

INVESTIGATIONS ON PRODUCTION TRAITS IN BROILER CHICKENS WITH EXPERIMENTAL AFLATOXICOSIS

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Summary

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Several production traits (live body weight, daily weight gain, feed conversion, daily feed consumption) and relative weights (g/100 g body weight) of visceral organs (liver, kidneys, heart, bursa of Fabricius, thymus, spleen, pancreas, gizzard and proventriculus) were investigated in broiler chickens with experimentally induced aflatoxicosis B₁. The experiments were carried out with five groups of ten 7-day-old Cobb broiler chickens in each. The groups were as followed: group I – control (fed standard compound feed according to the age and species); 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 duration of the experiment was 42 days. The dynamics of live weight, daily weight gain, daily feed consumption and conversion were followed out on 21, 35 and 49 days of age. The differences between relative visceral organ weights between control and experimental groups were determined after the trial's end. Lower live body weight, daily weight gain, daily feed consumption as well as increased feed conversion and higher relative weights of the liver, kidneys, heart, pancreas, spleen, gizzard and proventriculus were found out in groups III and IV. Simultaneously, the relative weights of the thymus and bursa of Fabricius were statistically significantly reduced. The supplementation of the feed of experimental group V with 1 g/kg Mycotox NG resulted in substantial reduction of negative effects of AFB₁ on production traits and visceral weights. There were no statistically significant differences between studied parameters between group II, receiving only mycosorbent, and controls.

Key words: aflatoxicosis B₁, chickens, Mycotox NG, production traits, relative weights

INTRODUCTION

Aflatoxins (AF) are extremely toxic biologically active compounds. They are secondary metabolites produced by the moulds from genus *Aspergillus* (*Aspergillus flavus* and *Aspergillus parasiticus*) (Tedesco *et al.*, 2004). These species are natural poultry feed contaminants (Leeson *et al.*, 1995). From the 18 identified AF, the main forms encountered in naturally

contaminated feeds are AFB₁, AFB₂, AFG₁ and AFG₂ (CAST, 1989; Hussein & Brasel, 2001; Tedesco *et al.*, 2004). The most toxic compound is aflatoxin B₁ (Hussein & Brasel, 2001), with very strong hepatotoxic, hepatocarcinogenic, teratogenic and mutagenic potential (Mishra & Das, 2003). Aflatoxins contaminate cereal crops in the field before

harvesting and then, during their storage in storehouses, transportation and after technological processing and packaging. The synthesis of AF in animal feeds increases when ambient temperatures are higher than 27 °C, the humidity higher than 62% and feed humidity – over 14% (Royes & Yanong, 2002). According to EC legislation, the allowed amounts of AF in compound poultry feeds are up to 20 µg/kg (EEC, 1991). From domestic poultry, growing turkeys, quails, ducklings, goslings and chickens are particularly susceptible to the toxic effect of aflatoxins (Arafa *et al.*, 1981). The intake of AF-contaminated feeds in growing chickens causes serious economic losses to poultry industry from reduced feed consumption, lower weight gain, increased feed conversion ratio and altered weights of visceral organs (Miazzo *et al.*, 2000; Oguz & Kurtoglu, 2000; Denli *et al.*, 2009; Shi *et al.*, 2009). At the same time, they cause haematological and blood biochemical changes (Basmacioglu *et al.*, 2005; Denli *et al.*, 2009), suppress the immunity (Oguz *et al.*, 2003) and impair the normal morphology of liver and kidneys (Ortatali & Oguz, 2001).

The livestock husbandry uses various measures to reduce the toxic effects of aflatoxins on animals through methods (physical, chemical and biological) for processing AF-contaminated feeds (CAST, 1989). Unfortunately, most of these methods are expensive, time-consuming and only partially efficient. At present, one of the most promising and practice-friendly approaches for decontamination of affected feeds is the use of adsorbents. Added to AF-contaminated feeds, mycosorbents bind to toxins during the digestion and thus, reduce their absorption in the gastrointestinal tract (Ramos *et al.*, 1996; Miazzo *et al.*, 2000).

