EFFECT OF AFLATOXIN B₁ ALONE OR CO-ADMINISTERED WITH MYCOTOX NG ON THE RENAL FUNCTION AND MORPHOLOGY OF TURKEY BROILERS

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Summary

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The changes in relative weight of kidneys, blood plasma uric acid, urea, creatinine, calcium and inorganic phosphate, and the morphology of kidneys were investigated in turkey broilers with experimentally induced aflatoxicosis B₁ (AFB₁) treated or not with a mycosorbent (Mycotox NB). 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 changes in blood parameters were assayed on day 21 and 42. Blood analysis in groups III and IV on day 21 showed increased urea and creatinine concentrations and reduction in blood uric acid, calcium and inorganic phosphate. These changes tended to become more pronounced on day 42. The relative weight of kidneys was increased in groups III and IV after the end of the experiment. Morphological alterations of renal parenchyma in Groups I and II were not present. In birds from group III, desquamation of epithelial cells from the basement membrane, dilated glomerular subcapsular space and round-cell proliferation were noted. In Group V, these changes were far more severe and comprised granular and fatty dystrophy, karyolysis and plasmolysis, necrobiotic to necrotic processes, haemorrhages, congestion. The supplementation of the feed of groups V and VI with the tested toxin binder reduced the severity of reduction of kidneys' relative weight, magnitude of changes in blood parameters, and the frequency and severity of histological lesions.

Key words: aflatoxin B₁, turkey broilers, kidneys, Mycotox NG

INTRODUCTION

Mycotoxins are low-molecular secondary toxic metabolites of some filamentous

fungi as *Aspergillus*, *Fusarium* and *Penicillium*. The commonest mycotoxins in

poultry compound feeds are aflatoxins, fumonisins, ochratoxin A, trichothecenes and zearalenone (Katole *et al.*, 2013).

So far, more than 500 mycotoxins are identified, and aflatoxins are among the most toxic causing considerable economic losses to poultry industry and health problems due to the frequent contamination of feeds (Chand *et al.*, 2011). Feed contamination with aflatoxins is a global concern for agricultural producers. Aflatoxins (AF) are heterocyclic toxic metabolites of fungi from the genus *Aspergillus* (*Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*) (Shlej *et al.*, 2015) and some species of genera *Penicillium*, *Rhizopus*, *Mucor* and *Streptomyces* (Verma *et al.*, 2004).

The four most important forms from the 18 identified aflatoxins found in naturally contaminated feeds are AFB₁, AFB₂, AFG₁ and AFG₂. Aflatoxins are distinguished on the basis of their chemical structure, chromatographic and fluorescence behaviour: aflatoxins B₁ and B₂ emit blue fluorescence, while G₁ and G₂ emit green fluorescence (Pier, 1992). Aflatoxin B₁ is the most frequently occurring aflatoxin in naturally contaminated feeds and at the same time, the most toxic for domestic fowl and animals as well as men. AFB₁ accounts for 75% of all aflatoxins in naturally contaminated animal feeds and foodstuffs for human consumption (Saad & Abdel-Fattah, 2008). The toxicity of AFG₂, AFB₂ and AFG₁ is equal to 10%, 20% and 50% of AFB₁ toxicity (Leeson *et al.*, 1995). Cereal crops (corn, wheat, sorghum, millet and rice), some oily crops (soybean, peanuts, sunflower, cotton), milk and dairy products are the most commonly contaminated foodstuffs consumed by humans and animals (Lopez *et al.*, 2002). In an EC report, 30% of cereals and agricultural products were con-

taminated with mycotoxins, and 90% of contaminated products – with aflatoxins (Moss, 1996).

