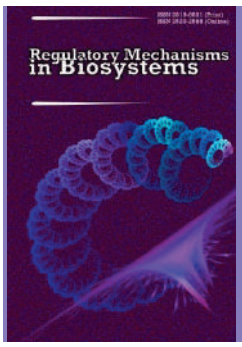

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Morphofunctional changes in the internal organs of laying hens affected by chronic thiamethoxam intoxication

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Insecticides play an important role in agriculture, general sanitary and veterinary practices, providing protection of the plants and yield from harmful insects and preventing the spread of arthropods which cause diseases in people and animals. Therefore, the objective of our study was to analyze the morphofunctional changes in the internal organs of laying hens affected by chronic intoxication with Actara 25 WG (thiamethoxam). Identification of the toxic action of theamethoxam was carried out in 150 day-old laying hens. The chronic intoxication with the insecticide was modeled by feeding mixed feed treated with the preparation in the doses that were calculated in mg of the active compound per 1 kg of body mass. The birds of the one group were the control and received mixed feed with no supplements. The hens of the first experimental group were given mixed feed that contained the insecticide in the dose of 360 mg/kg of body mass, and hens of the second experimental group consumed mixed feed containing the preparation in the dose of 180 mg/kg per body mass. We determined that laying hens of Experimental Group 1 had significant 1.24-fold decrease in the ventriculus and significant 1.39-fold increase in the spleen. Laying hens of Experimental Group 2 were observed to have increase in the absolute mass of the heart, measuring 1.36-fold compared with the control and 1.34-fold compared with Experimental Group 1. At the same time, the absolute masses of the spleen, liver, and ventriculus in Experimental Group 2 were 1.20, 1.46, and 1.19 times lower than in Experimental Group 1. Compared with the control, the absolute mass of the liver and ventriculus, was 1.54 and 1.48 times lower, respectively. Intake of feed with thiamethoxam by laying hens of the experimental groups led to decrease in the coefficient of relative mass of the liver and ventriculus. Those results significantly correlated with the absolute mass values of those organs, indicating the toxic impact of the insecticide on laying hens, with the digestive organs being the first to react. In Experimental Group 1 chickens, we observed dystrophic-necrotic changes in the liver, round-cell infiltration of the portal tracts; dystrophic-necrotic changes in epitheliocytes of the nephrons of the kidneys'; granular dystrophy of cardiomycetes, plethora of the capillaries, and stasis and edema of the stroma in the myocardium; pericellular edemas in the brain; mucous dystrophy, desquamation of the epithelium of the mucous membrane, decrease in lymphocytes in the lymphoid structures, and atrophy of the epithelium of the glandular structure in the stomach; hyperemia and necrosis of the villus tips, and round-cell infiltration of the crypt region in the thin intestine; and reproduction of cellular elements of the connective tissue between the crypts in the thin intestine. The insecticide in the dose of 180 mg/kg of body mass caused dystrophic-necrobiotic changes in the liver and kidneys; hyperemia and edema in the myocardium; pericellular edema, swelling, and vacuolar dystrophy of neurons in the brain; necrobiotic changes in the mucous membrane epithelioctes in the proventriculus; and deformation of the villi and edema of the mucous membrane in the small intestine.

Keywords: neonicotinoids; poultry; toxicity; absolute and relative mass; microscopic structure; pathoanatomic changes.

Introduction

A substantial role in modern systems of chemical protection of agricultural crops from harmful arthropods is played by neonicotinoid insecticides. The wide array of neonicotinoid insecticides on the market necessitates toxicological research on their effects on the environment and toxicity for agricultural animals, as well as people (Sekun, 2012; Cimino et al., 2017; Buszewski et al., 2019; Jactel et al., 2019).

Neonicotinoids are a new class of synthetic insecticides that have been developed based on the structure of nicotine for protection of a number of crops from harmful insects (Li et al., 2011). The range of active components and preparation forms made based on them is quite broad. Since their development in the 1980s and introduction in the 1990s, they have acquired massive popularity and now account for a quarter of the pesticide market (Bass et al., 2015; Hladik et al., 2018). In particular, the List of Pesticides and Agrochemicals Allowed for Use in Ukraine includes the following insecticides with active neonicotinoid compounds: thiamethoxam, imidacloprid, thiacloprid, clothianidin, and acetamiprid (Sekun, 2012). The wide popularity of neonicotinoids is evidenced in the broad spread and detection of compounds of this group in various environments, such as soil (Huseth & Groves, 2014) and water (Schaafsma et al., 2015). Furthermore, residual amounts of neonicotinoids have been

found in bees (Blacquière et al., 2012), crustaceans (Butcherine et al., 2019), and birds (Hamid et al., 2017).

Drugs based on neonicotinoids belong to hazard classes 2 and 3 (average- and low-toxic compounds) for mammals and class 1 (highly toxic) for bees. Because of the chemical structure of molecules and different sensitivity of mammals and insects, the toxic action of neonicotinoid-based drugs is selective. The electron-donating group of neonicotinoids poorly bonds with post-synaptic nicotine-sensitive receptors of mammals, but bonds well with those receptors of insects.

Neonicotinoids are neurotoxic poisons, and the mechanism of their toxic impact has been well studied on insects. They exert anticholinesterase activity and function as agonists of nicotine-sensitive receptors of post-synaptic membranes, leading to prolonged opening of sodium channels and death of insects as a result of convulsions and paralysis (Kumar et al., 2014; Wang et al., 2019).

Toxicological studies revealed that neonicotinoids increased oxidative stress and impacted the reproductive development in mice (Wang et al., 2018), the behavior and physiological function of honey bees (Pisa et al., 2021), and in median lethal doses caused chronic toxicity in mammals (Hafeza et al., 2016). Studies conducted in human medicine detected neonicotinoids and their metabolites in urine, blood serum, and other biological samples of people from different countries all around the world (Dzerzhynskyi et al., 2013; Zhang et al., 2019), demonstrating the threat they pose to health (Xiao et al., 2022).

Our previous reports have presented results pertaining to median lethal doses of Actara 25 WG and Mospilan RP for white mice, their chronic toxicity for white mice, their impact on the egg productivity of laying chickens and the quality of chicken meat (Bazaka et al., 2018; Dukhnytsky et al., 2019, 2020, 2021).

To identify pathogenesis and assess the severity of pathology in laying hens suffering from chronic poisoning with Actara 25 WG, besides clinical and hematological studies, it is important to conduct morphological studies.

