Alteration on the histological structure of some organs induced by low levels of Don mycotoxin in weaned piglets

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ABSTRACT
Deoxynivalenol is a mycotoxin produced by fungi of the Fusarium genra, which are abundant in various cereal crops and processed grains. In order to establish the morpho-physiological changes caused by the mycotoxin DON on various tissue types, we have taken samples from duodenum, liver, kidney, heart and spleen from 9 piglets belonging to three experimental groups: group I – control, group II (experimental I – 0.5 ppm DON) and group III (experimental II – 1.5 ppm DON). Microscopic studies are revealing a series of morphostructural changes, for the experimental groups, as follows: desquamation processes in the villositar epithelium and some denudation (epithelium together with subjacent chorion) of the mucous arias too, hepatic dystrophic processes and enlargement of the capillaries with hemorrhagic arias in the liver, glomerulites and tubulonephrites accompanied by blood vessel enlargement with local hemorrhages in the renal parenchyma, miocardiocytes mild miolysis accompanied by endothelial cells alteration with the local blood extravasations, stroma development in spleen.

Key words: Deoxynivalenol, Fusarium, duodenum, liver, kidney, heart, spleen, microscopic analysis.

INTRODUCTION
Deoxynivalenol is a mycotoxin produced by fungi of the Fusarium genra, i.e. Fusarium culmorum and Fusarium graminearum, which are abundant in various cereal crops (wheat, maize, barley, oats and rye) and processed grains (malt, beer and bread). The mycotoxin is a very stable compound, both during storage/milling and food processing/cooking and it does not degrade at high temperatures (Rotter et al., 1996; Ehling et al., 1997; Eriksen and Alexander, 1998). DON inhibits the synthesis of DNA and RNA and protein synthesis at the ribosomal level. Moreover, piglet sensibility to DON action increases during the weaning period, a critical period which suppose the adaptation to a new environment (housing and temperature), diet changes, mixture with other pigs,
removal of their mother, alteration of the intestinal micro-environment (pH, microflora) and the loss of the maternal milk (immune protection). Acute/subacute toxicity of DON is characterized by vomiting (vomiting is seen in pigs, whereas delayed gastric emptying has been observed in rats and mice), feed refusal, weight loss and diarrhoea. After acute intoxication necrosis in various tissues such as gastrointestinal tract, bone marrow, lymphoid tissue, kidney, spleen, pancreas is also observed (Eriksen and Alexander, 1998; Bergsjø et. al., 1992, 1993).

MATERIAL AND METHODS

In order to establish the morpho-physiological changes caused by the mycotoxin DON upon various tissue types, we have taken samples from duodenum, liver, kidneys, heart and spleen from 9 piglets belonging to three experimental groups: group I – control, group II (experimental I – 0.5 ppm DON) and group III (experimental II – 1.5 ppm DON). The fragments taken were fixed in a solution of ethylic alcohol 80° and in neuter formalin 10%, submitted to dehydration, clarification, drench and inclusion into histological paraffin, and then they were sectioned at the thickness of 5μ and coloured in order to differentiate optically the tissue and cell structures. We have applied three colouring techniques: HE, the trichromic method Mallory and the.

RESULTS AND DISCUSSION

At group I (control) the small intestine has the wall consisted of four layers: mucous, submucous, muscular and serous.

The mucous presents numerous circular rids and villosities and it is consisted of an epithelium, a chorion and the mucous muscular. The epithelium is prismatic mono-bedded, generally consisted of high prismatic cells, among which there is a small number of goblet cells. At villosities` surface, the epithelium is endowed with an evidently ribbed plateau (Fig. 1). The chorion from the villostiy axe contains evident sanguine cells, macrophages and migratory lymphocytes. The basal chorion is comprised of lax conjunctive tissue spread around the intestinal glands Lieberkühn, which have a relatively wide lumen and open at the villosities` base. The glandular epithelium is predominantly comprised of prismatic cells, but also contains a relatively high number of goblet cells. Within the interglandular chorion, we may remark the fine conjunctive stroma, comprising numerous sanguine capillaries with wide lumen and a rich-leukocyte infiltrate. Frequently, the leukocyte cells pass through the glandular epithelium and concentrate within the gland lumen.

Mucous muscular is formed of flat muscular fibers disposed in a circular layer and a longitudinal one. The thin muscular fascicles within the mucous muscular are prolonged between the intestinal tubular glands, too.
The submucous is comprised of a lax conjunctive tissue consisted of numerous serous acinous glands – the Brunner glands. The vascular network is very well developed, represented by numerous arterioles, venules and sanguine capillaries.

The muscular membrane is consisted of two layers of flat muscular fibers: an internal-circular layer and an external-longitudinal one.