The sensitivity of animals to aflatoxins depends on the species and age. Ducklings and turkey poult are more susceptible to toxic effects of aflatoxins than other birds species (Kamalzadeh *et al.*, 2009). Previous reports have documented deleterious AFB₁ effects on the health of birds including compromised performance (Oguz & Kurtoglu, 2000; Magnoli *et al.*, 2011); altered relative weight of visceral organs (Abousadi *et al.*, 2007; Valchev *et al.*, 2017); liver and kidney lesions (Ortatatli *et al.*, 2005; Ekhlal, 2012; Valchev *et al.*, 2014a,b); altered morphology of immunocompetent organs (Ekhlal, 2012; Valchev *et al.*, 2015); immunosuppression (Marin *et al.*, 2003; Sakhare *et al.*, 2007; Valchev *et al.*, 2014c), changes in haematological and blood biochemical parameters (Allameh *et al.*, 2005; Sakhare *et al.*, 2007; Valchev, 2015; Zhao *et al.*, 2010); increased infectious and parasitic morbidity rates (Oguz *et al.*, 2003).

The amount of mycotoxins in poultry feeds is controlled by various methods e.g. use of feed ingredients maintaining low aflatoxin levels, storage under proper conditions or use of toxin binders. One of the most appropriate methods for detoxication of mycotoxin-contaminated feeds is the use of non-nutritional toxin binders that bind toxins and inhibit their absorption from the gastrointestinal tract, and thus, minimise their toxic effects on animals and their occurrence in animal organs and animal foodstuffs (Oguz, 2012). A good toxin binder should be able to restore the nutritional value of aflatoxin-contaminated feeds. Its quality is assessed by four main parameters: binding capacity, absorption efficiency, activation time and binding extent (Van Kessel & Hiang-

Chek, 2004). The process of aflatoxin binding by mycosorbents is based on the electric polarity principle. The negative electrical charge of the mycotoxin is attracted by the positive charge of the mycosorbent and thus, mycotoxins are immobilised and subsequently, removed from the animal body. The effects of toxin binders as aluminosilicates, active charcoal and yeasts are extensively studied (Mabbet, 2005; Oguz, 2012).

The aim of this study was to investigate the toxic effects of experimental aflatoxicosis B₁ on kidney function and morphology in turkey broilers whose feed was supplemented or not with the mycosorbent Mycotox NB.

MATERIALS AND METHODS

Experimental design

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).

All birds were fed a complete compound feed according to their age category. The experimental design comprised: Group I – control; Group II – whose feed was supplemented with 0.5 g/kg Mycotox NG (micronised yeasts, montmorillonite, thymol); Group III – whose feed contained 0.2 mg/kg aflatoxin B₁; Group IV – whose feed contained 0.4 mg/kg aflatoxin B₁; Group V – supplemented with 0.2 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG; Group VI – supplemented with 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG.

The aflatoxin B₁ used in this experiment was produced by *Aspergillus flavus* (99% purity) purchased from Sigma-Aldrich, Germany. All turkey poults were kept under optimum microclimatic parameters according to Ordinance 44/2006 (Anonymous, 2006).

Blood samples were collected from *v. metatarsalis medialis* on post treatment days 21 and 42 in sterile heparinised containers (FL medical, Italy) for assay of urea, creatinine, uric acid, calcium and inorganic phosphate levels. Within 30 min after blood collection, samples were centrifuged for 10 min at 1500×g and then stored at –20 °C until the analysis on an automated biochemical analyser (BS-120, Mindray, China). Sampling days (day 21 and 42) were selected according to literature data of other researchers having studied the toxic effects of aflatoxin B₁.

After the end of the experiment, control and experimental turkeys were euthanised by cervical dislocation. Kidney specimens for histological examination were fixed in 10% neutral formalin and embedded in paraffin after dehydration in ascending ethanol series. Paraffin blocks were cut on a Leica RM 2235 microtome, section width was 5 µm. Cross sections were stained routinely with haematoxylin/eosin.

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

Statistical analysis

The statistical analysis of data was done by one-way ANOVA, and the level of statistical significance – with the Tukey-Kramer test ($P < 0.05$). Results are presented as mean $\pm$ standard error of the mean (SEM).

