Investigation of the level of endogenous intoxication of the body and the characteristics of the saliva crystallization in poultry with feed toxicosis

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Abstract: In the course of the experiments, it was found that during the reproduction of feed toxicosis in broiler chickens caused by prolonged intake of mycotoxins (T-2 toxin and aflatoxin B1), clinical signs of intoxication were recorded starting from the 5th day of the experiment. Disturbances in the biochemical profile of the bird's blood were manifested by changes in protein metabolism, as well as in indicators reflecting the functioning of the liver and kidneys. The level of endogenous intoxication in the body of broiler chickens with combined mycotoxicosis was significantly higher than the data of intact control at a spectrophotometer wavelength $\lambda = 254$ nm - by 14.8%, at $\lambda = 280$ nm - by 36.6%, AWM distribution index (DI) 280/254 increased by 18.9%. Comparison of the saliva facies of healthy chickens and chickens with mycotoxicosis revealed differences between the studied crystallograms.

1. Introduction

The anthropogenic load on the environment is increasing every year, which negatively affects the environmental situation around the world, directly affecting agriculture. One of the most disastrous consequences of this is toxic poisoning of animals, which reduces the efficiency of the livestock industry. The most common fodder toxicosis of animals, including poultry, is mycotoxicosis, which are widespread throughout Russia, causing multiorgan pathological changes in the body. It has been proved that the membrane toxic effect of mycotoxins is based on changes in the structure of phospholipids and the formation of intermediate hydrolysis products. In this case, a violation of the metabolic processes of the body occurs, which causes the accumulation of products of normal and abnormal metabolism. When the content of toxins exceeds the capacity of the body's detoxification systems, structural and metabolic disorders occur in cells, manifested by the development of endogenous intoxication [1-4].

Endogenous intoxication is understood as a complex of symptoms of pathological states of organs and body systems caused by the accumulation of endotoxins in tissues and biological fluids. Endogenous intoxication accompanies diseases and complications associated with increased tissue breakdown, enhanced catabolic processes, as a result of which microcirculation is impaired with damage to cells and body systems [5].

As a molecular marker of endogenous intoxication of the body, the indicator of concentration of average weight molecules (AWM) is most often used. To date, the biological effect of AWM has been studied in sufficient detail, many of which have neurotoxic activity, inhibit the processes of protein biosynthesis, are able to suppress the activity of a number of enzymes, uncouple the processes of
oxidation and phosphorylation, change the transport of ions through membranes, erythropoiesis, phagocytosis, microcirculation, lymphodynamics, cause the state of secondary immunosuppression. It was found that in the process of effective therapy, a decrease in the level of AWM outpaces the period of elimination of clinical signs of the disease, therefore, the determination of their concentration in the biological media of the body is one of the most informative and accessible ways to assess the severity of intoxication and the effectiveness of treatment in many pathological conditions [6].

Traditionally, two fractions of AWM are determined – at wavelengths of 254 and 280 nm. The 254 nm AWM fraction is a toxic fraction represented by hydrophobic toxins and incomplete protein breakdown products. The intensity of UV absorption at 280 nm is mainly determined by the presence of aromatic chromophores, and its increase is due to the accumulation of tyrosine- and tryptophan-containing peptides. This may be due to the loss of aromatic amino acids by proteins as a result of oxidative modification and fragmentation of molecules. Quite informative is the estimate of the coefficient of the distribution index (DI) of AWM 280/254 nm - an increase in this indicator may indicate an increase in catabolic processes, stimulation of lipid peroxidation and immunogenesis [7, 8].

New research methods are emerging very rapidly in the modern world, especially in medicine. The morphology of biological fluids, including saliva, as a fundamentally new scientific direction in the field of clinical diagnostics, is now developing at an extremely fast pace. Methodically, it is based on the transfer of a biological fluid into a solid phase and subsequent study of the facies (film) using an optical microscope. The use of the results of the structural analysis of biofluids (blood, saliva, bile, tears, etc.) allows obtaining fundamentally new information about the body's homeostasis and the nature of the course of pathological processes, which turned out to be very informative in the early diagnosis of many diseases [9-11].

In this regard, the aim of the work was to study the level of endogenous intoxication of the body and the characteristics of crystallization of poultry saliva in experimental mycotoxicosis.

2. Materials and methods
The studies were carried out on 40 Ross 308 broiler chickens aged 18 days with an average body weight of 680.4 ± 2.16 g, divided into 2 groups (1 – experimental and 2 – control, n = 20). In the experiments, we used birds that had passed the quarantine regime of the vivarium of the Krasnodar Research Veterinary Institute and had no external signs of disease. To obtain statistically reliable results, the groups were formed according to the principle of paired analogs.

