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Original article

CLOACAL BURSA MORPHOLOGY IN TURKEY BROILERS CHALLENGED WITH AFLATOXIN B_1 ALONE OR CO-ADMINISTERED WITH MYCOTOX NG

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Summary

Grozeva, N., I. Valchev, L. Lazarov, Ts. Hristov, D. Kanakov, K. Dimitrov & R. Binev, 2020. Cloacal bursa morphology in turkey broilers challenged with aflatoxin B_1 alone or co-administered with Mycotox NG. *Bulg. J. Vet. Med.,* **23**, No 1, 121–129.

Aflatoxins are toxic metabolites of moulds from the genus *Aspergillus* (*Aspergillus flavus* and *Aspergillus parasiticus* being the main producers). The aim of the present investigation was to evaluate the toxic effects of aflatoxin B_1 on bursa of Fabricius morphology. Also, the possibility for prevention of toxic effects of AFB_1 by feed supplementation of a mycosorbent (Mycotox NB) was studied. Experiments were carried out with sixty 7-day-old female turkey broilers (meat TM strain) divided into one control and five treatment groups (n=10). Groups were as followed: Group I – control (fed standard feed according to the species and age of birds); Group II – experimental, whose feed was supplemented with 0.5 g/kg Mycotox NG, Group III– experimental, whose feed contained 0.2 mg/kg aflatoxin B_1, Group IV – experimental, whose feed contained 0.4 mg/kg aflatoxin B_1, Group V – experimental, supplemented with 0.2 mg/kg aflatoxin B_1 and 0.5 g/kg Mycotox NG and Group VI – experimental, supplemented with 0.4 mg/kg aflatoxin B_1 and 0.5 g/kg Mycotox NG. The duration of the experiments was 42 days. The changes in bursal morphology in control and treated groups were followed out after the end of the study. In birds from experimental groups III and IV, atrophy and degenerative changes have occurred in the bursa of Fabricius: reduction of lymphoid cell - populations in lymphoid follicles along with dystrophy. Feed supplementation with the tested toxin binder (Groups V and VI) resulted in partial neutralisation of deleterious effects of AFB_1 on severity of histological lesions: interfollicular oedema, considerably lower lymphoid follicle rarefaction.

Key words: aflatoxin B_1, turkey broilers, bursa of Fabricius, Mycotox NG, histopathological changes

INTRODUCTION

Aflatoxins are produced by *Aspergillus* moulds (*A. flavus, A. parasiticus*). They contain a dihydrofurofuran moiety and emit light: aflatoxin B_1 (AFB_1) and aflatoxin B_2 (AFB_2) have blue fluorescence while aflatoxin G_1 (AFG_1) and G_2

(AFG2): yellow-green fluorescence (Verma *et al.*, 2004). AFB_1 is the most spread and most toxic aflatoxin in plant substrates. It is often present at high concentrations in cereal crops and peanut flour (Gowda *et al*., 2004). It is also the most potent natural hepatocarcinogen (Wilson & Payne, 1994). The toxicity of AFB_2, AFG_1 and AFG_2 amounts to 10%, 20% and 50% of AFB_1 toxicity, respectively (Leeson *et al.,* 1995).

The toxic effects of aflatoxins in domestic fowl are extensively studied and their carcinogenic, teratogenic, mutagenic and growth inhibiting effects are documented (Oğuz *et al.,* 2000; Sur & Celik, 2003). Haematological, blood biochemical, immunological and morphological changes are also described (Kiran *et al.,* 1998; Qureshi *et al.,* 1998; Oğuz *et al.,* 2000). All poultry species are sensitive to aflatoxins. Although feed levels are not high enough to induce death, their continuous intake is deleterious. Growing birds, ducklings and turkey poults in particular, are extremely sensitive to aflatoxins

