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IMMUNOSUPPRESSIVE ACTION OF DEOXYNIVALENOL OF THYMUS IN CHICKENS

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ABSTRACT. Deoxynivalenol (DON, vomitoxin) is a type *B-trichothecene*, naturally occurring contaminants of animal feed, being implicated in several mycotoxicoses in farm livestock. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye, and maize, and less often in rice, sorghum, and triticale. Deoxynivalenol is potent nefrotoxic**,** hepatotoxic and immunosuppressant. High doses of trichothecenes promote rapid onset of leukocyte apoptosis (programmed cell death), which is manifested as immunosuppression. The study aimed to prove the immunosuppressant action of deoxynivalenol in chickens experimentally treated each day, from the 7th day of life, using 5,4 mg/kg b.w in E group for 28 days (since 35 days of life). Histopathology studies of thymus were made on 7th, 14^{th,} 21st and 28th days of experiment. In E group small lesions of thymus were observed even after 7th day of poisoning but intense lesions, hydropic degeneration, necrotic foci and moderate lymphoid depletion was observed after the 14th and 21st day of poisoning. After 28th day a marked proliferation of stromal cells in the reticulum network, in medulla zone, presence of mucous cells, small mucous cysts and haemorages were observed.

Key words: Trichotecens; Broiler chickens; Immunosupression.

REZUMAT. Efectul imunosupresor al deoxinivalenolului asupra timusului la puii broiler. Deoxinivalenolul (DON, vomitoxină) face parte din categoria trichotecenelor de tip B, contaminanți naturali ai furajelor, implicați în producerea unor micotoxicoze la animalele de fermă. Aceste micotoxine se întâlnesc, predominant, în boabe de cereale, grâu, orz, ovăz, secară şi porumb, mai rar în orez, sorg şi triticale. Deoxinivalenolul este puternic nefrotoxic**,** hepatotoxic şi imunosupresor. Dozele mari de trichothecene determină apoptoza rapidă (moartea celulară programată) a leucocitelor, manifestată prin imunodepresie. Studiul are ca scop demonstrarea efectului imunosupresor al deoxinivalenolului la puii broiler, tratați experimental cu DON, în doză de 5,4 mg/kg/zi, timp de 28 zile (de la vârsta de 7 zile până la 35 zile). S-au studiat leziunile histologice ale timusului prin sacrificarea randomizată a unor grupe de câte cinci pui în zilele a 7-a, a 14-a, a 21-a şi a 28-a ale

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experimentului. La lotul experimental s-au observat uşoare leziuni ale timusului, începând din ziua a 7-a a intoxicaţiei, dar acestea au devenit mult mai intense în a 14-a şi a 21-a zi, constând în degenerare hidropică, focare necrotice şi depleţie limfoidă moderată. După cea de-a 28-a zi s-au constatat proliferarea marcantă a celulelor stromale reticulare şi din zona medulară, prezenţa de celule mucoase, mici chişti mucoşi şi hemoragii.

Cuvinte cheie: deoxinivalenol; pui broiler; efect imunosupresor.

INTRODUCTION

Deoxynivalenol (DON) is one of the most abundant and important trichothecenes in food and feed, and it is a significant contaminant due to its frequent occurrence at toxicologically relevant concentrations worldwide (Awad *et al.*, 2012). The trichothecenes are a group of over 180 structurally related sesquiterpenoid mycotoxins produced *by Fusarium, Stachybotrys* and other moulds growing on basic commodities used in animal feeds, foods or in the environment (Grove, 1993; Grove, 1998; Grove, 2000).

Experimentally, oral exposure to low to moderate dose of trichotecenes cause diarrhea and gastroenteritis, whereas higher doses cause severe damage of the lymphoid and epithelial cells of the gastrointestinal mucosa resulting in hemorrhage, endotoxemia and shock (Ueno, 1984). Other targets include bone marrow and thymus which can contribute to generalized immunosupression.

Inhibition of protein synthesis is considered to be the primary toxic effect of trichothecenes. Rocha *et al*. (2005) reported multiple inhibitory effects for trichothecenes on eukaryotic cells including disruption of normal cell function by inhibiting RNA, DNA, and inhibition of cell divisions, stimulation of ribotoxic stress response, and activation of mitogen-activated protein kinases. The latter enzymes catalyze reactions in signal transduction related to proliferation, differentiation, and apoptosis (Pestka *et al.,* 2005).

