

## Effect Of Bee Brood And Zeolite On Broiler Chickens Exposed By Mycotoxin T-2

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### ABSTRACT.

Mycotoxins cause a concern in the poultry industry as they can reduce bird productivity and increase susceptibility to diseases. One of the most important trichothecene mycotoxins affecting poultry is T-2 toxin. Since it is not possible to avoid mycotoxins in feed, one of the most practical ways to reduce the risk of mycotoxin contamination in poultry is to use effective mitigation programs that can limit the bioavailability of mycotoxins in the animal's digestive tract. It seems interesting to use alternative ways of protection – the use of natural products. The aim of this study was to estimate the effect of different concentrations of a mixture of zeolite and bee brood in broiler diets when exposed to mycotoxin T-2. In blood samples, total protein, albumin, globulins, glucose, hemoglobin concentration, erythrocyte and leukocyte content were determined and compared. We showed that the introduction of a mixture of zeolite and bee brood into the diet of broiler chickens had a significant positive effect on the increase in live weight and blood parameters, reducing the negative effect of mycotoxin. Thus, the addition of zeolite and the bee brood product to the diet has a certain protective effect when exposed to the T-2 mycotoxin produced by fungi of the genus *Fusarium*. Further research is needed to fully recognize the pharmacological activity and expand the use of bee products.

**Keywords:** mycotoxin T-2; broiler chickens; bee brood; zeolite, hematology; biochemical parameters.

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### INTRODUCTION

Mycotoxins are produced by microscopic fungi of the genera *Fusarium*, *Aspergillus*, *Claviceps*, *Penicillium*, *Alternaria* and others. Mycotoxins are metabolic products of several fungi that reproduce especially on cereal plants such as wheat, barley, sorghum, rice, corn, which are used in animal and human food (Presteset al., 2019).

Mycotoxins cause a concern in the poultry industry as they can reduce bird productivity and increase susceptibility to diseases (Wang et al., 2013). Feeding products prepared using mycotoxin-containing crops degrade nutritional value and pose a potential risk to animal and human health (Tinelliet al., 2019; Semenov et al., 2016). One of the most important trichothecene mycotoxins affecting the poultry industry is the T-2 toxin (Wu et al., 2017; Diaz et al., 2006; Singh et al., 2019).

Mycotoxins, which are small and fairly stable toxic molecules, are extremely difficult to remove or eradicate from agricultural and livestock products (Haque et al., 2020). Therefore, the development of effective strategies to reduce the toxicity of T-2 has caused a lot of interest in the last few decades. Since it is not possible to avoid mycotoxins in feed, effective mitigation programs must be used (Semenov et al., 2017; Kadikovet al., 2018; Karmanovet al., 2020; Smolentsevet al., 2020; Semenov et al., 2016).

One of the most practical methods to reduce the risk of mycotoxin contamination in poultry is to limit the bioavailability of mycotoxins in the digestive tract of the animal (Huwiget al., 2001; Karmanovet al., 2021).

It seems interesting to use alternative ways of protection – the use of natural products. Beekeeping products are natural pharmaceutical products that have a multidirectional effect on a living organism, including humans. Their pharmacologically active fractions are used in many areas of treatment and pharmacy as pharmacopoeial raw materials, food additives, and cosmetics (Mark et al., 2005; Ghosh et al., 2020). Therefore, the purpose of this study was to estimate the effect of different concentrations of adsorbent – zeolite and bee brood in broiler diets when exposed to mycotoxin T-2.

#### **MATERIALS AND METHODS.**

**Obtain of toxin.** By analogy with work (Yang et al., 2020), to obtain T-2 toxin, we used its producer – a strain from the collection of micromycetes of the Federal Center for Toxicological, Radiation and Biological Safety – *Fusarium sporotrichioides* 2m15.

**Preparation of contaminated feed.** Broiler chickens were fed with feed purchased in the same poultry farm where broiler chickens of the KOB 500 cross were purchased. The main diet was: 48% chopped corn, 13% wheat grain, 5% meat and bone meal, 1% vegetable fat, 18% sunflower meal, 8% barley grain, 3% feed yeast and 4% premix. The toxin was added to the main diet by stepwise mixing, so that the final concentration of the toxin was 400 µg / kg feed. A mixture of zeolite and bee brood (m/m 95/5) was also introduced by stepwise mixing. Special rations were compiled for feeding: A – the main diet; B – main diet + prophylactic mixture of zeolite and bee brood in a concentration of 1% of the mixture and 99% of the main diet; C – toxic diet (main diet + T-2 toxin); D – toxic diet 99% and 1% of the prophylactic mixture; E – toxic diet 99.5% and 0.5% prophylactic mixture; F – toxic diet of 99.5% and 0.5% zeolite.

