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# EXPERIMENTAL COMBINED MYCOTOXICOSIS IN PIGS AS AFFECTED BY INFECTION LOAD

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## Abstract

Animal and human mycotoxicoses occur due to the ingestion of metabolites of toxicogenic microfungi. The effect increases in case of the co-ingestion of several mycotoxins, their mix with another ecotoxicants and biological agents. However, published research data only partially cover the nature of mixed mycotoxicoses in infectious diseases. This work shows for the first time the effect on pigs of the infection load of *Clostridium perfringes* and the combined effect of T-2 toxin, zearalenone, and deoxynivalenol in low doses. Our goal was to study the chronic form of combined mycotoxicosis in weaned pigs with a persistent infection in herd on the animal productivity, blood morpho-biochemical and immunological parameters, pathological changes in organs and tissues. Combined experimental mycotoxicosis with infectious load was modeled under the conditions of the vivarium complex (the Federal Center for Toxicological, Radiation and Biological Safety, 2018) on the weaning Large White piglets (*Sus scrofa domesticus*) divided into three groups 3 pigs each. Group I received no mycotoxins, group II received dietary T-2 toxin (70 μg/kg feed), group III received mixed dietary mycotoxins (DON 1000 μg/kg, ZEN 50 μg/kg and T-2 70 μg/kg). All animals were orally administered a suspension of *Clostridium perfringes* No. 392 type C ($1\times10^6$ CFU/ml, 2 ml). On day 15, the animals were vaccinated intramuscularly in the posterior thigh with 1 ml of the associated vaccine against rota-, coronavirus and colorectal diarrhea of newborn piglets (FCTRB-VNIVI). Group I (control) was considered clinically healthy. Signs of intoxication, blood biochemical parameters (total protein, total bilirubin, glucose, malondialdehyde, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activity), blood morphology (counts of erythrocytes, leukocytes, the hemoglobin level) and immunological parameters (T- and B-lymphocytes, titer of antibodies to vaccine antigens) on day 10, 20 and 30. The antibody titers to the *Escherichia coli* vaccine strain were determined by the agglutination reaction, to the coronavirus vaccine antigen by the ELISA test using a Multiscan FC photometer (Thermo Scientific, USA), and to the rotavirus antigen by an indirect hemagglutination test. At the end of the experiment, pieces of organs were fixed in 10 % neutral formalin, followed by generally accepted pathomorphological processing for histological studies. Histopreparations were stained with hematoxylin and eosin. Feed contamination with mycotoxins combined with clostridiosis had an adverse effect on the clinical and immune status, blood morpho-biochemical parameters, and pathoanatomical patterns. The changes were more apparent in co-contamination with ecotoxicants. Average daily bodyweight gain in piglets of group II was lower by 20.5 % compared to the control ($p \geq 0.05$), of group III by 39.2 % ($p \leq 0.05$). In group III, by the end of the experiment, there was a decrease in the erythrocyte counts by 40 % ($p \leq 0.001$), in the level of hemoglobin by 20 % ($p \leq 0.01$), glucose by 57 % ($p \leq 0.001$), and total protein by 13 % ($p \leq 0.05$). The concentration of bilirubin increased 5.1-fold ($p \leq 0.001$), the activity of alanine aminotransferase and aspartate aminotransferase 2.2- and 1.8-fold ($p \leq 0.001$), respectively, the concentration of malondialdehyde 2.8-fold ($p \leq 0.001$), the activity of alkaline phosphatase decrease by 41.5 % ($p \leq 0.001$). Co-mycotoxicosis combined with an infectious load led to immunological changes. Titers of specific antibodies to rotavirus were 8 times lower, to

coronavirus 6.4 times lower ($p \leq 0.05$), to *Escherichia* 5 times lower ($p \leq 0.05$) compared to the control. Marked pathological changes in the internal organs also occurred. Therefore, the co-mycotoxicosis due to T-2 toxin-, deoxynivalenol- and zearalenone-contaminated feed combined with the persistence of *Clostridium perfringens*, the causative agent of intestinal infection lead to suppression of immunological parameters (a decrease in the titer of specific protective antibodies, the number of T- and B-lymphocytes), activation of lipid peroxidation, and pathological changes in tissues and organs of the piglets.