The immunity in birds relies on the ability of bursa of Fabricius, spleen and thymus to produce mature and active lymphocytes. The consumption of AFB_1-contaminated feeds during the growth of chickens provokes immune tissue atrophy and reduction of relative weights of immune organs (Ortatali & Oguz, 2005; Verma *et al.,* 2004). The intake of mycotoxin-contaminated feeds is the main reason for immune suppression and reduced resistance to infectious diseases. Aflatoxin B_1 exerts the strongest biological effect in lymphoid organs manifested with involution or hypoplasia of the thymus, spleen and cloacal bursa. The presence of AFB_1 in poultry feeds decreased substantially humoral and cell-mediated immunity, damages not only the immune system of adult birds, but also that of embryos suppressing phagocytosis in hatchlings and making them susceptible to various pathogens (Arulmozhi & Varghese, 2011). On the cell level, AFB_1 impairs innate and acquired immunity of domestic fowl (Hoerr, 2010).

Aflatoxin B_1 causes reduction of the weight of immunocompetent organs (bursa of Fabricius, thymus, spleen) (Raja *et al.,* 2017). Morphological changes in the bursa of Fabricius comprise impaired architectonics, thinning of the cortical layer, lymphocytolysis, interfollicular oedema, fibrosis, hyperplasia with epithelial corrugation (Ortatali & Oguz, 2005; Ekhlas, 2012).

Various strategies have been proposed to detoxicate feeds contaminated with mycotoxins – physical separation, heat inactivation, irradiation, microbial degradation, treatment with various chemicals. All these methods are economically inefficient (Saki *et al.,* 2018). Some methods of detoxication use various inert mycosorbents as hydrated sodium calcium aluminosilicate (HSCAS) (Neeff *et al.,* 2013), zeolites (Khadem *et al.,* 2012), bentonites (Bhatti *et al.,* 2017), active charcoal (Khadem *et al.,* 2012) bioproducts as live yeasts (*Sacchromyces cerevisiae*) (Wade *et al.,* 2018). Mycosorbents are among the most extensively studied approaches. They reduce the bioavailability of mycotoxins in blood and prevent their absorption from intestines. Nevertheless, some of them reduced also bioavailability of amino acids and/or minerals (Kumar *et al.,* 2015).

The aim of the present investigation was to evaluate the toxic effects of aflatoxin B_1 on bursa of Fabricius morphology and the possibility for prevention of toxic effects of AFB_1 by using Mycotox NB.

MATERIALS AND METHODS

Sixty 7-day-old female turkey broilers (meat TM strain) were divided into one control and five treatment groups (n=10). Group I was control. The experimental groups were supplemented with: Group II – 0.5 g/kg Mycotox NG (micronised yeasts, montmorillonite, thymol); Group III – 0.2 mg/kg aflatoxin B_1; Group IV – 0.4 mg/kg aflatoxin B_1; Group V – 0.2 mg/kg aflatoxin B_1 and 0.5 g/kg Mycotox NG; Group VI – 0.4 mg/kg aflatoxin B_1 and 0.5 g/kg Mycotox NG.

Aflatoxin B_1 with 99% purity used in this experiment was produced by *Aspergillus flavus* (Sigma-Aldrich, Germany). All turkey poults were kept under optimum microclimatic parameters according to Ordinance 44/2006 (Anonymous, 2006).

After the end of the experiment, control and experimental turkeys were euthanised by cervical dislocation, in compliance with Ordinance 20 on the minimum requirements for the protection and welfare of experimental animals and requirements to objects for use, cultivation and/or supply (State Gazette 87/9/11/2012). Specimens for histological examination were obtained from the bursa of Fabricius, fixed in 10% neutral formalin, dehydrated in ascending ethanol series and embedded in paraffin. Paraffin blocks were cut on a Leica RM 2235 microtome. Cross sections (3 μm) were stained routinely with haematoxylin/eosin.

The experiments were approved by the Bulgarian Food Safety Agency, permit 19218/06.11.2014.

RESULTS

The cloacal bursa of experimental groups showed degeneration of various intensity and depletion of lymphoid cells.