**Prevention means.** Bee brood was purchased from the “Ural” company (Russia). Chemical composition: moisture – 10%, protein – 50%, minerals – 10% (27 microelements including Ca, Cr, Al, Cu, Mg, P, Zn, Si, Ag, Mo, Fe), melanins – 20%, chitin – 10%.

Zeolite was used from the “Shantrashanit” field (Russia) and was purchased from the company “Zeolites of the Volga region”. Zeolite composition (%): SiO<sub>2</sub> – 56,77; TiO<sub>2</sub> – 0,3; Al<sub>2</sub>O<sub>3</sub> – 5,37; Fe<sub>2</sub>O<sub>3</sub> – 2,3; MnO<0,01; CaO – 14,9; MgO – 1,26; Na<sub>2</sub>O – 0,14; K<sub>2</sub>O – 1,24; P<sub>2</sub>O<sub>5</sub> – 0,11 and others (H<sub>2</sub>O+CO<sub>2</sub>) – 17,72.

**Experiment with chickens in vivo.** In the experiment, 60 individuals (30 males and 30 females) of 14 day old KOB 500 broiler chickens were used to study the preventive efficacy of bee brood and zeolite in case of poisoning of broiler

chickens with mycotoxin T-2. The experiment was approved by the Ethics Committee of the Federal Center for Toxicological, Radiation and Biological Safety (Russia).

Broiler chickens were randomly divided into 6 groups (5 males and 5 females in each group). At 15 days of age, normal food was replaced with the corresponding experimental food: group 1 – biological control, consumed diet A; Group 2 – consumed diet B; Group 3 – consumed diet C; Group 4 – consumed diet D; Group 5 – consumed diet E and group 6 – consumed diet F. The amount of feed corresponded to the recommendations for feeding the KOB 500 cross. Experimental broilers were weighed at the beginning and at the end of the experiment, the weight gain and feed consumption were recorded to calculate the feed conversion coefficients (kg/kg). At the beginning of the experiment, on the 14th and 28th days of the experiment, blood was taken from the axillary vein, contained in a vacuum tube with a K3EDTA tube, followed by centrifugation at 4000 g at 4°C for 15 min, in order to collect serum from a blood sample, and further storage at – 18°C until analysis. In blood serum samples total protein, albumin, globulins, glucose were determined using an automatic blood analyzer (Microlab 300). The concentration of hemoglobin (Hb) in the blood was assessed using the Sahli's method (Balasubramaniam, Malathi 1992). With the addition of 0.1 N HCl, hemoglobin was converted to acidic hematin. The resulting solution then was compared with a reference solution (i.e., a Sahli's hemoglobinometer). The content of leukocytes and erythrocytes was determined under a microscope.

**Statistical analysis.** For the results obtained, the values of the mean (M) and experimental standard deviation ( $\pm$  SEM) are given, calculated in accordance with the formula B.2.17 of the recommendations for the expression of uncertainty (JCGM 100: 2008 (2008)).

To assess the statistical significance of intergroup differences, the Kruskal-Wallis H-test was used; statistical significance ( $p$ ) was set at 0.05. If statistically significant differences were found in the Kruskal-Wallis H-test, the Mann-Whitney U-test was performed a posteriori.

The Mann-Whitney U-test was carried out according to two protocols: 1) the biological control group (group 1) was compared in pairs with all other groups; 2) the toxic control group (group 3) was compared in pairs with groups 4 – 6. In both cases, the  $p$  value was corrected taking into account the Bonferroni correction (the values  $p \approx 0.010$  and  $p \approx 0.017$  were taken, respectively).

**Results and Discussion.** Based on the data obtained in the study, it was found that the introduction of a mixture of zeolite and bee brood into the diet of broiler chickens had a positive effect on the increase in live weight, reducing the negative effect of mycotoxin. Chick production indicators are presented in table 1.