Keywords: mycotoxins, pigs, blood, morpho-biochemical parameters, immune suppression, histological study

Mycotoxins, the secondary metabolites of microscopic fungi represent a serious problem for animal husbandry [1, 2]. Trichothecene mycotoxins [3] with cytotoxic and cytostatic properties [4] interfere with protein synthesis [5], and damage parenchymal organs, nervous and immune systems [6], are a health hazard for both humans and animals.

T-2 toxin is the most toxic of the secondary metabolites produced by *Fusarium* micromycetes [7]. As early as 1973, the World Health Organization (WHO) included T-2 toxin among the inevitable contaminants that contaminate feed and agricultural products [8]. Zearalenone (ZEN) is a mycotoxin that contributes to the development of estrogen syndrome [9]. Deoxynivalenol (DON) can cause a variety of symptoms, including necrosis of the intestinal tract, liver, and bone marrow with leukopenia, vomiting, and diarrhea [10, 11]. The issue of the allowed and toxic content of DON still remains an unaddressed problem [12].

To better assess animal and human health risks, it is important to study the toxicological effects of mycotoxin combinations. The vast majority of studies on this topic were carried out on cell cultures in vitro and focused on the study of the toxic effect of two mycotoxins [13-15], a number of authors note an increase in the toxic effect of several mycotoxins [16-19], as well as in combination with other ecotoxicants [20, 21].

Under production conditions, there are frequent cases of the presence in feed of such mycotoxins as T-2 toxin, DON and ZEN [21-22]. Pigs are thought to be sensitive to mycotoxins [23]. Studies on the combined effect of three mycotoxins at once on these animals are few [24-26]. There are few data on the study of combined mycotoxicoses under infectious diseases [27, 28]. Mycotoxins adversely affect immune parameters, increase the susceptibility of animals to pathogenic agents of a bacterial and viral nature [29]. We have not seen any works devoted to the combined effects of mycotoxins against the background of an infectious load of *Clostridium perfringes* in pig models.

This paper shows for the first time the effect on pigs of infection load of *Clostridium perfringes* and the combined effect of mycotoxins T-2 toxin, zearalenone and deoxynivalenol at low dosage. Consumption of feed containing T-2 toxin, deoxynivalenol and zearalenone under persistence of C. perfringens, a causative agent of intestinal infection in piglets, is accompanied by inhibition of the immune system function (decrease in the titer of specific protective antibodies, the number of T- and B-lymphocytes), activation of lipid peroxidation (LPO), the development of pathological processes in tissues and organs.

Our goal was to study the effect of the chronic form of combined mycotoxicosis on the background of infection persisting in the herd in weaned piglets on the productivity of animals, morpho-biochemical, immunological blood parameters, and the pathoanatomical picture of organs and tissues.

*Materials and methods.* Experimental combined mycotoxicosis under an infectious load was modeled in the conditions of the vivarium complex of the Federal Center for Toxicological, Radiation and Biological Safety (FCTRBS-VNIVI) in 2018 on Large White piglets (*Sus scrofa domesticus*) of weaning age (35 days) divided into three groups of 3 pigs each. Feeding and maintenance was carried out in a

group way. We used complete feed for feeding piglets (GOST 34109-2017. M., 2017).

Animals received a diet that did not contain mycotoxins (group I), that contained T-2 toxin (70 rg/kg of feed, group II), three mycotoxins (DON at 1000 μg/kg, ZEN at 50 μg/kg and T-2 at 70 μg/kg, group III). Access to water was not restricted. Mycotoxins (purity 96.7-99.8%, obtained at the FCTRBS-VNIVI) were introduced into the feed using sequential and stepwise mixing. The experiment lasted 30 days. Pathogenic isolate *Clostridium perfringes* No. 392 type C (collection of FCTRBS-VNIVI) was used as an infectious agent. All animals were orally administered a suspension of *Clostridium perfringes* ($1\times10^6$ CFU/ml) in 2 ml. The amount of *C. perfringens* No. 392 type C microbial cells taken by us was previously estimated as a dose that does not cause clinical manifestations but ensures the carriage of clostridium. On day 15 of the experiment, the animals were vaccinated intramuscularly (in the back of the thigh in a 1 ml volume) with the associated vaccine against rota-, coronavirus and escherichiosis diarrhea of newborn piglets (FCTRBS-VNIVI).

