

# Protective effect of adsorbent complex on morphofunctional state of liver during chicken polymycotoxicosis

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## ABSTRACT

The presence of mycotoxins in food can lead to pathological effects ranging from acute intoxication to the effects of chronic exposure (such as carcinogenic, mutagenic, teratogenic) in both humans and animals. Mycotoxins are also a major problem in poultry farming and have harmful effects on birds. The experiment was conducted to determine the effect of adsorbents (shungite/zeolite/glucans 0.5% of the diet) added to a birds diet contaminated with mycotoxins on liver enzyme activity, liver weight and histological characteristics, and the antioxidant system. In experiment 60 Cobb 500 broilers of both sexes, divided into three equal groups (20 chicks in each group), live weight of 650-780 g were used. The experimental period was 20 days. The results showed that the addition of a complex of inorganic and organic adsorbents minimized the pathological effect of zearalenone, aflatoxin B<sub>1</sub> and T-2 toxin on the liver and the level of enzymes such as alanine transferase (ALT) and aspartate transferase (AST), and also contributed to the reduction of malone dialdehyde levels relative to the toxic control group. Thus, use of a complex of adsorbents offered by us is an effective and ecologically pure strategy of mycotoxin detoxication in protection of the end-user of animal production - the person.

**Keywords:** mycotoxins, adsorbents, aspartate transfer, alanine transfer, malone dialdehyde, bird

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## INTRODUCTION

The contamination of crops with mycotoxins leads to significant economic losses for grain producers. People and animals are constantly exposed to different levels of mycotoxins (aflatoxins, zearalenone, T-2 toxin, etc.) - toxic secondary metabolites produced by microscopic fungi (Richard, 2007, Semenov et al., 2016). Their presence in poultry feed can cause genotoxic, cytotoxic, carcinogenic and teratogenic effects (Hussar et al., 2020).

The most toxic trichothecene is T-2 toxin - type A trichothecene, which is naturally produced by fungi of the genus *Fusarium*, parasitic barley, wheat, etc. An important non-ribosomal action of T-2 toxin is the intensive formation of free radicals, DNA damage, and increased peroxidation (Chaudhary and Rao, 2010; Wu et al., 2014).

Zearalenone is a mycotoxin that promotes low fertility, reduced hatchability of chicks, and fruit resorption (Minervini and Dell'Aquila, 2008). T-2 toxin and zearalenone are subject to international and European guidelines on their maximum permissible levels in bird food.

Aflatoxins are mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Saleemi et al., 2020) and contribute to weight loss, acute mortality, poor nutrition, immunosuppression and clinical diseases (Bhatti et al., 2020; Sana et al., 2019).

Different methodologies have been adopted at different stages of production and storage to combat the mycotoxin problem. One of the most important methods is the use of toxic binders, so-called mycotoxin adsorbents, in the feed. Mycotoxin binders are the largest and most complex class of silicate minerals (Phillips et al.,

1995; Avantageggiato et al., 2007; Phillips et al., 2008; Li et al., 2018).

Tectosilicates include zeolites consisting of SiO<sub>4</sub> and AlO<sub>4</sub> tetrahedrons, which have a cellular structure infinite in three dimensions. Clay minerals have been used since the early 1970s and have high adsorption activity (Masimango et al., 1978; Yendluri et al., 2017; Tarasova et al., 2019).

Good results were obtained with the addition of shungite and zeolite to pigs' feed (Matrosova et al., 2020).

Significant ability to bind some mycotoxins in vivo was demonstrated by cell wall components of *Saccharomyces cerevisiae* yeast, such as glucans (Luo et al., 2018; Peng et al., 2018; Vila-Donat, 2018). It has been shown that an esterified glucomannan polymer, obtained from the cell wall of the yeast separately and in combination, binds aflatoxin, ochratoxin and T-2 toxin (Binder, 2007; Jouany, 2007). Glucans, in addition to binding mycotoxins, modulate immune operations and bind gastrointestinal pathogens (Santovito et al., 2018 a,b).

