EFFECTS OF EXPERIMENTAL AFLATOXICOSIS ON RENAL FUNCTION IN BROILER CHICKENS

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Summary


The morphological changes in kidneys and some blood parameters of the renal function were followed out in broilers chickens with experimental aflatoxicosis. The possibility for prevention of the toxic effects of aflatoxin B₁ (AFB₁) through supplementation of feed with the mycosorbent Mycotox NG was also investigated. The experiments were conducted with five groups of ten 7-day-old Cobb broiler chickens in each. The formed groups were as followed: group I – control, fed a standard compound feed; group II – experimental, whose feed was supplemented with 1 g/kg Mycotox NG, group III – experimental, receiving 0.5 mg/kg AFB₁; group IV – experimental, receiving 0.8 mg/kg AFB₁ and group V – experimental, supplemented with 0.5 mg/kg AFB₁ and 1 g/kg Mycotox NG. The trial’s duration was 42 days. Blood samples for analysis were collected on days 21 and 42. The results showed increased urea, creatinine and uric acid levels, as well as reduction in blood calcium, inorganic phosphate, sodium and potassium concentrations in groups III and IV. There were no morphological changes in the renal parenchyma of chickens from group I and II. In chickens from group III, the renal parenchyma showed cloudy swelling and vacuolated cytoplasm of tubular epithelial cells. Chickens from experimental group IV exhibited stronger desquamation of tubular epithelial cells, necrotic and necrobiotic changes and haemorrhages. The supplementation of poultry feed with Mycotox NG (group V) decreased the deviations in blood changes, as well as the incidence and severity of histological lesions (hyperaemia, epithelial cell disintegration and glandular dystrophy.

Key words: aflatoxin B₁, calcium, creatinine, kidneys, Mycotox NG, phosphorus, urea, uric acid

INTRODUCTION

Mycotoxins are secondary toxic metabolites with low molecular weight, produced by members of various mould genera (Aspergillus, Penicillium, Claviceps). They possess a broad spectrum of biological activity comprising carcinogenic, terato-
Aflatoxins are toxic phytopathogens produced by *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* fungi. They grow on a variety of nutritional substrates (cereals, leguminaceous plants, fruits) (Hagler et al., 1984; Banerjee, 2010). The contamination of cereal crops with aflatoxins could occur at each stage of the feed chain: in the field before harvest, during the harvest, during the transportation, technological processing and storage of grain (Giray et al., 2007). Aflatoxins possess teratogenic, mutagenic and carcinogenic effects (Wild et al., 2000; Sur & Celik, 2003). On the basis of their chemical structure, chromatographic and fluorescence features, they are divided into subtype B (B1 and B2) and subtype G (G1 and G2) (Pier, 1992). *Aspergillus flavus* and *A. parasiticus* produce aflatoxins within ambient temperature range between 24 and 35 ºC, air humidity over 7% and feed humidity over 14% (Santos et al., 2001). The amount of AFB1 in naturally contaminated feeds varies from 0 to 30 μg/kg, and that of total aflatoxin (AFB1, AFB2, G1 and G2) – from 0 to 50 μg/kg (FAO, 1995). According to EC legislation and the USA Food and Drug Agency, the total amount of aflatoxins in poultry feeds should not exceed 20 ppb (EEC, 1991).

Aflatoxins cause substantial economic losses to poultry industry, impeding the proper growth of birds, increasing feed conversion ratio and mortality, as well as reducing feed utilisation and egg production (Miazzo et al., 2000; Oguz & Kurtoglu, 2000). They are reported to cause anaemia (Oguz et al., 2000), suppressed immunity (Oguz et al., 2003), impaired liver and kidney function (Abdel-Wahhab et al., 2002; Mohamed & Mohamed, 2009; Zhao et al., 2010; Yildirim et al., 2011) and altered relative weights of viscera (Kubena et al., 1998; Ortatatli & Oguz, 2001; Sakhare et al., 2007; Yildirim et al., 2011).

Aflatoxins disturb the renal function through increasing the relative weight of kidneys (Quezada et al., 2000), inducing congestion in renal sinusoids (Hussain et al., 2008), degenerative and necrotic changes in renal tubular epithelium (Mollenhauer et al., 1989; Ortatatli & Oguz, 2001; Hussain et al., 2008; Yildirim et al., 2011) and reduce the glomerular filtration rate (Glahn et al., 1991). The toxic effects of AFB1 on blood biochemical parameters are exhibited through reduced concentrations of calcium, inorganic phosphate, sodium and potassium and increase in urea, creatinine and uric acid (Kubena et al., 1998; Sakhare et al., 2007; Soliman et al., 2008; Umar et al., 2012).