When forming groups of animals, the principles of humanity and sufficiency were taken into account, while trying to bring the experiment closer to production conditions. Animals of group I were considered as clinically healthy, and the parameters of the remaining piglets were compared with their performance.

The signs of intoxication in pigs, biochemical parameters (total protein, total bilirubin, concentration of glucose, malondialdehyde MDA, activity of alkaline phosphatase AP, aspartate aminotransferase AsAT, alanine amine transferase AlAT) in blood serum, hematological parameters (number of erythrocytes, leukocytes, hemoglobin content) and immunological indicators (the number of T-, B-lymphocytes in the blood, the titer of antibodies to vaccine antigens) were assessed. Blood for research was taken from the tail vein on days 10, 20, and 30 of the experiment.

Hematological studies were carried out on a Mythic 18 Vet analyzer (Orphee Geneva, Switzerland). Biochemical parameters were measured on an ARD-200 analyzer (OOO VITAKO, Russia) using special reagent kits (Chronolab Systems S.L., Spain). The number of T- and B-lymphocytes was determined by the method of spontaneous rosette formation with goat erythrocytes [30]. The accumulation of MDA in the reaction with 2-thiobarbituric acid was used to assess the intensity of lipid peroxidation [31].

The titers of antibodies to the vaccine strain of *Escherichia coli* were determined in the agglutination test (AT). The titer was the last dilution with clear agglutination observed. The result was assessed by the amount of sediment and the transparency of the supernatant after 18-20 h (OD at $\lambda$ = 490 nm). A positive result (titer) was taken as the ratio ≥2.0 of OD in the well with the studied serum to OD in the well with negative serum. The titers of antibodies to the rotavirus antigen of the vaccine were determined in the indirect hemagglutination test (IHA). The result of IHA was considered positive when the erythrocytes evenly covered the bottom of the well of the plate in the form of an umbrella for 2-4+. With a negative reaction, the accumulation of erythrocytes looked like a small disk ("buttons").

At the end of the experiment, animals of all groups were slaughtered in accordance with ethical standards. The samples for histological examination were fixed in 10% neutral formalin followed by processing by conventional pathomorphological methods. Histopreparations were stained with hematoxylin and eosin. Microphotography of histological preparations was carried out in transmitted light (Leica DM 1000 microscope, Leica DFC 320 camera, Leica Microsystems,

Germany) 200× magnification (10× objective, 20× eyepiece) and 600× (15× objective, 40× eyepiece).

During statistical processing, mean values (*M*) and standard errors of the means (±SEM) were calculated. Significance of differences was assessed by Student's *t*-test. Differences were considered statistically significant at $p \leq 0.05$; $p \leq 0.01$ and $p \leq 0.001$.

*Results.* The first signs of intoxication began to appear on days 6-8 of the experiment in animals treated with T-2 toxin, DON and ZEN. For several days there was a partial refusal of food. Subsequently, the animals consumed food in smaller quantities. The development of feed refusal syndrome is probably because trichothecenes disrupt the synthesis of liver proteins, cause hyperaminoacidemia, and increase the concentration of tryptophan and serotonin in the brain, and this affects the perception of satiety. Also, in piglets from group III, diarrhea and vomiting periodically occurred which are characteristic DON poisoning syndrome in pigs. Other researchers reported similar results [2, 32].

Clinical manifestations intensified in the second part of the experiment: we registered an increase in body temperature, the piglets huddled together, they were depressed, pursed their stomach, signs of gastrointestinal upset were noted. On day 23, one pig fell in group III. A *C. perfringes* No. 392 type C strain was isolated from organs of the piglets.

By the end of the experiment, pigs from group III had minor bleeding from the vagina. The bleeding was associated with the content of dietary zearalenone, an estrogenic mycotoxin. This is surprising given the low concentrations of ZEN. Zearalenone is a mycotoxin that contributes to the development of estrogen syndrome, including infertility, abortion, and resorption of fetuses [9]. Exposure of pregnant sows to ZEN reduces the number of follicles in piglets, leading to premature depletion of oocytes in adulthood [33]. Probably, the presence of trichothecene mycotoxins in the feed creates prerequisites for greater sensitivity of gilts to the action of zearalenone. Other researchers have also found that the effects of simultaneous exposure to mycotoxins are poorly recognized and difficult to predict [34]. Animals that received only T-2 toxin were more active and ate feed well.

