EFFECTS OF AFLATOXIN B₁ ALONE OR CO-ADMINISTERED WITH MYCOTOX NG ON PERFORMANCE AND HUMORAL IMMUNITY OF TURKEY BROILERS

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Summary

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The contamination of poultry feeds with aflatoxins is a global problem responsible for considerable losses to poultry industry. The aim of the present investigation was to evaluate the effects of aflatoxin B₁ and Mycotox NG, applied either independently or together, on performance, relative weights of visceral organs and antibody titres against Newcastle disease in turkey broilers. Experiments of 42-day duration were carried out with sixty 7-day-old female turkey broilers (meat TM strain) divided into one control and five treatment groups (n=10): 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 B₁, Group IV – experimental, whose feed contained 0.4 mg/kg aflatoxin B₁, Group V – experimental, supplemented with 0.2 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG and Group VI – experimental, supplemented with 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG. In Groups III and IV, production traits (live body weight, daily weight gain, feed intake) as well as antibody titres were reduced along with increased feed conversion and relative weights of liver, kidneys, heart, pancreas, proventriculus and gizzard. At the same time, relative weights of the spleen, thymus and bursa of Fabricius were statistically significantly lower. The supplementation of the feed of Groups V and VI with 0.5 g/kg Mycotox NG reduced and prevented some of deleterious effects of AFB₁ on production traits, antibody titres and relative weights of visceral organs.

Key words: aflatoxicosis B₁, turkey broilers, relative weights, humoral immunity, Mycotox NG

INTRODUCTION

The increased demands for poultry meat pose some risk for poultry health, some of

them associated to feed quality (Rawal *et al.*, 2010). Cereal crops which are among

the main components of poultry feeds could be contaminated with different moulds. Under favourable environmental conditions, some fungal strains are capable to produce specific metabolites termed mycotoxins (Pitt & Hocking, 2006). These compounds are structurally different and could cause various biological and toxicological effects (Fuchs *et al.*, 2008).

Moulds producing aflatoxins are ubiquitous in soil and common contaminant of feeds in parts of the world with warm and humid climate (Fowler *et al.*, 2014). Aflatoxins are a group of heterocyclic secondary toxic metabolites of fungi from the genus *Aspergillus* (*Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*) (Shlej *et al.*, 2015). They contaminate cereals (wheat, corn, sorghum, rice) and oil-bearing crops (sunflower, soybean, peanut and cotton flours), the most risky ingredients of compound feeds for turkey poult.

Aflatoxin B₁ is the most toxic among all other aflatoxins, and is frequently found at high concentrations in cereal crops and peanut flour (Gowda *et al.*, 2004). High dietary aflatoxin levels provoke acute aflatoxicosis with impaired coordination, vertigo, paresis, bloody diarrhoea, visceral haemorrhages, necrosis of hepatocytes, biliary epithelium hyperplasia, subcutaneous oedema, pale icteric skin, coma and death (Resanovic *et al.*, 2009). The consumption of feeds contaminated with low aflatoxin levels induce chronic aflatoxicosis which is usually associated to reduced productive performance and suppressed immunity. These changes are characterised with reduced weight gain, feed intake, increased feed conversion (Chibanga *et al.*, 2014), reduced production of eggs, meat, poorer technological properties of meat (Liu *et al.*, 2011); altered visceral organ weights

(Manafi, 2012; Manafi *et al.*, 2014; Kumar *et al.*, 2015). Aflatoxins are potent immunosuppressors, enhancing the susceptibility of birds to many secondary infections with other pathogens – fungi, bacteria, viruses, protozoa (Lawal & Bolu, 2014). The changes in haematological and blood biochemical parameters (Al-Daraji, 2012; Kana *et al.*, 2014); liver, heart and brain morphology (Ramdas *et al.*, 2013); immunocompetent organs (thymus, bursa of Fabricius, spleen) (Sakhare *et al.*, 2007; Ramdas *et al.*, 2013) and intestines (Aboutalebi, 2013) were studied. Among domestic fowl, most sensitive are growing ducklings, goslings, pheasants and broiler chickens (Leeson *et al.*, 1995).