MATERIALS AND METHODS

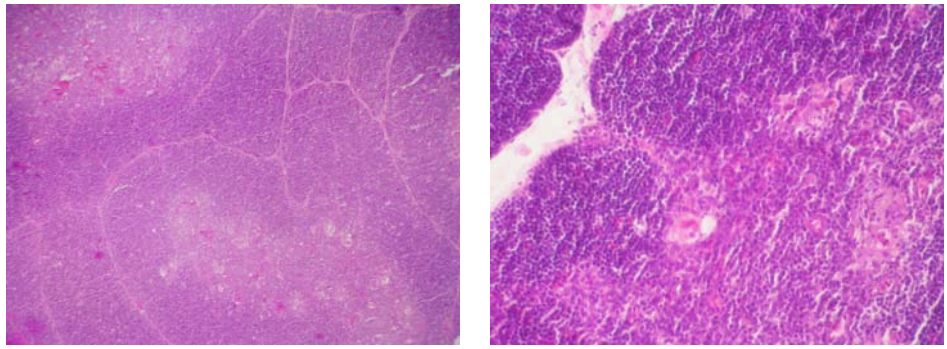
Experiment were used 40 chickens, which after a period of one week of accommodation to living conditions provided, were randomly divided in two groups: experimental (E) and control (C). Chickens were reared on sawdust litter, were provided specific microclimate conditions for age, room temperature gradually decreasing from 28°C, to 18°C. Commercial-type food, free of DON was administered ad libitum. E group received daily by gavage deoxynivalenol (DON-Sigma Chemicals Co.) eluted in sterilized sunflower oil at a dosed 5,4mg/ kg b.w. The control group received only eluent (sterilized sunflower oil). At the end of each week during the experiment, chicks were individually weighed. Five chickens were selected by random from each group and were killed at 7^{th}, 14^{th} , 21^{st} and 28^{th} day of the experiment.

Histopathology was performed on thymus fragments fixed in 10% formalin solution, embedded in paraffin, sectioned at 5μm and stained by HEA and PAS.

RESULTS

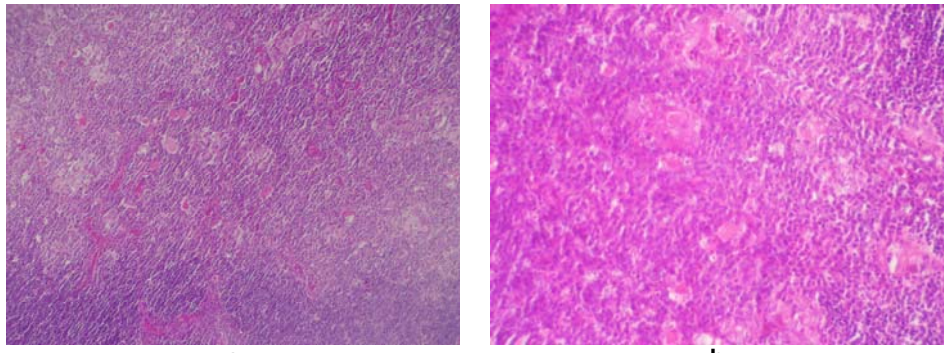
After 7 days of DON poisoning in E group, the lesions of thymus were not evident, a clear distinction between cortex and medulla being observed. The thymus cortex and medula become less atrophic (*Fig.1a*), with hydropic degeneration of residual cells and necrotic foci. Moderate lymphoid depletion is seen in the thymus starting with the cortical lymphocytes, but stroma cells are not affected. Stroma cells were proliferated (*Fig. 1b*).

After 14 days of exposure to DON the limit between cortical and medullar zone is in distinguished, reticular cells from medullar zone became secretory and also into this zone small PAS positive zones consisting from colloid secretory reticular cells were observed. Lymph cells into this region are reduced (*Fig. 2a, b*).



a b

Figure 1 - Thymus at 7th day of experimentaly intoxication with DON. HEA stain x60 (a); PAS stain x 400 (b)

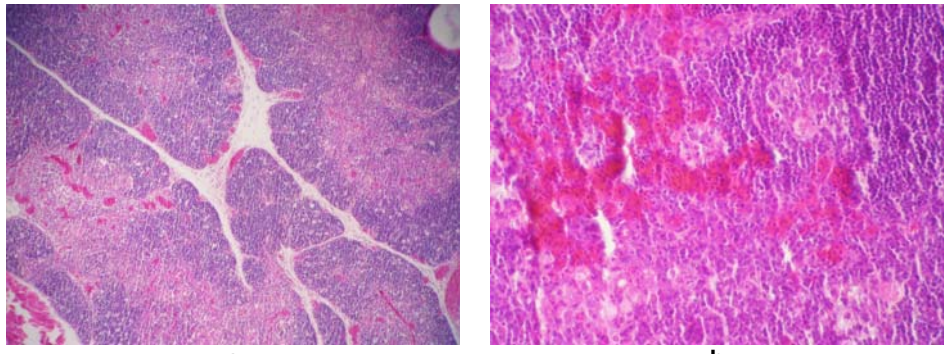


a b

Figure 2 - Thymus at 14 days of experimentaly intoxication with DON. HEA stain x100 (a); PAS stain x 200 (b)

