

INVESTIGATION ON PANCREAS MORPHOLOGY IN TURKEY BROILERS WITH EXPERIMENTAL AFLATOXICOSIS B₁

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ABSTRACT

The aim of the present investigation was to evaluate the toxic effects of aflatoxin B₁ on pancreas morphology. Also, the possibility for prevention of toxic effects of AFB₁ by feed supplementation of a mycosorbent (Mycotox NB) was studied. Experiments were carried out with 60 7-day-old female turkey broilers (meat TM strain) divided into one control and five treatment groups (n=10): Group I – control (0 mg/kg AFB₁ not supplemented with Mycotox NG); Group II (0.5 g/kg Mycotox NG), Group III (0.2 mg/kg AFB₁), Group IV (0.4 mg/kg AFB₁), Group V (0.2 mg/kg AFB₁ and 0.5 g/kg Mycotox NG) and Group VI (0.4 mg/kg AFB₁ and 0.5 g/kg Mycotox NG). The duration of the experiments was 42 days. Histopathologically, the pancreatic changes in turkey broilers treated at 0.2 mg/kg AFB₁ comprised generalised vascular hyperaemia, interlobular oedema, initial disorganisation and disintegration of glandular acini. Birds treated at 0.4 mg/kg AFB₁ exhibited complete glandular acini disorganisation, haemorrhages mononuclear cell infiltrations as well as necrobiotic changes. The supplementation of the feed of groups V and VI with the tested toxin binder reduced the severity and frequency of observed histological lesions (slight interlobular oedema, the disintegration of acinar cells was insignificant and initial granular dystrophy of the cytoplasm of Langerhans islet cells).

Key words: aflatoxin B₁, pancreatic lesions, Mycotox NG, turkey broilers.

Introduction

The contamination of agricultural products with aflatoxins is a serious threat to human health (Reddy et al., 2010). Production of mycotoxins in cereal crops occurs in inadequate temperature and humidity both in the field in storehouses (Singh and Mandal, 2014). Mycotoxins are secondary toxic metabolites produced by moulds and are still causing huge economic losses (Pappas et al., 2016). Moulds from the genus *Aspergillus* are widely prevalent in the environment (air, soil, plants and organic matter) at a global scale. The three species outlined with their ability to produce highly toxic mycotoxins are *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Jia et al., 2016). *Aspergillus* genus, and especially *A. flavus* and *A. parasiticus* are the main producers of mycotoxins in poultry feeds and their ingredients (Saleemi et al., 2012; Khan et al., 2017). Four types of aflatoxins are found in naturally contaminated feeds (AFB₁, AFB₂, AFG₁ and AFG₂) (Tedesco et al., 2004). Among them, AFB₁ is the most toxic; its deleterious effects ranged from milk digestive troubles to carcinogenesis. AFB₁ has been identified as group 1 carcinogen by the International Agency for Research on Cancer (IARC). In animals and domestic fowl, AFB₁ is a potent hepatotoxic and nephrotoxic agent exhibiting immunosuppressive, mutagenic and teratogenic effects (Khan et al., 2014; Hassan et al., 2012). Aflatoxicosis is an important problem for the poultry industry due to its wide spread and high toxicity (Saleemi et al., 2015). Even amounts equal to parts of billion (ppb) in animal fodders could cause significant losses (Bhat and Vasanthi, 2003). The contamination of cereal crops with aflatoxins is a global problem, as every year, 25% of world cereals become contaminated (CAST,

1989). Aflatoxins decrease the resistance of birds to infectious and parasitic diseases and suppress post vaccination immunity (Diekman and Green, 1992).

Growing birds – ducklings, goslings, pheasant poults, quails and broiler chickens are the most sensitive to the toxic effects of aflatoxins (Diaz and Murcia, 2011; Lawal et al., 2014). The latter are extensively studied by evaluation of their teratogenic (Sur et al., 2003), mutagenic, carcinogenic and growth inhibiting effects (Oguz and Kurtoglu, 2000). Toxic effects on complete blood counts, blood biochemical (Basmacioglu, et al., 2005) and immunological parameter (Zaghini et al., 2005) as well as morphological changes in viscera (Ortattali and Oguz, 2001) are acknowledged. The toxic effects of aflatoxin B₁ on pancreatic morphology have been reported in previous studies of ours with mule ducklings and broiler chickens (Valchev et al., 2012; 2015).