RESULTS

Kidney weight and blood biochemical analyses

The toxic effects of AFB₁ with or without Mycotox NG on relative weight of kidneys, blood urea, creatinine and uric acid

Table 1. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on blood plasma urea, creatinine and uric acid in turkey broilers (mean ± SEM; n=10)

Groups	Relative weight of kidneys		Urea (mmol/L)	
	42 days of age	21 days of age	42 days of age	42 days of age
I (control)	0.47±0.09	1.08±0.038	1.22±0.031	
II (0.5 g/kg Mycotox NG)	0.46±0.01	1.01±0.043	1.25±0.047	
III (0.2 mg/kg AFB ₁)	0.62±0.06 ^{1c,2c}	3.06±0.13 ^{1c,2c}	4.52±0.17 ^{1c,2c}	
IV (0.4 mg/kg AFB ₁)	0.68±0.010 ^{1c,2c,3b}	3.90±0.16 ^{1c,2c,3c}	4.95±0.19 ^{1c,2c,3a,4c}	
V (0.2 mg/kg AFB ₁ + Mycotox NG)	0.52±0.013 ^{1a,2b,3c,4c}	2.43±0.14 ^{1c,2c,3a,4c}	3.25±0.13 ^{1c,2c,3c,4c}	
VI (0.4 mg/kg AFB ₁ + Mycotox NG)	0.59±0.010 ^{1c,2c,4c,5b}	2.63±0.13 ^{1c,2c,3c,4c}	3.67±0.14 ^{1c,2c,3c,4c}	
Groups	Creatinine (μmol/L)		Uric acid (μmol/L)	
	21 days of age	42 days of age	21 days of age	42 days of age
I (control)	51.4±1.38	56.8±2.15	428.6±31.30	431.9±15.21
II (0.5 g/kg Mycotox NG)	50.8±1.92	55.4±2.38	437.7±8.00	440.6±13.73
III (0.2 mg/kg AFB ₁)	70.3±1.90 ^{1c,2c}	81.7±3.07 ^{1c,2c}	284.6±12.00 ^{1c,2c}	304.1±14.01 ^{1c,2c}
IV (0.4 mg/kg AFB ₁)	79.3±2.95 ^{1c, 2c, 3a}	86.4±2.91 ^{1c,2c,3c}	257.6±11.16 ^{1c,2c,3c}	264.8±14.00 ^{1c,2c}
V (0.2 mg/kg AFB ₁ + Mycotox NG)	62.4±3.36 ^{1a,2a,4c}	67.1±2.19 ^{1b,2c,3a,4c}	324.5±9.04 ^{1c,2c,3a,4c}	343.7±10.62 ^{1b,2b,4b}
VI (0.4 mg/kg AFB ₁ + Mycotox NG)	66.6±3.17 ^{1b,2b,4c}	70.7±1.70 ^{1a,2a,3b,4c}	332.00±13.30 ^{1c,2c,4b}	355.2±4.25 ^{1a,2b,4a}

^ap<0.05; ^bp<0.01; ^cp<0.001; 1 – vs group II; 2 – vs group III; 3 – vs group IV; 4 – vs group V; 5 – vs group VI.

are shown in Table 1. Increasing dietary doses of AFB₁ (0.2 and 0.4 mg/kg) in compound feed of Groups III and IV resulted in higher relative weight of kidneys vs control group ($P<0.001$). Blood analysis showed significantly higher urea concentrations at days 21 and 42 ($P<0.001$). In Groups III and IV, creatinine levels on both sampling days were substantially higher than control values ($P<0.001$). Uric acid concentrations in treated groups tended to decrease as compared to untreated birds on days 21 and 42 ($P<0.001$). Blood calcium and phosphate levels (Table 2) were also statistically significantly lower than controls on days 21 and 42 ($P<0.01$ – $P<0.001$).