**1. Live weight, feed conversion rate and survival of Large White weaned piglets (*Sus scrofa domesticus*) with experimental combined mycotoxicosis under infectious load** (*M*±SEM, Federal Center for Toxicological, Radiation and Biological Safety, 2018)

| Parameter | Group | | |
|---|---|---|---|
| | I ($n = 3$) | II ($n = 3$) | III ($n = 3$) |
| Weight at the beginning of the experiment, kg | 14.9±0.2 | 15.2±0.2 | 14.6±0.2 |
| Weight at the end of the experiment, kg | 22.2±0.3 | 21.0±0.4 | 19.1±0.6* |
| Average daily increase in live body weight, g | 244.0 | 194.0 | 148.3 |
| feed conversion | 3.55 | 4.06 | 5.07 |
| Survival rate of piglets, % | 100 | 100 | 66.6 |

N o t e. For a description of the groups, see the "Material and methods" section.

* Differences from control are statistically significant at $p \leq 0.05$.

The average daily live weight gain vs. control in piglets of group II was lower by 20.5% ($p \leq 0.05$) and from group III by 39.2% ($p \leq 0.05$). The deterioration in feed conversion occurred in group III (it was 1.4 times higher than in the group of biological control, $p \leq 0.05$) (Table 1).

Long-term consumption of toxic feed adversely affects morphological, biochemical, and immunological blood parameters (Table 2). Hematological parameters and blood biochemistry in the piglets of group I who received orally a suspension of *C. perfringes* No. 392 type C and did not receive mycotoxins, were

mostly within the normal physiological values for healthy animals. Thus, the number of erythrocytes was $5.45\pm0.15\times10^{12}$/l vs. physiologically normal values of $5.1\text{-}6.8\times10^{12}$/l. Leukocytes accounted for $16.47\pm0.51\times10^{9}$/l vs. $11\text{-}22\times10^{9}$/l, protein for 63.2±0.87 g/l vs. 58-83 g/l. We observed similar patterns in other parameters, including more labile traits, such as the activity of serum enzymes AlAT (compared to normal values of 22-47 U/l), AsAT (compared to normal values of 15-55 U/l) (see Table 2). The data obtained indicate that the animals of group I were clinically healthy.

**2. Morphological, biochemical and immunological blood parameters of Large White weaned piglets (*Sus scrofa domesticus*) with experimental combined mycotoxicosis under infectious load, depending on the time from the beginning of the experiment** (*M*±SEM, Federal Center for Toxicological, Radiation and Biological Safety, 2018)