Maximum levels of aflatoxins B₁, B₂, G₁, G₂ and M₁ in food stuffs are specified in commission regulation (EC) No 1881/2006 amended by commission regulation (EC) No 165/2010 and No 1058/2012. Maximum permissible levels are determined for aflatoxin B₁, aflatoxin M₁ and total aflatoxin (B₁, B₂, G₁ and G₂). As per EC legislation, in growing birds it should not exceed 20 ppb in complete feedingstuffs and 0.005 ppb in complementary feedingstuffs (Directive 2002/32/EC).

The prevention of the growth of moulds and contamination of feeds with aflatoxins is important, but when it is not possible, decontamination of aflatoxins should be preliminary done. Feed manufactures and researchers attempts to develop and efficient system of prevention and decontamination to reduce to a minimum the toxic effects of aflatoxins. Apart prevention, other management approaches include physical, chemical and biological methods for removal of aflatoxins from contaminated feeds. Most of them are of little practical value and are potentially dangerous due to the loss of nutritional value, high costs of equipment and formation of toxic residues or derivatives (Kabak et al., 2006). The proper approach to the problem includes the used of non-nutritional inert adsorbents which are added to the ration, bind aflatoxins and thus reduce their absorption from the gastrointestinal tract. Since the early 1990s, the experiments with adsorbents as zeolites and aluminosilicates turned out to be successful, but they have raised some concern when added at high levels and considering the possible potential interactions with feed nutrients (Philips, 1999; Rossa et al., 2001). Among the different tested methods and substances used for detoxication of animals are the utilisation of inert mycosorbent as hydrated sodium calcium aluminosilicate (HSCAS) (Jindal et al., 1993), zeolites (Miazzo et al., 2000), bentonite (Santurio et al., 1999), activated charcoal (Edrington et al., 1997) or live *Saccharomyces cerevisiae* yeasts (Aravind et al., 2003). These substances act by decreasing the bioavailability of mycotoxins in the blood and prevent their absorption in the intestines. Nevertheless, some of them decrease the bioavailability of dietary amino acids and/or minerals (Dawson, 1999).

The aim of the present investigation was to evaluate the toxic effects of aflatoxin B₁ on pancreas morphology in turkey broilers challenged with different dietary aflatoxin levels, either alone or combined with a mycosorbent (Mycotox NB).

Materials and methods

Experimental design. The experiment was performed with sixty 7-day-old female turkey broilers from the meat TM strain randomly divided into six groups (n=10).

Control and treated birds were fed standard feed according to the species and age, produced by a feed factory. The experimental design comprised the following groups: Group I received only control ration, while the feed of Groups II–VI was supplemented as followed: Group II: with

0.5 g/kg Mycotox NG (micronised yeasts, montmorillonite, thymol; Ceva Sante Animale, France); Group III: 0.2 mg/kg AFB₁; Group IV: 0.4 mg/kg AFB₁; Group V: 0.2 mg/kg AFB₁ and 0.5 g/kg Mycotox NG; Group VI: 0.4 mg/kg AFB₁ and 0.5 g/kg Mycotox NG.

Aflatoxin B₁ used in the experiment was produced by *Aspergillus flavus* (99% purity) and obtained from Sigma-Aldrich (Germany). Control and experimental turkey poults were kept under optimum microclimatic parameters according to Ordinance 44/2006.

After the end of the trial, all birds were euthanised by cervical dislocation as per 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).

Pancreas specimens for histology were fixed in 10% neutral formalin and embedded in paraffin after dehydration in ascending alcohol series. Paraffin blocks were cut on a Leica RM 2235 microtome. Cross sections of 3 µm were routinely stained with haematoxylin/eosin.

The experiment was approved by the Bulgarian Food Safety Agency – permit No 19218/06.11.2014.

Results

Histopathological evaluation of the pancreas in turkey broilers treated with 0.2 mg/kg AFB₁ demonstrated diffuse hyperaemia of blood vessels (Fig. 1), interlobular oedema (Fig. 2), signs of disintegration with initial disorganisation of glandular acini. The epithelial cells of the exocrine part of the pancreas had vacuolar degeneration and reduced number of secretory granules. The nuclei of most cells were of smaller size, hyperchromatic (karyopyknosis) and occasionally, cells with indistinct borders and poorly stained nuclei (karyolysis) were seen. In the endocrine part of the gland, the cells of islets of Langerhans exhibited also signs of cytoplasmic vacuolation and granular disintegration, and most of nuclei were with karyolysis (Fig. 3).