The prevention, detoxication and decontamination of feed ingredients contaminated with aflatoxins are of particular significance. The effects of various inert mycosorbents – hydrated calcium sodium (HSCAS) (Jindal *et al.*, 1993), zeolites (Miazzo *et al.*, 2000), bentonites (Santurio *et al.*, 1999), activated charcoal (Edrington *et al.*, 1997) and live yeasts cultures (*Saccharomyces cerevisiae*) (Aravind *et al.*, 2003) were investigated but the results were not promising. Toxin binders are capable to bind aflatoxins reducing their absorption by the gastrointestinal tract and their bioavailability in blood. Nevertheless, some of them also reduce the bioavailability of amino acids and/or minerals (Dawson, 1999).

The aim of the present investigation was to evaluate the effects of aflatoxin B₁ and Mycotox NG, applied either independently or together, on performance (live body weight, weight gain, feed intake, feed conversion), relative weights of visceral organs (liver, kidneys, heart, bursa of Fabricius, spleen, thymus, pancreas, gizzard and proventriculus) and

antibody titres against Newcastle disease in turkey broilers.

MATERIALS AND METHODS

Toxin and adsorbent

Aflatoxin B₁ used in this experiment (produced by *Aspergillus flavus*, 99% purity) was obtained from Sigma-Aldrich (Germany). Mycotox NG (Ceva Sante Animale, France) and contained micronised yeasts, montmorillonite, thymol.

Experimental design

The experiment was approved by the Bulgarian Food Safety Agency – permit No 19218/06.11.2014. It was performed with 60 7-day-old female turkey broilers (from the meat TM strain) randomly divided into six groups (n=10). All birds were fed standard feed according to the species and age, produced by a feed factory. The experimental design comprised: Group I – control; 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 B₁; Group IV – experimental, whose feed contained 0.4 mg/kg aflatoxin B₁; Group V – experimental, supplemented with 0.2 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG; Group VI – experimental, supplemented with 0.4 mg/kg aflatoxin B₁ and 0.5 g/kg Mycotox NG.

All turkey poults were kept under optimum microclimatic parameters according to Ordinance 44/2006.

Live body weight, daily feed intake, daily weight gain and feed conversion ratio were determined on 14th, 28th and 42nd experimental days by weighing. Feed conversion was evaluated as ratio of feed intake and average daily gain. Relative weights of visceral organs (liver, kidneys,

heart, bursa of Fabricius, spleen, thymus, pancreas, gizzard and proventriculus) were determined after euthanasia of birds 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) as percentages of respective organ weight to body weight.

At day-old, turkeys have been vaccinated with live lyophilised vaccine Nobilis® ND C2 against Newcastle disease (ND) applied via aerosol route. At 14 days of age, immunity boost was done through revaccination with lentogenic NDV vaccine (Nobilis ND Clone 30, Intervet) applied via eye drop.

Blood samples for antibody titre determination were collected from *v. metatarsalis medialis* on post treatment days 21 and 42 in sterile plain containers (FL medical, Italy). Sera were harvested and stored at –20°C until analysis. Antibody titres were assayed by haemagglutination inhibition (HI) test (Anonymous, 2012).

Statistical analysis

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

RESULTS

Growth performance

The live weight of birds from Groups III and IV (Table 1) was statistically significantly lower ($P < 0.001$) at the three studied time intervals (14, 28 and 42 days of age) by 22.35% and 27.66%, 20.13% and 27.61%, 18.84% and 24.65% respectively compared to control group ($P < 0.001$). The addition of toxin binder to the feed of

Table 1. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on body weight of turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean ± SEM; n=10