Turkey poults treated with 0.2 mg/kg AFB_1 (Group III) demonstrated mainly follicular cell depletion and degeneration, mostly karyopyknosis and karyolysis (Fig. 1A). Vascular haemolysis was occasionally seen (Fig. 1B). Some of birds showed haemorrhages, single necrotic foci, plasma infiltration of the peribursal follicular tissue (Fig. 1C).

Turkey poults treated with 0.4 mg/kg AFB_1 (IV group) exhibited high-grade lymphocytolysis (Fig. 2A), multiple haemorrhages and necrotic foci. The normal architectonics was impaired. The cortex was strongly thinned and destroyed at some places (Fig. 2B), whereas other zones were with hyperplastic simple prismatic epithelium (Fig. 3A). Apart the interfollicular oedema (Fig. 3B), many areas showed also strong interfollicular fibrous tissue proliferation (Fig. 2A).

Pathomorphological changes in the bursa of turkey poults from groups V and VI, supplemented both with aflatoxin and 0.5 g/kg Mycotox NG were less intense. In the group that received 0.2 mg/kg AFB_1 and 0.5 g/kg Mycotox NG, degenerative changes and lymphoid cell depletion were rather smaller compared to birds from Group III (Fig. 4A). Birds fed 0.4 mg/kg AFB_1 + 0.5 g/kg Mycotox NG (Group VI) showed moderate extent of degeneration and follicular cell depletion. Interfollicular oedema was still visible, and some vascular walls were thickened (Fig. 4B).

DISCUSSION

Mycotoxicoses are diseases resulting from intake of mould-contaminated feed ingredients. Among the various mycotoxins, AFB_1 is the prevailing primary metabolite in animal feeds (Nilipour *et al.,* 2012). Aflatoxins are an increasing concern for

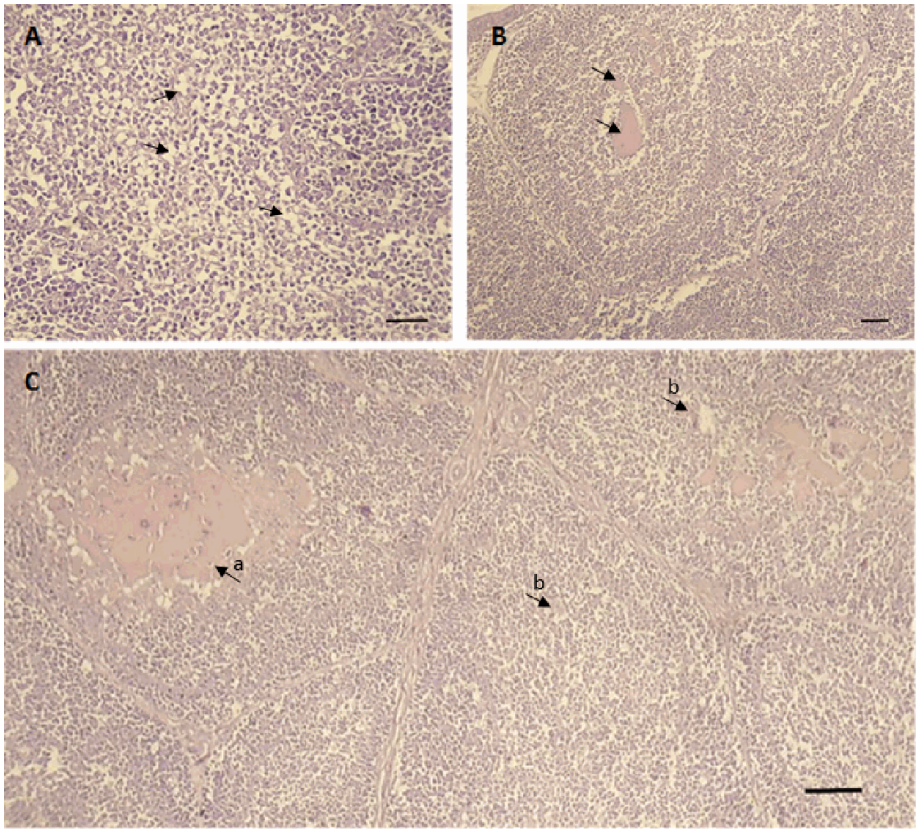

Fig. 1. Bursa of Fabricius' follicles in turkeys treated with 0.2 mg/kg AFB_1 (Group III). A) Degeneration and reduction of cells, H/E, Bar=20 µm; B) Generalised haemolysis in blood vessels, H/E, Bar= 50 µm; C) Haemorrhages (a) and necrosis (b), H/E, Bar=50 µm.

