

Pathomorphological changes in the organs of neonatal piglets during feed contamination with mycotoxins for sows

Orazali Mullakaev, Viktor Usenko, Irina Konstantinova*, and Elvira Bulatova

Kazan State Academy of Veterinary Medicine, Kazan 420029, Russia

Abstract. Pathomorphological study of the neonatal period piglets of ontogenesis from the pig breeding complex obtained from sows that were given feed contaminated with mycotoxins during pregnancy and after parturition was carried out. Pathological changes in the internal organs of piglets, characterized by various dystrophic and inflammatory processes resulting from the influence of mycotoxins, were established. Edema and congestion in the lungs of piglets is noted as a sign of circulation failure in the body. Signs of interstitial pneumonia are detected with areas of chronic alveolar emphysema and atelectasis. Signs of granular dystrophy were noted in the heart in cardiomyocytes. Interstitial connective tissue has signs of edema; the swelling of blood vessel walls is noted. The morphofunctional state of the thyroid gland in piglets, taking into account changes in the organ structure, is characterized as a macro-microfollicular proliferating colloid goiter. The border smoothness between white and red pulp, hyperemia of blood vessels and the small number of lymphatic nodules with a size decrease are noted in the spleen. The demarcation clarity of white pulp into separate structural zones is not diagnosed in lymphatic nodules. Signs of catarrhal enteritis and chronic catarrhal colitis with a tendency to its transition to atrophic catarrh are detected in the small and large intestines of the gastrointestinal tract. Hepatocytes have mild signs of granular dystrophy in the lobules of the piglets' liver. Protein hepatosis signs interspersed with areas of necrobiosis are identified in separate lobules of the organ against the background of general atrophy of hepatocytes. Granular dystrophy signs with the phenomena of its desquamation in certain areas are revealed in the convoluted tubules of kidney nephrons in epithelial cells. Part of epithelial cells of the nephron tubule is subject to necrobiotic processes. Thickening of the basic capillary membrane with glomerulitis is noted in the glomerulus of the renal corpuscle. Edema and mild lymphocyte-macrophage infiltration with the phenomena of the mucoid swelling of blood vessel walls is observed in the interstitial organ tissue.

1 Introduction

One of the urgent problems of modern pig breeding is to increase the resistance of animals' body. The feed contamination with micromycetes, and later contamination of feed by mycotoxins, should be attributed to negative factors that negatively affect pig production in general. Microscopic fungi, as currently known, produce several hundreds of different mycotoxins [7, 9, 12], which being ingested with animal food cause poisoning, affecting the nervous, endocrine and immune systems.

The mycotoxins formation in feed occurs at various stages of the feed production chain. Moreover, the production of mycotoxin-contaminated feed from high-quality raw materials is not excluded at the plant. Another aspect of this problem is the transformation of mycotoxins as a result of biochemical reactions in the body. This leads to more severe consequences of poisoning, since mycotoxins can be metabolized to more toxic derivatives and form substances that are not detected by conventional research methods [14].

In addition, a decline in the efficiency of feeding animals is noted due to a decrease in the amount of consumed food, and sometimes a complete rejection takes place. Thus, a more active development of the pathological process is provided not only in the gastrointestinal tract organs but also affects the reproductive abilities of sows and their productivity, and contributes to the development of hypo- and agalactia [2, 11].

Mycotoxins in lactating sows enter the milk and then into the gastrointestinal tract of newborns, reducing resistance to infectious and non-communicable diseases. Pigs are the most susceptible to poisoning by various mycotoxins [4]. According to experts [1, 5, 14], a safe level of mycotoxin content in feed does not exist, since various types of mold are often detected in them, and not one. Each of them is able to produce several types of mycotoxins simultaneously [10, 17]. That is why the synergistic effect is very often observed in practice, when even a small content of individual mycotoxins in feed ultimately has a strong negative effect on the animal body [15, 16].