The purpose of the present study was to follow out the changes in some blood biochemical parameters specific for renal function, as well as disorders in kidney morphology after experimental aflatoxicosis B1 in broiler chickens. Additionally, the study aimed at testing the possibility for prevention of toxic effects of AFB1 by supplementation of poultry feed with the mycosorbent Mycotox NG (Ceva Sante Animale, France).

**MATERIALS AND METHODS**

The experiments were approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Trakia University (permit No. 49/29.09.2012).

Fifty 7-day-old Cobb broiler chickens from both genders were divided into five groups of ten birds each:
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- Group I – control (fed balanced compound feed according to the age produced by Provimi feed plant, Stara Zagora);
- Group II – experimental – the feed of birds was supplemented with 1 g/kg Mycotox NG (Ceva Santé Animale, France);
- Group III – experimental – the feed of birds was supplemented with 0.5 mg/kg aflatoxin B1;
- Group IV – experimental – the feed of birds was supplemented with 0.8 mg/kg aflatoxin B1;
- Group V – experimental – the feed of birds was supplemented with 0.5 mg/kg aflatoxin B1 and 1 g/kg Mycotox NG.

The tested aflatoxin B1 was produced by Aspergillus flavus (99% purity) and purchased by Sigma-Aldrich, Germany. All birds were housed under optimum microclimatic parameters, identical for all groups, with compliance with Ordinance 44/2006 (Anonymous, 2006).

Blood samples were collected from v. metatarsalis medialis on experimental days 21 and 42 in sterile heparinised vacutainers (FL medical, Italy) for analysis of urea, creatinine, uric acid, calcium, phosphorus, sodium and potassium concentrations. Within 30 min after blood collection, blood samples were centrifuged at 1,500×g for 10 min. Plasma were harvested and stored at −20 °C until analysis. All biochemical analytes were assayed on an automated biochemical analyser BS–120, Mindray, China.

After the end of the experiment, control and treated chickens were euthanised by cervical dislocation. Kidney specimens for histological examination were fixed in 10% formalin and processed by routine histological methods. Specimens were embedded in paraffin blocks, 5 μm sections were cut on a Leica RM 2235 microtome and stained with haematoxylin/eosin.

Results were statistically processed by one-way analysis of variance and the Tukey-Kramer post hoc test (level of significance P<0.05).

RESULTS

Biochemical analyses

Table 1 presents the effects of AFB1 and Mycotox NG applied either independently or in combination, on blood urea, creatinine and uric acid concentrations after 21 and 42 days. Blood urea in chickens which received AFB1 at 0.5 and 0.8 mg/kg (groups III and IV) was statistically significantly higher on day 21 (by 20.73% and 42.39%) and day 42 (by 33.4% and 48.75%) compared to control group (P<0.001).

In chickens from groups III and IV, blood creatinine was considerably higher (P<0.001) on the 21st day (by 31.8% and 40.4%) vs untreated chickens. By the 42nd day, the observed changes became more pronounced with higher creatinine levels by 49.73% (group III) and by 56.41% (group IV) (P<0.001 vs controls).

Uric acid concentrations exhibited a similar pattern of change with more marked changes on day 21: increase by 37.65% and 54.14% in groups III and IV, respectively. Then the values declined and were higher by 17.54% in group III and by 26.07% in group IV vs group I (P<0.01–P<0.001).
Table 1. Effect of aflatoxin B1 (AFB1) only or co-administered with Mycotox NG on blood plasma urea, creatinine and uric acid in broiler chickens. Group I – control; group II – Mycotox NG; group III – 0.5 mg/kg AFB1; group IV – 0.8 mg/kg AFB1; group V – 0.5 mg/kg AFB1 + Mycotox NG. Data are presented as mean ± SEM; n=10