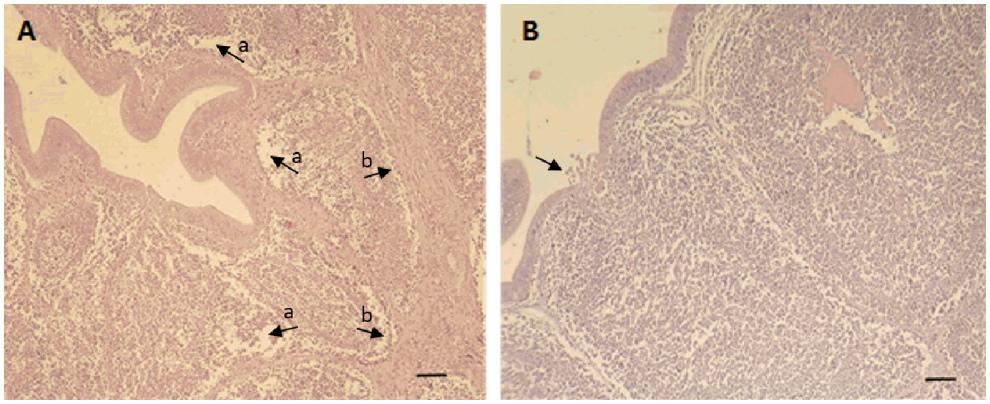

Fig. 2. Bursa of Fabricius in turkeys treated with 0.4 mg/kg AFB_1 (Group IV). A) Lymphocytolysis (a) and interfollicular connective tissue growth (b) in bursal follicles, H/E, Bar=50 µm; B) Thinning and injury of bursal epithelium, H/E, Bar= 50 µm.

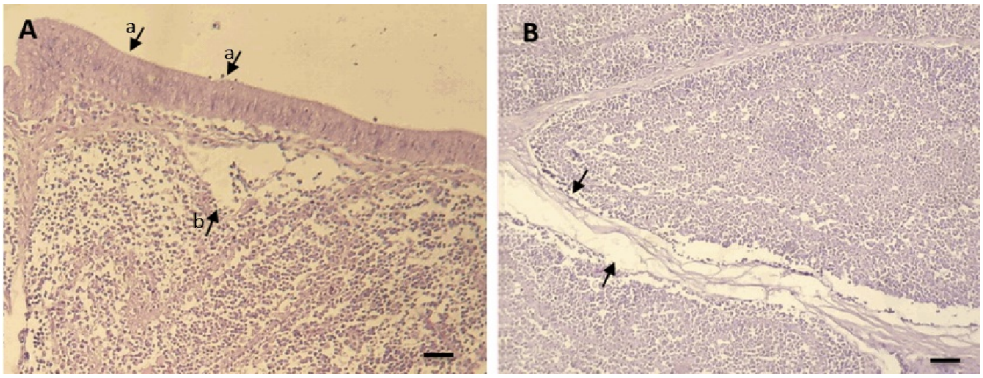

Fig. 3. Bursa of Fabricius in turkeys treated with 0.4 mg/kg AFB_1 (Group IV). A) Epithelial hyperplasia (a) and lymphocytolysis (b), H/E, Bar=50 µm; B) Interfollicular oedema, H/E, Bar= 50 µm.