Groups	Body weight (g)			
	Initial weight (g)	14 days of age	28 days of age	42 days of age
I	149.0±2.33	528±11.43	1565±51.96	2788±9.07
II	152.0±2.49	531±10.05	1564±28.68	2792±40.60
III	152.5±2.26	410±4.71 ^{1c,2c}	1250±16.53 ^{1c,2c}	2263±22.85 ^{1c,2c}
IV	151.8±2.43	382±6.29 ^{1c,2c}	1133±24.22 ^{1c,2c}	2101±33.96 ^{1c,2c,3a}
V	151.8±2.08	483±11.06 ^{1b,2c,3c,4c}	1380±27.36 ^{1c,2b,3a,4c}	2448±26.24 ^{1b,2c,3b,4c}
VI	150.3±2.33	465±10.35 ^{1c,2c,3b,4b}	1336±27.49 ^{1c,2c,4c}	2388±34.31 ^{1c,2c,4c}

*Difference from control group I; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

Table 2. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG daily weight gain of turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean ± SEM; n=10

Groups	Daily weight gain (g)		
	14 days of age	28 days of age	42 days of age
I	27.07±0.61	74.06±1.91	87.35±2.68
II	27.06±0.78	73.78±1.84	87.72±1.91
III	18.39±0.39 ^{1c,2c}	60.00±1.09 ^{1c,2c}	72.35±1.79 ^{1c,2c}
IV	16.44±0.49 ^{1c,2c}	53.64±1.51 ^{1c,2c}	69.13±1.63 ^{1c,2c}
V	23.71±0.63 ^{1a,2a,3c,4c}	64.07±1.94 ^{1a,2c,4c}	76.28±1.55 ^{1b,2b}
VI	22.47±0.63 ^{1b,2b,3b,4c}	62.21±1.93 ^{1c,2b,4a}	75.13±1.73 ^{1c,2c}

*Difference from control group I; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

Groups V and VI reduced partly the adverse effects of AFB₁ on live weight by 8.53% and 11.94% (day 14), 11.83% and 14.64% (day 28) and 12.2% and 14.35% (day 42) (P<0.01 – P<0.001).

On the 14th day, the daily weight gain (Table 2) was reduced by 32.07% in Group III and 39.27% in Group IV (P<0.001 vs Group I). By days 28 and 42, the weight gain was reduced by 18.99% and 17.18% respectively (Group III) and

by 27.58% and 20.86% respectively (Group IV) (P<0.001 vs control birds). After addition of mycosorbent to the feed of Groups V and VI the daily weight gain increased at a various extent (P<0.05–P<0.001). Yet, compared to untreated birds, the daily weight gain remained lower by 12.42% in Group V and 17.00% in Group VI on the 14th day; by 13.49% in Group V and 16.01% in Group VI on the 28th day and by 12.68% and 13.99% in

Groups V and VI, respectively on the 42nd day.

Daily feed intake (Table 3) declined substantially in Groups III and IV (by 12.79% and 15.55%, $P<0.001$) at day 14. During the second examination the reduction was by 5.4% and 7.86% ($P<0.001$), while during the third – by 3.63% and 5.47% respectively ($P<0.01$ – $P<0.001$). There were no significant changes in daily feed intake in Groups V and VI.

Feed conversion ratio in Groups III and IV increased significantly during the three studied time intervals (days 14, 28 and 42). In Group V, it was increased only by 10.17% by the 14th day of age. At the 28th day, feed conversion in Groups V and VI was by 12.30% and 15.38% respectively higher than that of controls ($P<0.05$ – $P<0.01$). By the 42nd day, the noted increase in these groups was respectively by 12.38% and 14.15% ($P<0.05$).