The essence of the method of reproduction of feed toxicosis consisted in the fact that for two weeks the birds of the 1st experimental group were fed with feed contaminated with mycotoxins, with the content of T-2 toxin – 0.029 mg/kg and aflatoxin B1 – 0.005 mg/kg. The concentration of mycotoxins individually did not exceed the MPL (that is, from the standpoint of the current regulatory documents, the feed was of high quality), but their combined effect on the poultry organism caused the development of mycotoxicosis. Chickens of the 2nd group were control and were fed high quality feed.

The immune-enzyme assay of feeds for the presence of mycotoxins was carried out on a Stat fax 2600 analyzer using test systems for indirect solid-phase competitive enzyme-linked immune-enzyme assay of Pharmatech CJSC.

During the clinical examination of the chickens throughout the experimental period, special attention was paid to the colour of the mucous membranes, the state of the feather cover, body temperature, etc. On the 15th day of experimental intoxication, from 5 chickens from each group blood was taken for research, in which the main biochemical parameters and the level of endogenous intoxication were determined (by the concentration of average weight molecules – AWM). Biochemical studies were carried out on an automated Vitalab Selectra Junior analyzer using reagents from ELITech Clinical Systems and Analyticon biotechnologies AG. The content of AWM in blood serum was determined using the screening method of N.I. Gabrielyan and V.I. Lipatova at three wavelengths λ = 237 nm (AWM 237), λ = 254 nm (AWM 254) and λ = 280 nm (AWM 280). To register the optical density in the ultraviolet region of the spectrum, an Ecoviw UV-1100 spectrophotometer was used. The distribution index of AWM was calculated using the formula: DI 280/254 = AWM 280 / AWM 254.
At the end of the experiment, mixed saliva in a volume of 20 μL was taken from 5 broilers from each group with an automatic micropipette from the oral cavity and immediately applied in the form of a drop onto a previously defatted glass slide. The drop was dried at $t = 20-25 ^\circ C$, relative humidity 65-70% and minimum mobility of the ambient air, strictly in a horizontal position. The duration of the drying period (before the analysis of the structure) ranged from 18 to 24 hours. The study of the dehydrated droplet – “facies” was carried out using an optical microscope “Mikmed-3” with a built-in digital camera “Canon” (5.0 Mpx). The resulting image was transmitted to the monitor screen. First, at low magnification, the entire surface of the dried droplet was scanned, and then, at high magnification, individual areas of the surface with different morphology were examined. Before saliva was collected, the birds were deprived of feed for 8 hours and kept in carefully cleaned wire-bottom cages. Watering of all birds was carried out from automatic drinkers, in free access.

Statistical data processing was carried out using the Statistica v. 10. The criterion of reliability was determined according to the Student's table.

3. Results and discussion

As a result of the studies, it was found that the development of combined mycotoxicosis in broiler chickens, starting from the 5th day of the experiment, was accompanied by a clinical manifestation of intoxication: suppression and decreased appetite; thirst and indigestion (diarrhea); ruffled and dull feather cover.

During the two-week period of the experiment, the death of 3 birds (15%) was recorded. At postmortem autopsy of the dead bird the following changes were found: corpses are anemic; point hemorrhages are visualized on the gastric mucosa; the liver is enlarged, with areas of hemorrhage on the capsule; the gallbladder is not full, the contents of the bladder are greenish-brown; the spleen is enlarged in size, its surface is smooth, the integrity is not broken; in the lumen of the small intestine, exudation is observed, the contents are watery, there are multiple punctate hemorrhages on the mucous membrane; the blind processes of the intestine are enlarged, with greenish contents, the mucous membrane is hyperemic; kidneys are slightly enlarged and with signs of hyperemia.

Gravimetric studies in groups revealed a positive increase in body weight, however, in the birds of the experimental group, the growth rate was noticeably lower in comparison with the control analogs, and by the end of the experiment the intergroup differences were 16.3%.

The results of a biochemical blood test (table 1) indicate a violation of protein metabolism and protein-synthetic function of the liver in birds of the experimental group, since the concentration of total protein by the 15th day of research was 14.1% lower than the data of the control group. In other indicators of protein metabolism - urea and creatinine, on the contrary, an increase in their level was revealed – by 27.7% ($p<0.05$) and 25.2% ($p<0.01$), respectively, which indicates the development of pathological processes in the kidneys of poultry with fodder intoxication.