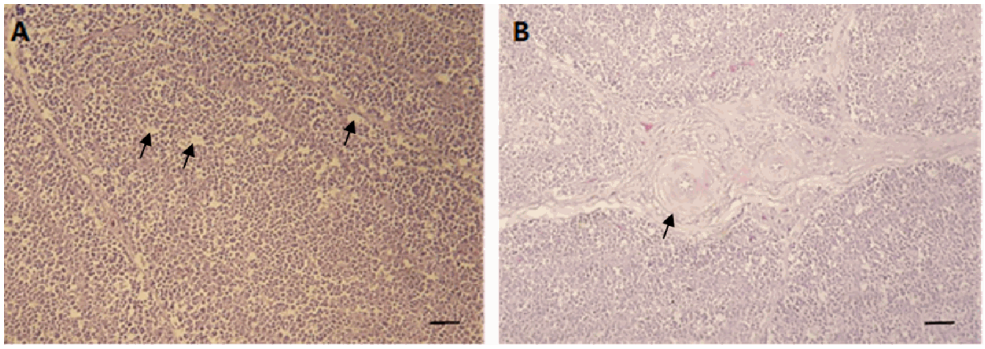

Fig. 4. Bursa of Fabricius in turkeys: A) treated with 0.2 mg/kg AFB_1 and 0.5 g/kg Mycotox NG (Group V) – mild degeneration and cell depletion. H/E, Bar=50 µm; B) treated with 0.4 mg/kg AFB_1 and 0.5 g/kg Mycotox NG (Group VI) – bursal vascular wall thickening, H/E, Bar= 50 µm.

poultry industry due to their high toxicity and common occurrence in feeds (Denli *et al.,* 2004; Tessari *et al.,* 2006). In most instances, dietary aflatoxin concentrations are higher than maximum allowed level of 20 $\mu g.kg^{-1}$ (Aravind *et al.,* 2003).

The immune system of birds is sensitive to irritants of various nature (nutritional, physiological, genetical and toxicological). Among the nutritional factors, the presence of mycotoxins or their metabolites in feeds raises an acute response from immunocompetent organs, resulting in damage and destruction of their histological structure. Aflatoxins are among the most throughly studied mycotoxins inducing damage by virtue of their direct effect on immunocompetent organs leading to necrosis, atrophy, reduction of lymphoid cell populations (Qureshi *et al.,* 1998). A previous study of ours (Valchev *et al.,* 2017) has provided evidence about lower weight of the bursa of Fabricius in turkeys challenged with AFB_1 only, consequently to the mycotoxin effect on lymphoid components of the bursa. In another study, this was manifested with lymphocytoloysis and bursa's size reduction (Lakkawar *et al.,* 2015). Aflatoxins induce immunosuppression and reduction of the relative

weight of the bursa of broiler chickens. Statistically significantly lower relative weights of the bursa of Fabricius were observed in relatively low levels total aflatoxin (200, 400, 600 μg/kg feed) (Ibrahim *et al.,* 2000).

Fish and birds are highly sensitive to AFB_1 and respond even to low doses of 15–30 ppb (Rawal *et al.,* 2010). Domestic turkey are also highly sensitive to toxic effects of AFB_1. The bioactivation of aflatoxins occurs in hepatocytes via microsomal enzyme systems cytochrome P450 (CYP450), to reactive aflatoxin-8,9-epoxide (AFBO) – the primary and most toxic metabolite. This compound inhibits protein synthesis, binds to DNA and RNA and incurs liver damage, immunosuppression and poor productive performance (Rawal et al., 2010). In these birds, liver glutathione S-transferases class alpha (GSTA) are not able to detoxicate AFBO, hence the higher sensitivity.