Although health effects of mycotoxins have been intensively studied in different types of poultry, much of the available information on toxicity has been obtained using a single mycotoxin as well as monoadsorbents (zeolites, bentonites, yeast cell wall) (Morgavi and Riley 2007; Grenier and Oswald, 2011; Bryden 2012; Murugesan et al., 2015; Magnin et al., 2016; Metayer et al., 2019).

Since the liver is one of the key metabolic organs, the main objective of our study was to study the effect of the mycotoxin complex (T-2, zearalenone, aflatoxin B<sub>1</sub>), which is most often detected in the Russian Federation,

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and the protective effect of the combined action of organic and inorganic adsorbents on the liver histology and weight characteristics, the activity of liver enzymes, residual amounts in the liver, as a factor directly affecting the safety of human consumption of chicken products.

### MATERIALS AND METHODS

The experiment is performed on 60 broilers of both floors, live weight 650-780 g, divided into three equal groups of 20 chicks each. The first group (biological control) received complete mixed feed, the second group (toxic control) received mixed feed contaminated with T-2 toxin (0.2 mg/kg), zearalenone (0.5 mg/kg) and aflatoxin B<sub>1</sub> (0.05 mg/kg). The third group in addition to toxic feed was given a mixture of adsorbents (zeolite of Shatrashan deposit, shungite of Zazhogin deposit, as well as yeast glucans in the amount of 0.5%/kg of feed. The experiment lasted 20 days.

Crystalline standards (Sigma) with mycotoxin purity of 90-95% were used for contamination of feeds.

The live weight of broiler chickens was determined by individual weighing. At the end of the experiment, the absolute and relative mass of the liver was determined.

The determination of residual amounts of mycotoxins in the liver was performed using standard methods (Cao et al., 2013).

For histological examination tissue samples were fixed with 10% neutral formalin. After dehydration, they were poured into paraffin. Histoscrees were obtained with the help of an arched microtom, hematoxylin Ehrlich was stained with water eosin. Microphotography was carried out on the following equipment: microscope Leica DM 1000, digital camera Leica DFC 320 (Germany).

Serum concentrations of alanine aminamin transferase (ALT) and aspartate amin transferase (AST) were analysed using the Microlab 300 biochemical analyzer.

MDA level was estimated spectrophotometrically in reaction with thiobarbituric acid by the value of optical density of the formed pink chromogen at 532 nm.

The digital material was processed by means of variation statistics using the Student criterion. The experiments were carried out in accordance with the humane attitude towards laboratory animals when performing research work in accordance with the laws and regulations of the Russian Federation, the Helsinki Declaration of 1975 and its revised version in 2000. The research was approved by the Ethics Committee of the Federal Centre For Toxicological, Radiation And Biological Safety of the Republic of Tatarstan (Minutes No.6 of 03.03.2020).

**Results and Discussion.** The combined effect of T-2 toxin, zearalenone and aflatoxin B<sub>1</sub> on the liver mass, absolute and relative mass is shown in Table 1.

**Table 1.** Liver mass, absolute and relative liver mass during polymycotoxicosis in broiler chickens.

Group	Live weight, g	Absolute liver mass, g	Relative liver mass, %
Biological control	750±12.8	54.4±3.1	7.3±0.5
Toxic control	695±26.8*	43.1±2.7**	6.2±0.2*
Preventive group	728±14.1	52.8±4.2	7.3±0.6

\* - p<0.05 \*\* p<0.01 \*\*\* p<0.001

As can be seen from Table 1, the live weight of broiler chickens, as well as absolute and relative liver weight decreased by 7.3 (p<0.05); 20.8 (p<0.01) and 15.1% (p<0.05) in the toxic control group, respectively. In the group using a complex of adsorbents these indicators had no statistically reliable deviations from the biological control group.