In turkey poultlets from groups V and VI, harmful effects of AFB₁ on kidney relative weight and blood renal parameters were partly counteracted by supplementa-

tion of the feed with 0.5 g/kg Mycotox NG. The toxin binder also reduced the increased relative weight of kidneys ($P<0.05$ – $P<0.001$ vs control birds). The increased blood urea and creatinine concentrations in groups V and VI on both sampling days were partly compensated after addition of the toxin binder ($P<0.05$ – $P<0.001$ vs controls). Less pronounced changes were detected in blood uric acid, calcium and inorganic phosphate ($P<0.05$ – $P<0.001$ vs untreated birds).

Kidney morphology studies

The kidney parenchyma of turkey poultlets treated with 0.2 mg/kg AFB₁ exhibited generalised hyperaemia, cloudy swelling, granular degeneration and vacuolation of the cytoplasm of tubular epithelial cells. The nuclei of most cells were vesicular with occasional karyolysis. Some cells

Table 2. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on blood plasma calcium and inorganic phosphate levels in turkey broilers (mean $\pm$ SEM; n=10)

Groups	Calcium (mmol/L)	
	21 days of age	42 days of age
I (control)	4.55 $\pm$ 0.11	4.01 $\pm$ 0.13
II (0.5 g/kg Mycotox NG)	4.57 $\pm$ 0.50	4.03 $\pm$ 0.12
III (0.2 mg/kg AFB ₁)	3.61 $\pm$ 0.23 ^{1b,2b}	3.27 $\pm$ 0.16 ^{1b,2c}
IV (0.4 mg/kg AFB ₁)	3.45 $\pm$ 0.18 ^{1c,2c}	3.15 $\pm$ 0.16 ^{1c,2c}
V (0.2 mg/kg AFB ₁ + Mycotox NG)	3.85 $\pm$ 0.17 ^{1b,2a}	3.70 $\pm$ 0.12 ^{1a,2a}
VI (0.4 mg/kg AFB ₁ + Mycotox NG)	3.70 $\pm$ 0.16 ^{1b,2b}	3.35 $\pm$ 0.14 ^{1a,2b}
Groups	Inorganic phosphate (mmol/L)	
	21 days of age	42 days of age
I (control)	2.91 $\pm$ 0.15	2.75 $\pm$ 0.18
II (0.5 g/kg Mycotox NG)	2.94 $\pm$ 0.096	2.77 $\pm$ 0.12
III (0.2 mg/kg AFB ₁)	2.37 $\pm$ 0.053 ^{1b,2b}	2.17 $\pm$ 0.073 ^{1b,2c}
IV (0.4 mg/kg AFB ₁)	2.19 $\pm$ 0.060 ^{1c,2c}	2.08 $\pm$ 0.013 ^{1c,2c}
V (0.2 mg/kg AFB ₁ + Mycotox NG)	2.47 $\pm$ 0.10 ^{1a,2a}	2.30 $\pm$ 0.035 ^{1a,2a}
VI (0.4 mg/kg AFB ₁ + Mycotox NG)	2.40 $\pm$ 0.074 ^{1a}	2.25 $\pm$ 0.060 ^{1a,2a}

^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$; 1 – vs group II; 2 – vs group III; 3 – vs group IV; 4 – vs group V; 5 – vs group VI.

Fig. 1. A) Degenerative changes and desquamation of epithelial cells from basement membranes of renal tubules in turkey poults treated with 0.2 mg/kg AFB₁; B) Focal mononuclear cell proliferation in the kidney interstitium in turkey poults treated with 0.2 mg/kg AFB₁; C) Generalised widening of the subcapsular space of renal glomeruli in turkey poults treated with 0.2 mg/kg AFB₁. H/E, Bar=15 µm.

Fig. 2. A) Fatty degeneration of the kidney in turkey poults treated with 0.4 mg/kg AFB₁; B) Haemorrhages and severe dystrophy and necrosis in the kidney parenchyma of turkey poults treated with 0.4 mg/kg AFB₁; C) Desquamation from basement membranes and necrosis of some epithelial cells of renal tubules in turkey poults treated with 0.4 mg/kg AFB₁. H/E, Bar=15 µm.

were disintegrated and desquamated from the basement membrane (Fig. 1A). Among the parenchyma, small foci of round cell proliferations were found out (Fig. 1B). The subcapsular space of most glomeruli was widened (Fig. 1C).

