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Influence on Mycotox® NG effects on relative weights of some internal organs in Pekin ducks with experimentally reproduced aflatoxicosis B_1

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Abstract. *Contamination of poultry feeds with mycotoxins is a global problem faced by poultry industry due to increased demands and availability of poor-quality cereals. The aim of the present study was to evaluate the beneficial effects of a mycotoxin binder (Mycotox NG) on relative weights of internal organs in Pekin ducks with experimental aflatoxicosis. The birds were divided into one control and six experimental groups (n=10) as follows: group I (0 mg/kg AFB_1 without Mycotox NG); group II (0.5 g/kg Mycotox NG); group III (1.0 g/kg Mycotox NG); group IV (0.2 mg/kg AFB_1); group V (0.4 mg/kg AFB_1); group VI (0.2 mg/kg AFB_1 + 0.5 g/kg Mycotox NG) and group VII (0.4 mg/kg AFB_1 + 1.0 g/kg Mycotox NG). Trial duration was 42 days. It was established that ducks fed AFB_1-contaminated feed had increased relative weights of liver, kidneys, pancreas, heart, gizzard and proventriculus compared to the control group. At the same time, the relative weights of immunocompetent organs (thymus, spleen and bursa of Fabricius) were reduced. The addition of Mycotox NG to the feed contaminated with AFB_1 compensated partly the changes in relative weights of visceral organs. The results from the present study demonstrated that the tested toxin binder could be effective for reduction of toxic effects of aflatoxins in domestic ducks.*

Keywords: aflatoxicosis domestic ducks, immunocompetent organs, toxin binder, viscera

Introduction

Mycotoxins, especially aflatoxins, are harmful for poultry farming due to frequent contamination of poultry feeds (Lakkawar et al., 2017). Aflatoxins are a class of secondary low-molecular toxic metabolites produced by the genus *Aspergillus*, mainly by *Aspergillus flavus and A. parasiticus*. Among all identified aflatoxins (AF), AFB_1, AFB_2, AFG_1 and AFG_2 are the four main forms found in naturally contaminated feeds (Monbaliu et al., 2010). The toxicity of AFG_2, AFB_2 and AFG_1 is equal to 10%, 20% and 50% of that of AFB_1, respectively (Leeson et al., 1995). The synthesis of aflatoxins occurs at ambient temperature of 24-35°C, air humidity over 7% and feed humidity over 14% (Santos et al., 2001). These mycotoxins are present in feeds worldwide and cause serious economic losses to poultry and livestock industries (Miller, 1995).

The sensitivity of the different domestic bird species to the toxic effects of aflatoxins is diverse. The most sensitive are ducklings, goslings, young pheasants, quails and chickens (Leeson et al., 1995). Aflatoxins have adverse effects on the health of birds and cause extensive economic losses (Kubena et al., 1993). Losses due to consumption of aflatoxin-contaminated feeds in domestic fowl come from poor growth performance, biochemical changes in blood, pathomorphological changes in various organs, increased death rates, reduced egg production (Lakkawar et al., 2015, 2017). A number of studies describe the adverse effects of aflatoxins in broiler chickens expressed in lower weight gain, feed conversion and feed intake, changes in blood serum and plasma biochemical parameters, relative weights of visceral and lymphoid organs, immune responses and morphological changes in organs (Ortatatli et al., 2005; Shi et al., 2006; Denli et al., 2009; Khan et al., 2010; Yalagod, 2014; Kumar et al., 2014). From the health point of view, aflatoxins are dangerous for people as poultry meat consumers, as they may accumulate in the human body and induce neoplastic processes (Istfaq et al., 2014). A general rule in domestic fowl feeding is to keep the total dietary aflatoxin amount below 20 µg (Coelho, 1990).

The producers of mycosorbents, researchers and governments focus their efforts on development of effective strategies for prevention and decontamination aimed at minimisation of toxic effects of aflatoxins (Rosa et al., 2001). The approaches used include physical, chemical, and biological treatment of contaminated feeds and feed ingredients. The successful detoxication could be economically profitable and eliminate the toxin from feeds without any residues, and at the same time, without deteriorating feed nutritional properties (Parlat et at., 1999). Adsorbents are preferred to most decontaminating

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agents. Their main advantages are efficiency, safety, and easy application with animal and poultry feeds (Lakkawar et al., 2017).