In the level of hepatoindicator enzymes in poultry of the experimental group, at the end of the studies, an increase in ALT was recorded to the level of the upper limits of the reference values. The difference between the groups for ALT was 47.2% ($p<0.01$) and for AST - 18.5%. With toxicosis, changes in the cholesterol profile were recorded, due to a decrease in cholesterol content beyond the lower limit of the norm ($2.45 \pm 0.05$ mmol / l) while here was a significant difference with the indicators of the control group by 21.2% ($p<0.01$). In poultry of the 1st group, there was a tendency to a decrease in the concentration of glucose in the blood serum, with a difference to the values of healthy broilers of 12.7%. In terms of mineral metabolism, no significant changes were found between the groups.
Table 1. Biochemical parameters of broiler chickens at experimental mycotoxicosis (M ± m; n = 5).

| Indicators            | Groups       |
|-----------------------|--------------|
|                       | experimental | control      |
| Total protein, g/l    | 33.4±0.58    | 38.9±0.77    |
| Urea, mmol/l          | 3.97±0.11 a  | 3.11±0.09    |
| Creatinine, µmol/l    | 32.3±0.17 b  | 25.8±0.14    |
| ALT, U/l              | 25.9±0.13 b  | 17.6±0.12    |
| AST, U/l              | 326.2±18.4   | 275.4±15.1   |
| Cholesterol, mmol/l   | 2.45±0.05 b  | 3.11±0.08    |
| Glucose, mmol/l       | 8.16±0.32    | 9.35±0.25    |
| Calcium, mmol/l       | 3.18±0.06    | 3.23±0.04    |
| Phosphorus, mmol/l    | 2.56±0.14    | 2.48±0.17    |

a - p≤0.05, b - p≤0.01 in relation to the control group.

When determining the level of endogenous intoxication in the body of broiler chickens with combined mycotoxicosis, it was found that all indicators of the level of AWM in the blood serum of the birds of the experimental group were higher than the data of the intact control (table 2).

Table 2. The level of AWM in the blood serum of broiler chickens with experimental mycotoxicosis (M ± m; n = 5).

| Indicators            | Groups       |
|-----------------------|--------------|
|                       | experimental | control      |
| AWM, 254 nm, AU       | 0.658±0.016**| 0.573±0.011  |
| AWM, 280 nm, AU       | 0.571±0.023* | 0.418±0.015  |
| AWM, 280/254 nm, AU   | 0.868        | 0.730        |

* p≤0.05, ** p≤0.01 in relation to the control group

When calculating, the significant difference was 14.8% for the spectrophotometer wavelength λ = 254 nm and 36.6% for λ = 280 nm. Analysis of IR 280/254 showed that, compared with the control in broiler chickens, after exposure to mycotoxins, this indicator increased by 18.9%.

At present, the use in veterinary medicine of crystallographic methods for studying biological fluids, namely, obtaining and describing crystallograms in some pathological conditions in animals, is in its initial stage. At the same time, in poultry, studies of saliva are generally sporadic, and information on the state of its solid-phase structures is not presented in the available literature. Therefore, our studies in this direction were purely descriptive, since for more statistically reliable results it is necessary to analyze a significantly larger number of facies images.

Comparison of the saliva facies of healthy and mycotoxic broiler chickens makes it possible to distinguish the following features: in a healthy bird, a low level of structure destruction is visualized, both in the center and at the periphery of the facies, as well as the presence of crystalline figures of the “fern” type; in case of mycotoxicosis in poultry with a high level of endogenous intoxication of the organism, the center of the facies is characterized by the uneven width of the main trunk of the “dendrite” and the asymmetry of the first-order branching.
Figure 1. Crystallographic saliva facies of broiler chickens (healthy and with mycotoxicosis).

4. Conclusion
Thus, the results of the work performed showed that the development of combined mycotoxicosis in broiler chickens, starting from the 5th day of the experiment, is accompanied by a clinical manifestation of intoxication. During the two-week period of the experiment, the death of 15% of the individuals in the group was recorded, and the decrease in the intensity of the increase in their body weight was 16.3%. Violations in the biochemical profile of blood manifested themselves mainly in changes in protein metabolism, as well as in indicators reflecting the functioning of the liver and kidneys. The level of endogenous intoxication in the body of broiler chickens with combined mycotoxicosis was significantly higher than the data of intact control. Comparison of the saliva facies of healthy and mycotoxic broiler chickens revealed differences between the studied crystallograms.

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