

INGLUVIAL, HEPATIC AND RENAL HISTO-PATHOLOGICAL CHANGES IN BROILERS CAUSED BY THE MYCOTOXINS DON, OTA AND MICOFIX PLUS

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This paper work aimed at the morphopathological highlight of the tissue, ingluvial, hepatic and renal lesions in broilers; broilers have received, within their feed, the product Microfix Plus (Biomim) and mycotoxins DON and OTA, for 42 days. The product MP (Biomim) was included within feed in order to annihilate the digestive adsorption and DON and OTA effects upon organism.

The most significant histopathological changes were noticed in the broilers from group T₂ (DON 1000 ppb and OTA 500 ppb), expressed through severe vascular, degenerative and necrosis changes within liver, kidneys and ingluvium. In the chicken from group T₃ and T₄ (DON 1000 ppb, OTA 500 ppb and Microfix Plus 0,5 – 1 ‰), the hepatic, renal and ingluvial changes were less intense and reversible, compared to group T₂.

Mycotoxins are metabolic products of the toxinogenous mycetes, which cause diseases in human and animals, being known as myco-toxicoses (3, 5). Dioxinivalenol or vomitoxin (DON) is a tricotecen, a fungi metabolite elaborated by mycetes from the genus *Fusarium* (*F. tricinctum*, *F. solani*, *F. roseum*, etc.), which causes the reduction of appetite and implicitly the refuse of animals of eating fodder. Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus ochraceus/A. alutaceus*, which develops well on coffee and cocoa beans, soy, peanuts, rice, maize and condiments, stored in improper conditions of temperature, humidity, etc. (2, 6). OTA is considered to be the strongest mycotoxin for poultry, having numerous pathogenic effects: nephrotoxic and hepatotoxic, delays sexual maturity and causes circulatory disorders, bone and intestinal fragility, it decreases egg production and increases mortality (1, 3, 4).

Materials and methods

The experiment was performed at the Discipline of Physiology, in 5 chicken groups (54 chicken/group), 9 chicken/cage (6 cages per experimental variant).

Experimental protocol

Variantă experiment	DON ppb	OTA ppb	Micofix ‰
T ₁	-	-	-
T ₂	1000	500	-
T ₃	1000	500	0.5
T ₄	1000	500	1.0
T ₅	-	-	1.0

The experiment has been performed during 1-42 days. Chicken killing was carried out in the day 42, in slaughterhouse. At killing, we have examined macroscopically the liver, kidneys and ingluvium of all chicken and we have taken

samples (fragments of tissue 2/0.5 cm of prismatic form) for the histo-pathological examination. These samples were fixed in formaldehyde solution 10%, included within paraffin, sectioned at 6 micrometers, colored through the methods HEA – for the general structure study, and Giemsa, for cell details. The histo-pathological preparations achieved were examined with the help of a research microscope MC5, with increasing magnification (x6, x10, x20, and x40). We have taken micro-photos of the most significant lesions.

Results and discussions

Chicken from groups T₁ and T₅

At the microscopic examination of the hepatic, renal and ingluvial samples, we have observed the normal architectonic structure: hepatic lobules – centrolobular vein, hepatic cordons, sinusoid capillaries, perilobular conjunctive tissue, porto-bile area (artery, vein, bile canaliculi), renal lobules – normal glomerulus, uriniferous tubules, interstitial area with conjunctive tissue and sanguine vessels, ingluvium – well developed corneous layers, central-epidermal layers, integer sub-mucous, muscular and serous, with the maintenance of the histological reports.

Chicken from group T₂ (DON 1000 ppb and OTA 500 ppb)

In the liver, macroscopically, we have observed:

- hepatocitary granular degenerations (mega-mitochondria – mitochondrial hypertrophies caused by the accumulation of serum albumin and serum globulin) and polymorph-cellular infiltrations in Disse areas;

- dissociations and fragmentations of the hepatic cordons;

- irreversible lipid degenerations; hepatocytes presented: in cytoplasm, numerous small vacuoles, optically empty, or a single big vacuole, optically empty, which occupied in most cases the whole cytoplasm; hepatocyte nuclei were small, edgy, hyperchromous or, in other cases, they were absent; vacuoles represented the quartering place for lipids, which were turn soluble with the paraffin technique; necrosis of the hepatic cells in focuses (Fig. 1);

- ectasia of the sinusoid capillaries; congestion of the central-lobular vein, with leukodiapedesis and erythrodiapedesis; perilobular edema.