* Corresponding author: irina.const@mail.ru

Moreover, it is precisely the low level of mycotoxins in the feed that does not allow identifying the causes of the animals' depressed state in good time, a decrease in their live weight and vitality, and an increase in susceptibility to various diseases [8].

Mycotoxins are fairly stable substances having teratogenic, mutagenic and carcinogenic effects that can disrupt protein, lipid and mineral metabolism in the body, and cause a suppression of the immune system. *Fusarium* fungi produce mycotoxins divided into several heterogeneous groups by their chemical and toxicological characteristics: trichothecene, zearalenone and fumonisins.

Trichothecene, zearalenone, fumonisins, aflatoxins and others are the most dangerous for pigs. Mycotoxins entering the body of pigs with food cause poisoning and dysfunction of organs of the gastrointestinal tract, liver, kidney, and etc. For example, trichothecene form a group consisting of more than 170 different substances characterized by a similar composition [18].

The aim of the study is to carry out a pathomorphological study of dead piglets of the ontogenesis neonatal period in chronic combined mycotoxin poisoning in pregnant and lactating sows.

2 Material and research methods

The study was conducted on pigs kept in the pig breeding farm "ROS-Bacon" of the Ulyanovsk region. Feed analysis was carried out in accordance with the guidelines for the mycotoxins rapid determination in grain, feed, and components for their production, approved by the Ministry of Agriculture of the Russian Federation on 10.10.2005 and GOST 31653-2012 [3]. Various molds and their secondary metabolites, including T-2 toxin, zearalenone, ochratoxin A, fumonisin B1, deoxynivalenol, were found out in compound feeds received by pregnant and lactating sows. Subclinical chronic combined poisoning of pigs with mycotoxins was revealed when they consumed compound feed contaminated with toxins.

The number of mycotoxins was defined with consideration to MPL in feed samples. It was revealed that the content of a number of toxins was in the MPL border zone, and some of them exceeded this level. Pathological material selected during the autopsy of piglets died from mycotoxicosis from the pig breeding complex was used for the research. The autopsy of three Landrace Yorkshire hybrid piglets aged 3-5 days was carried out with the extraction of organs: liver, kidney, lung, small and large intestine, spleen, and thyroid gland. Pathohistological examination studies were performed according to the generally accepted method, fixing pieces of organs in a 10% solution of neutral formalin according to Becker.

The compaction of the material was carried out by pouring in paraffin. The installation was implemented in the conventional manner, after which paraffin histosections were prepared with a thickness of 5-7 μm on a sliding microtome. Histosections were stained with hematoxylin and eosin to study the general structure of

organs and the degree of pathological changes in them [6]. Microphotography of individual organ structures was performed using an image visualization complex consisting of an MBI-3 microscope (Russia) and a Sony Cyber-shot DSC-W510 digital camera (Japan).

3 Results

Pathological changes in dead piglets were diverse and depended on the degree of mycotoxin poisoning. Visible mucous membranes of the oral and nasal cavities had a cyanotic appearance. The lungs had a bright red color at the autopsy of piglets, and a small amount of foamed pink liquid was observed in the incision in the bronchi lumen. A slight thickening of the interalveolar septa due to their infiltration by lymphoid cells and macrophages was observed during microscopic examination of the piglets lungs sections. The plethora, expansion and blood filling of the interalveolar septa capillaries and veins of the interlobular connective tissue were detected in the venous vessels.

An accumulation of homogeneous acidophilic stained contents was revealed in the cavities of individual alveoli. The connective tissue was swollen around the vessels and bronchi, collagen fibers were thickened. Thus, edema and congestion are observed during lungs microscopic examination as a sign of circulation failure in the organ. Interstitial pneumonia signs are found out with areas of chronic alveolar emphysema and atelectasis (Fig. 1A).