Materials and methods

The study was conducted at the Department of Pharmacology, Parasitology, and Tropical Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. The experimental studies on animals were carried out according to the methodological recommendations Technical Control of Novel Drugs for Animal Protection (Kosenko et al., 1997), and also the existing documents that regulate work with experimental animals, and with adherence to the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and Article 26 of the Law of Ukraine No. 5456-VI as of October 10, 2012 On Protection of Animals from Abuse.

Poultry of the control and experimental groups were held in the vivarium of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine according to the current Sanitary Rules of Structure, Equipment, and Maintenance of Experimental-Biological Clinics (Vivariums) at the temperature of 18–20 °C and the relative air humidity of 50–55%. The birds were fed a full mixed feed, according to the standard scheme and the norms for laying hens. The hens had free access to water.

The object of the study was the drug Actara 25 WG, which contains 25% of thiamethoxam.

The evaluation of the toxic effect of Actara 25 WG was performed on 150 day-old laying hens, with the body mass measuring 1,049 ± 50 g at the beginning of the experiment. Chronic intoxication with Actara 25 WG was modeled by feeding the mixed feed treated with this drug in the doses estimated in mg of the active compound (AC) per 1 kg of body mass. Birds of one group were the control (C) and received the mixed feed with no supplements. Hens of Experimental Group 1 (A1) were fed the mixed feed containing Actara 25 WG in the dose of 360 mg/kg of body mass, whereas hens of Experimental Group 2 (A2) received mixed feed containing Actara 25 WG in the dose of 180 mg/kg of body mass. To analyze the toxicity of Actara 25 WG, we employed the methods described in Preclinical Trials of Veterinary Drugs (Kotsyumbas, 2005). The intake of Actara-containing feed lasted for 30 days.

In the end of the experiment, the hens were euthanized and a necropsy was performed for the experimental and control groups (n = 7) using the method of partial evisceration. Then, we performed pathomorphological studies of the internal organs and tissues (Zon et al., 2009).

During necropsy, we removed the liver, spleen, heart, and ventriculus and proventiculus. The degree of toxicity was assessed during the macroscopic studies of the internal organs in the end of the experiment and according to the changes in coefficients of mass of the liver, spleen, lungs, kidneys, heart, and proventriculus and ventriculus.

The absolute mass of the organs was measured by weighing, and the relative mass was quantified using the ratio of absolute mass of the organ to body mass of a bird by multiplying it by 100%.

For the histological studies, we collected samples of material from those organs and fixated them in 10–12% aqueous solution of neutral formalin. They were dehydrated in alcohols of ascending concentrations and engulfed in paraffin. After deparaffinization, the 10 μm-thick sections were stained with the Carazzi's hematoxylin and eosin, and also using the method of Nissl (Horalskyi et al., 2019).

The histological preparations were analyzed under a Micros MCX 100 LED microscope (Austria). The names of morphological structures of the organs and tissue are given according to the international veterinary anatomical nomenclature (Khomych, 2005).

The obtained data were analyzed in the Statistica 6.0 software (StatSoft Inc., USA). The data are presented in tables as $x \pm SD$ (mean ± standard deviation). The differences between the values in the control and experimental groups were determined using the ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account the Bonferroni Correction).

Results

Necropsy was performed on the slaughtered laying hens of the first (A1) and the second (A2) experimental groups, which for 30 days consumed fodder, 1 kg of which contained 360 mg and 180 mg of the Actara 25 WG insecticide, respectively. During the procedure, we observed hemorrhages in the liver and its dark-cherry color, and the bladder overfilled with bile. In the thoracic-abdominal cavity, we found non-uniform staining of the lungs, expansion of the heart, petechial hemorrhages on the mucous membrane of the proventriculus and intestine, and blood filling of the vessels throughout the organism. In some laying hens, we saw distension of the small intestine.

According to the results of our research, laying hens of Experimental Group 1, after 30 days of intake of Actara 25 WG in their diet, were observed to have a tendency towards decline in the absolute mass of the heart, liver, and a significant, 19% ($P < 0.05$) decrease in the ventriculus. The absolute mass of the lungs and glandular part of the stomach, compared with the control had an upward tendency; and such of the spleen significantly, by 39% ($P < 0.05$), increased (Fig. 1). In Experimental Group 2, in the end of the experiment, the absolute mass of the organs also changed. Therefore, compared with the control and the first group, the absolute mass of the heart significantly ($P < 0.05$) increased by 26.6% and 25.4%, respectively. The absolute mass of the spleen of the Experimental Group 2 laying chickens changed non-uniformly: compared with the control, it had an upward tendency, and compared with Group 1, significantly ($P < 0.05$) decreased by 17.1%. The absolute mass of the liver and the venriculus in the second experimental group was significantly ($P < 0.05$) lower than in Control and Group 1 (Fig. 1).

In the both experimental groups, the relative masses of the heart, lungs, spleen, and proventriculus, as compared with Control, underwent almost no changes. However, the relative mass of the liver and ventriculus were significantly ($P < 0.05$) lower, especially in the Experimental Group 2 hens, compared with the control: 32.1% lower for the liver, 28.5% lower for the ventriculus, and significantly correlated with the respective parameters of their absolute mass (Fig. 2). This is direct evidence that the Actara 25 WG-caused changes in relative mass of the organs in hens of the experimental groups occur through decrease in the absolute mass of the organs.

In our experiment on laying hens of the experimental groups that consumed a diet with the Actara 25 WG insecticide, such morphometric indicators as absolute mass and relative mass of the organs were convincing evidence that the toxic action first of all provoked the reaction of the digestive organs, the relative mass of which significantly changed, thus being a sensitive indicator that reflects the morphofunctional condition of the organism and characterizes the degree of development of its intoxication.

According to the results of histological studies of the liver of the laying hens fed with a diet containing Actara 25 WG in the dose of 360 mg/kg of body mass, after staining the histosections with hematoxylin and eosin, we observed changes that manifested in cloudiness of the cytoplasm of hepatocytes and non-uniform (cleared at some places) color, and the contours of almost all hepatocytes were blurred. Such changes in hepatocytes against the background of a prolonged intake of Actara 25 WG on the hens resulted in deformation of the tubular structure of the hepatic lobules. The central vein of the hepatic lobules and their sinusoid capillaries in the central lobular area were enlarged. In the lumens of the sinusoids, we mostly observed rounded Kupffer cells. The Kupffer cells exhibited hyperplasia. The nuclei of hepatocytes were reduced in size, with numerous grains of chromatin, i.e. those that underwent hyperchromicity, and noticeable in other hepatocytes (Fig. 3). Therefore, according to the results of analysis of the histopreparations, the portal tract and the interlobular connective tissues of the parenchyma of the liver of laying hens, as a result of influence of Actara 25 WG, were infiltrated by cellular elements, and the lumens of veins were enlarged and overfilled with formed elements of blood. In the portal triad region, sites of lymphoid-histiocytic infiltrates were found (Fig. 3).

