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Nutrition and Physiology

Evaluation of effect of Mycotox® binder supplementation on production parameters in induced aflatoxicosis in Pekin ducks

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Abstract. *Aflatoxins are highly toxic fungal metabolites encountered in feed ingredients at various concentrations. The aim of this study was to investigate the effect of aflatoxin B1 administered either alone or with Mycotox NG on productive traits (body weight, weight gain, feed intake and feed conversion) in Pekin ducks. The birds were divided into one control and six experimental groups (n=10): group I (0 mg/kg AFB1 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 AFB1); group V (0.4 mg/kg AFB1); group VI (0.2 mg/kg AFB1 + 0.5 g/kg Mycotox NG) and group VII (0.4 mg/kg AFB1 + 1.0 g/kg Mycotox NG). Trial duration was 42 days; changes in productive traits were determined on 14th, 28th and 42nd day. Lower productive traits in terms of body weight, daily weight gain and feed intake along with higher feed conversion ratio were found out in ducklings from experimental groups IV and V. The addition of 0.5 g/kg or 1.0 g/kg Mycotox NG to the diet of groups VI and VIII reduced or prevented the deleterious AFB1 effects on studied productive parameters.*

Keywords: aflatoxicosis (B1), Pekin ducklings, Mycotox Ng, productive traits

Introduction

Mycotoxins are secondary toxic metabolites produced by some toxigenic molds under favourable environmental conditions (Elzupir et al., 2015). Mycotoxins are the second important problem for the poultry industry after the high cost of feeds. Aflatoxins (AF) contaminate ready poultry feeds and their ingredients around the world (Zainudin and Perumal, 2015). The presence of AF in feeds of domestic fowl and their consumption results in poorer growth performance, high mortality, reduced egg production, lower meat production and worse meat technological properties, higher susceptibility to stress (Liu et al., 2011; Ali et al., 2019). Aflatoxins are produced by molds from the genus *Aspergillus*, mainly by *Aspergillus flavus* and *A. parasiticus*. These molds are able to grow and contaminate cereals before and after crop harvesting, but also during storage, transportation and processing of feed ingredients. The growth of fungal spores is initiated when feed humidity exceeds 12%, at ambient temperature of 25-35°C, air humidity 80% and appropriate aeration (Singh, 2019). Out of all isolated AF, only types B_1, B_2, G_1 and G_2 are found in naturally contaminated feeds (Saki et al., 2018). Aflatoxins are fluorescence compounds: aflatoxins B_1 (AFB_1) and B_2 (AFB_2) have blue fluorescence, whereas aflatoxins G_1 (AFG_1) and G_2 (AFG_2) – yellow-green fluorescence under UV light (Verma et al., 2004). The sensitivity of animals to AF is species- and age-dependent. Ducklings, goslings and turkey poults are more sensitive to toxic effects of AF compared to other avian species (Kamalzadeh et al., 2009). Regardless of the detailed information on AF effects in other productive animal species, less information is available for ducklings, being the most sensitive domestic bird species (Diaz and Murcia, 2011). The European Economic Community (EEC) and Food and Drug Administration (FDA) have set minimum allowance of total AF in poultry feeds of 20 μg/kg (Lubna et al., 2018).

The most commonly used approach to detoxication of AF-contaminated feeds is use of sorbents for selective removal of toxin through adsorption before and after their passage through the avian gut (Ismail et al., 2018). Aflatoxin binders adsorb toxins from feeds or from intestines, and

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then they are excreted through faeces (Boudergue et al., 2009). Harmful effects of AF could be reduced to a minimum by supplementing feeds with inorganic or organic compounds as bentonites, zeolites, activated charcoal, yeasts, probiotics and antioxidants (Fouad et al., 2019).

The present study aimed to evaluate the possibility for effective alleviation of the toxic effects of AFB1 by feed supplementation of domestic ducklings with the mycosorbent Mycotox NG on productive traits (body weight, weight gain, feed intake and feed conversion) in Pekin ducks.