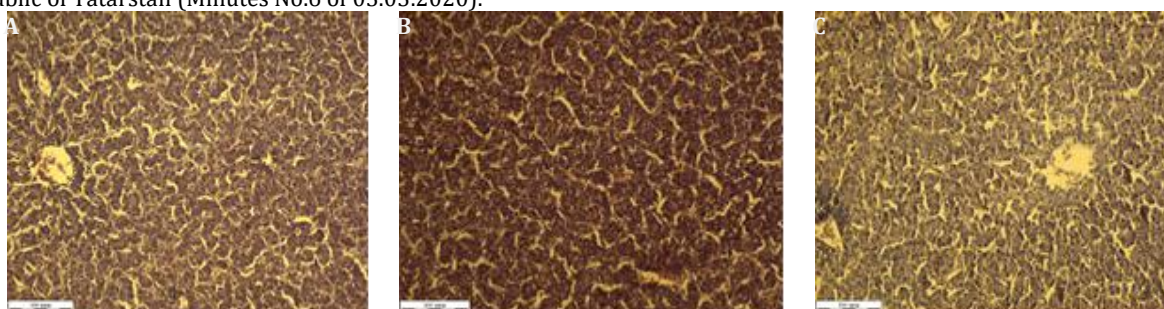
This trend, recorded in our studies in the prophylactic and toxic control groups, may be a consequence of depressed growth activity, as other researchers also described during feed contamination with mycotoxins (Edrington et al., 1997; Hashem and Mohamed, 2009).

The low body weight gain in birds during mycotoxicosis may be associated with low feed intake (Verma et al., 2004; Hussain et al., 2008; Yalcin et al., 2018; Saleemi et al., 2020).

The introduction of adsorbents in the feed leads to increased feed consumption and, accordingly, to a greater increase in body weight and indicates the ameliorative effect of binding mycotoxins.

Among the parenchymatous organs, the liver occupies a leading position due to its multifunctionality. It is for this reason that its morphofunctional state is a primary indicator, especially under the impact of a complex of adverse factors of different etiologies. In this regard, the liver is a priority in our research.

In chickens of the biological control group the liver has an unexpressed division into slices, which is typical for normal morphology of this gland in domestic chickens (Fig). Hepatocytes have clear contours, a well-detectable basophilic nucleus. Cooper cells are small and equidistant.



**Fig.** Liver fragment of broilers (A - biological control; B - toxic control; C - prophylactic group). Coloring with hematoxylin Ehrlich, eosin water, lens X 20

Cetrolobular veins are dilated, filled with erythrocytes and eosinophilic masses irregularly. In the triads, the lumen of the vessels is widened, the walls have no features. The veins are thin. Trabecules are fuzzy structures. Microphages are distributed evenly throughout the drug. Connecting fibres are located between the trabeculars, thin and delicate.

The greatest degree of disturbance of the morphological structure of the liver was observed in the group of broiler chickens of the toxic control. That was expressed by redistribution of hepatocytes in the slice, occurrence of non-nuclear hepatocytes, cytoplasm irregularly coloring, vacuum grained (polymorphic dystrophy). The use of sorbents in a given dose showed the negative impact of the sum of mycotoxins on the liver. The histology of the hepatic parenchyma matched that of the biological control group. However, slight stroma swelling has been noticed in some areas of the parenchyma of the liver. The contamination of feed with mycotoxins leads to pathological lesions in various organs (Karaman et al., 2005; Denli et al., 2009; Hussain et al., 2016; Ortatatlı and Oguz, 2001).

Similarly, we observed lesions of the liver, with histological changes virtually absent in the group with zeolite, schungite and glucans compared to the group without adsorbents. Similar phenomena were noted by a number of authors (Ahmed et al., 2009) who reported improvement in gross and microscopic changes in the liver of broilers in birds against aflatoxicosis and adsorbent use.

Thus, it can be concluded that zeolite, schungite and glucans have hepatoprotective effects in combined experimental mycotoxicosis, as in the present study there were noticeable effects in minimizing the liver pathology caused by mycotoxins.

At the next stage, studies were conducted on the content of residual amounts of mycotoxins (T-2, zearalenone and aflatoxin B<sub>1</sub>) in the liver of toxic control birds and the prophylactic group. Table 2 shows the level of mycotoxins in the liver.