In the kidneys of laying hens of this group, according to results of the histological studies, the vascular bundles of the renal corpuscles were mostly rounded and elongated, with deformed capsule and enlarged lumens of the vascular plexuses, with thickened basal membrane of the capillaries. The interstitium around the glomeruli was moderately swollen, in some places infiltrated by cellular elements (Fig. 4a). The lumen of individual nephrons was narrowed due to swelling of the nephrothelium, while in others, it was enlarged, likely as a result of increased strain on the kidney's reabsorptive function. Often, we observed indistinctiveness of the contours of the nephrothelium cells, the cytoplasm of which appeared mostly translucent and swollen. In some nephrons, as a result of swelling and clearing of the nephrothelium cytoplasm, the intact nuclei were shifted to the periphery of cytoplasm. The contours of the cells were blurred, and the bordering was fragmented. In some cells, the nuclei exhibited lysis, while in others, they showed karyorrhexis. In the distal renal nephrons, we saw swelling and granularity of cytoplasm of epitheliocytes, with nuclei shifted towards the periphery and karyolyses.

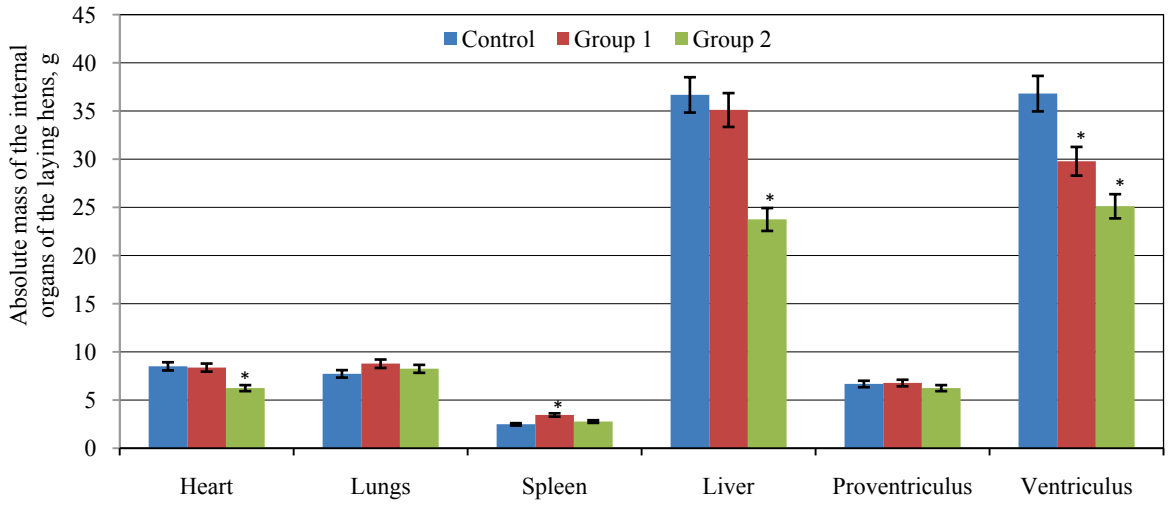

Fig. 1. Absolute mass of the internal organs of the laying hens after prolonged intake of a diet with Actara 25 WG (g, x ± SD, n = 7): * – P < 0.05 compared with the control group (taking into account the Bonferroni Correction)

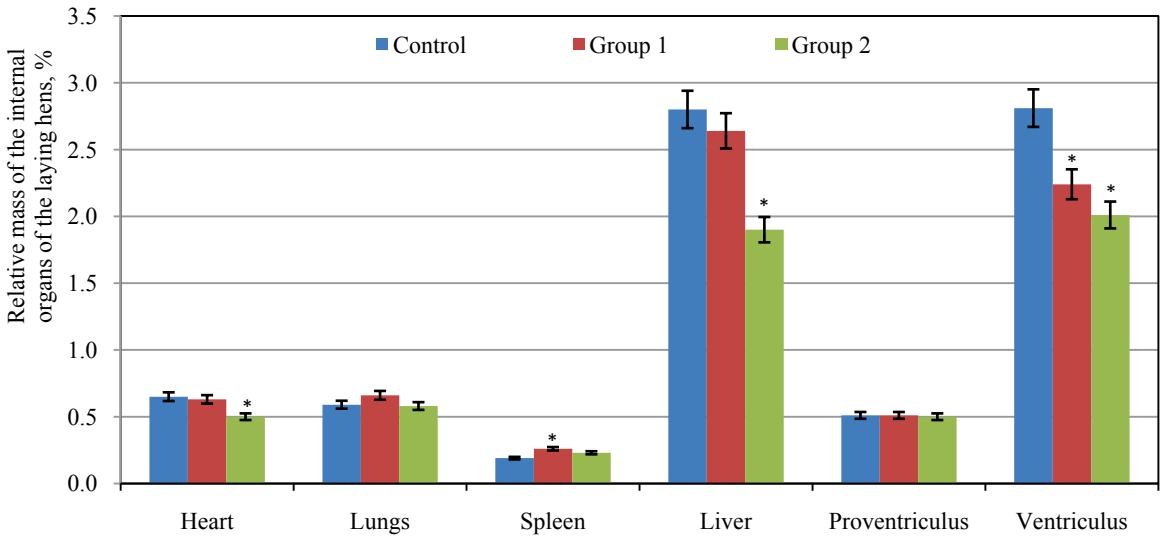

Fig. 2. Relative mass of the internal organs of the laying hens after prolonged intake of a diet with Actara 25 WG (%, x ± SD, n = 7): * – P < 0.05 compared with the control group (taking into account Bonferroni's Correction)