Table 3. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG daily feed intake of turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean $\pm$ SEM; n=10

Groups	Daily feed intake (g)		
	14 days of age	28 days of age	42 days of age
I	44.97 $\pm$ 0.048	143.33 $\pm$ 0.077	196.28 $\pm$ 0.088
II	44.77 $\pm$ 0.13	143.31 $\pm$ 0.095	198.42 $\pm$ 0.12
III	39.22 $\pm$ 0.016 ^{1c,2c}	135.60 $\pm$ 0.067 ^{1b,2c}	189.16 $\pm$ 0.054 ^{1b,2c}
IV	37.98 $\pm$ 0.14 ^{1c,2c}	132.07 $\pm$ 0.051 ^{1c,2c}	185.56 $\pm$ 0.076 ^{1c,2c}
V	42.18 $\pm$ 0.28 ^{4b}	139.15 $\pm$ 0.077 ^{4a}	192.72 $\pm$ 0.070 ^{4a}
VI	42.77 $\pm$ 0.024 ^{4a}	139.26 $\pm$ 0.048 ^{4b}	193.00 $\pm$ 0.018 ^{4a}

*Difference from control group I; ^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

Table 4. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on daily feed intake of turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean $\pm$ SEM; n=10

Groups	Feed conversion ratio (g feed/g weight gain)		
	14 days of age	28 days of age	42 days of age
I	1.67 $\pm$ 0.049	1.95 $\pm$ 0.04+	2.26 $\pm$ 0.069
II	1.67 $\pm$ 0.046	1.96 $\pm$ 0.045	2.27 $\pm$ 0.056
III	2.14 $\pm$ 0.046 ^{1c,2c}	2.27 $\pm$ 0.040 ^{1b,2b}	2.63 $\pm$ 0.074 ^{1b,2b}
IV	2.33 $\pm$ 0.073 ^{1c,2c,4c}	2.48 $\pm$ 0.069 ^{1c,2c}	2.69 $\pm$ 0.066 ^{1c,2c}
V	1.84 $\pm$ 0.067 ^{3b,4c}	2.19 $\pm$ 0.062 ^{1a,4b}	2.54 $\pm$ 0.057 ^{1a,2a}
VI	1.92 $\pm$ 0.054 ^{1a,2a,4c}	2.25 $\pm$ 0.060 ^{1b,2a,4b}	2.58 $\pm$ 0.060 ^{1a,2a}

*Difference from control group I; ^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

Table 5. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on relative weights of visceral organs of turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean ± SEM; n=10

Groups	Liver	Kidneys	Heart	Bursa of Fabricius	Thymus
I	1.98± 0.019	0.47± 0.09	0.46± 0.010	0.17± 0.004	0.25± 0.003
II	1.98± 0.031	0.46± 0.01	0.46± 0.009	0.18± 0.005	0.25± 0.004
III	2.51± 0.025 ^{1c,2c}	0.62± 0.06 ^{1c,2c}	0.58± 0.010 ^{1c,2c}	0.13± 0.003 ^{1c,2c}	0.17± 0.004 ^{1c,2c}
IV	2.71± 0.043 ^{1c,2c,3b,4c}	0.68± 0.010 ^{1c,2c,3b}	0.62 ± 0.003 ^{1c,2c,3c}	0.12± 0.003 ^{1c,2c}	0.15± 0.002 ^{1c,2c,3b}
V	2.30± 0.063 ^{1b,2c,3c,4c}	0.52± 0.013 ^{1a,2b,3c,4c}	0.51± 0.006 ^{1c,,2c,3c,4c}	0.15± 0.007 ^{1b,2b,4b}	0.19± 0.002 ^{1c,2c,3c,4c}
VI	2.43± 0.043 ^{1c,2c,4c}	0.59± 0.010 ^{1c,2c,4c,5b}	0.53± 0.009 ^{1c,2c,4c}	0.14± 0.005 ^{1b,2b,4a}	0.17± 0.003 ^{1c,2c,4c,5c}

Groups	Spleen	Pancreas	Proventriculus	Gizzard
I	0.132± 0.021	0.26± 0.004	0.34± 0.006	1.86± 0.030
II	0.136± 0.021	0.27± 0.004	0.35± 0.003	1.83± 0.023
III	0.094± 0.093 ^{1c,2c}	0.34± 0.006 ^{1c,2c}	0.50± 0.012 ^{1c,2b}	2.16± 0.012 ^{1c,2c}
IV	0.09± 0.009 ^{1c,2c}	0.38± 0.004 ^{1c,2c,3c,4c}	0.53± 0.008 ^{1c,2b}	2.26± 0.022 ^{1c,2c}
V	0.16 0.008 ^{3c,4c}	0.30± 0.006 ^{1c,2c,3c,4c}	0.46± 0.007 ^{1c,2c,4c}	2.02± 0.031 ^{1c,2c,3b,4c}
VI	0.15± 0.015 ^{3a,4a}	0.32± 0.005 ^{1c,3a,4c}	0.48± 0.013 ^{1c,2c,4b}	2.09± 0.020 ^{1c,2c,4c}