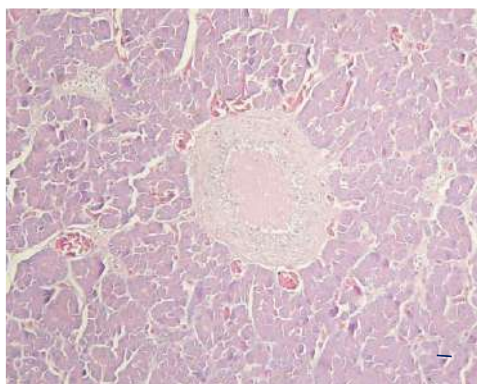


Figure 1: Diffuse vascular hyperaemia in turkey broilers treated with 0.2 mg/kg AFB₁. X/E. Bar 15 µm

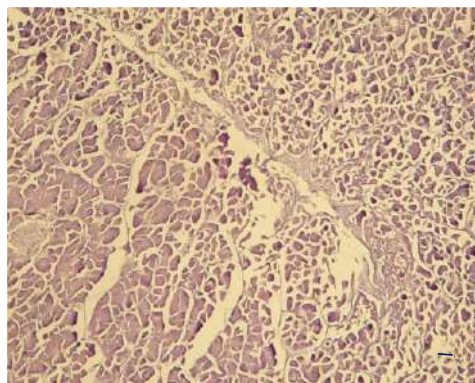


Figure 2: Interlobular oedema with disintegration of glandular acinar cells in turkey broilers treated with 0.2 mg/kg fodder AFB₁. X/E. Bar 15 µm

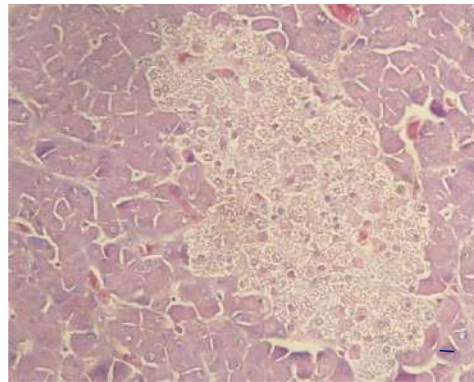


Figure 3: Vacuolation and granular disintegration of the cytoplasm, pyknosis and chromatolysis of some cell nuclei of the islets of Langerhans in turkey broilers treated with 0.2 mg/kg AFB₁. X/E. Bar 15 μm

The degenerative changes in the pancreas of turkey broilers treated with 0.4 mg/kg AFB₁, were very pronounced. Along with the complete disorganisation of glandular acini, necrobiotic to necrotic areas were observed (Fig. 4 and 5). At some areas, haemorrhages were also visible. Apart the vacuolar degeneration and granular disintegration of the cytoplasm and exocrine pancreatic cells, mononuclear cell infiltrations could be occasionally seen (Fig. 6).

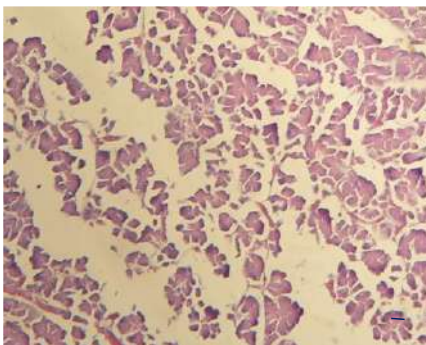


Figure 4: High-grade disorganisation of acinar pancreatic cells in turkey broilers treated with 0.4 mg/kg AFB₁. X/E. Bar 15 μm

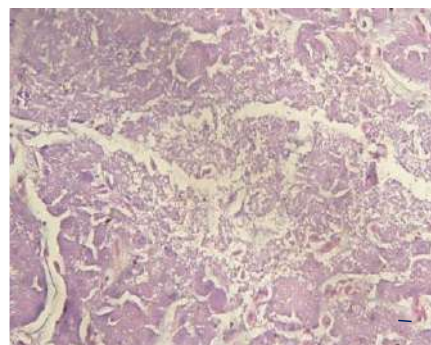


Figure 5: Necrobiotic to necrotic changes in glandular acini of the pancreas in turkey broilers treated with 0.4 mg/kg AFB₁. X/E. Bar 15 μm