The degenerative and necrotic changes observed in groups III and IV, lymphoid cell depletion, epithelium hyperplasia, cortical atrophy of follicles, haemorrhages, oedema and fibrous tissue growth are comparable with findings reported by other researchers in broiler chickens treated with various levels of mycotoxins. The established depletion of lymphoid cells with lysis of lymphoid cells, atrophy, necrosis, interfollicular fibrous and edema in cloacal bursa follicles were reported both in low dietary total aflatoxin levels (100 μg/kg) (Ortatatli *et al.,* 2005), 60, 80 or 100 μg/kg AFB_1 fed over 42 days (Arulmozhi & Varghese, 2011) as well as high concentrations (2.5 mg/kg feed) fed over 35 days (Ekhlas, 2012). Degeneration of epithelial cells of bursal follicles was documented in broiler chickens fed feed containing 2.5 mg/kg AFB_1 for 21 days (Celic *et al.,* 2000). Observed lesions were similar to those reported in previous studies of broiler chickens fed 1 mg/kg feed total aflatoxin for over 28 days (generalised lymphoid depletion) (Raja, 2009). Our findings were in line with histopathological alterations shown by Lakkawar *et al.,* (2015) in broiler chickens fed 0.5 or 1 mg/kg AFB_1 (depletion of lymphoid cells, follicular necrosis and hyperplasia of the epithelium) for 35 days.

Broilers that received 4 mg/kg total aflatoxin B showed depletion of lymphoid cells, follicular necrosis and haemorrhages of the thymus, cloacal bursa and spleen (Mohamed & Mohamed, 2009). Broilers fed 0.2 mg/kg aflatoxin B_1 (Sakhare *et al.,* 2007) had depletion of lymphocytes in bursal follicles, necrosis, fibrous tissue proliferation in interfollicular spaces, as well as lack of significant changes in the histostructure of the organ after supplementation of the fed with a mycosorbent. Lower lymphoid cell density, degeneration and necrotic changes in lymphoid cells, lymphocytolysis and fibrous proliferation were also reported by Arulmozhi & Varghese (2011) in broilers fed 20, 40, 60, 80 and 100 μg/kg AFB_1 fed over 45 days.

The detoxication of aflatoxin-contaminated feeds is a principal task for poultry industry. The feed producers and researchers have developed efficient decontamination technologies (Lakkawar *et al.,* 2015). During the last two decades, the detoxicating ability of various adsorbents has been tested (Abo-Noag *et al.,* 1995). Adsorbents e.g. silicates, aluminosilicates, bentonites. Mycosorb etc. reduce or prevent the deleterious effects of aflatoxins in animal feeds. These compounds should be able to bind physically to chemical substances and to prevent their absorption (Santurio *et al.,* 1999). The mechanism of action of toxin binders consists in attrac-

tion of negatively charged mycotoxin molecule by the positively charged toxin binder molecule; thus, toxins are immobilised and excreted from the animal body (Kana *et al.,* 2014).

The addition of the tested toxin binder to the feed of Groups V and VI succeeded partly to counteract the reduced weight of the bursa of Fabricius (Valchev *et al.,* 2017) and the severity of observed pathomorphological lesions. Reduced severity of histological lesions and significant increase in relative weight in immunocompetent organs was reported in broiler chickens whose aflatoxin-contaminated feed was supplemented with and natural zeolite (clinoptilolite) (Ortatali & Oguz, 2001; Ortatatli *et al.,* 2005), bentonite (Indresh *et al.,* 2013); silicate (diatomaceous earth) (Lakkawar *et al.,* 2015) or the semi-herbal mycosorbent Toxiroak (Sakhare *et al.,* 2007). The absorbing capacity of the components of tested mycosorbent Mycotox NG (micronised yeast, bentonite and thymol) was previously confirmed by other authors (Manafi *et al.,* 2014; Haider al., 2015; Ologhobo *et al.,* 2015).

In conclusion, the supplementation of the feed of turkey broilers with AFB_1 either at 0.2 mg/kg or 0.4 mg/kg impaired the morphology of the cloacal bursa (degeneration, necrosis, lymphoid cell depletion). The addition of 0.5 g/kg mycosorbent (Mycotox NG) to the diet of birds supplemented with 0.2 or 0.4 mg/kg AFB_1 was found able to compensate partly the harmful effects of the aflatoxin on bursal histology. This was manifested with lower intensity of degenerative processes and lower lymphoid cell depletion rate.

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