The aim of the present study was to investigate the effects of AFB₁ and Mycotox NG upon production traits and relative weights of visceral organs in broiler chickens after their independent intake with the compound feed. Also, we aimed to establish the potential of prevention of the toxic effects of AFB₁ via supplementation of 1 g/kg Mycotox NG to chickens whose feed was contaminated with 0.5 mg/kg aflatoxin B₁.

MATERIALS AND METHODS

The experiments were carried out with 50 7-day-old Cobb broiler chickens from both genders divided into five groups of ten birds each.

The experimental design was as followed:

- 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 Sante 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 average body weight in the beginning of the experiment was 131±2.21 g for group I, 130.5±2.17 g for group II, 130±2.10 g for group III, 131.5±2.36 g for group IV and 130±2.10 g for group V.

The used aflatoxin B₁ was produced by *Aspergillus flavus* (99% purity) and purchased from Sigma-Aldrich, Germany. In experimental groups, the feed was ground before being mixed with aflatoxin for better homogenisation. The dynamics of live weight, daily weight gain, daily feed consumption and conversion were followed out on 21, 35 and 49 days of age. Feed and water were offered *ad libitum*. Chickens were reared in conditions compliant to hygienic norms. Microclimatic parameters were optimal, equal for all groups according to Ordinance 44/2006 (Anonymous, 2006).

The weights of visceral organs (liver, kidneys, heart, bursa of Fabricius, thymus, spleen, pancreas, gizzard and proventriculus) were determined after euthanasia of chickens through cervical dislocation.

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

Data are presented as means and standard errors of means (n=10), and were processed statistically using one-way ANOVA and Tukey-Kramer test (P<0.05).

RESULTS

The effects of feed supplementation with either AFB₁ or Mycotox NG, as well as their combined oral administration on body weight, daily weight gain, feed conversion and daily feed consumption over 6 weeks are presented in Tables 1–4.

The analysis of live weight changes showed that during the three monitoring periods (21, 35 and 49 days of age), the groups having received 0.5 or 0.8 mg/kg AFB₁ exhibited statistically significantly lower body weight than controls (P<0.001). The weight gain compared to controls (by 21 days of age) was reduced by 22.51% and 27.58% in groups III and IV respectively (P<0.001). During the second (days 22–35) and third (days 36–

Table 1. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on body weight of 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

Groups	Body weight (g)						
	Initial weight (g)	21 days of age	%*	35 days of age	%*	49 days of age	%*
I	131.0±2.21	655±8.33	100	1412±7.42	100	2403±9.07	100
II	130.5±2.17	660±9.06	+0.76	1415±7.03	+0.21	2400±12.73	-0.13
III	130.0±2.10	536±5.41 _{1c,2c}	-18.17	1122±8.27 _{1c,2c}	-20.54	1943±13.08 _{1c,2c}	-19.15
IV	131.5±2.36	511±6.04 _{1c,2c}	-21.99	1053±19.61 _{1c,2c,3c}	-25.43	1851±17.15 _{1c,2c,3c}	-22.98
V	130.0±2.10	621±6.40 _{1a,2b,4c}	-5.20	1322±9.04 _{1b,2b,3c,4c}	-6.38	2240±21.02 _{1c,2c,3c,4c}	-6.79

*Difference from control group I; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs control group; 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 daily weight gain of 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