| Parameter | Group | | |
|---|---|---|---|
| | I ($n$ = 3) | II ($n$ = 3) | III ($n$ = 3) |
| | Day 10 | | |
| Erythrocytes, $\times10^{12}$/l | 5.4±0.1 | 5.3±0.1 | 6.1±0.1 |
| Leukocytes, $\times10^{9}$/l | 16.4±0.5 | 17.5±0.4 | 19.3±0.5* |
| Hemoglobin, g/l | 93.0±1.5 | 92.0±1.9 | 95.0±1.3 |
| Total protein, g/l | 63.2±0.8 | 62.2±0.9 | 62.6±0.8 |
| Bilirubin total, μmol/l | 1.5±0.2 | 4.4±0.5*** | 4.2±0.1*** |
| Glucose, mmol/l | 3.5±0.1 | 3.5±0.1 | 2.9±0.1* |
| AlAT, U/l | 33.2±1.6 | 28.8±1.7 | 46.3±1.9*** |
| AsAT, U/l | 36.9±2.4 | 35.2±2.6 | 60.2±2.2*** |
| ALP, U/l | 137.9±14.9 | 76.8±15.9 | 312.2±18.7*** |
| MDA, μmol/l | 1.9±0.2 | 3.3±0.1*** | 5.2±0.1*** |
| T-lymphocytes, % | 53.2±2.4 | 51.6±2.2 | 53.7±2.8 |
| B-lymphocytes, % | 26.5±1.4 | 26.0±1.8 | 28.6±1.5 |
| | Day 20 | | |
| Erythrocytes, $\times10^{12}$/l | 5.6±0.1 | 5.2±0.1 | 5.8±0.1 |
| Leukocytes, $\times10^{9}$/l | 15.9±0.5 | 19.4±0.5** | 26.9±0.2*** |
| Hemoglobin, g/l | 95.0±1.7 | 90.0±1.3 | 91.0±1.3 |
| Total protein, g/l | 63.3±0.7 | 60.6±0.7 | 58.1±0.8 |
| Bilirubin total, μmol/l | 3.2±0.2 | 6.3±0.3*** | 4.9±0.2*** |
| Glucose, mmol/l | 4.2±0.1 | 3.4±0.1* | 2.5±0.1*** |
| AlAT, U/l | 29.2±1.9 | 39.4±1.3*** | 80.6±1.5*** |
| AsAT, U/l | 35.2±2.3 | 43.4±1.9** | 75.2±2.2*** |
| ALP, U/l | 143.4±13.9 | 144.4±13.7 | 267.7±17.2*** |
| MDA, rmol/l | 2.8±0.1 | 5.6±0.1*** | 6.9±0.1*** |
| T-lymphocytes, % | 51.4±2.8 | 55.0±2.8 | 48.3±2.1 |
| B-lymphocytes, % | 25.3±0.9 | 28.6±0.4 | 26.1±0.4 |
| | Day 30 | | |
| Erythrocytes, $\times10^{12}$/l | 6.4±0.1 | 5.0±0.1** | 4.0±0.2*** |
| Leukocytes, $\times10^{9}$/l | 17.9±0.4 | 17.4±0.5 | 19.1±0.5 |
| Hemoglobin, g/l | 99.0±1.8 | 85.0±1.6* | 79.0±2.1** |
| Total protein, g/l | 63.9±0.9 | 57.3±0.9 | 55.5±0.9* |
| Bilirubin total, μmol/l | 2.5±0.1 | 7.3±0.1*** | 12.7±0.5*** |
| Glucose, mmol/l | 4.4±0.1 | 4.3±0.1 | 1.9±0.1*** |
| AlAT, U/l | 51.9±1.8 | 38.4±1.9*** | 114.8±1.9*** |
| AsAT, U/l | 55.3±2.5 | 48.8±2.6 | 99.5±2.5*** |
| ALP, U/l | 175.3±15.1 | 143.0±14.8* | 102.6±14.0*** |
| MDA, μmol/l | 3.0±0.1 | 7.3±0.2*** | 8.7±0.2*** |
| T-lymphocytes, % | 46.7±3.0 | 52.0±1.8 | 32.1±3.0*** |
| B-lymphocytes, % | 28.5±1.3 | 27.2±0.4 | 23.2±1.8* |

Note. ALT — alanine aminotransferase, AST — aspartate aminotransferase, ALP — alkaline phosphatase, MDA — malondialdehyde. For a description of the groups, see the "Materials and methods" section.

*, **, *** Differences from control are statistically significant at $p \le 0.05$, $p \le 0.01$, and $p \le 0.001$.

When compared to control, more pronounced deviations occurred in animals from group III. Thus, there was a 17.7% ($p \le 0.05$) increase in the number of leukocytes by day 10 of the experiment and a 69.2% ($p \le 0.001$) increase by day 20. In piglets of group II, the number of leukocytes by day 20 was 21.8% ($p \le 0.01$) higher than in the control group. Statistically significant changes in the number of erythrocytes and the hemoglobin level in animals from the experimental

groups occurred on day 30. In group III, on day 30, the erythrocyte number and hemoglobin level were 36.9 and 20.2% ($p \leq 0.01$) lower compared to control.

Progressive erythropenia and leukopenia and anemia indicated destructive changes in immunocompetent and hematopoietic organs known as a target for T-2 toxin [35]. This also occurred in chronic toxicosis caused by zearalenone and deoxynivalenol [36].