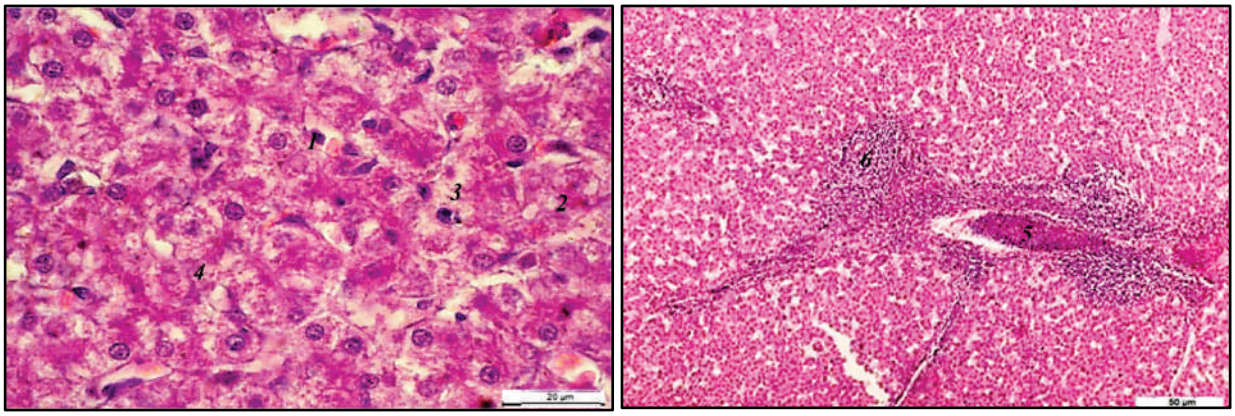

Fig. 3. Fragment of microscopic structure of the hepatic lobule of the Experimental Group 1 chicken: *1* – granular dystrophy of hepatocytes; *2* – necrobiosis of hepatocytes; *3* – deformation of hepatic sinusoids; *4* – karyolysis; *5* – enlarged vein, overfilled with blood cells; *6* – round cell infiltration of the portal tract; hematoxylin and eosin

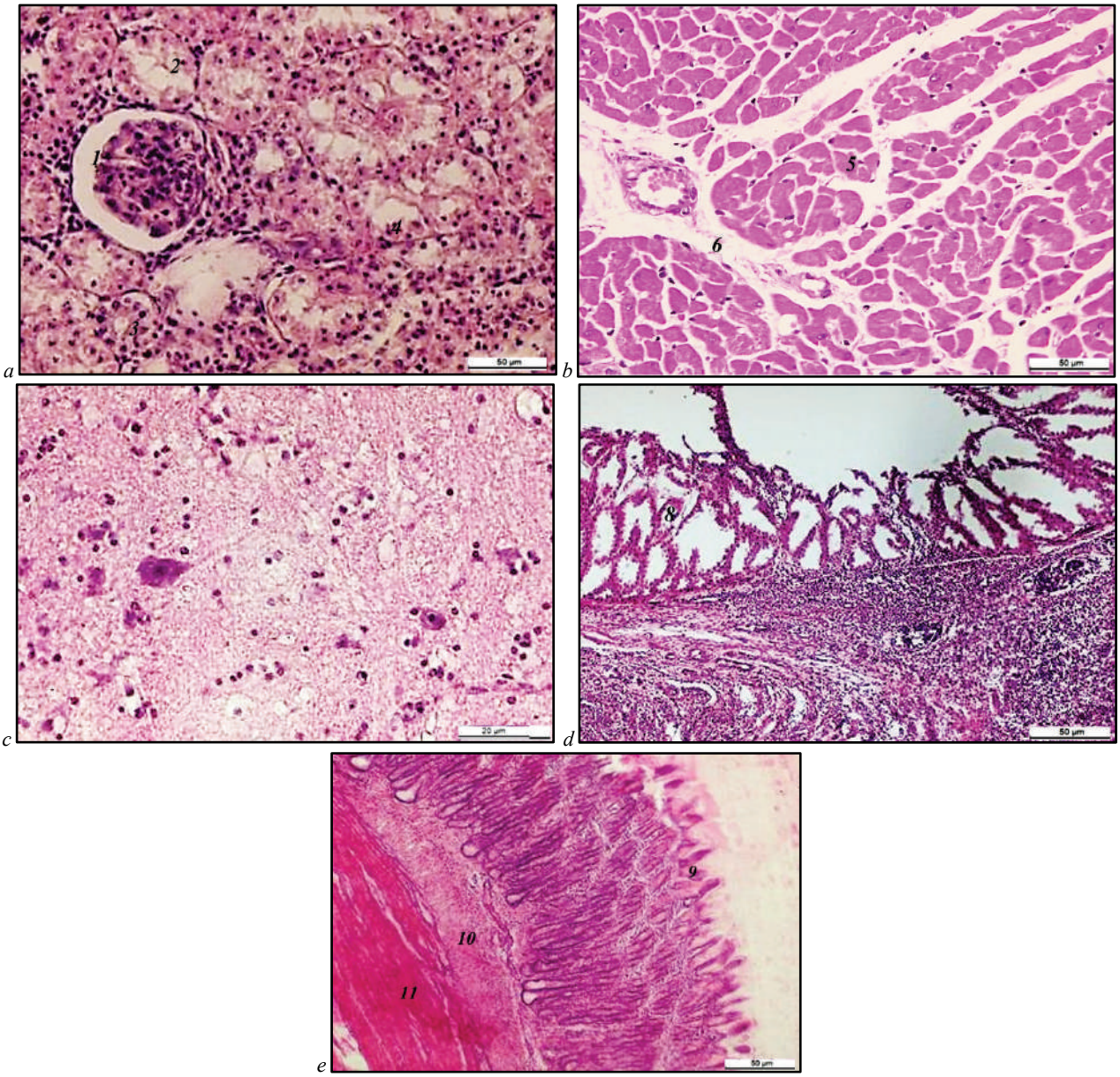

Fig. 4. The Experimental Group 1 hens: fragment of the microscopic structure: *a* – kidneys; *b* – myocardium; *c* – the brain; *d* – the proventriculus; *e* – the ventriculus: *1* – enlargement of renal corpuscle capsule; *2* – granular dystrophy of epitheliocytes of the renal convoluted tubules; *3* – separation of epitheliocytes of the basal membrane; *4* – ruination of epitheliocytes of the renal convoluted tubule; *5* – muscular fibers on a transverse section; *6* – transudate between bundles of muscular fibers; *7* – aggregated immune structures of the mucous membrane; *8* –disrupted structure of the superficial tubular glands; *9* – expansion of the connective tissue between the gastric pits; *10* – expansion of the connective tissue in the submucous layer; *11* – focal edema in the muscularis externa. Carazzi's hematoxylin and eosin

The histological studies of the heart of the Experimental Group 1 hens found no changes in the epicardium and endocardium. However, in the middle layer (myocardium), we observed dyscirculatory impairments accompanied by increased vessel permeability. The endothelial cells were bloated, their cytoplasm vacuolized, and the nuclei in some cells were in the state of lysis. Against the background of hyperemia and bile sludge of the erythrocytes, accumulations of transudate were observed in the interstitium between the muscle fibers. At the same time, the muscular fibers were in some places swollen, significantly misaligned, in the state of granular dystrophy (Fig. 4b).