**Table 2.** Residual amounts (µg/kg) of mycotoxins in the liver of broiler chickens

Name of toxin	Toxic control	Preventive group
T-2 toxin	9.1±0.25	2.1±0.13***
Aflatoxin B <sub>1</sub>	0.9±0.04	0.2±0.01***

\*\*\* p<0.001

The content of mycotoxins in the liver of the prophylactic group decreased reliably relative to the group of toxic control. Thus, the T-2 toxin content was lower by 76.9% (p<0.001) in the group with adsorbents and aflatoxin B<sub>1</sub>-77.8% (p<0.001) respectively. In the study of liver samples, it was determined that daily administration of zearalenone mycotoxin with feed did not lead to its accumulation in the liver. Thus, our data show a significant decrease in functional cumulation of T-2 and aflatoxin B<sub>1</sub> due to the introduction of binding agents into the diet.

The content of malone-dialdehyde (MDA) in blood was studied as a marker of oxidative stress (Table 3).

The statistically reliable increase of MDA level in chicken broilers of toxic control group by 22.2% (p<0.01) was

shown, in the prophylactic group the increase was not so significant and amounted to 5.6%.

**Table 3.** MDA content in the blood of experimental animals

Indicator, µmol/l	Biological control	Toxic control	Preventive group
MDA	1.8±0.11	2.2±0.15**	1.9±0.12

\*\* p<0.01

Similar trends were noted by other researchers. Thus, in most cases, T-2 toxin significantly increased the level of active oxygen intermediates (ROI) and caused changes in the antioxidant status of cells (Chaudhari et al., 2009; Bócsai et al., 2015), while the intake of contaminated feed containing deoxynivalenol and zearalenone in combination significantly reduced glutathione peroxidase activity and increased MDA levels in liver tissue (Borutova et al., 2008).

The liver is the main target organ for mycotoxicosis, which causes acute biochemical changes, while disrupting the metabolism of lipids, amino acids, vitamins, nucleic acids and liver enzymes (Ellis et al., 1991).

Liver enzymes (ALT and AST) are associated with liver parenchyma cells. ALT is found mainly in the liver and AST in both liver and heart, skeletal muscles and other tissues.

The influence of mycotoxins on liver enzymes is presented in Table 2.

**Table 4.** Biochemical indicators (liver enzymes) of broiler chicken blood serum

Indicator, u/l	Biological control	Toxic control	Preventive group
ALT	33.9±2.8	43.4±1.6**	36.6±1.4
AST	184.1±6.5*	239.5±8.3***	203.8±0.2*

\* - p<0.05 \*\* p<0.01 \*\*\* p<0.001

According to the literature, liver enzyme levels (AST and ALT) increased in chicken and turkey aflatoxicosis and showed the extent of liver damage from mycotoxicosis (Cheng et al., 2000, Quist et al., 2000).

The level of liver enzymes also increased compared to the normal value for T-2 toxicosis (Ulaiwi, 2018). These data are relevant to our research showing increased liver enzyme levels. Thus, ALT and AST in the second group increased by 28.1 (p<0.01) and 30.1% (p<0.001), respectively. The use of zeolite, schungite and glucanes as binding mycotoxins contributed to the increase of aspartate and alanine transfer within smaller limits (10.7(p<0.05) and 8.1%, respectively).

Our data on elevated concentrations of AST and AST enzymes in the toxic control group are consistent with the pathologistological data of liver tissue because elevated values of these enzymes indicate liver degeneration (Hashem and Mohamed, 2009).

## CONCLUSION

Despite the high demand for mycotoxin adsorbents, imported mycotoxin binders are expensive. Thus, there is an urgent need to develop integrated adsorbents consisting of inexpensive endogenous ingredients that create less economic burden for poultry producers. The

combination of organic and inorganic components will reduce the cost of the adsorbent without compromising its efficiency.

The obtained results proved the effectiveness of our proposed combination of sorbents, which is shown by the example of the morphogistological state of the liver as the main organ-targeting mycotoxins, as well as minimization of residual amounts of mycotoxins and reducing the impact on the oxidative status of poultry and liver enzymes. The use of a complex of adsorbents offered by us is an effective and ecologically pure strategy of mycotoxin detoxication in the protection of the end-user of animal production - the person.

#### Conflict of interest

The authors declare that there is no known conflict of interest associated with this publication.

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