In kidneys:

Histopathologically, we have observed the following lesions:

- parenchimatous and membranous tubulonephrosis, expressed through the affection of the nephrocytes covering up the uriniferous ducts; nephrocytes were unstuck from the basal membrane and fallen into lumen; renal corpuscle hypertrophy and reduction of the filtration area (Fig. 2).

In chicken from group T₃ (DON 1000 ppb, OTA 500 ppb and Micofix Plus 0.5 ‰)

Within the kidney structure, we have observed the following lesions: vascular changes expressed through active congestions; granular degenerations of the

renal cells; renal corpuscles were hyper-chromatic, and the filtration area was slightly increased (Fig. 3).

Within the liver structure, we have noticed the following lesions:

- congestion of the central-lobular vein and of the sanguine vessels from the portobilliar areas; hepatic cordons were intact, with little hepatocitary granular degeneration and with intact nuclei in the hepatic cells, well emphasized and located in the centre of the cell.

On the whole, from the chicken from group T₃ (DON, OTA + Micofix 0.5 ‰) we have observed less severe lesions than in the group T₂, and the kidneys are more affected than the liver. Liver and the hepatic lobules have maintained their architectonic, with the isolated presence of granular degenerations (reversible lesions) and active congestion lesions (Fig. 4).

In chicken from group T₄ (DON, OTA + Micofix 1 ‰) we have observed the following renal lesions: unstucking on small areas of the uriniferous tubules epithelium, less severe than in group T₃, the cytoplasm of the renal cells is granular, with intact nuclei, located in the center.

Within the liver structure, we have noticed the following lesions: focal granular hepatoses, with the maintenance of the integrity of the hepatic cordons; active congestion of the central-lobular veins and of the sanguine vessels from the perilobular areas.

We may state that: renal lesions are present, but less severe than in the individuals from group T₃; they are represented especially by granular degenerations and active vascular congestions, justifying the benefic role exerted by the product Micofix upon organism.

The association between OTA and DON does not allow a clear identification of the lesions caused by each one of these toxins. Although the literature of specialty specifies the process of necrosis produced by DON in various tissues, renal lesions are caused especially by OTA, which are present in the uriniferous tubules due to a low protein fraction, binding OTA in a more specific way than the plasmatic albumins. The hepatic lesions are caused by OTA and by DON as well. The other studies presented in literature show that lesions caused by DON are especially contact lesions, a fact also supported by the histo-pathological changes observed in samples. These have a bigger rate of incidence in group T₂, in which the irritative and necrotic effects were supported by an exaggerate cornification of the superficial epithelial structures from ingluvium, and by small superficial ulcers within the epithelium (Fig. 5); ectasia of the sanguine vessels from sub-mucous and inter-fascicular edema and diffuse leukocitary infiltrate in sub-mucous and muscular.

Within the ingluvium, in the case of the chicken from group T₃, we have observed: a hypertrophy in the epithelium, with the moderate keratinization of the superficial layers, congestion and perivascular edema in sub-mucous and fibrino-leukocitary exudates on small areas (Fig. 6).

In the chicken from group T₄, the lesions within ingluvium are less severe, the central-epidermal layers are intact (cells have their nuclei in various stages of mitosis), and the vascular lesions within sub-mucous are represented by congestion, leuko- and erythro-diapedesis, fibrin network in sub-mucous and perivascular edema in muscular.

We may also state that the association between Micofix, OTA and DON causes the adsorption of mycotoxins in a quantity direct proportionally with the dose of Micofix (0.5 ‰ and 1 ‰), reducing in this way the amount of mycotoxin available for intestinal adsorption, and the hepatic, renal and ingluvial lesions are less intense compared to those belonging to the broilers from group T₂.