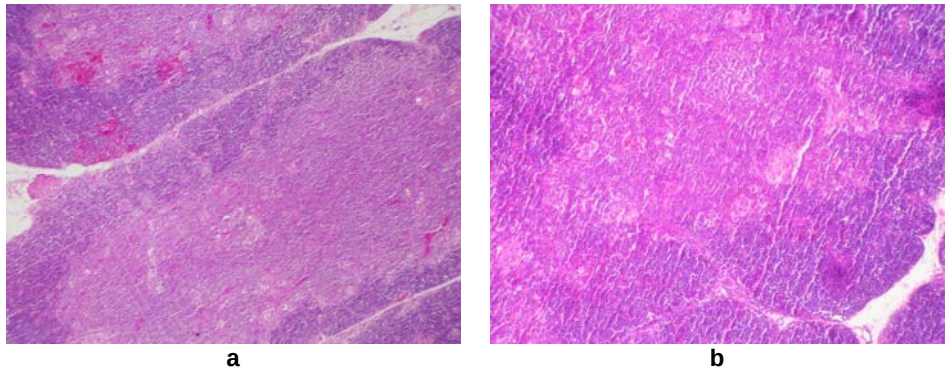
Intense histological lesions of thymus appeared at 21 days after experimentaly intoxication and involved a marked proliferation of stroma cells in the reticulum network, in medulla zone (*Fig.3a*), the presence of mucous cells and more small mucous cysts and haemorrages (*Fig.3b*).

More severe histological lesions of thymus appeared at 28 days of experimentaly intoxication and involved a marked proliferation of non-epithelial cells in the reticulum network, in medulla zone (*Fig.4a*), the presence of mucous cells and more small mucous cysts and haemorrages (*Fig.4b*).



a **b**

Figure 3 - Thymus at 21 days of experimentaly intoxication with DON. HEA stain x 60 (a); PAS stain x 400 (b)



a **b**

Figure 4 - Thymus at 28 days of experimentaly intoxication with DON. HEA stain x100 (a); x 200 (b)

DISCUSSION

Normally, the thymus has distinct cortical and medullar regions that demonstrate thymocytes at serial stages of maturation. Thymocytes undergo positive and negative selection on the basis of their T cell receptor specificities and the majority of cortical thymocytes die by

apoptosis. The mature thymocytes that survive accumulate in the medulla (Eguchi *et al.,* 1992). Lymphoid depletion and increased number of stromal cells hyperplasia was observed in all trated chickens.

Numerous studies have been conducted in a variety of cell lines to assess the cytotoxic effects of DON. In mouse thymocytes *in vivo*, DON [0.5–8.0 mg/kg (92–27) μmol/kg] dose-dependently induced significant increases in apoptosis rates compared to controls. In addition, DON [4 and 8 mg/kg (13–27 μmol/kg)] significantly decreased the proliferation indexes of the treated cells (Bony *et al*., 2007; Ouyang *et al*., 1996). Explants from weanling pigs were exposed to 0, 0.2, 1, 5 μM DON in the culture medium for 4 h. Preliminary cultures had shown that 10 and 30 μM DON induced necrosis of the explants after 4 h of incubation (Kolf-Clauw *et al*., 2009).

Groups of 10 female white Leghorn chicks, 1 day old, were fed with diets containing uncontaminated wheat or naturally contaminated wheat containing deoxynivalenol at a concentration of 18 mg/kg, equivalent to 2.25 mg/kg bw per day, for 18 weeks. The contaminated diet resulted in a suppressed antibody response to Newcastle disease vaccine given at week 14. When groups of three 1-day-old broilers were fed a diet containing 50 mg/kg, equivalent to 6.25 mg/kg bw per day, a suppressed lymphocyte blastogenesis response was seen (Harvey *et al.,* 1997).

The ability of deoxynivalenol to alter the expression of cytokines transiently is important because such effects can disrupt normal regulation of a wide variety of immune functions.

High doses of trichothecenes promote rapid onset of leukocyte apoptosis, which is manifested as immunosuppression. Flow cytometry was used to demonstrate that deoxynivalenol inhibits or enhances apoptosis in a concentration-dependent manner in T cells, B cells, and IgA^+ cells isolated from spleen, Peyer patches, and thymus. Induction of apoptosis was dependent on the lymphocyte subset, tissue source, and glucocorticoid induction (Pestka *et al.,* 1994).

CONCLUSIONS

Intense histological lesions of thymus appeared at 14, 21 and 28 days after experimentaly intoxication with DON, consisting in hydropic degeneration, necrotic foci and moderate lymphoid depletion, followed by lymphocyte necrosis.

Marked proliferation of non-epithelial cells in the reticulum network of thymus, in medulla zone suggest that DON induces reticuloendothelial hyperplasia.

At 21^{st} and to 28^{th} day of exposure to DON the presence of mucous cells and more small mucous cysts and small necrotic foci and haemorrages were observed.

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