A pronounced decrease in the content of protein and glucose in animals treated with mycotoxins was recorded on day 30. In group III, the total protein decreased by 13.1% ($p \leq 0.05$) and the glucose level by 55.8% ($p \leq 0.001$). In groups II and III, by the end of the experiment, the level of bilirubin was 2.9 and 5.1 times higher vs. control ($p \leq 0.001$), respectively. In group III on day 20, the activity of blood ALP, AlAT, and AsAT was 1.9, 2.8, and 1.7 times higher ($p \leq 0.001$) vs. control. In group III vs. group I on day 30, the activity of ALP was 41.5% lower ($p \leq 0.001$), the activity of AlAT and AsAT was 2.2 and 1.8 times higher ($p \leq 0.001$). The data obtained indicate the destructive effect of the combination of mycotoxins on hepatocytes.

Activation of lipid peroxidation plays an important role in the pathogenesis of mycotoxicoses [37]. In groups II and III on day 30, the content of MDA, a quantitative marker for assessing the degree of lipid peroxidation increased 2.4-fold and 2.8-fold ($p \leq 0.001$), respectively, compared to control. Data of interest are changes in immunological parameters under the infectious load. On day 30, the number of T-lymphocytes in animals of group III was 31.3% lower ($p \leq 0.001$) than in the control.

The immunosuppressive effect of mycotoxins has long been known [38-40]. The decrease in the body's resistance to infectious diseases in mycotoxicoses is due to immunosuppression and the direct damaging effect of mycotoxins on the gastrointestinal tract [41].

In the gut, mycotoxins are metabolized and their toxicity decreases until a concentration is accumulated that can affect the intestinal mucosa [42]. Mycotoxins damage mucosal tissues, increase the permeability of the intestinal epithelial barrier, and cause a syndrome of poor absorption (or malabsorption). DON and T-2 toxin can directly damage mucosal tissues. Intestinal epithelial cells are the main targets for DON and T-2 toxin [43]. Deoxynivalenol has also previously been reported to reduce nutrient absorption [44]. Some studies indicate that the mechanisms of action of ZEN on intestinal function are unknown [12], while others indicate an increase in apoptosis and a decrease in the proliferation of Peyre's patches lymphocytes [45].

When the intestinal epithelial barrier is disrupted, pathogens are translocated under the action of mycotoxins, as, for example, in salmonellosis [46], pathologies caused by *C. perfringens* [47] and *Helicobacter* sp. [48]. DON-induced toxicosis leads to leak of plasma amino acids into the intestinal lumen, providing the necessary growth substrate for *C. perfringens* [49], which is a risk factor for intestinal disease and increases vaccination costs [50].

Determination of titers of specific antibodies in experimental animals confirmed the immunosuppressive effect of the combination of mycotoxins in the studied doses in the presence of a pathogenic bacterial pathogen in the herd (Table 3).

Severe intestinal infections (clostridium, rota-, coronavirus infections, escherichiosis) can be triggered by exposure to mycotoxins. Mycotoxins entering the body with food prevent the formation of specific antibodies in the required amount, which reduces the intensity of post-vaccination immunity. In our experiments, this was especially true for animals from group III which consumed a diet artificially contaminated with T-2 toxin, ZEN and DON. Also, in these piglets,

the titers of specific antibodies compared to group I (biological control) were lower, 8-fold ($p \leq 0.05$) to rotavirus, 6.4-fold ($p \leq 0.05$) to coronavirus, and 5-fold ($p \leq 0.05$) to *Escherichia*. Antibody titers in group I were typical for healthy animals. In group II, there was a tendency to decrease in titers, but the indicators remained within those for healthy animals.

**3. Serum specific antibody titers in vaccinated Large White weaned piglets (*Sus scrofa domesticus*) with experimental combined mycotoxicosis under infectious load** ($M \pm$SEM, Federal Center for Toxicological, Radiation and Biological Safety, 2018)

| Group | Titer (dilution) of antibodies | | |
|---|---|---|---|
| | to rotavirus | to coronavirusy | to *Escherichia coli* |
| I ($n = 3$) | 8533.0±2090.0 | 170.6±52.0 | 213.3±65.0 |
| II ($n = 3$) | 7680.0±3135.0 | 128.0±0.0 | 160.0±0.0 |
| III ($n = 3$) | 1066.6±261.0* | 26.7±6.5* | 46.6±21.6* |

N o t e. For a description of the groups, see the "Material and methods" section.

