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ORIGINAL ARTICLE

The influence of HamekoTox on the morphological and biochemical indices of laying hens blood in spontaneous fumonisin toxicosis

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The most practical methods of detoxication of mycotoxins in animals and poultry are based on damaged feed sorbents. The search aims to study the influence of HamecoTox on the morphological and biochemical indices of the blood of laying hens in spontaneous fumonisin toxicosis. Laying hens (ferrets, aged 78 weeks) of cross Highsex White, with an average live weight of 1.5 kg, were used in the experiment. Three groups were formed: I – a group of laying hens served as a control, in the experimental II, III, groups of poultry was characteristic clinical signs of fumonisin toxicosis. Experiment III (E_2) was fed with HamecoTox feed for 21 days. In the research of morphological indices of the blood of laying hens under conditions of fumonisin toxicosis and with the use of HamecoTox on the 21st day of the experiment, an increase in the number of erythrocytes and hemoglobin content was found in comparison with sick poultry. The number of leukocytes in the blood of laying hens, which used HamekoTox, reached physiological values on the 21st day of the experiment. HametoTox contributed to the recovery of the protein-synthesizing function of the liver by reducing the negative influence of fumonisins. After the release of the organism from fumonisins, the action of toxins on the liver ends up, and the inflammatory processes disappear accordingly. Under conditions of spontaneous fumonisin toxicosis in poultry, the additional introduction of HamecoTox to laying hens during the 21st day contributed to the normalization of biochemical indices of the blood of experimental poultry. To prevent the fumonisin toxicosis, it is recommended to add HamekoTox feed additive at a dose of 4 kg/t of feed to mycotoxin-contaminated feed.
Key words: fumonisins, toxicosis, poultry, laying hens, HamekoTox, blood.

Introduction

In foreign and domestic scientific literature, much attention is paid to controlling grain contamination and feeding with mycotoxins. The latter are considered dangerous of feed contaminants and food products under normal conditions and are included in the list of dangerous natural ecological toxicants. Mycotoxicosis is a multifactorial pathological process based on systemic tissue hypoxia with all its complex metabolic consequences (Guerre, 2015; Guta, 2016; Brezvyn et al., 2018).

The peculiarity of the danger of mycotoxins for human health and animal health is their ability to act in ultraminimal doses, which are often not exposed to modern detection methods. That is why mycotoxicosis in animals is one of the most current issues in veterinary medicine.

Fumonisins belong to a large group of mycotoxins produced by microscopic fungi of the genus *Fusarium*. Fumonisin B_1 occurs most often in natural conditions, and fumonisin B_2 and B_3 occur much less frequently and in smaller quantities (Brezvyn et al., 2013). Fumonisin poisoning is poorly studied, and its DL_{50} has not been established. It is known, for example, that the dose of 18 mg/kg body weight, introduced twice into the digestive tract of pregnant females of *Syrian cavia* porcellus, does not harm them, but causes pathological disorders of fetal development (Fravalo et al., 2013; Grenier et al., 2016).

Fumonisins have a nephrotoxic effect, cause encephalomyelitis, and changes in the leukocyte composition of the blood. Also, they destroy cell membranes, which primarily leads to damage to the liver and kidneys of farm animals. In poultry, fumonisins

often lead to the development of the so-called toxic feed syndrome, including movement disorders and growth slowdown (Kim et al., 2002; Fodor et al., 2006; Hoister et al., 2013; Guerre, 2015).

It is defined that the primary mechanism of toxic action of fumonisins is the blocking of the process of lipid synthesis in the biological membranes of cells. They are specific inhibitors of ceramides synthetase - the central enzyme in the chain of formation of ceramides and more complex sphingolipids - the leading group of lipids that are part of the cell membrane (Berezovskyi et al., 2014). We note that the maximum allowable levels of fumonisins in Ukraine are not regulated for all species of animals, and determining their influence on specific biochemical processes requires in-depth toxicological research. Therefore, in the scientific aspect, it is actually to assess the criteria for toxicity of fumonisins and prevent fumonisin toxicosis in animals and poultry, as there are no effective specific treatments and prevention.