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 trial 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 0.5 g/kg Mycotox NG (Ceva Sante Animale, France- micronized brewers' yeasts, thymol – group II); 1.0 g/kg Mycotox NG (group III); 0.2 mg/kg AFB_1 (group IV); 0.4 mg/kg AFB_1 (group V); 0.2 mg/kg AFB_1 + 0.5 g/kg Mycotox NG (group VI) and 0.4 mg/kg AFB_1 + 1.0 g/kg Mycotox NG (group VII), respectively.

Table 1. Composition and nutritional value of compound feed

Ingredients	Compound feed: Starter (0-4 weeks of age)	Compound feed: 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 44/2006.

Live body weight, daily feed intake, daily weight gain and feed conversion were followed out on day 14, 28 and 42 by weighing. The weight gain for the three periods was calculated by subtracting the initial weight from the final weight for the period. Feed conversion ratio was determined by the formula:

$$FCR = \frac{\textit{Feed in take}}{\textit{average daily gain}}$$

Feed was offered *ad libitum*. The non-consumed feed was weighed on the next morning. Thus, feed intake for each group was obtained by subtracting the non-consumed feed from the amount of offered feed. The daily feed intake per bird was obtained by dividing the total amount of feed consumed over 24 hours by each group to the number of birds in the group.

Experiments were approved by the Bulgarian Food Safety Agency (permit No.225/17.01.2019).

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

Body weight

The results showed statistically significantly lower body weight in ducklings from groups IV and V, fed only AFB_1 during all three survey periods (day 14, 28 and 42), compared to the control group ($p<0.001$) (Table 2). At the end of the trial period, body weight in these groups was lower by 27.89% and 42.09%, respectively. The addition of mycosorbent (Mycotox NG) at 0.5 g/kg and 1.0 g/kg feed to ducklings' ration (groups VI and VII) was the reason for higher values of this parameter as compared to groups that received only AFB_1 (groups IV and V). At the end of the period, however, the body weight of birds from groups VI and VII was lower than that of controls – by 17.73% and 23.71%, respectively.

Table 2. Effect of aflatoxin B1 (AFB1) only or co-administered with Mycotox NG on body weight of Pekin ducks

Groups	Live body weight (g)				
	Initial weights (g)	14 days of age	28 days of age	42 days of age	Difference %
I	43.0±0.81	341.5±4.94	1235±10.02	2274±8.71	100
II	43.5±1 .50	341.5±6.28	1237±10.75	2270±11.74	-0.18
III	43.0±1.10	340.5±7.12	1243±14.14	2278±18.18	+0.17
IV	43.2±0.89	257±10.08[1c,2c,3c]	826±29.25[1c,2c,3c]	1640±37.11[1c2c,3c,]	-27.89
V	42.0±0.81	219.5±9.90[1c,2c,3c,4b]	743±18.07[1c,2c,3c]	1317±29.81[1c,2c,3c,4c]	-42.09
VI	43.2±1.29	305.3±4.24[1a,2a, 3a, 4c,5c]	1010±21.02[1c,2c,3c,4c,5c]	1871±18.52[1c,2c,3c,4c,5c]	-17.73
VII	43.0±1.85	300.2±5.92[1b,2b, 3b, 4b,5c,6c]	909±24.83[1c,2c,3c,5b,6a]	1735±42.17[1c,2c,3c,4c,5c]	-23.71

*Data are presented as mean±SEM; n=10 ducks per group; ap<0.05; bp<0.01; cp<0.001; 1 - control group (0 mg/kg AFB1 not supplemented with Mycotox® NG) vs group II (0.5 g/kg Mycotox® NG); 2 - vs experimental group III (1.0 g/kg Mycotox® NG); 3 - vs experimental group IV (0.2 mg/kg AFB1); 4 - vs experimental group V (0.4 mg/kg AFB1); 5 - vs experimental group VI (0.2 mg/kg AFB1 and 0.5 g/kg Mycotox® NG); 6 - vs experimental group VII (0.4 mg/kg AFB1 and 1.0 g/kg Mycotox® NG)