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea, mmol/L</th>
<th>Creatinine, µmol/L</th>
<th>Uric acid, µmol/L</th>
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<tbody>
<tr>
<td></td>
<td>Days of age</td>
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<tr>
<td></td>
<td>21</td>
<td>42</td>
<td>21</td>
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<tr>
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<td>4.43±</td>
<td>3.49±</td>
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<td></td>
<td>0.12</td>
<td>0.10</td>
<td>1.27</td>
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<tr>
<td>II</td>
<td>4.33±</td>
<td>4.40±</td>
<td>3.54±</td>
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<td>1.47</td>
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<tr>
<td>III</td>
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<td>4.60±</td>
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<td></td>
<td>0.19±</td>
<td>0.20±</td>
<td>1.43±</td>
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<tr>
<td>IV</td>
<td>6.18±</td>
<td>6.59±</td>
<td>4.90±</td>
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<td></td>
<td>0.18±</td>
<td>0.19±</td>
<td>2.13±</td>
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<tr>
<td>V</td>
<td>4.64±</td>
<td>4.88±</td>
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<td></td>
<td>0.14±</td>
<td>0.17±</td>
<td>1.46±</td>
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* Level of significance: *P<0.05; *P<0.01; *P<0.001; 1 – vs control group I; 2 – vs group II; 3 – vs group III; 4 – vs group IV.

Table 2. Effect of aflatoxin B1 (AFB1) only or co-administered with Mycotox NG on blood plasma calcium, inorganic phosphate, sodium and potassium, creatinine and uric acid in broiler chickens. Group I – control; group II – Mycotox NG; group III – 0.5 mg/kg AFB1; group IV – 0.8 mg/kg AFB1; group V – 0.5 mg/kg AFB1 + Mycotox NG. Data are presented as mean ± SEM; n=10

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Inorganic phosphate, mmol/L</th>
<th>Sodium, mmol/L</th>
<th>Potassium, mmol/L</th>
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<tr>
<td></td>
<td>Days of age</td>
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<td>21</td>
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<tr>
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<td>0.11</td>
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<td>0.06</td>
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<tr>
<td>III</td>
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<td>1.80±</td>
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<td>0.04±</td>
<td>0.05±</td>
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<tr>
<td>V</td>
<td>2.77±</td>
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<td>0.10±</td>
<td>0.06±</td>
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* Level of significance: *P<0.05; *P<0.01; *P<0.001; 1 – vs control group I; 2 – vs group II; 3 – vs group III; 4 – vs group IV.

Blood calcium concentrations (Table 2) were substantially lower at both studied time intervals: by 16.02% and 28.47% on day 21 and by 24.82% and 30.83% on day 42 (P<0.05–P<0.001). Blood inorganic phosphate levels were also reduced by 14.52% (P<0.01) in group III and by 16.54% in group IV (P<0.001) as compared to the untreated group. On day 42, observed changes were more pronounced...
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with values lower by 14.47% and 23.41% in the groups treated with 0.5 and 0.8 mg/kg AFB₁, respectively (P<0.001).

In groups III and IV, sodium concentrations (Table 2) declined statistically significantly on day 21 (by 4.81% and 7.15%) and day 42 (by 10.46% and 14.74%) compared to group I (P<0.001). Chickens that received aflatoxin only in their feed, exhibited also considerably lower blood potassium concentrations on both sampling periods – day 21 (by 12.29% and 19.8%) (P<0.05 – P<0.001) and day 42 (by 14.48% and 23.57%), vs controls (P<0.001).

There were statistically significant differences in the values of studied blood biochemical parameters between group I (control) and group II (supplemented with 1 g/kg Mycotox NG). In chickens of group V, the deleterious effects of AFB₁ on studied parameters were prevented by Mycotox NG co-administration. At the same time, the severity of morphological changes in kidneys was reduced.

Morphological studies

The kidney parenchyma of birds treated with 0.5 mg/kg AFB₁ exhibited generalised hyperaemia, cloudy swelling, and cytoplasmic vacuolation of tubular epithelial cells. Some of cells were disintegrated and desquamated from the basement membrane (Fig. 1).

In chickens treated with the higher dose (0.8 mg/kg AFB₁), the dystrophic changes of kidneys were more intensive. The epithelial cells of all tubules were desquamated and were within their lumens. At some locations, necrobiosis to necrotic foci, impregnated with erythrocytes (haemorrhages) could be observed (Fig. 2).

Chickens treated with 0.5 mg/kg AFB₁ and Mycotox NG showed a significantly
milder dystrophy of renal parenchyma cells compared with chickens from the groups treated with either 0.5 mg/kg or 0.8 mg/kg AFB1. No haemorrhages were observed, only vascular hyperaemia and disintegration of tubular cells, some of them exhibiting granular dystrophy (Fig. 3).