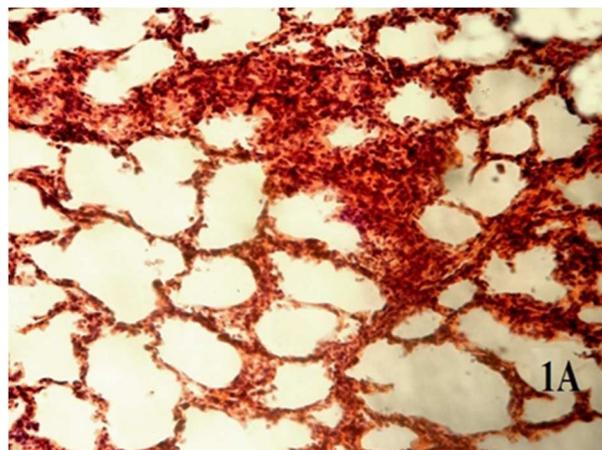


Fig. 1A. Slight thickening of the interalveolar septa. Alveolar emphysema. Interstitial pneumonia (Fig. 1A). Hematoxylin and eosin stain. x100

A slightly enlarged heart and poorly coagulated blood was observed in its cavities by microscopic examination. Punctate hemorrhage was visible on the epicardium; myocardium was distinguished by its flabbiness. Myocardial cardiomyocytes were loosened by microscopic examination of heart sections; small foci of hemorrhage were present in separate areas. Signs of granular dystrophy were noted in cardiomyocytes. Cell polymorphism was observed with the presence of large and smaller nuclei. Interstitial connective tissue had signs of edema; swelling of the blood vessel wall was noted. Small accumulations of lymphocytic macrophage

infiltrates were defined in separate areas of the myocardium in the interstitial tissue.

The microstructure of the thyroid parenchyma was formed by follicles of various sizes filled with colloid. The follicles size ranged from large cystiform, in which the wall was formed by squamous epithelium, to small. Papillary growth of epithelium was observed in most small follicles. This gives grounds to qualify the morphofunctional state of the thyroid gland in dead piglets as a macro-microfollicular proliferating colloid goiter (Fig. 1B).

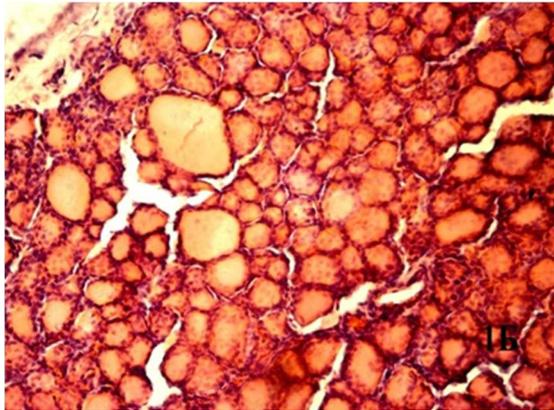


Fig. 1B. Thyroid gland (Fig. 1B). Macro- and microfollicular colloid struma. Hematoxylin and eosin stain. x200.

The capsule and trabeculae thickening due to the proliferation of the connective tissue is diagnosed by the spleen microscopic examination. The border smoothness between white and red pulp, hyperemia of blood vessels and the small number of lymphatic nodules with a size decrease are also noted in the spleen. The clarity of differentiation into individual structural zones is not observed in the lymphatic follicles.

The bright centers of lymphatic nodules include a few medium and small lymphocytes with weakly stained nuclei. Reticular cells and single lymphoblasts are identified in the bright center and periarterial zone. The absence of mitosis figures indicates mild proliferative cell activity. The lumen of the nodule central artery is slightly reduced; its wall has signs of swelling. Exfoliating of endotheliocytes and prolapse of their nuclei into the vessel lumen is detected in some of them. A change in the state of reticular tissue due to its homogenization and substitution of collagen fibers of the connective tissue is observed in the red pulp.

The blood vessels in the mesentery were full-blooded when examining the gastrointestinal tract. The mucous membrane of the small intestine was swollen and reddened, and had folds; punctate hemorrhages were observed in its individual areas. Catarrhal enteritis with changes in the structures of the mucous membrane, in which there was a disturbance of the shape, size and the villi deformation with epithelial cells desquamation, was revealed by microscopic examination of the small and large intestines. The mucous membrane in the duodenum was moderately preserved.