At group II (experimental I) the microscopic analysis carried out upon sections of small intestine taken from the individuals from this group shows desquamation processes in the villositar epithelium, present on large areas. Dystrophic processes are also present at glandular level, where we may notice processes of epithelial degeneration accompanied by the expansion of the periglandular areas. Within the villositar and interglandular chorion, we may observe a process of lympho-histiocitary hyperplasia and numerous processes of trans-epithelial leukopedesis. Within the submucous, the microscopic analyses reveal the development of lymphoid formations, of the periacinar conjunctival-muscular tissue, degeneration processes at the acinar epithelial cell level and slight lesions in the capillary endothelium.
At group III (experimental II) mucous and submucous, hypertrophic, form numerous circular ribs with dense, high villosities, with a rectangular aspect, only a few with a triangular aspect. At villosity level, there are frequent desquamation processes on some mucous denudation areas, too (the epithelium together with the subjacent chorion). The dystrophic processes occur in the glandular epithelium, too, which is separated from the basal membrane. The periglandular areas are wide, and we may observe extravased sanguine cells (erythrocytes), due to degeneration of the capillary walls. Within the villositary chorion and the interglandular one, the hyperplasic processes are emphasized, at sanguine capillaries level, and also at the level of the leukocyte infiltrate. The wide interglandular areas are occupied in this way by the leukocyte infiltrate. Within submucous, in the interacinar conjunctival tissue, the collagen fibers are disposed and among them there are fibroblast-type cells, and also numerous leukocyte infiltrative cells, concentrated in more or less extended areas. The peri- and interacinar vascular network is extremely evident.

In the case of group I, the microscopical studies reveal the fact that liver is wrapped in the Glisson’s capsule, consisted of a condensation of collagen fibres and elastic fibres, among which fibroblasts. The capsule’s conjunctive tissue is continued within the organ with fine perilobular conjunctival tissue, which is richer in the perilobular or portal areas (Kiernan areas) and covers sanguine vessels (arteriola and hepatic venule) and the interlobular bile duct. In the hepatic lobules structure, hepatocytes disposed in cordons, uniform in size, with a polygonal aspect, presenting a central nucleus, slightly eccentric or totally eccentric, with an evident nucleolus, and the cytoplasm has a fine granular aspect. We may frequently see binuclear hepatocytes. Between the hepatocyte cordons, there are sinusoid capillaries with a large lumen, which converge towards the centrilobular vein.
In group II, within the hepatic cells located at the periphery of the hepatic lobules and periportal, we may observe cellular dystrophic processes expressed through hypertrophies, cytoplasmatic vacuolizations, picnosis and caryorexis at cell level. Hepatocytes have a dark color, because the cytoplasm is loaded with granulations, heterogenous in size. The sinusoidal capillary network is hyperplasic, and the capillaries have a very long lumen; their endothelium suffers, on small areas, lesions leading to small hemorrhage.

In group III, we may also remark a slight stromal fibrosis. Hepatic lobules are delimited by conjunctive walls, thicker than in the anterior groups. We may also notice within the interportal areas a bigger quantity of conjunctive tissue, from which some sept forming the lobular stroma, the support of the conjunctive tissue, got detached. Hepatocytes are hypertrophic, their cytoplasm being loaded with numerous big granulations, and the nucleus is frequently disposed eccentric. Towards the lobule periphery, there is a big number of hepatocytes, with clear cytoplasm, with vacuolar aspect and hyper-chromatic nucleus.

The transversal sections performed in the kidneys taken from group I have showed the presence of the covering capsule, consisted of collagen fibers, numerous fibroblasts and blood vessels.

Within the parenchyma cortical area, we have noticed the renal corpuscles, heterogenous as size. They are consisted of the renal glomerule and the Bowman capsule, consisted of two foils: visceral, which moulds on the capillary glomerule, and parietal. The space between the two foils belonging to the Bowman capsule, the capsular space, is slightly increased in a big number of renal corpuscles, and the renal glomerules are easily atrophied, but with a reduced frequency.

In group II, the general aspect of the kidney sections is characterized by glomerular atrophies, expressed through the compression of the vascular ball and the presence of wide capsular areas, and the Bowman capsule foils are thickened on more or less extended areas. At uriniferous tubule level, we may see slight retentions, epithelial dystrophies on small areas and an evident peritubular capillary network. Nephrocytes detach from the basal membrane, hypertrophied, their cytoplasm turn into vacuoles, and step-by-step we may observe picnosis at nucleus level, followed by caryorexis and caryolysis.