Kidney degeneration was significantly more pronounced in birds treated with 0.4 mg/kg AFB₁. Along with granular degeneration, fatty degeneration was also detected although less frequently (Fig. 2A). Epithelial cells of all tubules demonstrated karyolysis and plasmolysis, were desquamated from the basement

membranes and cast off in the lumen (Fig. 2B). Occasionally, necrobiotic to necrotic areas imbibed with red blood cells (haemorrhages) as well as congestion signs were identified (Fig. 2C).

In turkeys treated with 0.2 mg/kg AFB₁ and Mycotox NG, kidney parenchyma dystrophy was considerably milder compared to those in birds treated only with 0.2 mg/kg AFB₁. The findings comprised vascular hyperaemia and disintegration of tubular cells; some of them had also granular dystrophy of the cytoplasm (Fig. 3A).

Fig. 3. **A)** Overfilling of blood vessels of kidney parenchyma, congestion, mild disintegration and desquamation of the epithelium of basement membranes of tubules in turkey poults treated with 0.2 mg/kg AFB₁ + 0.5 g/kg Mycotox NG; **B)** Mild granular dystrophy and insignificant disintegration of renal tubular epithelium in birds treated with 0.2 mg/kg AFB₁ + 0.5 g/kg Mycotox NG. H/E, Bar=15 µm.

Fig. 4. Normal histology of kidney parenchyma in control turkey poults (**A**) and of birds treated with 0.5 g/kg Mycotox NG (**B**). H/E, Bar=15 µm.

In birds supplemented with 0.4 mg/kg AFB₁ and Mycotox NG, apart the congestion, blood vessels' overfilling with blood and single haemorrhages, a mild granular dystrophy of kidney tubular epithelial cells was registered (Fig. 3B).

Control turkey poults and those given only toxin binder (0.5 g/kg Mycotox NG), showed no pathomorphological alterations of kidney parenchyma (Fig. 4).

DISCUSSION

Kidneys are sensitive to toxins due to their profuse blood supply. One third of the total blood volume is filtered through them and at the same time, 98–99% of total water and sodium chloride are reabsorbed through the kidneys. Various parts of kidney nephrons are exposed to the toxic impact of AFB₁ and its metabolites (Sharma *et al.*, 2011).

Morphological changes in kidneys are similar to those in layer hens after intake of feed contaminated with aflatoxin B₁ (parenchymatous degeneration of distal tubules with swelling of epithelial cells, cytoplasm's granulation, vacuolar and hydropic degeneration and desquamation of epithelial cells) (Dhanasekaran *et al.*, 2009). In an experiment with broiler chickens, Yildirim *et al.* (2011) investigated the effect of dietary 2 mg/kg total aflatoxin (AFB₁, AFG₁, AFB₂ и AFG₂) either alone or in combination with a mycosorbent (Mycosorb) on kidney parenchyma histology. They consisted in thickening of the basement membrane, hyaline drops within epithelial cells, presence of cylinders in tubular lumen and epithelial cells' dystrophy. It was also found out that the disturbance of excretory renal function was accompanied with increased blood urea in birds fed only AF. In broiler chickens supple-

mented with 0.2 mg/kg AFB₁, Sakhare *et al.* (2007) observed dystrophic changes in tubular epithelial cells, liquefaction of the cellular matter, desquamation of epithelial cells from the basement membrane, haemorrhages and necrosis of kidney parenchyma. Mohamed & Mohamed (2009) established round cell and neutrophil infiltration, thickening of the basement membrane of the Bowman's capsule and glomerular capillaries, coagulation necrosis and dystrophic changes in renal epithelial cells (karyorrhexis and karyopyknosis).