In the brain, there occurred impairments of hemodynamics in the form of plethora in the capillaries, stasis, resulting in the swelling of the organ, which was characterized by the formation of perivascular and pericellular lumens. Such changes, compared with the hypothalamus area, were more expressed in the molecular layer of the brain parencephala, cerebellum, and medulla oblongata. Also, we saw loosening of the subependymal tissue, which was expressed in the formation of cyst-like cavities, protrusion of the ependymal epithelium, and was accompanied by disruption of the structure of glial elements, and the most affected were the microvessels located nearby. The cytoarchitectonics of the neurons was not uniform; we observed distinct types of neurons with well-defined neurolemma, moderately stained neuroplasm, and centrally located rounded nuclei, as well as deformed nerve cells that exhibited intensely stained neuroplasm. Often, we found pynknotic, irregularly shaped neurons, which indicated their degeneration and death. The contours of other neurons were hardly noticeable, and their neuroplasm was translucent, the nuclei were shifted to the cell periphery, and often had disintegrated contours (Fig. 4c).

Significant histological changes were also seen in the gastrointestinal tract. Therefore, the lumen of the proventriculus contained a noticeable amount of cellular detritus. We observed changes in the structural organization of its mucous and muscular membranes, which manifested as swelling, pronounced mucous dystrophy, desquamation of the epithelium, and ruination of the mucous membrane. In the lamina propria, lymphocytes were found in the aggregated immune structures that were in the loosened condition. The relative number and size of the lymphoid nodules were reduced. The structure of the superficial tubular glands of lamina propria mucosae was affected: their alveoli were significantly enlarged, torn in some places, and the shared cavity was significantly enlarged (Fig. 4d). The loose fibrous tissue of the submucous layer was enlarged as a result of its moderate edema and infiltration by a high number of leukocytes. The alveoli of deep glands were enlarged, their epithelium was flattened, basophilous. The muscular membrane was also moderately swollen, and its cells were in the state of granular dystrophy.

In the ventriculus, we found infiltration of the connective tissue between the gastric pits by cellular elements, and also between the base of the gastric pits and the lamina muscularis mucosae. The latter was permeated with transudate. We also observed edema in the submucous layer (Fig. 4e). Furthermore, the muscularis externa manifested focal edemas of various sizes and forms.

The mucous membrane of the duodenum was swollen and exhibited moderate hyperemia, with singly located petechial hemorrhages, covered by thick light-brown mucus. We found mucous dystrophy of the epithelium, necrosis, and notable desquamation. On the surface of the villi, epithelial cells of the lamina epithelialis were absent. In the mucous membrane, we observed round cell infiltration. In the area of the crypts, the mucous membrane was swollen and infiltrated by cellular elements (Fig. 5a).

A portion of the jejunum crypts was ruined. In such areas, we observed a large number of fibroblasts that formed thin bundles (Fig. 5b). In some places, atrophy of the crypts was recorded, which resulted in relatively small areas of mucous membrane of the jejunum having no crypts at all. A number of epithelial cells of the crypts displayed necrosis. Necrosis of cells in the region of the villi and crypts was characterized by karyorrhexis (Fig. 5c).

Smooth muscular cells of the lamina muscularis mucosae, similarly to the smooth muscular cells of the muscular membrane were in the condition of grainy dystrophy. In the ileum, we observed edema of the submucosa and and lamina muscularis mucosae and pronounced hyperplasia of the goblet cells with formation of excessive mucus. Epitheliocytes of the villus tips were in the state of dystrophic-necrotic changes and were often desquamated (Fig. 6a).