In group III, a big number of glomerules present a hemorrhagic aspect; hematia are present within the capsular area, and also in the uriniferous tubule lumen. Within the proximal and distal contort tubules, we may remark a dystrophic process on large epithelial areas. Nephrocytes increase in volume, detach from the basal membrane, turn into vacuoles and then degenerate. The epithelial dystrophic processes occur in the renal medullar, too, where there are numerous ecstasy capillaries and local haemorrhage.
Fig. 6. Liver – group II (experimental I) (400x; trichromic Mallory)

Fig. 7. Liver – group III (experimental II) – cytoplasmic vacuolisations (400x; trichromic Mallory)

Fig. 8. Kidneys – group II (experimental I) – renal corpuscles with compressed glomerule and wide capsular area (400x, trichromic Mallory)

Fig. 9. Kidneys – group III (experimental II) – renal corpuscles with compressed glomerule and hematia within capsular area (400x, trichromic Mallory)

Fig. 10. Kidneys – group III (experimental II) – medullar area – epithelial dystrophic processes and local haemorrhages (400x, trichromic Mallory)
The microscopic study performed on myocardium provided from the group I (control) emphasized cardiac muscular fibres with a cylindrical aspect presenting one or two oval nuclei, central disposed, with an evident nucleolus, with a functional sarcoplasm who included a contractile well developed apparatus, represented on the fibre length through alternances of dark and clear disks. Both endomisium and perimisium are including numerous blood vessels: capillaries with large lumen loaded with erythrocytes in endomisium; capillaries, arterioles and venules in perimisium.

In the case of groups II (experimental I) and III (experimental II), on small surfaces, in the perinuclear space were been observed clear zones, of lypofuscinic pigment deposition, process accompanied by muscular fibres depigmentation, aspect who characterizes miolisis (with zones of characteristic myofibrilar striates disappearances). Interfibrilar is disposed conjunctive lax tissue, the support of a rich capillaries net. Are often observed capillaries dilatation, with endothelium alterations and blood local extravazation, these morphological aspects are similar with those of haemorrhagic miopathies.

![Fig. 11. Myocardium– group III (experimental II) – miolisis and blood local extravasation (400x, trichromic Mallory)](image)

The spleen is covered in a tinny conjunctivo-vascular capsule, from that are detached thick trabecules who penetrates the organ profoundness dividing in splenic lobules. The fibrouse capsule, with all the trabecules who are detaching from it, forms the conjunctive stroma who contains a reticuline fibres net and reticuloendothelial cells. Under the capsule is disposed the splenic parenchyma composed from white and red pulp. The white pulp is represented by splenic lymphonodules, lymphocytes agglomeration and macrophages disposed around nodular arteriole. On splenic lymphonodules periphery, the reticular stroma forms several concentric layers.

The red pulp is well represented occupying a great area from the splenic parenchyma. Is composed by numerous venules, around them are disposed cells and reticulin fibres, numerous eritrocytes, macrophages and lymphocytes.

In the case of groups II and III, microscopic studies pointed out the organ stroma development, in which are predominant the reticulin fibres,
reticuloendothelial cells and smooth miocytes. The splenic follicles are generally hypertrophied.

![Fig. 12. Spleen- group I – splenic lymphonodule (200x, trichromic Mallory)](image1)
![Fig. 13. Spleen- group III – splenic lymphonodule (200x, trichromic Mallory)](image2)

The results obtained in this microscopic study is according with the results obtained by Fioramenti et al., 1993 in mice and rats, as cited in Eriksen and Alexander, 1998 and Manolescu et. al., 1997 and represent an important characteristic in DON intoxication.

**CONCLUSIONS**

1. The microscopic analysis carried out upon sections of small intestine taken from the individuals from group II, shows desquamation processes in the villositar epithelium, present on large areas.

2. At group III (experimental II) at villosity level, there are frequent desquamation processes on some mucous denudation areas too (the epithelium together with the subjacent chorion).

3. Within the villositar and interglandular chorion, we may observe a process of lympho-histocitary hyperplasia and numerous processes of trans-epithelial leukopedesis.

4. Compared to group I, in case of groups II and III we have observed cellular dystrophic processes expressed through hypertrophies, cytoplasmic vacuolisations, picnosis and cariorexis at nuclear level, in the hepatic cells located at the periphery of the hepatic lobules and periportal.

5. In groups II and III, the sinusoidal capillary network is hyperplasic, capillaries have a very wide lumen, and their endothelium suffers on small areas from lesions leading to light hemorrhages.

6. In the case of group II, at kidney level we may observe evident glomerular atrophies and tubular nephrocitary atrophies.

7. In group III, the microscopical images reveal a big number of glomerules with hemorrhagic aspect, and within the capsular area and also in some of the uriniferous tubes lumen, we may notice hematia.
8. Epithelial dystrophic processes are expressed within the renal medullary, too, where we may notice numerous ectasied capillaries and local hemorrhages.

9. At cardiac muscular cells level, on small areas were been observed clear zones, of lipofuscinic pigment deposition, process accompanied by muscular fibre decoloration, aspect observed in miolysis.

10. Microscopic studies carried out on spleen sections coloured by trichromic Mallory method pointed out the organ stroma development, in which are predominant the reticulin fibres, reticuloendothelial cells and smooth miocytes. The splenic follicles are generally hypertrophied.

REFERENCES