* Differences from control are statistically significant at $p \leq 0.05$.

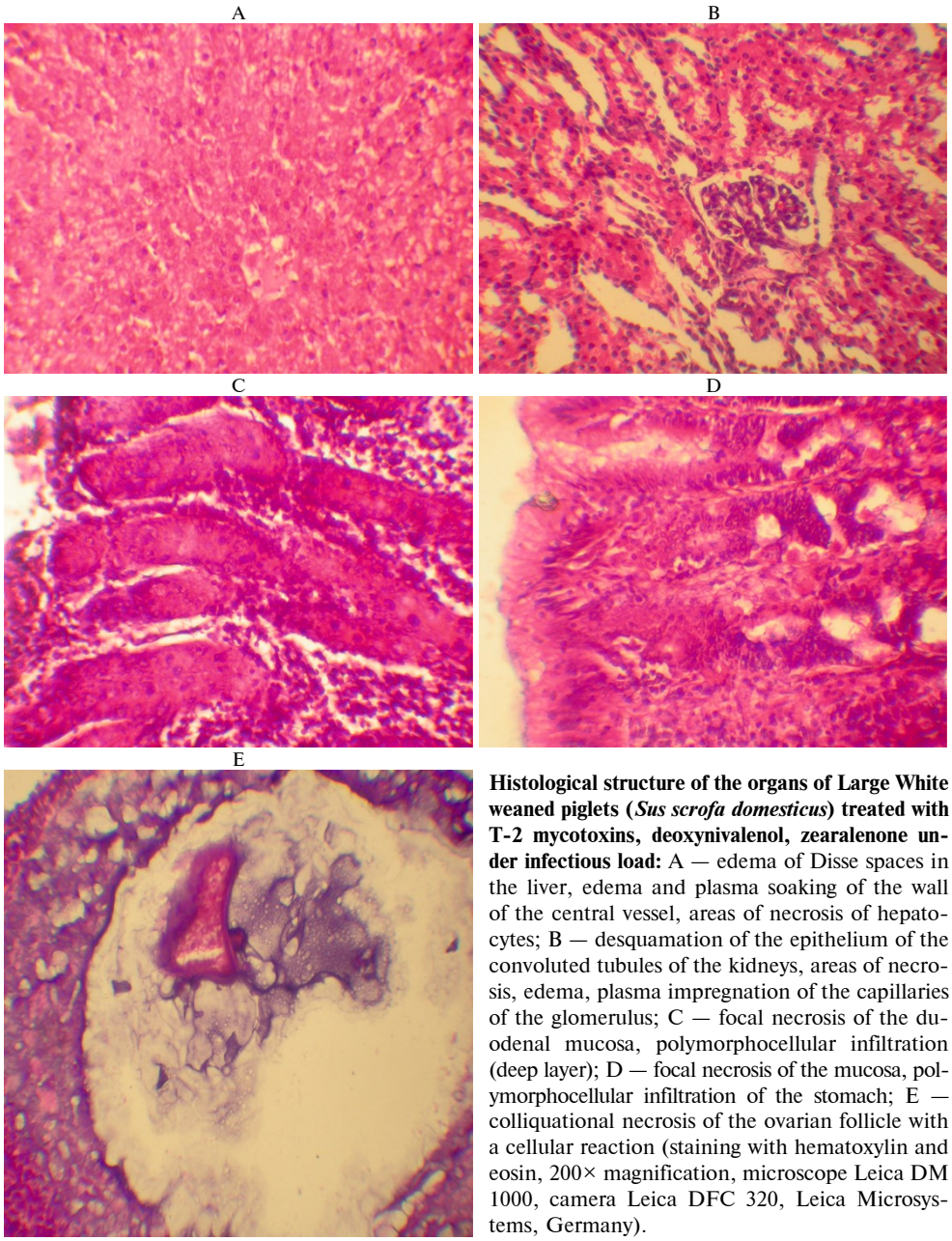
Our results indicate that mycotoxins, in particular, combination of T-2 toxin, ZEN and DON, can block the functions of the immune system of animals, preventing the synthesis of specific antibodies after vaccination. This increases the risk of reducing the protective effect of vaccination, which must be taken into account when organizing anti-epizootic measures.