For detoxication of aflatoxin-contaminated feeds, various adsorbents were reported to be useful: zeolite (Miazzo et al., 2000), bentonite (Rosa et al., 2001), hydrated sodium-potassium aluminosilicate (Abo-Norag et al., 1995), *Saccharomyces cerevisiae* (Celik et al., 2001) and activated charcoal (Jindal et al., 1994).

An important requirement to toxicological experiments is the possibility to evaluate the effects of xenobiotics on the function of specific organs. In many organs, this is done by macroscopic studies, measurement of organ weights and histological evaluation of tissue specimens (Bailey et al., 2004). The changes in organ weights may be the most sensitive parameter about the effect of tested compounds. Further, the comparison of organ weights between animals treated with xenobiotics and untreated controls could be often impeded by differences in live body weights of the compared groups. That is why, another parameter which is often used is the ratio between body weight and organ weight. This ratio is described as relative weight (e.g. the organ weight in relation to body weight). All mycotoxins, including aflatoxins, are known to cause substantial changes in the relative weight of internal and lymphoid organs (Lakkawar et al., 2017).

Despite the large body of data about toxic effects of aflatoxins in several poultry species, the available information in ducklings as the most sensitive avian species (Feng et al., 2017) is still scarce.

The present experiment aimed to evaluate the possibility for effective alleviation of the toxic effects of AFB_1 by feed supplementation of domestic ducklings with the mycosorbent Mycotox NG.

Material and methods

Experimental design

The experiment was carried out with 70 1-day-old Pekin ducklings from both sexes, divided randomly into 7 groups with 10 birds each. The duration of the experiments was 42 days. Ducklings from control and experimental groups were fed a pelleted feed according to their age, produced by Zara Furazhi AD, Stara Zagora (Table 1). The different study groups were as follows: group I (control diet); and experimental groups that received, respectively, Mycotox NG (Ceva Sante Animale, France) mixed with feed as follows: 0.5 g/kg feed (group II); 1.0 g/kg feed Mycotox NG (group III); 0.2 mg/kg feed AFB_1 (group IV); 0.4 mg/kg feed AFB_1 (group V); 0.2 mg/kg feed AFB_1 + 0.5 g/kg feed Mycotox NG (group VI) and 0.4 mg/kg feed AFB_1 + 1.0 g/kg feed Mycotox NG (group VII).

Table 1. Composition and nutritional value of compound feed

Ingredients	Compound feed	
	Starter (0-4 weeks of age)	Grower (5-7 weeks of age)
Corn, %	20	20
Wheat, %	45	52
Soybean meal – 46%	13	3.5
Sunflower meal – 34%	14	14
Wheat bran, %	2	5
Sunflower oil, %	1	0.5
Lysine, %	0.15	0.1
Oxyguard, %	0.01	0.01
Vitamin:mineral premix BK 2111, %	4.5	4
Nutritional value		
Crude protein, g/kg	180.20	149.07
Metabolisable energy, kcal/kg	2764.39	2784.07
Crude ash, g/kg	57.30	49.12
Crude fibre, g/kg	59.16	57.91
Crude fat, g/kg	30.88	26.32
Calcium, g/kg	10.33	9.20
Phosphorus, g/kg	7.04	5.80
Lysine, g/kg	9.40	7.89
Methionine+cysteine, g/kg	5.08	7.18
Threonine, g/kg	6.67	5.50
Tryptophan, g/kg	2.09	1.71

Tested aflatoxin B_1 with 99% purity was produced by *Aspergillus flavus* (Sigma-Aldrich, Germany). Ducklings from control and treated groups were reared under optimum microclimatic parameters in line with Ordinance No 44/2006.

For determination of relative weights of visceral organs (liver, kidneys, heart, bursa of Fabricius, spleen, thymus, pancreas, gizzard and proventriculus), birds were euthanised by cervical dislocation as per Ordinance No 20/2012 on the minimum requirements for the protection and welfare of experimental animals and requirements to objects for use, cultivation and/or supply. Experiments were approved by the Bulgarian Food Safety Agency (permit No 225/17.01.2019).

Relative organ weights (ROW) were calculated by the formula:

ROW, % = [Organ weight (g)/Body weight (g)].100

Results were statistically processed by one-way ANOVA and the level of significance: with the Tukey-Kramer test (p<>0.05). Results are presented as mean ± standard error of the mean (SEM).