Methods of detoxification of mycotoxins in livestock and poultry are based on the use of appropriate sorbents. The latter reduces the biological activity of mycotoxins, reduces their absorption in the digestive tract of animals.

Therefore, the development of effective schemes for treating animals and preventing mycotoxicosis, particularly fumonisin toxicosis, is promising in this direction. Carrying out research, in fact, in the aspect mentioned above, is relevant and timely.

The purpose of the work is to study the influence of HamekoTox on the morphological and biochemical blood counts of laying hens with spontaneous fumonisin toxicosis

Material and methods

The experiment used laying hens (aged 78 weeks) cross Highsex White, with an average live weight of 1.5 kg. Three groups were formed: I - a group of laying hens served as a control, in experimental II, III, groups of poultry was characterized by clinical signs of fumonisin toxicosis. Experiment III (E_2) was fed with HamecoTox feed for 21 days. The following clinical signs of this toxicosis were observed in the sick poultry, namely: decreased productivity, growth retardation, decreased level of feed absorption, diarrhea. All manipulations of chickens that were involved in the experiments were carried out following bioethical requirements for animals, corresponding to the Law of Ukraine "On protection of animals from cruel treatment" of 21.02.2006

The concentration of hemoglobin – hemoglobin cyanide method (with acetone cyanohydrin) was determined in the blood; the number of erythrocytes and leukocytes - by counting on the Goryaev grid counting chamber. The total protein content in the serum was determined using an IRF-22 refractometer. The activity of enzymes (ALT, AST) in serum was determined using a semi-automatic biochemical analyzer (HumaLyzer 3000). The analysis of research results was performed using the software package Statistica 6.0. Student's t-test evaluated the probability of differences. The results were considered plausible at $P \leq 0.05$.

Results and discussion

Blood composition is a relatively stable indicator that is one of the labile systems of the body of laying chickens. The physiological processes occurring in the body primarily affect the qualitative composition of the blood (Gutyj et al., 2016; Gutyj et al., 2017; Grynevych et al., 2018; Lesyk et al., 2020; Vasylyev et al., 2021). Morphological indicators of poultry blood under conditions of fumonisin toxicosis on the 14th day of use of HamekoTox are given in the table. 1. It is defined that under conditions of fumonisin toxicosis in the blood of chickens, the number of erythrocytes decreases by 7.2%, and the hemoglobin level is reduced by 12% relative to the control group of chickens. A decrease in hematocrit to 33.1±0.76% was also found.

The number of leukocytes in the blood of chickens affected by fumonisin toxicosis throughout the experiment was increased, ranging from 34.4±1.1 G/l, while in the blood of chickens of the control group, this figure was 29.5±1.4 G/l, i.e., 16.6% was considerably higher. The use of HamecoTox to chickens of the second experimental group was accompanied by a slight increase in the number of erythrocytes and hemoglobin content compared to the first experimental group. However, compared with the control group of chickens, it was found that the number of erythrocytes decreased by 5.8%, and the level of hemoglobin, respectively, by 9%. The number of leukocytes in the blood of the second experimental group increased to 33.2±0.9 G/l and, compared with the control group, increased by 12.5%.

Table 1. Morphological indicators of poultry blood under conditions of fumonisin toxicosis on the 14th day of use of HamekoTox (M±m, n=5)

Indicators	Groups of animals		
	Control	Experimental 1	Experimental 2
Hemoglobin, g/l	90.8 ± 2.85	79.8 ± 2.35*	82.6 ± 2.47
Erythrocytes, T/l	3.29 ± 0.20	3.05 ± 0.15	3.10 ± 0.16
Hematocrit,%	40.5 ± 1.37	33.1 ± 0.76*	33.9 ± 1.51*
Leukocytes, G/l	29.5 ± 1.4	34.4 ± 1.1	33.2 ± 0.9*