The limbic epithelium covered the villi and was mainly preserved. The connective tissue base of the villi

was weak and edematous with minor lymphocytic macrophage infiltration. The submucosa of the intestinal wall had signs of moderate edema and dissociation, vasodilation and plethora were noted (Fig. 2A). Pathological changes in the large intestine were characterized by the mucous membrane swelling; mild activity of crypt secretory epithelium was noted. Significant lymphoid-macrophage infiltrates involving reticulocytes were observed in the ileum's own layer. Signs of chronic catarrhal colitis with a tendency to the transition to atrophic catarrh were defined in the large intestine (Fig. 2B).

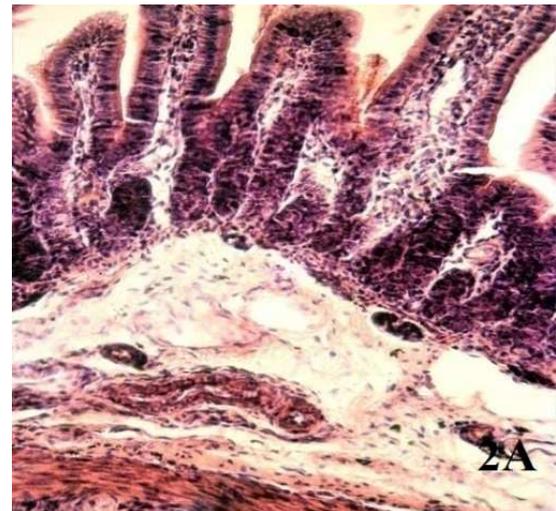


Fig. 2A. Deformation of the mucous membrane villi with signs of edema and dissociation in the submucosal layer of the duodenum (Fig. 2A). Hematoxylin and eosin stain. x200

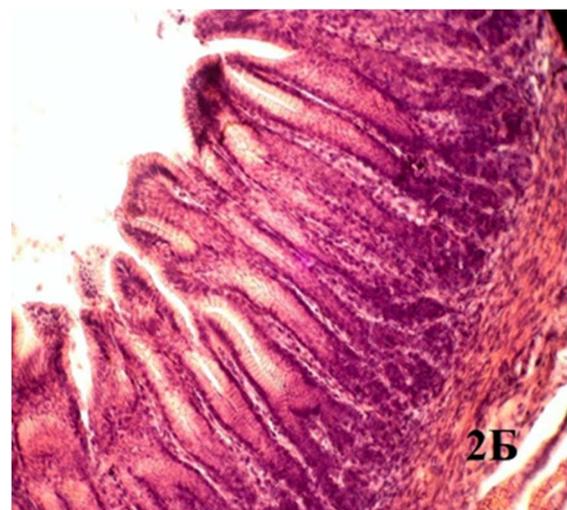


Fig. 2B. Mucous membrane of the ileum with lymphocytic macrophage infiltration in its layer (Fig. 2 B). Hematoxylin and eosin stain. x100.

An enlarged liver and the unevenness of its staining were marked during pathoanatomical autopsy of piglets, the consistency was flabby. The capsule covering the liver was slightly thickened. The gall bladder was slightly enlarged and moderately filled with yellow-brown bile. The liver was characterized by lobular structure, mainly maintaining the beam structure. Discomplexation of the beam structure was defined in

some areas and in separate lobules. Protein hepatosis signs interspersed with areas of necrobiosis were observed in some lobules against the background of general atrophy of hepatocytes.

The sinusoids of the liver lobules were dilated, plethorical, and numerous macrophages were located along the sinusoids. Lobular hepatocytes had mild signs of granular dystrophy. Some of the nuclei of hepatocytes were susceptible to karyopyknosis, karyorrhexis, and karyolysis (Fig. 3A).