Results and discussion

Aflatoxicosis induces abnormalities in some internal organs through increase in their relative weights (Mishra and Das, 2003). Relative weights of visceral organs (g/100 g body weight) are presented in Table 2. The liver is the target organ for toxic effects of aflatoxins as it is the site where aflatoxins undergo metabolic conversion to reactive Aflatoxin B1 8,9 epoxide, which binds to proteins and DNA forming adducts damaging liver structures and increases liver relative weight (Sridhar et al., 2015). Data in Table 2 show statistically significantly ($p<0.05$) higher relative weight of liver in birds that received aflatoxin B1 with feed, confirming earlier reports (Ortatatli et al., 2005; Shi et al., 2006; Denli et al., 2009; Khan et al., 2010; Yalagod, 2014; Lakkawar et al., 2015). Increased liver weight was probably due to accumulation of lipids and impaired hepatic lipid transport, induced by mycotoxins. Hepatic lipidosis is mainly mediated by inhibited synthesis of phospholipids and cholesterol, which consequently influences lipid transport through the organ (Manegar et al., 2010). Vascular degeneration and inflammation induced by aflatoxins in visceral organs may be responsible for increased weights of organs. Histological lesions, fatty dystrophy, lipidosis and inflammatory response were reported in several previous studies (Yalagod, 2014; Lakkawar et al., 2015; Valchev et al., 2014a; 2020).

Table 2. Effect of aflatoxin B_1 (AFB_1) only or co-administered with Mycotox NG on relative weight of internal organs (g/100 g live weight %) of domestic ducklings

Groups	Liver	Proventriculus	Gizzard	Pancreas	Heart
I	2.54±0.026	0.43±0.005	2.18±0.008	0.35±0.003	0.57±0.006
II	2.56±0.036^{c}	0.45±0.005	2.17±0.008^{c}	0.36±0.00	0.56±0.003
III	2.54±0.023	0.46±0.004	2.18±0.015	0.37±0.003	0.56±0.004
IV	3.43±0.060^{1c,2c,3c}	0.62±0.010^{1c,2c,3c}	2.55±0.062$^{1c;2c,3c}$	0.54±0.012^{1c,2c,3c}	0.76±0.016^{1c,2c,3c}
V	4.21±0.032^{1c,2c,3c,4c}	0.74±0.001^{1c,2c,3c}	2.71±0.036$^{1c;2c,3c,4a}$	0.66±0.033^{1c,2c,3c,4c}	0.91±0.002^{1c,2c,3c,4c}
VI	2.99±0.100^{1c,2c,3c,4c,5c}	0.57±0.004^{1c,2c,3c,4b,5c}	2.50±0.023$^{1c;2c,3c,5c}$	0.50±0.012^{1c,2c,3c,4c,5c}	0.68±0.008^{1c,2c,3c,5c}
VII	3.28±0.046^{1c,2c,3c,4c,5c,6a}	0.61±0.011^{1c,2c,3c,5c,6c}	2.60±0.036$^{1c;2c,3c}$	0.50±0.006 ^{c,2c,3c,6c}	0.75±0.012^{1c,2c,3c,5c,6a}

Groups	Kidney	Thymus	Spleen	Bursa of Fabricius
I	0.69±0.013	0.27±0.004	0.15±0.002	0.16±0.002
II	0.69±0.073	0.27±0.003	0.16±0.003	0.16±0.006
III	0.71±0.010^{1}	0.26±0.002	0.16±0.002	0.16±0.003
IV	0.96±0.032^{1c,2c,,3c}	0.19±0.004^{1c,2c,,3c}	0.11±0.002^{1c,2c,3c}	0.10±0.003^{1c,2c,3c}
V	1.14±0.029^{1c,2c,,3c,4c}	0.17±0.006^{1c,2c,,3c}	0.09±0.002^{1c,2c,,3c,4c}	0.09±0.003^{1c,2c,,3c,4a}
VI	0.99±0.016^{1c,2c,,3c}	0.21±0.003^{1c,2c,,3c,4c}	0.13±0.002^{1c,2c,,3c,4c,5c}	0.11±0.003^{1c,2c,,3c,5c}
VII	1.08±0.021^{1c,2c,,3c,4b,5c}	0.22±0.006^{1c,2c,,3c,4c}	0.12±0.003^{1c,2c,,3c,4b,5c}	0.10±0.003^{1c,2c,,3c}

*Group I- control; group II- 0.5 g/kg Mycotox NG; group III- 0.5 g/kg Mycotox NG; group IV- 0.2 mg/kg AFB_1; group V- 0.4 mg/kg AFB_1; group VI- 0.2 mg/kg AFB_1 + 0.5 g/kg Mycotox NG; group VII- 0.4 mg/kg AFB_1 + 1.0 g/kg Mycotox NG; Data are presented as mean ± SEM; n=10; $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$.