Groups	Daily weight gain (g)					
	21 days of age	%*	35 days of age	%*	49 days of age	%*
I	37.42±0.61	100	54.06±0.92	100	70.78±0.94	100
II	37.81±0.56	+1.04	53.92±0.75	-0.26	70.35±0.65	-0.61
III	29.00±0.30 1c,2c	-22.51	41.85±0.71 1c,2c	-22.59	57.56±0.87 1c,2b	- 18.68
IV	27.10±0.44 1c,2c	-27.58	38.70±0.19 1c,2c,3c	-28.42	56.99±1.26 1c,2c	- 19.49
V	35.06±0.49 1b,2c,4c	-6.31	50.06±0.65 1a,2a,3c,4c	-7.4	65.56±1.53 1b,2b,3c,4c	-7.38

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

Table 3. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on feed conversion ratio of 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

Groups	Feed conversion ratio (g/g)					
	21 days of age	%*	35 days of age	%*	49 days of age	%*
I	1.52±0.025	100	1.98±0.033	100	2.42±0.026	100
II	1.50±0.022	-1.32	1.98±0.028	0	2.43±0.022	+0.41
III	1.74±0.029 1c,2c	+14.47	2.44±0.041 1c,2c	+23.23	2.85±0.041 1c,2c	+17.76
IV	1.81±0.029 1c,2c	+19.07	2.51±0.089 1c,2c	+26.76	2.89±0.062 1c,2c	+19.42
V	1.63±0.021 1a,2c,3a,4c	+7.23	2.17±0.02/8 1a,2a,3a,4c	+9.59	2.63±0.070 1a,2a,3a,4c	+8.67

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

49) age periods, the weight gain decreased by 22.59% and 18.68 % (group III) and by 28.42% and 19.49% in group IV compared to controls (P<0.001).

The daily feed consumption was reduced in groups III and IV by 11.14% and 13.72% during the first, by 4.64% and

9.89% during the second and by 4.2% and 4.23% during the fourth monitoring periods (P<0.001). Feed conversion was higher in groups III and IV by 14.47% and 19.07% (first period), by 23.23% and 26.76% (second period) and by 17.76% and 19.42% (third period) (P<0.001).

Chickens from group V in which aflatoxin (0.5 mg/kg AFB₁) was co-administered with 1 g/kg mycosorbent (Mycotox NG) showed milder negative effects of AFB₁ on live body weight, daily weight gain and feed conversion (P<0.05–P<0.001). There were not statistically significant difference between that group and the control group.

Also, during the entire experimental period, body weight, weight gain and daily feed consumption and conversion did not differ statistically controls and birds from group II which received 1 g/kg Mycotox NG only.

The relative weights of viscera (g/100 g body weight) (liver, kidneys, heart, spleen, bursa of Fabricius, thymus, pancreas, gizzard and proventriculus) are shown on Table 5. Chickens from experimental groups III and IV whose feed was supplemented with either 0.5 or 0.8 mg/kg AFB₁ showed considerably increased relative weights of liver, kidneys, heart, spleen, pancreas, gizzard and proventriculus vs controls (P<0.001). In addition, the

relative weights of the thymus and bursa of Fabricius were lower than those in controls (P<0.001). The supplementation of the ration of group V with mycosorbent reduced statistically significantly the negative toxic impact of AFB₁ on relative visceral weights (P<0.05–P<0.001). It has been also shown that the addition of 1 g/kg Mycotox NG to the compound feed of group II was not deleterious with regard to relative weights of aforementioned organs.

DISCUSSION

Aflatoxins are mycotoxins of particular importance for poultry industry due to their high toxicity and the high rates of poultry feed contamination with them (Denli *et al.*, 2004; Tessari *et al.*, 2006). The high toxicity of aflatoxins in domestic fowl was reported by many researchers (Kubena *et al.*, 1991; Abousadi *et al.*, 2007; Mogadam & Azizpour, 2011). The lower body weight, weight gain, feed con-

Table 4. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on daily feed consumption of 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