Daily weight gain

The dynamics of daily weight gain (Table 3) in groups IV and V (supplemented, respectively, with 0.2 and 0.4 mg/kg AFB_1 only) demonstrated statistically significantly lower values ($p<0.001$) for the three measurement periods (days 14, 28 and 42). The addition of AFB_1 at 0.2 mg/kg feed resulted in reduction of daily weight gain by 28.17% (on the 14th day), by 36.17% (on the 28th day) and by 23.48% (on the 42nd day). Considerably higher reducing effect on this parameter was shown in ducklings from group V that received higher AFB_1 dose: 0.4 mg/kg feed: by 41.96% (day 14), by 41.3% (day 28) and by 47.24% (day 42). Ducklings from group VI (0.2 mg/kg feed AFB_1 + 0.5 g/kg feed Mycotox Ng) and group VII (0.4 mg/kg feed AFB_1 + 1.0 g/kg feed Mycotox Ng) exhibited statistically significantly better daily weight gain compared to groups IV and V ($p<0.001$), yet it was lower compared to control group throughout the experiment ($p<0.05$ – $p<0.001$). At trial's end, daily weight gain of ducklings from groups VI и VII was by 18.13% and 22.73% lower vs controls.

Table 3. Effect of aflatoxin B1 (AFB1) only or co-administered with Mycotox NG on daily weight gain of Pekin ducks

Groups	Daily weight gain (g)			
	14 days of age	28 days of age	42 days of age	Difference %
I	21.31±0.35	63.67±0.83	75.98±1.39	100
II	21.08±0.49	63.96±0.91	73.78±1.35	-2.9
III	21.24±0.52	64.46±1.07	73.92±1.92	-2.72
IV	15.32±0.71[1c,2c,3c]	40.64±1.49[1c,2c,3c]	58.14±2.25[1c,2c,3c]	-23.48
V	12.37±0.64[1c,2c,3c,4b]	37.38±1.17[1c,2c,3c]	40.09±2.63[1c,2c,3c,4c]	-47.24
VI	18.79±0.33[1a,2a,3c,4b,5c]	50.33±1.67[1c,2c,3c,4c]	62.21±1.38[1c,2c,3c,5c]	-18.13
VII	18.37±0.36[1b,2b, 3b,4b,5c]	44.77±2.00[1c,2c,3c,5b]	58.71±1.32[1c,2c,3c,5c]	-22.73

*Data are presented as mean±SEM; n=10 ducks per group; [a]p<0.05; [b]p<0.01; [c]p<0.001; 1 - control group (0 mg/kg AFB_1 not supplemented with Mycotox® NG) vs group II (0.5 g/kg Mycotox® NG); 2 - vs experimental group III (1.0 g/kg Mycotox® NG); 3 - vs experimental group IV (0.2 mg/kg AFB_1); 4 - vs experimental group V (0.4 mg/kg AFB_1); 5 - vs experimental group VI (0.2 mg/kg AFB_1 and 0.5 g/kg Mycotox® NG); 6 - vs experimental group VII (0.4 mg/kg AFB_1 and 1.0 g/kg Mycotox® NG)

Feed intake

Data about the average daily feed intake are presented in Table 4. Ducklings that were treated with AFB_1 only at 0.2 mg/kg feed (group V) and 0.4 mg/kg feed (V group) had lower feed intake during all three periods vs controls. At the end of the experiment, feed intake in these groups was decreased by 9.97% and 20.65%, respectively, vs controls. The supplementation of feed with tested fungal inhibitor in groups VI and VII resulted in statistically significantly increased feed intake as compared to groups IV and V ($p<0.05$ – $p<0.01$). However, with regard to controls, feed intake on the 42nd day was by 5.36% and 7.9% lower, respectively. This difference was not significant vs controls ($p>0.05$).