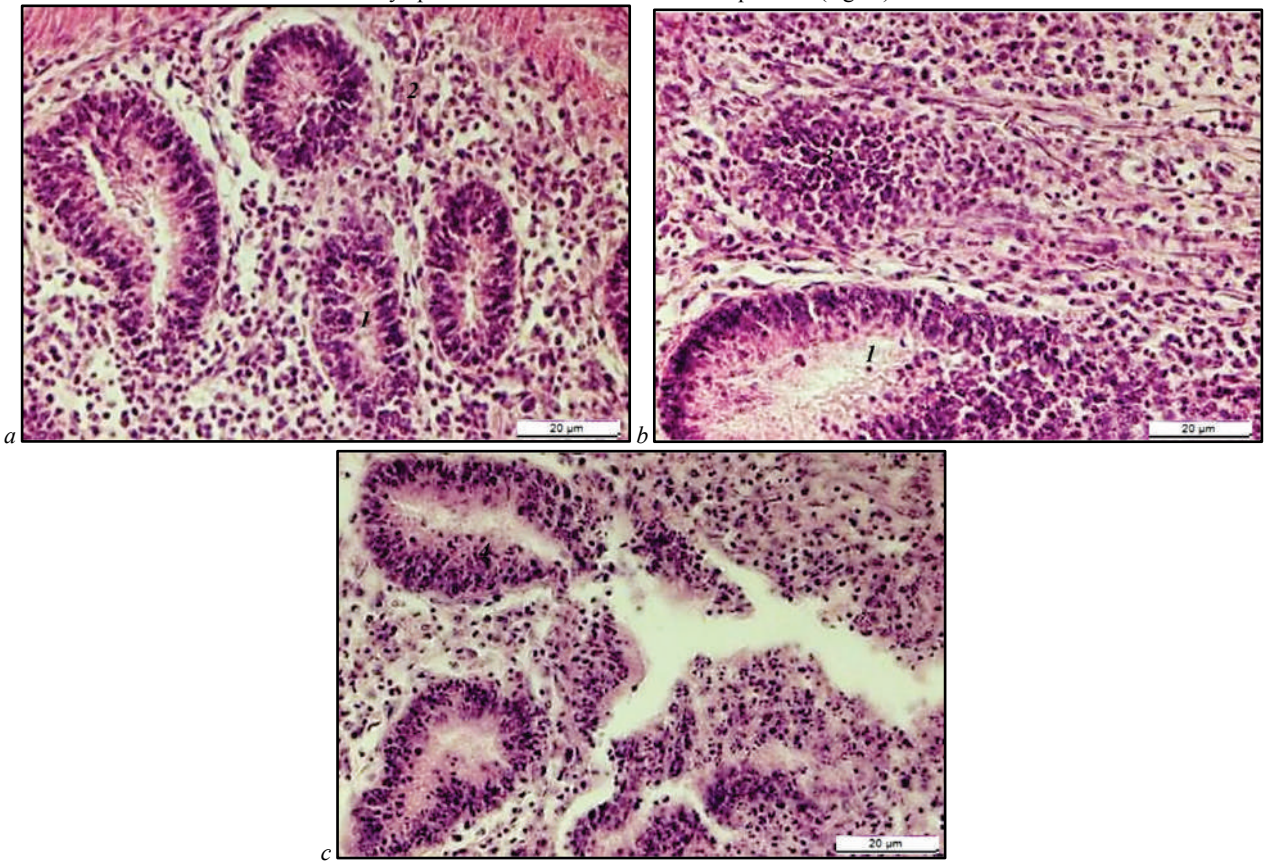

Fig. 5. The region of the crypts: *a* – duodenum of the Experimental Group 1 hens; *b*, *c* – the intestinal cavity of the Group 1 hen: *1* – crypt; *2* – infiltration of the mucous membrane by leukocytes; *3* – ruination of the crypt; *4* – karyorrhexis of the crypt epitheliocytes; Carazzi's hematoxylin and eosin

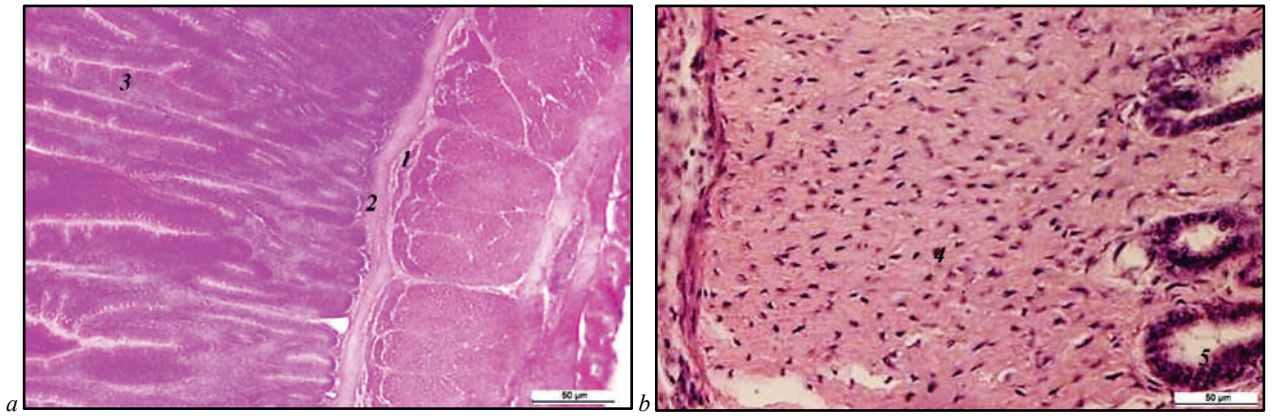

Fig. 6. Microscopic structure: *a* – the ileum of the Group 1 hens; *b* – the jejunum of the Group 1 hens: *1* – edema of the submucosa; *2* – edema of the lamina muscularis; *3* – hyperplasia of the goblet cells; *4* – fibroblasts; *5* – cubic epithelium in the area of the crypts; Carazzi's hematoxylin and eosin

In the large intestine of the Experimental Group 1 hens, unlike the small intestine, no ruination of the mucous membrane was observed. Instead, there was detected a quite notable reproduction of the cellular elements of the young connective tissue between the crypts in its lower and middle regions and highly pronounced increase in the same tissue between the bottoms of the crypts and lamina muscularis mucosae (Fig. 6b).

Study of the non-formed fibrous connective tissue between the bed of the crypts and lamina muscularis mucosae found numerous focal edemas of various sizes and shapes. The bed of the crypts and their middle part were covered by cubic epithelium. The upper part of the crypts was covered mostly by flat epitheliocytes.

In our opinion, this suggested the inhibition of the mitotic activity of cambial cells in the area at the base of the crypts, resulting in a decrease in the general number of epitheliocytes in each crypt, which forced them to elongate along the crypts. Also, in the area of the upper parts of the crypts, there was seen atrophy of the mucous membrane, resulting in sections between the crypts reducing in size.

Thus, in the conditions of long (for 30 days) intake of Actara 25 WG with fodder in the dose of 360 mg/kg of body mass, in the liver of laying hens, dystrophic-necrotic changes and round cell infiltration of the portal tracts developed ; in particular, dystrophic-necrotic changes in the epithelium of the renal tubules in the kidneys; in the myocardium, there was plethora of the capillaries, stasis, and edema of the myocardium stroma and granular dystrophy of cardiomycetes; the brain had impaired hemodynamics and pericellular edemas; the stomach presented mucous dystrophy and desquamation of the epithelium of the mucous membrane, decrease in lymphocytes in the lymphoid structures and atrophy of the glandular structures; the small intestine exhibited hyperemia and necrosis of villus tips and round cell infiltration of the area of the crypts; the large intestine showed signs of cellular element reproduction typical of young connective tissue between the crypts.

Intake of a diet with Actara 25 WG in the dose of 180 mg/kg of body mass by the Experimental Group 2 hens inflicted damage to the tubular structure of the lobules and their structure in general. In this case, hepatocytes were swollen, and the cytoplasm was translucent and poorly absorbed staining agents. Nuclei of some hepatocytes were rounded, with low content of chromatin, and in most hepatocytes they were in a state of lysis, indicating prevalence of necrotic processes (Fig. 7a). In some regions, we saw infiltration of the organ by leukocytes and granular dystrophy of hepatocytes.