The established increase in relative weights of the gizzard and proventriculus ($p<0.05$) is attributed, on one hand, to the direct cytotoxic effect of AFB_1 on digestive organs (Abousadi et al., 2007; Valchev et al., 2013), and on the other, results from AF-induced irritation of gastrointestinal mucosa with consequent inflammation and thickening (Abd El-Ghany and Hatem, 2013).

Increased relative weight of the pancreas ($p<0.001$) was a result from thickening of interlobular septae among acinar cells, cell proliferation and congestive events (Abd El-Haleem and Mohamed, 2011; Valchev et al., 2015).

The statistically significant increase in the relative weight of the heart ($p<0.05$) corresponded to previous investigations in broiler chickens (Abousadi et al., 2007; Valchev et al., 2013).

The data presented demonstrate statistically significant ($p<0.001$) increase in the relative weight of kidneys in birds fed aflatoxins-contaminated ration. Increased kidney weight in this study was in line with earlier findings and is attributed to vascular reaction and degenerative changes in renal parenchyma during aflatoxicosis (Valchev et al., 2013, 2014b; Yalagod, 2014; Lakkawar et al., 2015).

In this study, the relative weight of the thymus, spleen and bursa of Fabricius were considerably ($p<0.05$) reduced in ducklings fed diet contaminated with aflatoxin B_1 (similarly to other previous data (Kumar et al., 2014; Yalagod, 2014; Lakkawar et al., 2015). Reduced relative weights of lymphoid

organs may be attributed to the toxicity of AFB_1 on the lymphoid component inducing lymphocytolysis and thus, reduced size of lymphoid organs (Peng et al., 2015; Grozeva et al., 2019). Contrary to the findings of this study, some authors reported considerably higher relative weight of spleen in broiler chickens fed aflatoxin-contaminated feed (Bailey et al. 2006; Shi et al., 2006; Valchev et al., 2014c). Differences in splenic relative weight could be due to various factors, e.g. aflatoxins concentration and exposure time.

The supplementation of the ration of group VI and VII with mycosorbent reduced statistically significantly the negative toxic impact of AFB_1 on relative visceral weights ($p<0.05$). These data are in concordance with our research (Valchev et al., 2013, 2014c) and those reported by other authors having used brewers' yeasts (*Saccharomyces cerevisiae*) (Celik et al., 2001); bentonite (*montmorillonite*) (Shi et al., 2006); zeolites (Miazzo et al., 2000); silicate (*Diatomaceous earth*) (Lakkawar et al., 2015). Studies were carried out to reduce the toxic effects of aflatoxins through binding and adsorption in the gastrointestinal tract (Nabi et al., 2018). The main advantages of adsorbents are efficacy of costs, easy application with feeds. The results from this study confirmed that the tested amount of Mycotox NG was able to bind AFB_1 molecules in the digestive tract of birds. The aflatoxin molecules possess aromatic hydrophilic structure with high binding affinity to the surface of mycosorbents (Boudergue et al., 2009). The binding of aflatoxins to toxin binders occurs on electrical polarity principle. Negatively charged mycotoxins bind to positively charged toxin binders, and thus toxins are immobilised and eliminated from the animal bodies (Kana et al., 2014). The formation of stable complexes of aflatoxins with mycosorbents in the proventriculus, gizzard and intestines and their excretion through avian cloaca reduce toxin absorption (Saminathan et al., 2018).

Conclusion

Finally, it could be concluded that the addition of 0.2 mg/kg feed AFB_1 and 0.4 mg/kg feed AFB_1 to the compound feed of Pekin ducklings causes a significant increase ($p<0.05$) in the relative weights of liver, kidneys, spleen, heart, pancreas, proventriculus and gizzard and reduces the relative weights of thymus and bursa of Fabricius. The addition of 0.5 g/kg feed Mycotox NG and 1.0 g/kg feed Mycotox NG to contaminated rations reduces the toxic effects of aflatoxins on relative weights of internal organs.

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