

# The Complex Effect Of Retinyl Acetate And Zeolite On The Resistance Of Rabbits With Aflatoxicosis

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## ABSTRACT

The article presents the results of studying the effect of a combination of zeolite from the Tatar-Shatrashansky deposit of the Republic of Tatarstan and retinyl acetate in rabbit aflatoxicosis on the indicators of natural resistance of animals. The duration of the experiment was 55 days. Animals of the first group received the main diet, of the second group received a toxic diet, animals of the third group were additionally injected retinyl acetate with a toxic diet at a dose of 1500 IU per individual, the rabbits of the fourth group, in addition to the toxic diet, received retinyl acetate at a dose of 1500 IU per individual and zeolite at a dose of 2% of the diet. Studies showed, that the complex use of retinyl acetate and zeolite is highly effective in case of aflatoxicosis, contributing to the normalization of clinical, biochemical indicators and non-specific resistance factors.

**Keywords:** aflatoxin, retinyl acetate, zeolite, resistance, rabbits.

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## INTRODUCTION

More recently, the growing dynamics of feed contamination by mycotoxins has been causing increasing danger (Semenov *et al.*, 2016). At least a quarter of the world's grain production contains mycotoxins (Cheli *et al.*, 2013). The main reasons of aflatoxin contamination of raw materials and food products are non-compliance with sanitary requirements in the production technology and storage conditions of food products (El-Hamaky *et al.*, 2016).

Close attention is drawn to microfungus from the genus *Aspergillus*, in particular, *A. flavus*, *A. parasiticus*, which secrete mycotoxins, aflatoxins, which are dangerous for the health of animals and humans, which have an immuno-inhibitory effect on animal organisms (Do *et al.*, 2007). Aflatoxins belong to hepatotropic poisons, that have teratogenic, mutagenic, carcinogenic properties, the study of which is the subject of many studies (Marai *et al.*, 2008). Studies of low levels of aflatoxins in animal diets, that do not cause changes in the clinical condition of the animal or pathological changes, serious tissue injury, showed, that this leads to significant changes in peripheral blood lymphocyte levels, which can lead to more serious problems (Sur *et al.*, 2012).

The consumption of aflatoxin contaminated feed by animals leads to the formation of aflatoxicosis. The problem of animal aflatoxicosis is widespread, and as a result, scientists around the world consider various options for inhibiting the negative effects of aflatoxins on the body. There is no specific treatment for aflatoxicosis. The main preventative measure is to prevent the feeding of aflatoxin contaminated feed to animals, therefore, feeds on farms should be constantly monitored. As a preventative measure, it is possible to mix food, affected by aflatoxins with benign, however, this can lead to the development of chronic aflatoxicosis. For the prevention of mycotoxicosis, antioxidants and immunomodulators are widely used. To

combat aflatoxicosis and reduce the toxic effect of aflatoxicosis, preparations are being developed, including those, based on sorbents, to bind toxins from the gastrointestinal tract. Many studies were conducted on the prevention of aflatoxicosis, which showed, that the addition of a sorbing substance into the diet, contaminated with aflatoxin, restores the clinical, hematological and biochemical parameters of animal blood (Abdel-Fattah *et al.*, 2016; Semenov *et al.*, 2016). The development of new, affordable and effective drug for the prevention and treatment of animal mycotoxicosis is still relevant. Perhaps, the complex use of zeolite and retinyl acetate will become one of the best preventative procedures in case of aflatoxicosis.

## MATERIALS AND METHODS.

In the experiments Chinchilla rabbits with a live weight of 3.5-4 kg were used, of which 4 groups were formed on the principle of analogues (three experimental groups and one biological control) 6 animals each. The experimental work was conducted in vivarium conditions on the basis of the FSBSI "FCTRB-RSRVI". The conditions for keeping rabbits were in accordance with generally accepted zootechnical standards. Toxic feed was prepared by contamination of the main diet with aflatoxin B1 (75 µg / kg). The experiment was conducted in accordance with the scheme, presented in table 1.

Crystalline aflatoxin, previously produced in the laboratory of mycotoxins of the FSBSI "FCTRB-RSRVI" was used for experimental intoxication of animals. As a preventative drug, zeolite from the Tatar-Shatrashansky deposit of the Republic of Tatarstan and retinyl acetate were tested.

**Table I:** Scheme of the conduction of the experiment on rabbits

Group	The amount of toxin, µg / kg	The amount of retinyl acetate, IU per individual	The amount of zeolite, % of diet
Biological control No.1	0	0	0
Experimental No.2	75	0	0
Experimental No.3	75	1500	0

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Experimental No.4	75	1500	2
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During the experiment, the clinical condition, the dynamics of changes in live weight were recorded. Blood for biochemical and immunological studies was drawing from the marginal vein of the ear. Blood serum total protein was determined refractometrically. The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) was determined on an EXPRESS PLUS analyzer. The condition of natural resistance of rabbits was estimated by the analysis of lysozyme activity of blood serum, phagocytic activity of neutrophils. The functional capacity of neutrophils was determined by phagocytosis indicators: phagocytic activity; phagocytic index; phagocytic number; phagocytic capacity. Blood serum lysozyme activity was determined by the nephelometric method.