Histopathological changes in renal parenchyma were not detected in chickens from the untreated group, as well as in birds receiving 1 g/kg Mycotox NG in their feed.

DISCUSSION

Kidneys are the major excretory organs and about 20–25% of the total amount of circulating toxins, including mycotoxins, reach them (Harriet, 2003). The demands of kidneys for nutrients and oxygen are high because of their extensive functional load. Approximately one-third of the total blood volume is filtered through kidneys and at the same time, 98–99% of systemic water and sodium chloride are reabsorbed. The different parts of nephrons are exposed to the toxic effect of AFB1 and its metabolites before being excreted, resulting in nephrotoxicity (Coulombe, 1994; Sharma et al., 2011). In birds exposed to the toxic effect of AFB1, haemorrhagic and fatty kidney syndrome, thickening of glomerular membranes, degenerative changes in renal epithelial cells and congestion of renal parenchyma are reported (Mollenhauer et al., 1989; Coulombe, 1994; Hussain et al., 2008). At the same time, the toxic effects of AFB1 on glomerular membranes and renal tubules in rats consisted in reduced glomerular filtration rate, glucose reabsorption, tubular electrolyte and organic ion transportation rate (Manseld Grunert & Kautna, 1989).

The morphological changes in kidneys observed in the present study (parenchymal degeneration of distal tubules, characterised with epithelial cell swelling, cytoplasmic granulation, vacuole degeneration and epithelial cell desquamation) were similar to those occurring in layer hens fed aflatoxin B1–contaminated feed (Ortattati & Oguz, 2001; Dhanasekaran et al., 2009). In an experiment with broiler chickens, Yildirim et al. (2011) evaluated the effects of supplementing the compound feed with 2 mg/kg total aflatoxin (AFB1, AFG1, AFB2 and AFG2) without or with mycosorbent (Mycosorb) on renal parenchyma histological changes. They consisted in basement membrane thickening, hyaline droplets in epithelial cells, presence of cylinders in tubular lumens and epithelial cell dystrophy. The authors established that damaged excretory renal function was accompanied by increased blood urea in birds, whose feed was supplemented with AF. Sakhare et al. (2007) observed that the dietary intake of 0.2 mg/kg AFB1 from broiler chickens resulted in dystrophic changes in epithelial cells of renal tubules, karyopyknosis, detachment of epithelial cells from the basement membrane, renal parenchyma haemorrhages and necrosis. Mohamed & Mohamed (2009) demonstrated round cell and neutrophil infiltration, basement membrane and Bowman capsule thickening, coagulation necroses and dystrophic lesions in renal epithelial cells (karyorrhexis and karyopyknosis). In broiler chickens with experimental aflatoxicosis, hyperaemia and focal mononuclear leukocytic infiltration of the intertubular tissue was reported (EL-Lethy & El-Zorb, 2004). Tubular degeneration was observed in broiler chickens, whose feed was supplemented with 0.1 mg/kg total aflatoxin up to 42 days of age (Ortattati et al., 2005). The AFB1–induced generation of reactive oxygen species (ROS) occurs
mainly in the mitochondria of hepatocytes and renal epithelial cells and results in damage of important biomolecules as DNA, proteins and lipids (Hwang & Kim, 2007). Aflatoxins increase lipid peroxidation in liver and kidney tissues and induce cellular damage causing impaired morphology of organs (Verma & Chakraborty, 2008; Darwish et al., 2011). Despite that AFB$_1$ is eliminated mainly through the kidneys, the accumulation of a relatively high concentration of toxin impairs the excretory function and leads to congestion with subsequent morphological disorders (Glahn et al., 1991). Low activity of glutathione-S-transferases involved in conjugation, detoxication and excretion of aflatoxins is reported in birds. The deficiency or the total lack of functional activity of these enzymes with affinity for binding to the major AFB$_1$ metabolite (AFBO) in birds is the mechanism of renal toxic effect of aflatoxins during their excretion (Klein, 2000). Aflatoxins inhibit the protein synthesis, forming adducts with DNA, RNA and proteins, inhibit RNA synthesis via binding DNA-dependent RNA polymerase, degranulate endoplasmatic reticulum and thus, cause a number of alterations in many organs – liver, kidneys, skeletal muscles and heart (Wangikar et al., 2005; Mohammed & Metwally, 2009; Sharma et al., 2011).