*Difference from control group I; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

Throughout the experiments, body weight, daily weight gain, daily feed intake and feed conversion ratios were similar in control group and Group II (receiving only 0.5 g/kg Mycotox NG).

The relative weights of visceral organs (g/100 g body weight) are presented in Table 5. Groups III and IV demonstrated increased relative weights of the liver by 26.76% and 36.86%; kidneys: by 31.91%

and 44.68%; the heart: by 26.08% and 34.78%; the pancreas: by 30.76% and 46.15%; the proventriculus: by 47.05% and 55.88% and the gizzard: by 16.12 and 21.50% as compared to control group (P<0.001). Lower relative weights were established for the bursa of Fabricius (by 23.53% and 29.42%); the thymus (by 32% and 40%) and the spleen (by 28.79% and 31.82%) than in controls (P<0.001). The

addition of mycosorbent to the feed of Groups V and VI compensated to some extent ($P<0.05$ – $P<0.001$) increased relative weights of the liver (by 16.16% and 22.72%); kidneys (by 10.63% and 25.53%); the heart (by 10.86% and 15.21%); the pancreas (by 15.38% and 23.07%); the proventriculus (by 35.29% and 41.17%) and the gizzard (by 8.6% and 12.36%). Furthermore, the reduction in the bursa of Fabricius and the thymus was less expressed ($P<0.01$ – $P<0.001$). There was no statistically significant difference in the relative spleen weight of Groups V and VI vs controls. The addition of 0.5 g/kg Mycotox NG to compound feed of turkey broilers (Group II) did not have any adverse effect on the weights of studied organs ($P>0.05$).

Immunological studies

In aflatoxin-treated groups (Table 6) antibody titres after vaccination against Newcastle disease were statistically significantly lower by the 21st and 42nd day of age as compared to unchallenged group I ($P<0.001$). Feed supplementation with 0.5 g/kg Mycotox NG (Group V) had a positive effect on antibody production at both

intervals ($P>0.05$ vs controls). In Group VI, the addition of Mycotox NG reduced the immunosuppressive effect of AFB₁ only partly ($P<0.05$ – $P<0.01$).

DISCUSSION

Aflatoxin B₁ is the prevailing fungal metabolite detected in animal feeds (Nilipour *et al.*, 2002). This toxin raises a serious concern in poultry industry due to its high toxicity and common occurrence in poultry feeds (Tessari *et al.*, 2006). Broilers are fed diets made from various ingredients produced under different agrometeorological conditions. Many compound feeds include corn and soybean flour, which in most instances are imported; so the contamination of these ingredients with aflatoxins occurs during transportation and storage. The content of aflatoxins in poultry feeds often exceeds the maximum allowances of 20 µg.kg⁻¹ (Aravind *et al.*, 2003).

Birds and fish are highly susceptible to the toxic effects of AFB₁, even at low doses within the range 15–30 ppb (Rawal *et al.*, 2010). The bioactivation of aflatoxins to reactive aflatoxin-8,9-epoxide

Table 6. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on haemagglutination titres against Newcastle disease in turkey broilers. Group I – control; 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 + Mycotox NG; group VI – 0.4 mg/kg AFB₁ + Mycotox NG. Data are presented as mean ± SEM; n=10

Groups	Antibody titres (log ₂ ¹⁰)	
	21 days of age	42 days of age
I	7.10±0.031	8.1±0.23
II	7.28±0.038	8.0±0.39
III	4.30±0.44 ^{1c,2c}	5.9±0.34 ^{1c,2c}
IV	4.10±0.34 ^{1c,2c}	5.0±0.36 ^{1c,2b}
V	6.00±0.51 ^{2a,4a}	6.6±0.34 ^{2b,4a}
VI	5.10±0.60 ^{1a,2b,3a}	6.0±0.44 ^{1b,2b,4a}

*Difference from control group I; ^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$; 1 – vs control group; 2 –vs group I; 3 – vs group II; 4 – vs group III; 5 – vs group IV.