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

Groups	Daily feed intake (g)			
	14 days of age	28 days of age	42 days of age	Difference %
I	34.66±0.36	126.41±0.89	182.66±2.58	100
II	34.14±0.52	125.67±1.03	181.51±2.58	-0.63
III	34.51±0.18	127.46±0.92	181.12±3.444	-0.85
IV	29.68±0.228[1c,2c,3c]	103.20±1.49[1c,2c,3c]	164.45±4.89[1a,2a,3a]	-9.97
V	27.58±0.20[1c,2c,3c,4c]	102.94±1.74[1c,2c,3c]	144.95±7.11[1c,2c,3c,4a]	-20.65
VI	33.10±0.20[4c,5c]	116.68±2.35[1b,2b,3c,4c,5c]	172.88±3.25[4a,5c]	-5.36
VII	33.74±0.18[4c,5c]	111.42±2.40[1c,2c,3c,4a]	168.24±2.56[5b]	-7.9

*Data are presented as mean±SEM; n=10 ducks per group; [a]p<0.05; [b]p<0.01; [c]p<0.001; 1 - control group (0 mg/kg AFB_1 not supplemented with Mycotox® NG) vs group II (0.5 g/kg Mycotox® NG); 2 - vs experimental group III (1.0 g/kg Mycotox® NG); 3 - vs experimental group IV (0.2 mg/kg AFB_1); 4 - vs experimental group V (0.4 mg/kg AFB_1); 5 - vs experimental group VI (0.2 mg/kg AFB_1 and 0.5 g/kg Mycotox® NG); 6 - vs experimental group VII (0.4 mg/kg AFB_1 and 1.0 g/kg Mycotox® NG)

Feed conversion ratio

Feed conversion ratio (Table 5) measured on days 14, 28 and 42 in ducklings from groups IV and V was the highest. Feed conversion ratio in group IV was increased by 22.36% (day 14), by 29.29% (day 28) and by 22.5% (day 42). In ducklings from group V, the respective rates were by 42.23%, 14.64% and 52.08%. Ducklings from groups VI and VII, whose ration was supplemented with AFB_1 and 0.5 g/kg Mycotox NG, feed conversion ratio was substantially lower than those in groups IV and V (p<0.001), yet significantly exceeded control FCR values (p<0.001) during the 2nd and 3rd periods. At the end of the trial, feed conversion ratio in this group was by 15.41% and 20.41% higher than that in controls (p<0.001).

Table 5. Effect of aflatoxin B1 (AFB1) only or co-administered with Mycotox NG on feed conversion rate of Pekin ducks

Groups	Feed conversion rate (g feed/g weight gain)			
	14 days of age	28 days of age	42 days of age	Difference %
I	1.61±0.023	1.98±0.015	2.40±0.013	100
II	1.60±0.032	1.98±0.017	2.46±0.012	+2.5
III	1.63±0.033	1.97±0.023	2.45±0.019	+2.08
IV	1.97±0.096[1b,2b,3b]	2.56±0.083[1c,2c,3c]	2.94±0.067[1c,2c,3c]	+22.5
V	2.29±0.106[1c,2c,3b]	2.77±0.068[1c,2c,3c]	3.65±0.091[1c,2c,3c,4c]	+52.08
VI	1.77±0.025[3c,5c]	2.33±0.049[1c,2c,4a,5c]	2.77±0.027[1c,2b,3c,4c]	+15.41
VII	1.73±0.035[5c]	2.51±0.065[1c,2c,5b]	2.89±0.061[1c,2c,3c,4c,5c]	+20.41

*Data are presented as mean±SEM; n=10 ducks per group; [a]p<0.05; [b]p<0.01; [c]p<0.001; 1 - control group (0 mg/kg AFB_1 not supplemented with Mycotox® NG) vs group II (0.5 g/kg Mycotox® NG); 2 - vs experimental group III (1.0 g/kg Mycotox® NG); 3 - vs experimental group IV (0.2 mg/kg AFB_1); 4 - vs experimental group V (0.4 mg/kg AFB_1); 5 - vs experimental group VI (0.2 mg/kg AFB_1 and 0.5 g/kg Mycotox® NG); 6 - vs experimental group VII (0.4 mg/kg AFB_1 and 1.0 g/kg Mycotox® NG)

Discussion

Aflatoxins are common contaminants of poultry feeds and feed ingredients at a global scale (Kana et al., 2014). It is acknowledged that fish and domestic fowl are extremely sensitive to AF toxicity and react to levels as low as 15-30 µg/kg feed (Rawal et al., 2010). Ducklings are believed to be the most sensitive domestic birds (Chen et al., 2016). The high sensitivity of ducks to AF toxicity as compared to chickens, turkeys, quails is due to higher activity of the enzyme cytochrome P450 (CYP450) that performed bioconversion of aflatoxins to reactive aflatoxin-8,9-epoxide (AFBO) – the main and most toxic metabolite (Diaz and Murcia, 2011). The AFBO inhibits the protein synthesis, causing liver damage and reduces growth performance (Rawal et al., 2010).