Groups	Daily feed consumption (g)					
	21 days of age	%*	35 days of age	%*	49 days of age	%*
I	56.96±0.036	100	106.88±0.038	100	171.20±0.075	100
II	56.94±0.034	-0.04	106.90±0.039	+0.01	171.16±0.055	-0.03
III	50.62±0.024 _{1c,2c}	-11.14	101.93±0.032 _{1c,2c}	-4.64	164.01±0.042 _{1c,2c}	-4.20
IV	49.15±0.015 _{1c,2c}	-13.72	96.31±0.028 _{1c,2c,3c}	-9.89	163.96±0.048 _{1c,2c}	-4.23
V	57.00±0.027 _{3c,4c}	+0.07	108.45±0.020 _{3c,4c}	+1.46	171.8±0.070 _{3a,4c}	+0.35

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

Table 5. Effect of aflatoxin B₁ (AFB₁) only or co-administered with Mycotox NG on relative weights of visceral organs of 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

Groups	Liver	Kidneys	Heart	Bursa of Fabricius	Thymus
I	2.08±0.012	0.44±0.012	0.47±0.015	0.20±0.006	0.26±0.012
II	2.07±0.013	0.46±0.010	0.46±0.014	0.22±0.006	0.27±0.012
III	2.68±0.025 1c,2c	0.61±0.013 1c,2c	0.62±0.010 1c,2c	0.14±0.004 1c,2c	0.17±0.007 1c,2c
IV	2.83±0.037 1c,2c,3c	0.67±0.014 1c,2c,3b	0.70±0.015 1c,2c,3b	0.12±0.004 1c,2c	0.15±0.011 1c,2c
V	2.22±0.032 1b,2b,3c,4c	0.52±0.012 1a,2a,3c,4c	0.53±0.014 1c,2b,3c,4c	0.18±0.006 1a,2c,3c,4c	0.22±0.007 1c,2c,3a,4c

Groups	Spleen	Pancreas	Proventriculus	Gizzard
I	0.10±0.003	0.22±0.010	0.35±0.011	1.71±0.021
II	0.11±0.002	0.22±0.011	0.37±0.013	1.70±0.040
III	0.16±0.003 1c,2c	0.33±0.012 1c,2c	0.48±0.011 1c,2b	2.03±0.025 1c,2c
IV	0.17±0.003 1c,2b,3c	0.39±0.010 1c,2c,3c	0.52±0.012 1c,2b	2.23±0.037 1c,2c
V	0.12±0.004 1b,2a,3c,4c	0.27±0.010 1a,2a,3b,4c	0.43±0.012 1b,2b,3a,4c	1.86±0.025 1b,2b,3b,4c

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

sumption and the higher feed conversion ratio in broilers whose feed was supplemented with 0.5 or 0.8 mg/kg AFB₁ agree with results from previous studies and confirm the inhibiting effect of aflatoxins on production traits (Afzal & Saleem, 2004; Girish & Devegowda, 2006; Abousadi *et al.*, 2007). This depression results from inhibition of protein synthesis, reduced absorption of nutrients and lower rate of pancreatic enzymes release (Swamy & Devegowda, 1998; Oguz & Kurtoglu, 2000; Oguz *et al.*, 2000; Safameher *et al.*, 2008). The impaired liver function and the decreased utilisation of proteins, nucleic acids, carbohydrates and fats influence the weight gain and the general

health of poultry (Parlat *et al.*, 1999; Miazza *et al.*, 2000; Ortatatli & Oguz, 2001; Allameh *et al.*, 2005). Aflatoxins provoke a nutritional deficiency and hence, inhibition of weight gain, hepatomegaly and increased weights of visceral organs (Kubena *et al.*, 1990; Basmacioglu *et al.*, 2005; Abousadi *et al.*, 2007). The suppressed appetite in aflatoxicosis is due to the impaired liver metabolism consequently to the impaired liver structure (Johri & Majumdar, 1990; Verma *et al.*, 2004).