Kidneys are the organs through which urea and creatinine are eliminated and electrolyte are reabsorbed into the blood – a mechanisms that regulates their excretion. The filtration occurs in glomeruli, while reabsorption – in renal tubules (Mayne, 2005). Urea is the end product of protein metabolism synthesised in the liver urea cycle. Increase blood urea and creatinine concentrations indicate low glomerular filtration rate secondary to impaired glomerular function (Fapohunda *et al.*, 2012).

Increased blood serum creatinine in mice challenged with AFB₁ suggests enhanced transformation of muscle phosphocreatine into creatinine, and thus, sign of decreased utilisation of phosphocreatine in muscle contractions. When renal function is normal, creatinine is rapidly removed from kidneys. Increased blood plasma concentration is probably due to enhanced release from muscles and/or reduced renal excretion rate (Mathuria & Verma, 2008). Increased blood urea and creatinine as markers of impaired kidney function in aflatoxicosis was reported in chicks, rats and rabbits (Soliman *et al.*, 2008; Mohamed & Mohamed, 2009; Hassan *et al.*, 2012). Higher relative weight of kidneys was

probably due to enhanced fat deposition e.g. lipaemia (Sharghi & Manafi, 2011); hypertrophy of proximal renal tubules with infiltration of lymphoid cells (Nataraj *et al.*, 2004) or increased blood uric acid concentrations with subsequent deposition in renal tubules (Pandey & Chauhan, 2007). It is assumed that increased urea and creatinine in blood were due to impaired transport function of epithelial cells in collecting ducts and diffusely impaired function of proximal tubules (Umar *et al.*, 2012). Other researchers believe that alterations in blood urea and creatinine result from kidney parenchymal necrosis (Sakhare *et al.*, 2007; Mohamed & Mohamed, 2009).

Uric acid, the main ultimate protein metabolism product in birds, is synthesised in the liver and excreted through the kidney tubular apparatus (Hochleithner, 1994). Hypouricaemia in this study confirms other reported results in chickens that received AF with the feed (Oguz *et al.*, 2000; Maciel *et al.*, 2007; Safameher *et al.*, 2008). From one part, hypouricaemia is a result of liver damage with reduced conversion of dietary proteins (Ledoux *et al.*, 1999; Zao *et al.*, 2010). On the other hand, lower blood uric acid is probably resulting from worse feed conversion e.g. lower utilisation and metabolism of proteins and/or protein synthesis inhibition (Safameher *et al.*, 2008).

Lower blood calcium and inorganic phosphate concentrations were found out by Umesh *et al.* (1995), Safameher (2008) and Zhao *et al.* (2010). They result either from kidney tubules' damage or from decreased intestinal absorption of calcium and phosphorus, decreased parathormone in the circulation, impaired vitamin D metabolism or reduced renal sensitivity to this hormone (Yildirim *et al.*, 2011). The severity of observed kid-

ney lesions in aflatoxicosis are the cause of the disturbed calcium and phosphate homeostasis (Soliman *et al.*, 2008; Yildirim *et al.*, 2011). Glahn *et al.* (1991) investigated the effects of AFB₁ on the kidney function in broiler chickens and concluded that it decreased circulating blood vitamin D and consequently, parathyroid hormone synthesis, reduced the feedback between parathormone and intestines and kidneys and thus, calcium and phosphorus absorption was reduced. The glomerular apparatus is the target of the toxic action of aflatoxins on kidneys (Glahn *et al.*, 1991).

In conclusion, the consumption of feeds contaminated with AFB₁ for 42 days induced histopathological changes in kidneys. Impaired kidney function was responsible for the increased relative weight of kidneys and blood biochemical changes. In this study, the supplementation of the feed of turkey poult challenged with 0.2 or 0.4 mg/kg AFB₁ with 0.5 g/kg Mycotox NG could prevent the changed in analysed blood biochemical parameters and reduce the severity of kidney histological lesions.

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