Note: the degree of probability compared to the data of the control group - $p<0.05$ - *, $p<0.001$ - **

Based on the research of the content of total protein in the blood of laying hens of the experimental groups, its decrease in the first experimental group to 45.2±1.64 g/l, while in the control group during the experiment, this figure ranged from 58.4±1.75 g/l. After using HamekoTox to chickens of the second experimental group, a decrease in total protein by 15.8% relative to the control group was found out. The decrease in total protein in the blood of the first and second experimental groups was due to a decrease in albumin levels, where in the first experimental group, the level of albumin was 28.5±0.63%, while in the blood of the second experimental group, this figure was 30.3±0.57%. After researching the level of globulins in the blood of chickens

of the experimental groups, it was found that its highest level was in the blood of the first experimental group, where, respectively, it was 71.5±2.44%, in the second experimental group, this figure ranged from 69.7±3.21%. On the background of general hypoproteinemia, a significant disproportion between albumin and globulin in the serum of sick chickens was set up, which indicates a decrease in the value of the A/G ratio to 0.40 in sick chickens against 0.54 in clinically healthy poultry (Table 2). The smaller it is than optimal, the more reduced the protein-synthesizing is the function of the liver. After using HamekoTox to sick poultry, it was found that on the 14th day of the experiment, the protein-synthesizing function of the liver of chickens of the second experimental group was not completely restored. Low levels of albumin indicate this, and the presence of inflammatory processes, as indicated by increased levels of globulins.

Table 2. Biochemical indicators of poultry blood under conditions of fumonisin toxicosis on the 14th day of HamekoTox use (M±m, n=5)

Indicators	Groups of animals		
	Control	Experimental 1	Experimental 2
Total protein, g/l	58.4 ± 1.75	45.2 ± 1.64**	49.2 ± 1.80**
Albumins, %	35.1 ± 0.75	28.5 ± 0.63**	30.3 ± 0.57**
Globulins, %	64.9 ± 3.18	71.5 ± 2.44	69.7 ± 3.21
Coefficient A/G	0.54	0.40	0.44
ALT, mkkat/l	0.38 ± 0.04	0.54 ± 0.05*	0.49 ± 0.04*
AST, kkat/l	0.95 ± 0.02	1.12 ± 0.03*	1.05 ± 0.03*
Alkaline phosphatase, mmol/g	2.71 ± 0.11	2.96 ± 0.13*	2.89 ± 0.15
Lipids total, g/l	9.5 ± 0.47	7.6 ± 0.32*	8.0 ± 0.43*

Note: the degree of probability compared to the data of the control group - p<0.05 - *, p<0.001 - **

In our experiments (Table 2), in chickens affected by fumonisins in the serum, there is increased activity of enzymes. It is caused by an increase in the permeability of cell membranes and the entry of intracellular enzymes into the bloodstream. After the use of HamecoTox for the treatment of poultry affected by fumonisins, gradual normalization of aminotransferase activity and phosphatase activity in blood serum was found (Table 2). After researching the activity of aminotransferases in the serum of laying hens affected by fumonisins, ALT activity was 42% higher compared with clinically healthy birds. After the use of HamecoTox for treatment, the enzyme activity in the blood of chickens of the second experimental group was higher than the control group by 29%, respectively. AST activity in the serum of sick laying hens of the first experimental group was 18% higher than clinically healthy. It was slightly increased in the serum of the second experimental group, i.e., in the poultry used HamekoTox. After researching alkaline phosphatase activity, it was found that in the serum of the first experimental group, it ranged from 2.96±0.13 mmol/g, and in the second experimental group - 2.89±0.15 mmol/g, while in the control group, this figure ranged from 2.71±0.11 mmol/g.