(AFBO) – the main and most toxic metabolite is done in hepatocytes by microsomal enzyme systems – cytochrome P450 (CYP450). The AFBO inhibits the protein synthesis, causing liver damage, immunosuppressive effects and reduces growth performance (Rawal *et al.*, 2010). Domestic turkeys are highly susceptible to the toxic effects of AFB₁. The liver glutathione S-transferases alpha class (GSTA) in this species is not able to detoxify AFBO, which is probably the main factor for their high sensitivity (Rawal *et al.*, 2010; Monson *et al.*, 2016).

The results of the present study indicated that the supplementation of compound feeds with increased amounts of AFB₁ (0.2 mg/kg or 0.4 mg/kg) reduced substantially the live weight, weight gain and feed intake and consequently, increased feed conversion. Our results are in line with other (Afzal & Saleem, 2004; Suksombat *et al.*, 2011; Yang *et al.*, 2012; Zuo *et al.*, 2013; Chibanga *et al.*, 2014) reporting that the consumption of feeds contaminated with low amount of aflatoxins in broiler chickens worsened significantly growth performance.

The contamination of feed ingredients with mycotoxins deteriorates the quality of feeds which is manifested with altered nutritional profile and organoleptic features (Markovic *et al.*, 2005). Reduced feed intake established in this study is due to inappetance, a protective mechanism in aflatoxicosis (Rauber *et al.*, 2007) or to impaired liver metabolism secondary to impaired liver morphology (Shareef & Omar, 2012). Reduced feed utilisation is also a result from the decreased activity of a number of enzymes involved in carbohydrate, proteins, nucleic acids conversion, to reduced intake or nutritional deficiency (Makinia, 2014). On the other hand, the lower feed intake is attributed to

increased blood ammonia concentrations following reduced glomerular filtration rate (Saei *et al.*, 2013). The lower weight gain is due to reduced protein synthesis rate, impaired lipogenesis, reduced protein and lipid utilisation from feeds, enhanced faecal excretion of lipids. Aflatoxins reduce protein synthesis (total protein and albumin) through interruption of mRNA transcription and amino acid transport (Chibanga *et al.*, 2014). The impaired absorption of nutrients results from damaged small intestine, lower rate of pancreatic digestive enzymes secretion (amylase, lipase, trypsin) are other reasons for the reduced ability of birds to utilise energy from aflatoxin-contaminated feeds (Rauber *et al.*, 2007; El-Ghany *et al.*, 2013; Nemati *et al.*, 2015).

Our data about the reduced appetite and increased feed conversion ratio are supported by data of Beura *et al.* (1993) in broiler chickens, fed diets containing >100 ppb total aflatoxin. Increased feed conversion is attributed to poorer utilisation of feed nutrients (Kana *et al.*, 2014), anorexia, inhibited lipogenesis and protein synthesis (Dhanapal *et al.*, 2014).

The relative weights of the liver, kidneys, pancreas, heart, gizzard and proventriculus in Groups III and IV were statistically significantly higher vs controls along with lower relative weights of immunocompetent organs (thymus, spleen, bursa of Fabricius). Higher relative weights of visceral organs result from reduced body weight (El-Ghany *et al.*, 2013). Higher relative weights established in the present study are compatible with results of other researchers in broilers fed AFB₁ supplemented feed (Abousadi *et al.*, 2007; Kumar *et al.*, 2015).