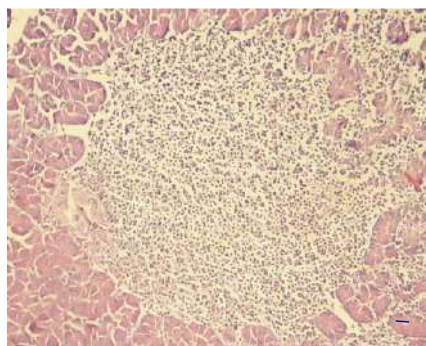


Figure 6: Round cell mononuclear infiltrations in exocrine pancreatic areas in turkey broilers treated with 0.4 mg/kg AFB₁. X/E. Bar 15 μm

The turkey broilers that received 0.2 mg/kg or 0.4 mg/kg AFB₁ combined with Mycotox NG, exhibited considerably milder changes in the cells of the pancreas than birds fed with aflatoxin B₁ alone at both doses and comprised mild interlobular oedema, diffuse hyperaemia /Fig. 6/ and initial granular degeneration of the cytoplasm of Langerhans islets' cells (Fig. 7, 8 and 9).

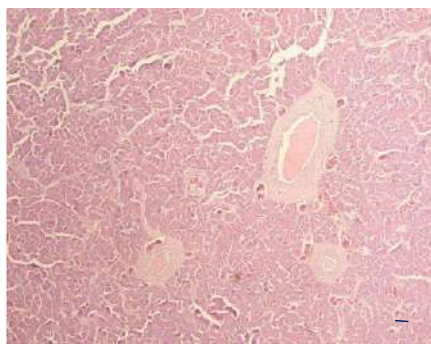


Figure 7: Vascular congestion and hyperaemia in turkey broilers treated with 0.2 mg/kg AFB₁ and Mycotox NG were less intense compared to birds from experimental groups III and IV. X/E. Bar 20 µm

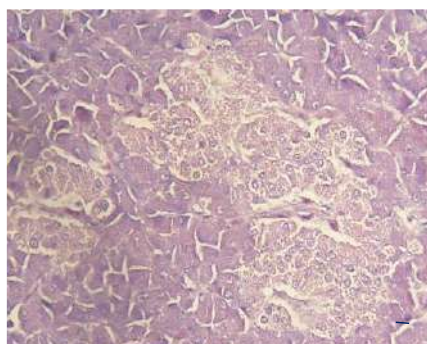


Figure 8: Initial granular dystrophy of the cytoplasm of cells of islets of Langerhans in turkey broilers treated with 0.2 mg/kg AFB₁ and Mycotox NG. X/ E. Bar 15 µm

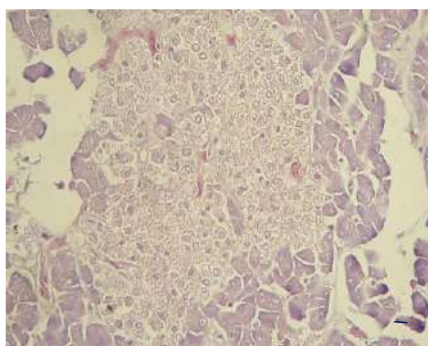


Figure 9: Degeneration in the cytoplasm and nuclei in cells of islets of Langerhans were less pronounced in in turkey broilers treated with 0.4 mg/kg AFB₁ and Mycotox NG as compared to in turkey broilers from experimental group IV and were demonstrated with cytoplasmic vacuolation and pyknosis of nuclei. X/E. Bar 15 µm

Control turkey broilers and birds from the group fed only 0.5 g/kg Mycotox NG did not show any histopathological deviations in pancreas morphology.

Discussion

The pancreas is an important organ involved in the utilisation of carbohydrates, proteins and lipids through its enzymes and hormones. The disturbances in the pancreatic function could directly influence the quality of life and productive performance of animals (Simsek et al., 2007).

Impaired morphology of the pancreas has been observed in quails that received 2.5 or 5.0 mg/kg total aflatoxin (AF) throughout 3 weeks (Simsek et al., 2007). Light microscopy study of the pancreas showed vacuolar degeneration of epithelial cells and mononuclear cell infiltrations in exocrine pancreas in the group fed 2.5 mg/kg AF. For comparison, the groups supplemented with

5.0 mg/kg AF in the feed had significantly more severe lesions (necrotic foci). The reported morphological changes were comparable to those observed by us.

Morphological changes of the pancreas were in line with those seen in broiler chickens whose feed was supplemented with 0.5 mg/kg T-2 toxin (infiltration with mononuclear cells, vacuolar degeneration and reduced number of secretory granules) (Krishnamoorthy, et al., 2007).