After researching the morphological indicators of the blood of laying hens under conditions of fumonisin toxicosis and for the use of HamecoTox for 21 days of the experiment, an increase in the number of erythrocytes and hemoglobin content compared to a sick bird was found were, respectively, the number of erythrocytes and the content of hemoglobin in the blood of the second experimental group were 3.21±0.15 T/l and 84.8±2.30 g/l. In contrast, in the first experimental group, these values were probably low and were 2.96±0.17 T/l and 77.6±2.41 g/l, respectively (Table 3). Analogical changes were found after the research of hematocrit, which compared with the control group was decreased by 23% in the blood of the first experimental group and by 9% - in the blood of the second experimental group. The number of leukocytes in the blood of laying hens, which used HamekoTox, reached physiological values on the 21st day of the experiment. Changes in protein metabolism are an essential objective indicator of the organism's state of laying hens, both regular and in pathology. The content of protein and protein fractions characterize the degree of resistance of the organism. As can be seen from the data in table 3, chickens of the first experimental group, which did not use HamekoTox, found a tendency to reduce total protein and albumin, respectively, by 27 8% the values of the control group. Thus, HametoTox contributed to restoring the protein-synthesizing function of the liver by reducing the negative influence of fumonisins. After the release of the organism from fumonisins, the action of toxins on the liver passes away, and the inflammatory processes disappear accordingly.

Table 3. Morphological indicators of poultry blood under conditions of fumonisin toxicosis on the 21st day of use of HamekoTox (M±m, n=5)

Indicators	Groups of animals		
	Control	Experimental 1	Experimental 2
Hemoglobin, g/l	91.2 ± 2.90	77.6 ± 2.41*	84.8 ± 2.30
Erythrocytes, T/l	3.31 ± 0.21	2.96 ± 0.17*	3.21 ± 0.15
Hematocrit,%	40.7 ± 1.40	31.4 ± 0,66**	37.1 ± 0.86
Leukocytes, G/l	29.7 ± 1.2	35.7 ± 1.1*	31.4 ± 1.3

Note: the degree of probability compared to the data of the control group - p<0.05 - *, p<0.001 - **

Under the conditions of the experimental preparation use on the 21st day of the experiment in chickens of the second group, the studied indicators were at the control group level. Under these conditions, there was a decrease in aminotransferases and alkaline phosphatase activity, respectively, by 33.9, 17.2, and 6.7%, compared with the values of the first experimental group.

The level of total lipids in the blood of the second experimental group on the 21st day of use of HamecoTox ranged from 8.6±0.33 g/l, whereas in the first experimental group, this figure was 7.1±0.30 g/l.

Table 4. Biochemical indicators of poultry blood under conditions of fumonisin toxicosis on the 21st day of use of HamekoTox (M±m, n=5)

Indicators	Groups of animals		
	Control	Experimental 1	Experimental 2
Total protein, g/l	58.6 ± 1.61	42.7 ± 1.35**	58.2 ± 1.58
Albumins, %	35.3 ± 0.72	27,8 ± 0.58**	33.9 ± 0.61
Globulins, %	64.7 ± 3.12	72.2 ± 2.55*	65.8 ± 2.40
Coefficient A/G	0.55	0.39	0.52
ALT, mkkat/l	0.37 ± 0.03	0.62 ± 0.04**	0.41 ± 0.05
AST, kkat/l	0.93 ± 0.02	1.16 ± 0.03**	0.96 ± 0.03
Alkaline phosphatase, mmol/g	2.74 ± 0.12	2.99 ± 0.10	2.79 ± 0.13
Lipids total, g/l	9.4 ± 0.38	7.1 ± 0.30*	8.6 ± 0.33

Note: the degree of probability compared to the data of the control group - $p<0.05$ - *, $p<0.001$ - **

Conclusions

Under conditions of spontaneous fumonisin toxicosis in poultry, the additional introduction of HamecoTox to laying hens during the 21st day contributed to the normalization of morphological and biochemical indicators of the blood of experimental poultry. To prevent the fumonisin toxicosis, it is recommended to add feed additive HamekoTox at a dose of 4 kg/t of feed to mycotoxin-contaminated feed.

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