Creatinine is the final metabolite of creatine conversion and a major marker of kidney function. Increased blood serum creatinine in AFB$_1$-treated mice indicates enhanced conversion of muscle phosphocreatine to creatinine, therefore, for reduced utilisation of phosphocreatine for muscle contractions. When the kidney is functioning normally, creatinine is rapidly excreted. According to Mathura & Verma (2008) increased blood plasma creatinine in mice treated orally with 750 or 1500 μg/kg total aflatoxin over 45 days was possibly due to enhanced release by muscles and/or reduced renal excretion (Mathura & Verma, 2008). Increased urea and creatinine as indices of impaired kidney function in aflatoxicosis were reported in chickens and rats (Kubena et al., 1998; El-Letey & El-Zorb, 2004; Mathuria & Verma, 2008; Mohamed & Mohamed, 2009; Yildirim et al., 2011; Hassan et al., 2012). The cause for elevated urea concentrations and higher weight of kidneys is the nephrotoxicity of AFB$_1$ (Kubena et al., 1998). Increased blood urea and creatinine in broiler chickens whose feed was supplemented with 3 mg/kg AFB$_1$ from 2 to 6 weeks of age (Umar et al., 2012) suggest inflammatory and dystrophic processes in renal tubules (Benjamin, 1978). It is assumed that higher urea and creatinine levels are due to disturbed transportation function of epithelial cells in collecting tubules and diffuse impairment of proximal tubules’ function (Ortatli et al., 2005; Umar et al., 2012). According to other researchers, the changes in blood urea and creatinine could be secondary to necrotic changes in renal parenchyma (Pier, 1987; Manseld Grunert & Kautna, 1989; Jindal & Mahipal, 1994; Fung & Clark, 2004; Hanif et al., 2006; Sakhare et al., 2007; Mohamed & Mohamed, 2009).

Uric acid is the primary end product of protein metabolism in birds. It is synthesized in the liver and excreted through the kidney tubules. Increased blood uric acid concentrations along with increased urea and creatinine suggest impaired kidney function in both birds and mammals (Hochleithner, 1994). Increased blood uric acid and creatinine is reported in egg-laying chickens whose feed was supplemented with 0.5 ppm AF over 12 weeks after hatching (Gounalan et al., 2006).
Comparable results are demonstrated by other research teams in broiler chickens after dietary treatment with aflatoxin B₁ (EL-Lethy & El-Zorb, 2004; Sakhare et al., 2007; Mohamed & Mohamed, 2009). Increased blood uric acid and creatinine occurred in broiler chickens receiving 50, 150 and 300 mg/kg aflatoxin in their feed over 42 days (George et al. 2006). A partly reduction of the toxic effects on kidney function was achieved by adding mycosorbents to contaminated feeds (Sakhare et al., 2007; Soliman et al., 2008; Yildirim et al., 2011).

Lower blood calcium and inorganic phosphate levels are reported in the studies of Fernandez et al., (1994) in stock layers and broiler chickens by Harvey et al. (1993); Bailey et al. (1998); Kubena et al. (1998); Stanley et al. (2004), Safameher (2008); Zhao et al. (2010). They were attributed to renal tubular damage or reduced intestinal absorption of calcium and phosphorus, lower blood parathormone circulation rate, impaired metabolism or lower renal sensitivity to vitamin D (Glahn et al., 1991; Yildirim et al., 2011). The severity of kidney lesions in aflatoxicosis caused disturbances in calcium and phosphate homeostasis (Soliman et al., 2008; Yildirim et al., 2011). The kidney glomerular apparatus is the target of the renal toxic effects of aflatoxins (Glahn et al., 1991). The addition of mycosorbent (clinoptilolite) to aflatoxin-contaminated poultry feed was able to increase phosphate concentrations at a certain extent (Oguz et al., 2000). The reduction of blood levels of macroelements could be attributed to nephrotoxic effects of aflatoxins and confirmed by renal histological changes (Soliman et al., 2008).

In conclusions, the presented results demonstrated that the intake of rations contaminated with AFB₁ by broiler chickens over 42 days induced histopathological changes in kidneys. The disturbed renal function triggered changes in studied biochemical blood parameters. In this investigation, the addition of 1 g/kg Mycofix NG to poultry feed contaminated with 0.5 mg/kg AFB₁ was able to prevent the alterations in studied parameters and to alleviate the severity of histological lesion caused by aflatoxicosis.

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