In the kidneys of the Group 2 laying hens, which had been consuming a diet with Actara 25 WG in the dose of 180 mg/kg of body mass, the microscopic changes in the renal corpuscles were similar to such in the Group 1 hens (Fig. 7b). Epitheliocytes of most of the proximal sections of the nephrons were in a bloated condition. In some epitheliocytes, the cytoplasm appeared clear, while in others it was clouded. Nuclei of the epithelial cells were shifted to the periphery, suggesting the development of vacuolar dystrophy of nephrocytes. Typically, we observed lysis of nuclei and complete ruination of the cells of proximal tubules. In some regions, we observed total necrosis of epithelicocytes of the nephrons, and scaling and formation of a structureless mass. In the myocardium of most laying hens, there were observed enlargement and permeation of the stroma by transudate. The cardiomyocytes lost their alignment, became somewhat swollen and were in the condition of protein dystrophy (Fig. 7c). Similarly to the Group 1 hens, the brain of the Group 2 hens was observed to have a diffusive edema of the gray matter and white matter. Moreover, we saw pronounced edema around glia, and also pericellular edemas around some nerve cells of the gray matter. Furthermore, there was vacuolar dystrophy of nephrons. Cytoplasm contained various-sized, translucent blisters, which mostly were located on the periphery (Fig. 7d). The cells were of irregular shape, ballooned, the chromatophil matter of hyaloplasm in the area around the nuclei was disintegrated, and other cells underwent complete chromatolysis.

In the gastrointestinal tract of the Group 2 laying hens, the microscopic changes were similar to such in the Group 1 hens, but much more pronounced. Therefore, in the proventriculus, we observed necrobiotic changes in epitheliocytes of the lamina epithelialis mucosae and edema of the mucous membrane. Furthermore, in the alveoli of regular tubular glands, the cubic epithelium was ruined, desquamated, and filled the enlarged lumens of the regular glands (Fig. 8a).

In the small intestine, we observed notable deformation of the villi, proliferation of the epithelium of the villi in the mucous membrane, and excessive mucus secretion. In some regions, we found edema of the submucosa and necrobiosis and desquamation of epithelium of villi. In this case, contours of the villi were poorly visible, which indicated development of acute process (Fig. 8b). In the small intestine, the microscopic changes were also similar to those in the Experimental Group 1 hens.

Discussion

A crucial role in researching the biological reaction of a body to action of toxic compounds belongs to morphological and morphometric methods of study, as they provide insights into the patterns and severity of the course of a pathological process induced by these substances. Morphometric analysis of morphological structures is objective, accurate, and provides an opportunity of a more logical interpretation of study results (Horalskyi et al., 2022, 2023a, 2023b). Likewise, morphological methods (macroscopic, histological, morphometric) of research, which provide valuable data and are widely utilized, are necessary for evaluation of the effects of drugs, pesticides, and other chemical compounds, and allow a more in-depth and concrete reveal of pathogenetic mechanisms of intoxication of examined and experimental animals (Bazaka et al., 2018; Goralskyi et al., 2020; Horalskyi et al., 2020; Gutyj et al., 2022; Koreneva et al., 2023).

Important data regarding the development and morphofunctional condition of the organs and tissues in animals can be obtained by organometric studies, which can reveal and help determine the quantitative characteristics of an organ at the organ and tissue levels in the process of onto- and phylogenetic development of animals subject to environmental factors (Gibbons et al., 2015; Gobeli et al., 2017; Muktyaz & Vishram, 2017; Gul et al., 2020).

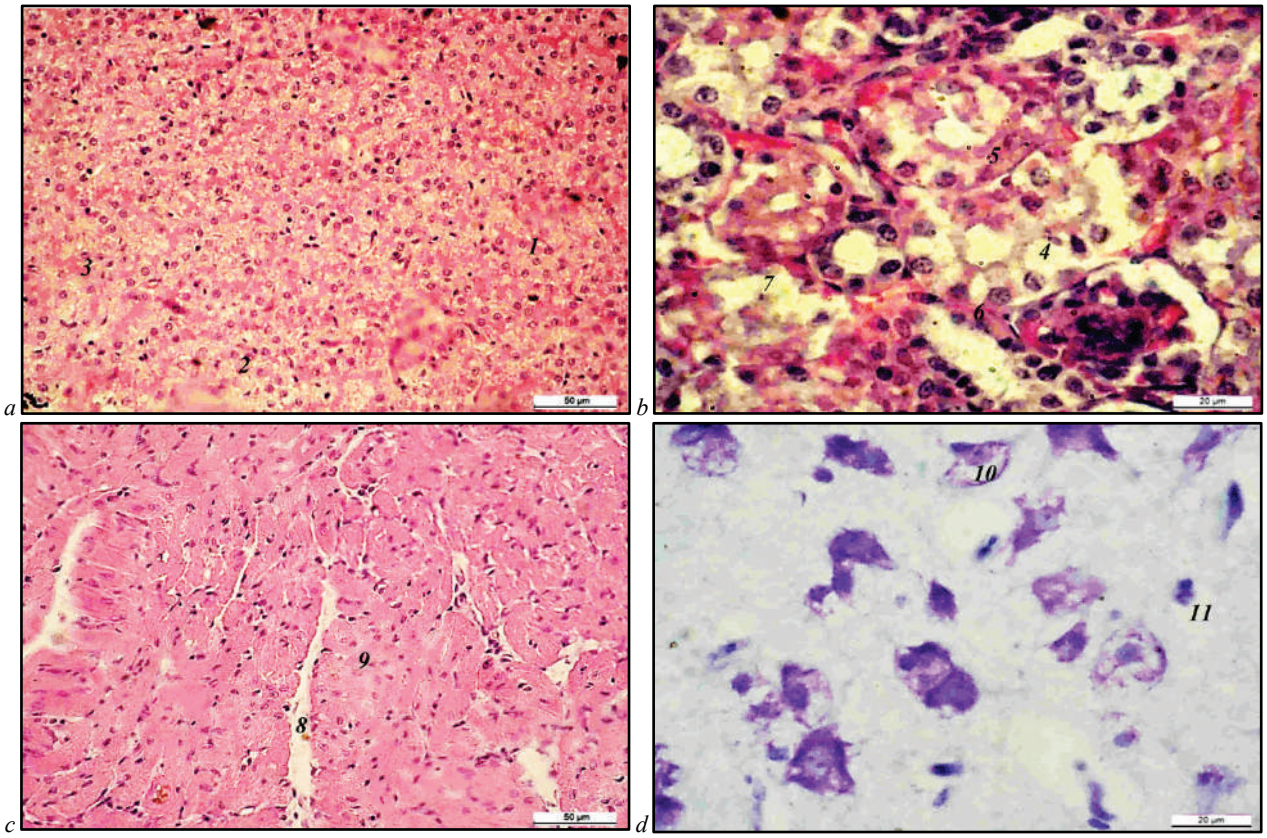

Fig. 7. Microscopic structure: *a* – the liver of the Experimental Group 2 hen; *b* – the kidneys of the Experimental Group 2 hen; *c* – myocardium of hen of Experimental Group 2; *d* – the brain of the Experimental Group 2 hen: *1* – swollen hepatocytes; *2* – translucent cytoplasm; *3* – lysis of hepatocyte nuclei; *4* – vacuolar dystrophy of epitheliocytes of the nephron; *5* – ruination of eptheliocytes of renal corpuscule; *6* – ruination of the basal membrane of the renal convoluted tubule; *7* – microcavity; *8* – loosened and transudate-permeated stroma; *9* – non-uniformly stained cardiomycetes; *10* – vaculolar dystrophy of nerve cell; *11* – edema around the glial cells; Carazzi's hematoxylin and eosin