The kidneys were slightly enlarged, flabby, gray-pink in color with hemorrhages. A weakly pronounced border between the cortical and brain layers of the organ parenchyma was identified by histological examination in the kidneys. Granular dystrophy signs with the phenomena of its desquamation in certain areas were detected in the convoluted tubules of nephrons in epithelial cells, and part of the tubules epithelial cells of the nephron was exposed to necrobiotic processes. Thickening of the basic capillary membrane with glomerulitis is noted in the glomerulus of the renal corpuscle. Edema and mild lymphocyte-macrophage infiltration with the phenomena of the mucoid swelling of blood vessel walls was observed in the interstitial connective tissue of the organ (Fig. 3B).

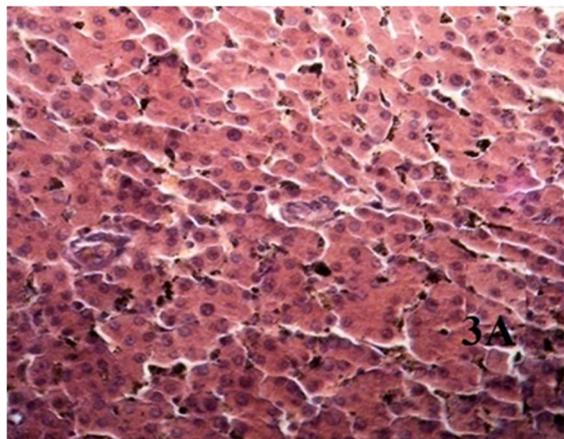


Fig. 3A. Piglet liver segment with dilated sinusoids and macrophages located along them (Fig. 3A). Hematoxylin and eosin stain. x100

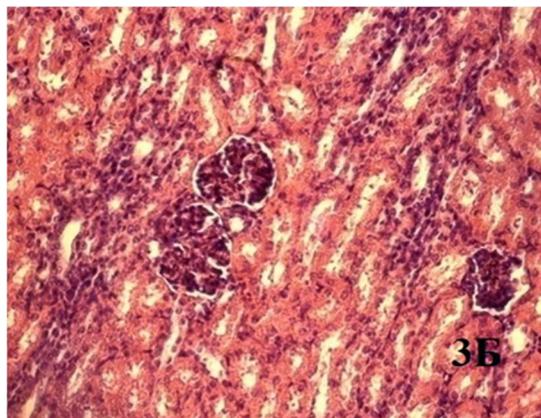


Fig. 3B. Cortical substance of a pig kidney with granular dystrophy signs and desquamation of epithelial cells into the lumen of tubules (Fig. 3B). Hematoxylin and eosin stain. x200

4 Conclusion

Thus, eating feeds contaminated with secondary metabolites of microfungus in pregnant sows leads to disruption of piglets' prenatal development, to the newborns with pathological disturbance in development, which are further aggravated by the production of colostrum contaminated with mycotoxins. Based on the autopsy results, we have noted that the most profound changes in the body are associated with a dysfunction of the morphofunctional state of the liver, kidneys and gastrointestinal tract organs.

Signs of granular dystrophy, phenomena of necrobiotic processes and cell desquamation, as well as structural changes in the nephron associated with a thickening of the basement membrane of hemocapillaries with glomerulitis and a disorder of the structural organization of interstitial tissue with the development of edema and mucoid swelling of the vessel walls have been identified. Signs of catarrhal enteritis and chronic catarrhal colitis with a tendency to its transition to atrophic catarrh have been detected in the small and large intestines of the gastrointestinal tract.

Aggravating causes of the pathology development in the body of piglets with mycotoxin poisoning are changes in the structural organization of the cardiovascular system and lungs, in which edema and congestion have been noted as a sign of circulatory disorders. The development of pathological changes in the form of interstitial pneumonia, and granular dystrophy in cardiomyocytes were also revealed. The insult of the body of piglets with mycotoxins occurs at the level of the most important system – the immuno-neuroendocrine.