### RESULTS AND DISCUSSIONS.

Observation of the clinical condition of rabbits showed, that there were no significant differences in the general condition in all groups in the first days of the experiment. Animal groups of the biological control freely consumed feed and water and were active. Starting from the 9th – 11th day of feeding, the rabbits of the second experimental group showed frank clinical signs of toxicosis, which manifested by refusal from feed and water, depression, weakening of muscle tone, and gastrointestinal upset in the form of diarrhea. Similar deviations in the clinical status in rabbits of the third experimental group were observed 3-5 days later. The combined use of retinyl acetate and zeolite in animals of the fourth group prevented the appearance of external signs of poisoning. Analyzing the dynamics of live weight, it should be noted, that in the group of animals, being received a toxic diet, there was a decrease in weight by 10.3% of the initial data, and in the retinyl acetate preventive group by 4.9%. In this case, the body weight of rabbits of the fourth group was higher relative to the seed control by 22.1%.

An important parameter for the diagnosis of diseases, associated with metabolic diseases, is the content of total protein in the blood serum. Plasma proteins form the most important blood buffer system and maintain blood pH in the normal condition. Albumin performs a transport function (Wu, 2013). Our studies indicate a decrease in total protein in animals, treated with toxic feed by 26.0%; in rabbits of the third group by 20.8%;

and in the retinyl acetate and zeolite preventive group, the level of the studied indicator was 10.6% lower in comparison with the biological control group. A decrease in serum albumin content in animals of the second group was found to be 11.8%; in the third group - 9.0%; in the fourth group - 1.6% in comparison with the biological control group. This trend is confirmed by the data of researchers from the Selcuk University of Konya, Turkey (Abdelhady *et al.*, 2017).

The results of studies of the activity of serum enzymes, namely aspartate aminotransferase, showed an increase by 10.0; 21.5 and 30.8% respectively in the group of animals receiving "toxic feed" in comparison with the biological control group at 35, 45, and 55 days of the experiment. Analysis of the alanine aminotransferase indicator in the same periods of studies showed an increase by 6.6; 11.0 and 18.8%, respectively. That is also confirmed by the studies of scientists from Kyrgyzstan (in a joint study with scientists from Turkey) (Eisa *et al.*, 2011).

In animals of the third group, the activity of the studied enzymes (AST and ALT) changed insignificantly by day 35, since the level of AST was lower by 1.1%, ALT was higher by 0.2%; at the 45th and 55th day of the experiment, an increase in AST activity was observed by 3.7 and 10.1%, respectively; at the same time, the increase in ALT activity was 1.2 and 6.1%, respectively. AST activity in animals of the fourth retinyl acetate and zeolite preventive group was higher by 0.3, 4.4, and 7.0% at 35, 45, and 55 days relative to the control group. ALT activity in the same group was higher by 1.6; 2.6 and 6.0%, respectively. The effect of aflatoxin on a living organism is accompanied by inhibition of indicators of nonspecific resistance (Boonyaratpalin *et al.*, 2001). Our studies also found a decrease in non-specific resistance indicators. As it can be seen from table 2, the most significant deviation of nonspecific resistance indicators was recorded in rabbits of the second group, so the decrease in phagocytic activity was 11.6%; phagocytic index - 5.4%; phagocytic number - 12.7%; phagocytic capacity of neutrophils - 22.6%; lysozyme activity - 20.5%.

**Table II :** Indicators of nonspecific blood resistance of rabbits with aflatoxicosis on the background of the use of retinyl acetate and zeolite

Indicator	Groups of animals			
	Biological control No.1	Experimental No.2	Experimental No.3	Experimental No.4
Phagocytic activity, %	64.27±2.75	56.84±2.63	59.84±2.89	61.26±2.68
Phagocytic number	4.72±0.20	4.12±0.15*	4.36±0.24	4.50±0.19
Phagocytic index	7.47±0.28	7.07±0.31	7.29±0.32	7.39±0.26
Phagocytic capacity, %	44.35±2.00	34.32±2.10*	37.82±1.82*	40.62±1.74
Lysozyme activity, %	35.26±1.49	28.02±1.41*	30.92±1.32	32.17±1.54

Note: \* differences with control are accurate,  $p < 0.05$ ; \*\* -  $p < 0.01$

In animals of the third group, the phagocytic activity of neutrophils decreased by 6.9%; phagocytic index - by 2.4%; phagocytic number - by 7.6%; phagocytic capacity of neutrophils - by 14.7%; lysozyme activity - by 12.3%.

The decrease in natural resistance in rabbits, which received retinyl acetate and zeolite at the same time with the seed, was less pronounced. So, phagocytic activity was lower by 4.7%; phagocytic index - by 1.1%;

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phagocytic number - by 4.7%; phagocytic capacity - by 8.4%; lysozyme activity - by 8.8%.

### Summery

Thus, the ingestion of aflatoxin in an amount of 75 µg / kg for 25 days causes chronic intoxication, characterized by a decrease in the intensity of the body's metabolic processes, negatively affects the liver, as evidenced by the results of studies of the protein and enzymatic blood profile of animals, manifested by a decrease in the content of total protein and albumin, an increase in the activity of hepatospecific enzymes. Poisoned animals have decrease in natural resistance, which is expressed by a decrease in the phagocytic activity of neutrophils, phagocytic number, phagocytic index, phagocytic blood capacity and lysozyme activity. It was found, that the complex use of retinyl acetate in an amount of 1,500 IU / individual and zeolite in an amount of 2% of the diet is highly effective in case of aflatoxicosis, prevents protein imbalance and favorably affects the functional condition of the liver. Conducted studies showed, that after their combined use, clinical, biochemical indicators are normalized and the body's natural resistance increases.

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