**Table 1.** Increase in live weight and feed conversion of broiler chickens when using zeolite and bee brood during the T-2 toxicosis

Group	Live weight, g		Absolute gain, g	Average daily gain, g	Feed conversion
	Beginning of	End of experiment			

	experiment				
1	517±11,2	2931±43,9	2414±37,2	80,4±9,1	1,53
2	567±19,1	3140±41,1	2573±38,4	85,7±13,1	1,44
3	510±20,3	2465±45,1	1955±39,4	65,1±4,3*	2,40
4	502±11,8	3015±49,4	2513±40,7	83,7±9,6**	1,50
5	494±11,8	2794±45,5	2300±42,5	76,6±8,5***	1,84
6	509±10,2	2502±44,3	1993±41,6	66,4±11,4*	1,97

Note: \* – statistically significant differences from group 1; \*\* – statistically significant differences from group 3; \*\*\* – statistically significant differences from both group 1 and group 3.

It is possible that the mixture contributes to the normalization of the processes of assimilation of nutrients, due to which there is a growth-stimulating effect. According to table 1, it can be concluded that by the end of the experiment, the absolute increase in live weight of broilers of group 3 (toxic diet) was 1955 g, while in the experimental groups, where the test mixture was added to the toxic diet in doses of 1 and 0.5% and zeolite 0.5% of the diet, it was 2513, 2300 and 1993, which is higher than the indicators of toxic control by 28.5, 17.6 and 1.9%, respectively. In the biological control group with the addition of the mixture at a dose of 1% of the diet, there was a noticeable increase in absolute gain by 6.5%, which confirmed the possibility of stimulating growth.

The calculations also showed that according to the average daily gain, the values of the chickens of the 3rd experimental group significantly decreased by 19.1%. In 5 and 6 experimental groups, the indicators also decreased, but less significantly – by 17.5 and 4.8%. In group 4, the average daily gain increased by 4.1%. When using zeolite and bee brood in the biological control group, the average daily gain was 85.7 g, which is 6.5% higher than the indicator of the biological control group.

The feed conversion ratio, which depends on the digestibility and absorption of nutrients, is the ratio of the amount of feed consumed to the unit of production received. The value of this coefficient gives information about the quality of the feed used and the degree of its assimilation in the animal body.

The lowest feed conversion ratio (1.44) was registered in group 2 (biological control with the use of feed additives). The highest feed conversion ratio (2.40) was observed in the toxic control group, indicating a high feed intake and a decrease in production from broiler chickens. The use of feed additives in groups 4, 5 and 6 had a softening effect on the bird organism under the action of the toxin. The conversion rate in these groups was 1.5, 1.84 and 1.97, which is better than the values of the group with a toxic diet. The closest value to the indicator of the biological control group (1.53) is the coefficient of group 4 (1.50), in which a mixture of zeolite and bee brood was used at a dose of 1% of the diet for T-2 toxicosis.

**Table 2.** Hematological parameters of the blood of broiler chickens when using zeolite and bee brood during the T-2 toxicosis.

Investigation time, days	Group	Erythrocytes, 10 <sup>12</sup> /l	Leucocytes, 10 <sup>9</sup> /l	Hemoglobin, g/l
<b>Beginning</b>	1	2,21±0,05	41,53±0,95	118,14±2,71
	2	2,19±0,07	41,38±1,35	117,74±3,84
	3	2,25±0,06	42,12±1,19	120,00±3,38
	4	2,18±0,07	41,01±1,23	116,90±3,52
	5	2,19±0,07	41,00±1,34	117,43±3,83
	6	2,20±0,06	41,13±1,42	117,60±3,31
<b>14</b>	1	2,71±0,06	42,93±0,99	121,96±2,80
	2	2,70±0,09	42,80±1,40	121,53±3,96
	3	2,63±0,07	41,76±1,40	118,58±3,98
	4	2,69±0,08	42,35±1,27	121,00±3,64
	5	2,68±0,09	42,22±1,38	120,74±3,94
	6	2,66±0,07	42,27±1,19	120,00±3,38
<b>28</b>	1	2,51±0,06	39,82±0,06	121,36±2,79
	2	2,52±0,08	39,78±0,09	121,64±3,97
	3	2,30±0,08	34,76±0,06*	114,60±3,23
	4	2,48±0,07	39,63±0,08**	120,30±3,62
	5	2,47±0,09	39,40±0,09**	119,64±3,90
	6	2,41±0,07	38,37±0,07	117,90±3,32

Note: \* – statistically significant differences from group 1; \*\* – statistically significant differences from group 3; \*\*\* – statistically significant differences from both group 1 and group 3.

According to the table of hematological parameters of the blood of broiler chickens, it can be concluded that erythrocytes and hemoglobin on days 14 and 28 did not have statistically significant differences from the values of the control groups of animals ( $p > 0.05$ ). In the situation with leukocytes, it can be seen that on the 28th day in the toxic control group the indicator was reduced by 12.2% in comparison with the control ( $p < 0.05$ ). It can be concluded that the addition of a mixture of zeolite and bee brood in all studied dosages to groups with a toxic diet and biological control did not cause negative changes in the blood parameters of broiler chickens throughout the experiment.