The haemorrhages and congestion in the pancreas occurring after experimental reproduction of aflatoxicosis in quails (Jakhar and Sadana 2004) corresponded to morphological changes demonstrated in experimental groups III and IV, treated with 0.2 mg/kg or 0.4 mg/kg AFB₁. Haemorrhages, congestion, necrotic foci and mononuclear cell infiltration in the pancreas were also reported in pigs treated with 100 µg/kg toxin over 16 weeks (Elias et al., 2005).

In an experiment with broiler chickens whose fodder was contaminated with 1 mg/kg AFB₁ and 20 mg/kg cyclopiazonic acid (CPA), alone or together from hatching to 28 days of age, Kumar and Balachandran (2009) found out depletion of secretory granules in pancreatic acinar cells, dissociation, acinar disorganisation, necrosis, mononuclear cell infiltration. Similar morphological results, apart aforementioned ones – vascular hyperaemia, interlobular oedema, karyopyknosis, karyolysis, vacuolar degeneration, necrobiotic to necrotic areas and mononuclear cell infiltrations – were present in this study in turkey broilers fed increasing amounts of AFB₁ (0.2 or 0.4 mg/kg). The severity of observed histopathological changes in this experiment depended on the tested AFB₁ amount.

Morphological changes observed in experimental groups III and IV (vacuolar degeneration and degenerative changes in the epithelial cells of glandular acini) were similar to those reported in ducks (Agag, 2004).

Some plant mycosorbents as ginger (Abd El-Haleemet al., 2011) were able to adsorb mycotoxins and to reduce the toxic effect of AFB₁ on pancreas morphology (hyperaemia, interlobular oedema, vacuolar and granular degeneration). Similar histological changes were found out in the present study in turkey broilers treated with 0.2 or 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG.

The observed morphological changes in pancreatic acinar cells induced by AFB₁ could be attributed to its inhibition of protein synthesis, formation of DNA, RNA and protein adducts, inhibited RNA synthesis through binding to DNA-dependent RNA polymerase, degranulation of endoplasmatic reticulum: all these are mechanisms that alter the structure of a number of tissues (liver, kidneys, skeletal muscle, heart, pancreas etc.) (Sharma et al., 2011). The observed morphological changes in the pancreas could be explained with ultrastructural changes in mitochondria caused by aflatoxins, leading to reduction of their function and apoptosis (Vermeulen et al., 2003; Hornsby, 2007). Also, aflatoxins decrease the length of telomeres in chromosomes and consequently, impair the normal cellular cycle and normal tissue morphology (Hornsby, 2007). The formation of reactive oxygen species (ROS) under the toxic influence of AFB₁ occurs mainly in mitochondria of hepatocytes and renal epithelial cells and damages important biomolecules as DNA, proteins and lipids (Hwang and Kim, 2007). It is known that aflatoxins enhance lipid peroxidation (LPO) and indices cellular damage with impairment of the normal morphology of parenchymal organs (Verma and Chakraborty, 2008; Darwish et al., 2011).

Domestic turkeys are highly sensitive to the toxic effects of AFB₁. The bioactivation of aflatoxins is done in hepatocytes via microsomal enzyme systems – cytochrome P450 (CYP450) to reactive aflatoxin-8,9-epoxide (AFBO), the primary and most toxic metabolite. This reactive

metabolite inhibits protein synthesis from one part, binds to DNA and RNA and consequently, damage parenchymal organs, induces immunosuppression and deteriorates production traits (Rawal et al., 2010). In domestic turkeys, liver glutathione S-transferases alpha (GSTA) are not capable for AFBO detoxication, which is probably the main reason for their high sensitivity.

Conclusion

In conclusion, the supplementation of increases doses of AFB₁ at 0.2/0.4 mg/kg to compound poultry feed induces disturbed pancreatic morphology (vascular hyperaemia, interlobular oedema, dissociation and disorganisation of glandular acini, vacuolar dystrophy, karyopyknosis and karyolysis of epithelial cells' nuclei, haemorrhages, mononuclear cell infiltrations, necrobiotic to necrotic changes). In this experiment, the addition of 0.5 g Mycotox NG per 1 kg of the ration containing 0.2 mg/kg or 0.4 mg/kg AFB₁, exerted a reducing effect on histological pancreatic lesions attributed to aflatoxicosis (hyperaemia, milk interlobular oedema, early granular degeneration).

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