Increased liver weight was probably due to increased fat deposition consequently to impaired fat metabolism. Fatty

liver is mainly mediated by inhibited synthesis of phospholipids and cholesterol, hence the fat transportation through the liver (Manegar *et al.*, 2010). Higher relative weights of the gizzard and proventriculus are attributed to the direct cytotoxic effect of aflatoxins on digestive organs (Abousadi *et al.*, 2007), as well as to the irritation of gastrointestinal mucosa by aflatoxins, provoking inflammation and thickening (El-Ghany *et al.* 2013). Increased relative weight of kidneys was probably due to lipaemia (increased fat deposition) (Sharghi & Manafi, 2011); hypertrophy of proximal renal tubules with infiltration of lymphoid cells (Nataraj *et al.*, 2004) or increased blood uric acid concentration leading to its deposition in renal tubules (Pandey & Chauhan, 2007). The relative weight of the pancreas increased subsequently to thickening of interlobular septa among acinar cells, cell proliferation and congestive events (Jakhar & Sadana, 2004; Abd El-Haleem *et al.*, 2011). The established higher relative weight of the heart in the present study was most probably a result of myocardial congestive events (Jakhar & Sadana, 2004).

Lower relative weights of the thymus, spleen and bursa of Fabricius in turkeys challenged with aflatoxin only compared to untreated control birds could be due to necrosis and reduced density of lymphoid cells (Perozo & Rivera, 2003).

The sensitivity of immune system to mycotoxin-induced immunosuppression is a reflexion of the sensitivity of continuously proliferating and differentiating immune cells involved in immune response and regulating the complex communication among the components of cellular and humoral immunity (Pestka & Bondy, 1994). Immunosuppressive effects of aflatoxins are associated to protein synthesis inhibition, including specific pro-

teins as immunoglobulins IgG and IgA, inhibited migration of macrophages, and impaired haemolytic activity of the complement, lower lymphocyte counts in bursa of Fabricius and the thymus and lower rate of cytokine synthesis by lymphocytes (Ibrahim *et al.*, 2000). Lower antibody titres are due to protein and DNA synthesis inhibition. *In vitro*, aflatoxin B₁ inhibits RNA polymerase and the synthesis of albumin, globulins and immunoglobulins is disturbed (Makinia, 2014). As aflatoxins reduce the density of lymphoid follicles in immunocompetent organs, antibody titres against Newcastle disease and infectious bursitis decreases (Ali, 2004). Similar results were demonstrated in this study with turkeys vaccinated against ND as well.

The addition of toxin binders to poultry feeds was aimed at prevention or reduction to a minimum of free mycotoxins content. The proper storage of feed ingredients and feeds are factors essential for minimisation of mycotoxin production (Saif *et al.*, 2003). The inclusion of Mycotox NG to the ration of birds from Groups V and VI reduced the deleterious effects of AFB₁ on production traits, relative weights of the visceral organs and humoral immunity. Mycosorbents are able to bind aflatoxin molecules in the gastrointestinal tract and thus, to reduce their absorption by domestic fowl. The results agree with data from other studies with mycosorbents as clinoptilolite (Oguz *et al.*, 2003), hydrated calcium aluminosilicate (HSCAS), sodium bentonite, montmorillonite (Ologhobo *et al.*, 2015), essential oils (Saei *et al.*, 2013), antioxidants (resveratrol) (Sridhar *et al.*, 2015) and probiotics (Zuo *et al.*, 2013).

In conclusion, the addition of increased doses of AFB₁ (0.2 mg/kg or 0.4 mg/kg) to the compound feed of turkey

broilers decreased growth performance (live body weight, weight gain, feed intake, feed conversion), relative weights of visceral organs and antibody titres against Newcastle disease. The supplementation of AFB₁-contaminated rations with Mycotox NG reduced or prevented the toxic effects on production traits relative weights of visceral organs and altered humoral immunity. According to our results, the utilisation of toxin binders containing essential oils could reduce the toxic effects of aflatoxins in poultry. The results could serve as a background for further studies on protective influence of essential oils on poultry health, the safety and quality of poultry products.

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