From the data of the presented table 3 of glucose concentration and protein metabolism, it is possible to distinguish a tendency of the positive effect of zeolite and bee brood on the organism of broiler chickens.

**Table 3.** Data of protein metabolism and glucose content of broiler chickens with T-2 toxicosis and the use of zeolite and bee brood.

Investigation	Group	Glucose,	Total protein,	Albumins,	Globulins,
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time, days		mmol/l	g/l	g/l	g/l
<b>Beginning</b>	1	13,17±0,30	31,70±0,75	13,9±0,33	17,80±0,42
	2	13,13±0,43	31,76±1,04	13,83±0,45	17,85±0,58
	3	12,70±0,36	29,75±0,84	12,6±0,35	19,20±0,54
	4	13,0±0,39	31,40±0,95	13,8±0,42	18,12±0,55
	5	12,83±0,42	31,22±1,02	13,63±0,44	18,62±0,61
	6	12,7±0,36	29,98±0,84	13,1±0,37	18,70±0,53
<b>14</b>	1	13,98±0,32	33,68±0,77	14,88±0,34	18,80±0,43
	2	13,93±0,45	33,57±1,10	14,73±0,48	18,75±0,61
	3	11,79±0,40*	29,37±0,98*	13,19±0,44*	20,53±0,69
	4	13,80±0,42	33,10±1,00	14,6±0,44	19,05±0,57
	5	13,33±0,43	32,67±1,07	14,13±0,46	19,78±0,65
	6	12,2±0,34*	30,70±0,86	13,7±0,39	20,06±0,56
<b>28</b>	1	12,47±0,29	31,07±0,71	14,48±0,33	16,59±0,38
	2	12,53±0,41	31,88±1,04	14,43±0,47	16,54±0,54
	3	9,06±0,26*	26,04±0,73*	11,8±0,33*	19,04±0,54*
	4	12,3±0,37**	30,31±0,91**	14,1±0,42**	17,06±0,51
	5	11,82±0,39**	29,94±0,98**	13,63±0,44**	18,14±0,59
	6	11,0±0,31***	27,90±0,79*	13,1±0,37	18,49±0,52*

Note: \* – statistically significant differences from group 1; \*\* – statistically significant differences from group 3; \*\*\* – statistically significant differences from both group 1 and group 3.

According to table 3, it was found that on days 14 and 28 in the group with a toxic diet, the most pronounced decrease in glucose concentration was by 14.5 and 26.9%. These changes indicate disturbances in energy metabolism, pancreas and liver functions. Also, the concentration differed from the biological control group in group 6, namely, on the 14th day by 8.1%, on the 28th day by 10.7%. The rest of the parameters of the experimental groups throughout the experiment were within the values of the control group.

The total protein in the blood serum of broiler chickens indicates the intensity of protein metabolism in the organism. According to the data presented in Table 4, it can be concluded that the addition of zeolite and bee brood at T-2 toxicosis indicates a positive effect of prophylactic agents. Whereas the indicators of total protein in the group receiving the toxic diet had statistically significant differences from the control group. This indicator decreased on the 14th day by 12.1% and on the 30th day by 15.7%. On the 28th day, the total protein content in the biological control group increased with the addition of feed additives by 3%, which indicates the stimulation of protein exchange reactions, the restoration of liver functions and a decrease in intoxication.

Also, the speed and direction of protein metabolism were inferred from the content of albumin in the blood serum of chickens. On the 28th day, there was a significant decrease in the content of albumin in the third group by 17.8% in comparison with the control group. A decrease in the level of albumin in broilers indicates inhibition of tissue protein synthesis and impaired liver function. In the chickens of the remaining experimental groups, during the use of prophylactic agents, the normalization of the albumin content indicators, and, consequently, the liver function, was recorded.

The fraction of globulins in the blood of chickens of the toxic control group significantly increased on day 20 by 9.9% ( $p < 0.05$ ) and on day 30 – by 15.4% ( $p < 0.05$ ). Also, on the 30th day, there was an increase in groups 6 and 7 by 12.1% ( $p < 0.05$ ) in each. The consequence of an increase in the number of globulins is a violation of protein assimilation and protein starvation in mycotoxicosis. The rest of the parameters of the experimental groups did not differ significantly from the control group.