The results of our experiments are comparable to other reports about reduced production traits in broiler chickens after dietary intake of low amounts of AF between 0.04 and 0.5 mg/kg with feed

(Afzal & Saleem, 2004; Hesham *et al.*, 2004; Denli & Okan, 2006; Abousadi *et al.*, 2007; Shi *et al.*, 2009; Suksombat *et al.*, 2011; Khadem *et al.*, 2012).

The addition of mycosorbent (Mycotox NG) to the feed of group V had a fairly protective effect with respect to the inhibiting effect of AFB₁ on the growth of chickens and feed conversion. Similar results were found out in chickens, whose contaminated feed was supplemented with mycosorbents such as hydrated sodium potassium aluminosilicate (Kubena *et al.*, 1990; Ledoux *et al.*, 1999; Denli *et al.*, 2009), zeolites (Miazza *et al.*, 2005; Girish & Devegowda, 2006), bentonites (Santurio *et al.*, 1999; Rosa *et al.*, 2001) and clinoptylolites (Oguz & Kurtoglu, 2000; Oguz *et al.*, 2000). Mycosorbents are able to bind aflatoxins in the gastrointestinal tract and thus, to prevent or decrease their absorption (Abdel-Wahhab *et al.*, 1999; Ortatatli & Oguz, 2001; Aravind *et al.*, 2003; Oguz & Parlat, 2004).

The liver is considered to be the target organ for the toxic effect of aflatoxins in domestic fowl. The relative weight of the liver is reported to increase significantly compared to other organs after intake of low aflatoxin doses (Kubena *et al.*, 1993). The hepatotoxicity of AF entails disturbances in lipid, carbohydrate and protein metabolism (Kubena *et al.*, 1993; 1998; Ledoux *et al.*, 1999), as well as in haematopoiesis (Oguz *et al.*, 2000). The increased relative weights of the liver, kidneys, heart, pancreas, gizzard and proventriculus confirm previous results reported in broilers after oral intake of AFB₁ (Kubena *et al.*, 1990; 1993; 1998; Ledoux *et al.*, 1999; Abousadi *et al.*, 2007; Sakhare *et al.*, 2007; Sharghi & Manafi, 2011). The irritating effect of AF on alimentary organs is the cause for increase in

their relative weights. This irritation further causes inflammation of the gizzard and proventriculus mucosa and its thickening (Abousadi *et al.*, 2007). The higher relative weight of the liver is due to the excessive fat accumulation consequently to the impaired fat metabolism (Abousadi *et al.*, 2007; Sharghi & Manafi, 2011). The increased relative weight of the kidneys is a result of the lipidaemia and the resulting fat deposition (Sharghi & Manafi, 2011).

Immune organs of birds – thymus, spleen and bursa of Fabricius – are responsible for the humoral and cell immunity and remain functionally active over a lifetime (Qureshi *et al.*, 1998; Sakhare *et al.*, 2007). Lower relative weights of the thymus and bursa of Fabricius in groups treated with AFB₁ only are due to atrophy, sclerosis, necrosis and reduction in lymphoid cell counts (Ortatatli & Oguz, 2001; Sakhare *et al.*, 2007). The higher relative weight of the spleen is interpreted as a compensatory mechanism to the reduced functional activity and weights of the bursa of Fabricius and the thymus (Hesham *et al.*, 2004; Khadem *et al.*, 2012).

CONCLUSIONS

According to the results of the present study, the supplementation of poultry compound feed with 0.5 mg/kg or 0.8 mg/kg AFB₁ exerted an adverse effect on production traits manifested with lower live body weight, daily weight gain, daily feed consumption as well as increased feed conversion. At the same time, the relative weights of the liver, kidneys, heart, pancreas, spleen, gizzard and proventriculus were higher vs controls, and the relative weights of the thymus and bursa of Fabricius were reduced. The supplementation of the feed of experimen-

tal group V (containing 0.5 mg/kg AFB₁) with 1 g/kg Mycotox NG resulted in partial reduction of negative effects of AFB₁ on production traits and visceral weights.

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