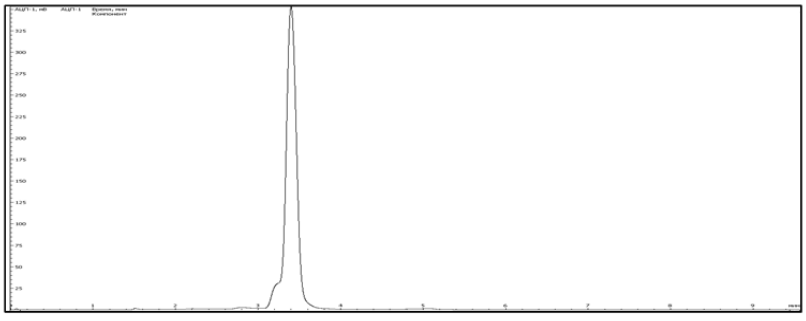
Table 3 - The vitamin A content in the serum of rabbits (mg %).

Research period, days	Animals group			
	the first	the second	the third	the fourth
Profile	0.057±0.03	0.058±0.04	0.060±0.03	0.059±0.03
5	0.058±0.04	0.056±0.03	0.076±0.04	0.091±0.03
15	0.056±0.03	0.057±0.03	0.073±0.03	0.088±0.02
30	0.054±0.02	0.059±0.03	0.078±0.04*	0.092±0.03
35	0.056±0.03	0.054±0.04	0.077±0.03*	0.093±0.04*
45	0.054±0.03	0.047±0.03	0.072±0.02*	0.089±0.03*
55	0.055±0.02	0.040±0.02*	0.066±0.03	0.083±0.03*

Important: * - P<0.05; ** - P<0.01



Picture 1 - Chromatograms of vitamin A release in rabbit liver (the second group)



Picture 2 - Chromatograms of vitamin A release in rabbit liver (the fourth group)

In comparison with the control group, the research results of the serum enzymes aspartate aminotransferase and alanine aminotransferase activity showed an increase by 10.0; 21.5 and 30.8% and at 6.6; 11.0 and 18.8% respectively at 35, 45 and 55 days of experience in the group of animals receiving “toxic food”.

The AST and ALT activity of the third and fourth groups animals using retinyl acetate at 55 days was higher compared to the control group by 14.7 and 9.2% and by 10.1 and 6.1%, respectively.

Table 3 presents the changes dynamics of the vitamin A content in the serum of rabbits, which increased significantly in the third and fourth groups. It is possible due to the fact that retinyl acetate was added to the rabbits ration of these groups. In these groups the level of vitamin A goes up 1.4 and 1.7 times relative to the control group by 30 days.

Starting from the 30th day, the intake of aflatoxin B_1 in animals caused a decrease of the vitamin A level in the blood serum of the second group rabbits by 55 days of experience by 27.3% relative to the control group. By the 55th day of the experiment, the vitamin A amount of the third and fourth groups also tended to decrease, however, the data of the biological control group remained 1.2 and 1.5 times higher, respectively.

Analysis of the content of vitamin a in the liver showed that the amount of vitamin A of the seed group rabbits having aflatoxicosis B_1 at the 55th day of the test was lower by 3.7% relative to control. At the same time, the level of the studied index of the third and fourth group animals remained significantly higher compared to the control by 6.5 and 13.6 times, respectively.

Chromatograms of vitamin A release in rabbit liver are shown in picture 1 and 2.

CONCLUSION.

Rabbit poisoning with aflatoxin B1 at a dose of 75 mcg / kg of animal feed without additional introduction of vitamin A into the diet leads to a decrease of the vitamin A amount in the blood serum already from the fifth day of animal feed consumption containing aflatoxin B1. By the 15th day of the consumption of mictoxin, there is a significant, relative to control, decrease of vitamin A. At the same time, a decrease of vitamin A content with the additional vitamin introduction begins only after 2 weeks of intoxication, but at the same time, its content exceeds the level in the control. The results of the experiment confirm the negative effect of aflatoxin B_1 on the hematological and biochemical parameters of rabbits blood and at the same time show that the use of retinyl acetate has a positive effect on these indicators. The tested 2 levels of retinyl administration allow us to conclude that 750 IU / per individual can be administered to prevent aflatoxicosis, and in the case of forced feeding of substandard food, it is advisable to increase the vitamin level to 1,500 IU to create a vitamin A supply in the body and protect it.

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