Early studies in poultry showed that T-2 toxin causes decreased feed intake and decreased weight gain, oral cavity lesions, coagulopathy, behavioral changes and distorted plumage (Wyatt et al., 1975; Wyatt et al., 1972; Doerret al., 1981; Hoerret al., 1982; Huff et al., 1988). Therefore, the criterion for estimating the effectiveness of protection of zeolite and bee brood from T-2 toxin, we considered these indicators first of all. Throughout the experiment, there was a decrease in body weight and feed conversion in chickens. These data on decreased body weight and feed intake were due to T-2 toxicosis, which is consistent with earlier reports of T-2 toxin feeding (Raju et al., 2000; Nesicet al., 2011; Arvind et al., 2003).

The suppressive effects of mycotoxin on growth can be primarily caused by their inhibitory effect on protein synthesis and nutrient utilization (Bamburg, Strong 1971).

In our study, birds that consumed a diet with T-2 toxin and feed additives showed that body weight and feed absorption were higher than without the use of feed additives.

Data related to various biochemical parameters such as total protein, serum enzyme activity, glucose were statistically analyzed and the mean values are presented in tables. The present study showed that food contamination with T-2 toxin at a level of 400  $\mu\text{g} / \text{kg}$  feed resulted in a decrease in protein content. These results are consistent with previous reports (Pande et al., 2006) in the literature, which show that the decrease in whey protein concentration is proportional to the dietary level of T-2 toxin. In addition, T-2 toxin is a well-known protein synthesis inhibitor due to its ability to bind and inactivate peptidyl transferase (ribosomal unit 60S) (Adhikari et al., 2017).

The beneficial effects of adsorbents as mycotoxin utilizers in birds are widely known (Bunzen, Haese 2006). Studies have shown the ability of the adsorbent of modified hydrated sodium and calcium aluminosilicate (HSCAS), obtained from natural zeolite ore, to reduce the toxicity of T-2 toxin in broilers (Wei et al., 2019).

Several previous *in vitro* and *in vivo* studies (Vila-Donatet al., 2018) have demonstrated the adsorption capacity of mineral clays such as hydrated sodium calcium aluminosilicate (HSCAS) against aflatoxins (AF), bentonites

(montmorillonites) against AF, ZEN, ochratoxin A (OTA) and fumonisins (FB), and zeolites against AF and FB, as well as other inorganic mineral adsorbents (diatomite, sepiolite) against AF.

Previous studies have shown that adsorbents contain aluminosilicates such as bentonite (Santurioet al., 1999), montmorillonite (Deshenget al., 2005) and zeolite (Miazzoet al., 2000; Tarasovaet al., 2020), demonstrating the ability to effectively protect against zearalenone (Abbes et al., 2006), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and fumonisin B<sub>1</sub> (FB<sub>1</sub>) (Mitchell et al., 2014) in several farm and experimental animals.

However, in the present study, we used zeolite mixed with an alternative component, bee brood, which positively changed the production system using broiler chickens as an experimental model.

The basis for the comprehensive use of beekeeping products in human nutrition and treatment is their diverse and unique chemical composition, including the content of many characteristic substances with a bioactive effect (Krell et al., 1996). Biologically active compounds of beekeeping products include bactericidal agents and antioxidants (Gheldofet al., 2002).

The rich chemical composition of beekeeping products contributes to a high degree of biological activity and has a beneficial effect on the human body. Due to the high content of protein, vitamins and hormones, for example, the drone brood effectively prevents the processes of cellular aging and many diseases. This therapeutic effect has been widely described by scientists from Romania, Slovakia, Ukraine and Russia (Czerkasowa, Prochoda 2006; Lebidiew, Legowicz 2003; Lazaryan 2002). In vivo studies carried out to date using both animals and humans indicate a positive effect of bee products in the treatment of hypothyroidism, liver diseases, it is also used in adaptogenic therapy and in the treatment of infertility. The ability to increase the body's nonspecific immunity (Vasilenkoet al., 2005) and to improve the physical and psychic resistance of experimental animals (Kryłowet al., 2007) has been established. Further research is needed to fully understand the pharmacological activity and expand the use of bee products (Sidor, D'zuga 2020).

## **CONCLUSION.**

Thus, the addition of zeolite and the bee brood product to the diet has a certain protective effect when exposed to the T-2 mycotoxin produced by fungi of the genus *Fusarium*. Further research is needed to fully recognize the pharmacological activity and expand the use of bee products.

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**Conflicts of Interest.** The authors have no conflicts of interest to declare. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

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