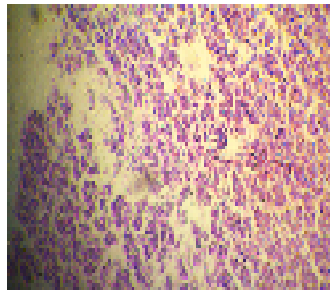


Fig. 1.
Necrosis of the hepatic
cells in foci (lot T₂)
Col. HEA x 100

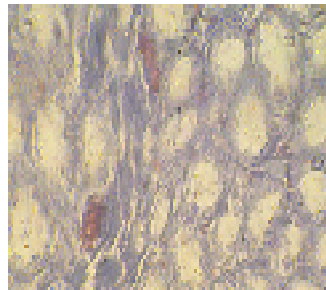


Fig. 2.
Parenchymatous and membra-
nous tubulonephrosis (lot T₂)
Col. HEA x 200

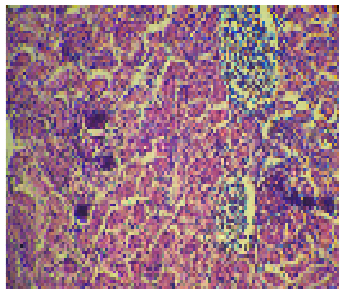


Fig. 3. Active congestions;
granular degenerations
of the renal cells (lot T₃)
Col. HEA x 100

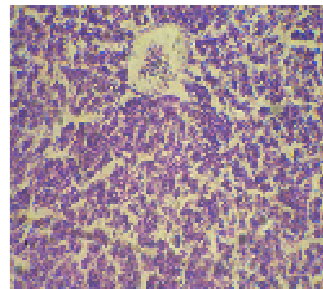


Fig. 4. Liver-active congestions
and granular degenerations
(lot T₃)
Col. HEA x 100

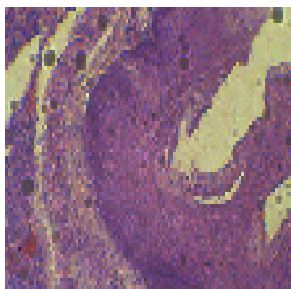


Fig. 5. Ingluvium-superficial
Ulcers within the epithelium
(lot T₂)
Col. HEA x 100

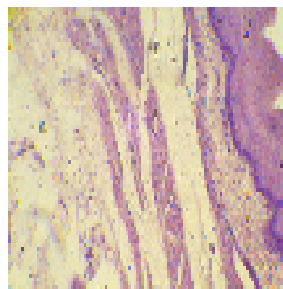


Fig. 6. Ingluvium-congestions,
perivascular edema and
fibrinoleukocitary exsudates
on small areas (lot T₃ and T₄)
Col. HEA x 100

Conclusions

- In chicken from group T₁ (without DON, OTA and Micofix plus) and group T₅ (Micofix Plus 1‰) we have not determined any hepatic, renal and ingluvial changes.
- In chicken from group T₂ (DON 1000 ppb and OTA 500 ppb), we have noticed:
 - hepatic lesions: hepatocitary granular degenerations; fragmentations and dissociations of the hepatic cordons; coagulation necrosis, in focuses, of the hepatic cordons; vascular changes, ectasis, congestions, leuko- and erythro-diapedesis, perilobular edema;
 - renal lesions: granular tubulo-nephrosis, granular-vacuolar; degenerations and/or necrosis of the renal corpuscles and the reduction of the area of glomerular filtration;
 - ingluvial lesions: epithelial hypertrophy with the keratinization of the superficial corneous layers, small superficial ulcers in mucous and vascular changes in sub-mucous and muscular (ectasia, congestions, edema and fibrino-leukocitary infiltrate).
- In chicken from groups T₃ and T₄ (DON, OTA and Micofix Plus), the hepatic, renal and ingluvial lesions are less intense and reversible, compared to group T₂, which presented dystrophy and necrosis lesions, irreversible.
The seriousness of lesions may be expressed as following: T₂ > T₃ > T₄.

References

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