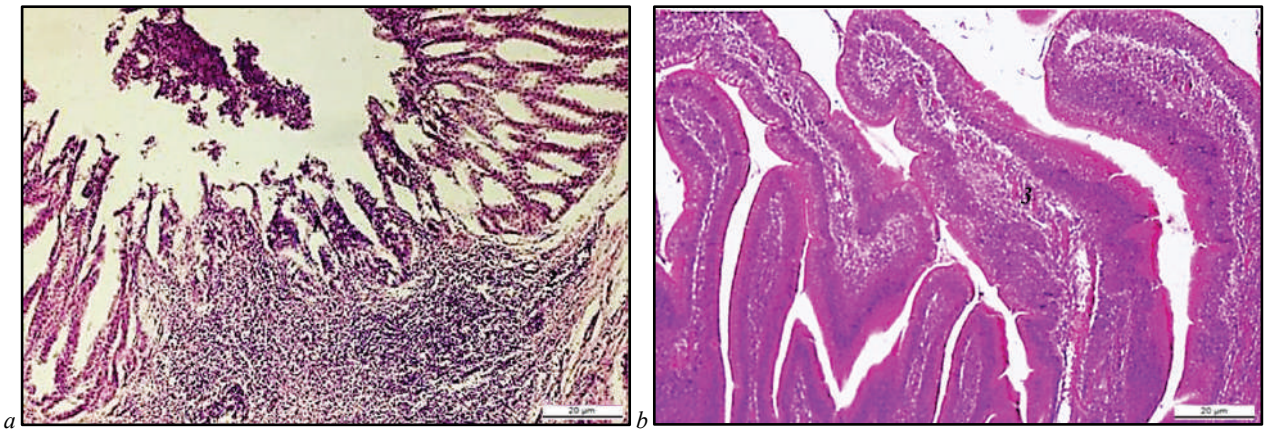

Fig. 8. Microscopic structure of the proventriculus of the Experimental Group 2 hens (*a*), the jejunum of the Experimental Group 2 hens (*b*): *1* – ruination of the alveoli of the regular tubular glands; *2* – edema of the lamina propria mucosae; *3* – deformation of the villi of the mucous membrane; Carazzi's hematoxylin and eosin

In this case, informative indicators of the physiological condition of an animal are the absolute and relative masses of the organs and systems, which are important in clinical medicine and prophylaxis. They are reliable and are used for assessment of the condition of the internal organs (Goralskyi et al., 2022). The results of the studies suggest that absolute and relative masses are directly dependent on age, species, and breed of an animal (Horalskyi et al., 2017; Goralskyi et al., 2020) and change significantly when subject to pathological processes. Thus, they are important indicators that reflect the degree to which toxicants have impacted the body (Butsiak et al., 2019; Gutyj et al., 2019; Bashchenko et al., 2020; Karpenko et al., 2022; Martyshuk et al., 2022; Varkholiak et al., 2022).

The relative mass of the organs directly correlates with mass of the animal body and absolute mass of the organs, the absolute values of which change over the process of ontogenetic development, in the conditions of experiment, and under the influence of anthropogenic surrounding factors, development of pathological processes, etc. (Horalskyi et al., 2021).

Our studies revealed that the absolute masses of the organs in laying hens from the experimental groups - exposed to long-term Actara 25 WG treatment – differed significantly from those in the control group. The differences were clearly reflected in the relative masses of these organs, serving as indicators of morphofunctional changes and the extent of intoxication (Bazaka et al., 2018).

Metabolites of chemical drugs can concentrate on the walls of the vessels and stimulate the proliferation of cellular elements. Thus, prolonged toxic exposure to such preparations results in the interstitial tissues of the liver being infiltrated (Bazaka et al., 2018), as confirmed by our histological studies. Intake of a diet that contained Actara 25 WG in the dose of 180 mg/kg of body mass by the hens led to the following changes:

dystrophic-necrotic alterations in the liver and kidneys; hyperemia and edema in the myocardium; pericellular edema, swelling and vacuolar dystrophy of neurons in the brain, indicating neurotoxic action of the insecticide. At the same time, the proventriculus was observed to have necrobiotic changes in the epithelial cells of the mucous membrane, and in the small intestine, deformation of villi and edema of the mucous membrane prevailed.

Conclusion

Intake of a diet containing Actara 25 WG decreased the coefficients of relative masses of the liver and ventriculus of the stomach in the laying hens, which significantly correlated with the parameters of absolute masses of the respective organs, indicating the insecticide-induced changes, to which the digestive organs were the first to react.

In the hens of Experimental Group 1, the liver presented dystrophic-necrotic changes and round cell infiltration of the portal tracts. Also, there occurred dystrophic-necrotic changes in the epithelyocytes of the nephrons; granular dystrophy of cardiomycetes, plethora of the capillaries, stasis and edema of the stroma in the myocardium; pericellular edemas in the brain; mucous dystrophy, desquamation of the epithelium of the mucous membrane, decrease in lymphocytes in the lymphoid structures, atrophy of the epithelium of the glandular structures in the stomach; hyperemia and necrosis of the villus tips, round cell infiltration in the crypt area in the small intestine; reproduction of cellular elements of the connective tissues between the crypts in the large intestine.

Intake of the diet that contained Actara 25 WG in the dose of 180 mg/kg of body mass caused dystrophic-necrotic changes in the liver and kidneys; hyperemia and edema in the myocardium; pericellular edema, swelling, and vacuolar dystrophy of neurons in the brain; necrobiotic changes in the epitheliocytes of the mucous membrane in the proventriculus; and deformation of the villi and edema of the mucous membrane in the thin intestine.

The authors declare no conflict of interests.

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