During this experiment, no deaths have occurred in any of the groups. Similar findings were reported also by Subhani et al. (2018) in broiler chickens treated with 350 ppb AFB_1 applied either alone or together with 250 mg/kg feed or 500 mg/kg feed algae (*Chlorella pyrenoidosa*). The most relevant economic effect of aflatoxicosis in domestic poultry is poorer growth performance, reduced feed intake, increased feed conversion ratio (Valchev et al., 2017). Most researchers (Verma et al., 2002; 2004; Subhani et al., 2018; Singh, 2019) attributed the adverse effects of AFB_1 on weight gain of birds with lower utilisation of dietary energy and protein.

The worse productive traits identified in this study (e.g body weight and weight gain) in ducklings supplemented only with AFB_1 through feed (groups IV and V) ($p<0.01$) are in line with results from other studies (Chen et al., 2016). The observed reduction of growth parameters in broiler chickens fed diets contaminated with AF, could be attributed to the ability of aflatoxins to inhibit metabolism (Liu et al., 2016; Saminathan et al., 2018) and inhibit protein synthesis by competing with phenylalanine for specific binding site in phenylalanine-transfer RNA synthase. In comparison with the present study, Chen et al. (2016) found out lower weight gain in Pekin ducks after intake of 0.11, 0.14 and 0.21 mg/kg feed AFB_1 which were far lower as compared to those in broiler chickens. Numerous studies in broiler chickens have demonstrated that the minimum amount of AFB_1 that led to lower weight gain and reduced body weight, was 0.5 mg/kg feed (Verma et al., 2004; Manafi et al., 2012). It was shown that lower weight gain and body weight resulted in lower secretion of pancreatic digestive enzymes (Verma et al., 2002; Valchev et al., 2013; Singh, 2019). The reduced absorption surface of small intestine after intake of low amounts of AFB_1 was outlined as a possible cause for the lower body weight and weight gain by Galarza-Seeber et al. (2016)

Increased feed conversion ratio is associated with poorer utilisation of dietary nutrients (Kana et al., 2014; Valchev et al., 2014), anorexia, inhibited protein synthesis and lipogenesis (Dhanapal et al., 2014). Higher FCR was also found out in other studies on ducklings and broiler chickens supplemented with purified AFB_1 at doses from 40 to 200 µg/kg (Bintvihok and Davitiyananda, 2002; Chen et al., 2016).

Mycosorbents compensate the deleterious effects of aflatoxins (Nabi et al., 2018). According to the results from the present study, the tested amount of Mycotox NG was able to bind AFB_1 molecules in the gastrointestinal tract of birds. The structure of molecules of aflatoxins is aromatic hydrophilic, with high binding affinity vs mycosorbents' surface (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 stomach and intestines and their excretion through avian cloaca reduce toxin absorption (Saminathan et al., 2018). These results agree with those reported from other studies with mycosorbents, e.g. clinoptilolite (Oguz et al., 2003), hydrated sodium calcium aluminosilicate (HSCAS), sodium bentonite and montmorillonite (Ologhobo et al., 2015). These data are in concordance with our research (Valchev et al., 2014, 2017).

Conclusion

In conclusion, the supplementation of compound feed of Pekin ducklings with 0.2 and 0.4 mg/kg feed AFB_1 reduced growth performance parameters (live body weight, weight gain, feed intake) and increased feed conversion ratio; furthermore, changed the relative weights of internal organs. The addition of 0.5 g/kg or 1.0 g/kg Mycotox NG to AFB_1 diets reduced or prevented the deleterious toxic effects of mycotoxins on studied productive parameters.

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