After exposure to mycotoxins, severe intestinal illness caused by *C. perfringens* is more likely. At autopsy, gilts that received a diet with only T-2 toxin showed hyperemia of the mucous membrane in the stomach and in the small intestine. Pathological changes in internal organs with combined exposure to mycotoxins were more pronounced. Registered catarrhal and catarrhal-hemorrhagic lesions of the gastrointestinal tract, catarrhal inflammation of the lungs. The liver was enlarged, flabby, yellow in color. The muscles of the heart are flabby, the vessels of the brain are filled with blood. The kidneys are flabby, with a smoothed border. The spleen is enlarged, with blunt edges, cherry-red color. No such changes occurred in animals from groups I and II. The revealed histological changes confirm studies of long-term consumption of feed contaminated with DON and ZEN [51]. We also recorded polymorphocellular infiltration and focal necrosis in the mucosa of the wall of the stomach and duodenum (Fig.).

In animals exposed to three mycotoxins, protein dystrophy was noted in the kidneys, the epithelium of the tubules was with areas of necrosis. Protein dystrophy and foci of necrosis occurred in the liver, as well as the response of Kupffer cells in the form of an expansion of the Disse space, their enlargement and deformation. There was a depletion of the white pulp of the spleen. Serous edema of the interalveolar septa developed in the lungs. It is interesting to identify colliquatative necrosis of the ovarian follicle with a cellular reaction (see Fig.).

The intake of low levels of mycotoxins with feed is a serious problem for animal husbandry. In our studies, we tested the maximum allowable concentrations of mycotoxins. In the standard feed assessment scheme, their presence at the MPC level does not mean that the feed is potentially dangerous for pigs. It was of interest to us to methodically correctly reproduce this combination using a benign basic diet and the introduction of toxins against the background of persistent infection in the herd with the maximum exclusion of the influence of other significant toxins and xenobiotics.

Based on information about the individual toxicity of xenobiotics, it is not always possible to predict the effect of their combinations, as well as other influencing factors [52]. The relationship between mycotoxin exposure and infectious disease requires further study, as was noted previously [53].




**Histological structure of the organs of Large White weaned piglets (*Sus scrofa domesticus*) treated with T-2 mycotoxins, deoxynivalenol, zearalenone under infectious load:** A — edema of Disse spaces in the liver, edema and plasma soaking of the wall of the central vessel, areas of necrosis of hepatocytes; B — desquamation of the epithelium of the convoluted tubules of the kidneys, areas of necrosis, edema, plasma impregnation of the capillaries of the glomerulus; C — focal necrosis of the duodenal mucosa, polymorphocellular infiltration (deep layer); D — focal necrosis of the mucosa, polymorphocellular infiltration of the stomach; E — colliquational necrosis of the ovarian follicle with a cellular reaction (staining with hematoxylin and eosin, 200× magnification, microscope Leica DM 1000, camera Leica DFC 320, Leica Microsystems, Germany).

Thus, combined mycotoxicosis under an intestinal infection was accompanied by suppression of immunological parameters. The titers of specific antibodies to rotavirus decreased by 8 times ($p \leq 0.05$), to coronavirus by 6.4 times ($p \leq 0.05$), to *Escherichia* by 5 times ($p \leq 0.05$) compared to control. There was a decrease in the number of T-lymphocytes by 31.3% ($p \leq 0.001$), B-lymphocytes by 18.6% ($p \leq 0.05$). Due to activated lipid peroxidation, the concentration of malonic dialdehyde increased by 2.4-2.8 times ($p \leq 0.001$) in animals fed feed contaminated with T-2 toxin and three mycotoxins (T-2 toxin, deoxynivalenol and zearalenone) compared to control. Protein dystrophy and foci of necrosis were found in the liver and kidneys of piglets. Our findings indicate the negative impact of T-2 toxin, DON and ZEN combined intake at allowed concentrations on the clinical condition, morphobiochemical, and immunological parameters of piglets.

The data obtained can be used to diagnose animal diseases. In addition, these results should be accounted when carrying out anti-epizootic measures.

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