A deep structural change in the spleen, as a peripheral organ of the immune system, where the development of the white pulp and its differentiation into different zones are depressed with the development of individual lymphoid elements of the blood, gives evidence of this. Moreover, the development of macro-microfollicular proliferating colloid goiter has been observed in the thyroid gland. Its pathology is accompanied by an underdevelopment of newborn piglets, growth and development retardation of young animals, a decrease in its live weight, and dystrophic processes in the internal organs.

References

1. L.G. Burdov, L.G. Matrosova, *On the results of the feed analysis for mycotoxins content*, Veterinar., **2**, 7–9 (2011)
2. Iu.V. Gankina, A.A. Kudriashov, *Pathomorphological changes in piglets with mycotoxicosis*, Actual Issues of Veter. Biol., **3**, 28–31 (2009)
3. GOST 31653-2012. *Feed. Enzyme immunoassay for mycotoxins* (Publ. House of Standards, Moscow 2012), 16 p.

4. I.S. Elistratov, *Mycotoxins effect on the body of piglets*, Veter. Sci., **3**, 58–60 (1981)
5. A.V. Ivanov, M.Ia. Tremasov, K.Kh. Papunidi, *On the causes of massive animal mycotoxicosis*, Immunopathol., allergol., infectol., **1**, 192 (2010)
6. I.S. Konstantinova, E.N. Bulatova, V.I. Usenko, *Fundamentals of cytology, general histology and embryology of animals* (Lan, St. Petersburg, 2015), pp. 16–84
7. A.F. Kuznetsov, *Veterinary* (Lan, St. Petersburg, 2001), 406 p.
8. B.G. Orliankin, A.M. Mishin, T.I. Aliper, E.A. Nepoklonov, *Pig mycotoxicosis*, Pig Farm.: Indust. and Breed., **2**, 46–49 (2006)
9. V. Nevinnyi, I. Rubinskii, *Pig mycotoxicosis*, Animal Husbandry of Russ., **2**, 30–31 (2009)
10. F. Dzh. Neer, *Mycotoxins and their consequences for the body of gilts*, Pig Farm.: Indust. and Breed., **2**, 62–63 (2006)
11. I.A. Shkuratova, L.I. Drozdova, M.V. Riaposova, P.O. Busygin, I.V. Konopleva, *Pathological changes in tissues and organs under the influence of T-2 toxin*, Agrar. Bull. of the Urals, **9(115)**, 21–24 (2013)
12. M.Ia. Tremasov, V.P. Pavlov, *Mycotoxicosis of animals*, Veter. Sci., **11**, 15–16 (2000)
13. V.A. Antipov, V.F. Vasilev, T.G. Kutishcheva, P.V. Miroshnichenko, *Chronic combined animal mycotoxicoses in the Krasnodar Territory*, in: *Mater. of the Int. Symp. Scientific basis for the protection of animals from ecotoxicants, radionuclides and pathogens of dangerous infectious diseases*, vol. 1 (Kazan, 2006) p. 42
14. S.N. Shelamov, S.N. Sadovnikova, *Mycotoxicosis in pig farming: a problem that should not be underestimated*, Agricult. Sci., **9**, 22–25 (2018)
15. E. Concova, A. Lagiakova, G. Kovac, H. Seidel, *Fusarian toxins and their role in animal*, Veter. J., **165(3)**, 214–220 (2003)
16. R.J. Cote, R.H. Cox, *Handbook of Toxic Fundal Metabolites* (Academic Press, New York; London; Toronto; Sydney; San Francisco, 1981), 937 p.
17. M. Garies, B. Ertl, J. Bauer, B. Gedek, *Biotransformation of T-2-toxin and diacetoxyscirpenol in the isolated perfused rat liver*, Mycotoxin Res., **1**, 77–82 (1985)
18. H.S. Hussein, M. Brase, *Toxicity, metabolism and impact of mycotoxins on humans and animals*, Toxicol., **167**, 101–134 (2001)