Effects of Aflatoxin on the Performance of Broiler Breeders and Its Alleviation through Herbal Mycotoxin Binder

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ABSTRACT

This study was conducted to investigate the effect of Aflatoxin B_1 (AFB_1) on performance and egg quality in broiler breeders and the abatement of its deteriorative effect through its counteraction with Herbal Mycotoxin Binder (HMB). Thirty-six, 28-wk-old broiler breeder hens were allotted to one of the three treatments of: (1) basal diet (control), (2) control plus 500 µg kg^{-1} AFB_1 and (3) control diet plus 500 µg kg^{-1} AFB_1+0.2% HMB for three periods, each of a duration of three weeks and when from 28 to 36 weeks of age. Results revealed that 500 µg kg^{-1} AFB_1 significantly ($P< 0.05$) reduced feed consumption, feed efficiency, egg production as well as egg weight. Supplementation of HMB partially restored feed consumption and egg production alleviating some side effects of AFB_1.

Keywords: Aflatoxin B_1, Breeder hens, Internal egg parameters, Performance parameters.

INTRODUCTION

Cereal grains and their by-products are important ingredients in poultry diet. Global supplies of cereal grains intended for animal feed are frequently contaminated with mycotoxins (Gowda *et al.,* 2008). Among the mycotoxins, aflatoxins are ubiquitous in nature and in feed ingredients (Manafi *et al.,* 2009a). Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species (Manafi *et al.,* 2011). Aflatoxin B_1 (AFB_1) is the most toxic among all aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2). Either the presence or production of aflatoxins in agricultural commodities depends upon many factors, including the time and condition of harvesting, storage as well as transportation. The negative effects of these toxins in animals are influenced by a range of factors including concentration of aflatoxin, duration of exposure, species, gender, age and general health status of animals (Jewers, 1990; Manafi *et al.*, 2009b). In poultry, the economic losses associated with aflatoxin exposure include poor feed conversion and growth, increased mortality, decreased egg production, leg problems, and carcass condemnations (Smith and Hamilton, 1970; Hamilton and Garlich, 1971; Huff *et al.*, 1992).

Adsorbent compounds have been utilized to ameliorate aflatoxicosis in poultry diets. Among several adsorbents, aluminosilicate binders have been found beneficial (Phillips *et al.*, 1988; Gowda and Ledoux, 2008). Lipid peroxidation during aflatoxicosis plays a major role in damaging cell membrane due to free radical generation. These free radicals can be scavenged by dietary antioxidants and consequently prevent cellular damage (Galvano *et al.*, 2001).

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Certain plant compounds like flavonoids and curcuminoids possess antioxidant property, inhibiting the biotransformation of AFB_1 to their active epoxide derivatives (Lee *et al.*, 2001). Turmeric (*Curcuma longa*), a medicinal plant native to the Asian subcontinent, is known to have antimicrobial as well as antioxidant properties. The powder of dried roots and rhizomes of turmeric is traditionally used as an important spice in orient curries and in other cuisines. Curcuminoids are yellowish pigments present in turmeric powder, and have proven to be protective against AFB1 (Soni *et al.*, 1997; Manafi *et al.*, 2009c). The most recent dietary formulation approach to prevent mycotoxicosis in poultry is an incorporation of antioxidants with adsorbents (Surai, 2001). Herbal Mycotoxin Binder (HMB) is a unique combination of minerals (extra purified clay containing diatomaceous earth minerals), antioxidants (curcuminoids extracted from turmeric) and enzymes (Epoxidases and Esterases) at proportions of 15, 10 and 75%, respectively. It is claimed that incorporation of this product in poultry diets would effectively prevent DNA adduct formation and cellular damages in the biological systems through degrading peroxides, amides and lacto rings in such non-polar toxins as aflatoxins. The experiment was conducted at a Private Breeder Poultry Farm in Malayer, Iran. Herbal Mycotoxin Binder (Zeus Biotech Limited, Mysore, India.) used in the study was procured from local market. This study was undertaken to evaluate the efficacy of a Herbal Mycotoxin Binder for counteracting AFB_1 in experimentally contaminated broiler breeder diets.

MATERIALS AND METHODS

Experimental Design and Diets

Thirty-six, 28-wk-old broiler breeder hens along with eighteen 28-wk-old broiler breeder cocks of a commercial strain were weighed and randomly assigned to group cages of 3 replicates with 4 birds per replicate for three periods, each with a duration of three weeks from and 28 to 36 weeks of age. The cocks and hens were separately caged a receiving the corresponding diets. Artificial insemination was carried out twice weekly the semen being transferred from the corresponding males to the related females. The experimental procedures were all approved by the Ethical Committee of the Malayer University. Aflatoxin B_1 (AFB1) was produced using the pure culture of *Aspergillus parasiticus* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India) grown on potato dextrose agar. AFB_1 was produced on rice and the toxin extracted as described by Rukmini and Bhat (1978) then quantified through Thin Layer Chromatography (TLC) as detailed by AOAC (1995). Herbal Mycotoxin Binder is a property product of Zeus Biotech Limited, Mysore, India. Hens and cocks were fed maize (62.5%), soybean meal (19%) plus sunflower meal (10%) based standard breeder diets. Hens were assigned to one of the three treatments of: (1) control (basal diet), (2) basal diet plus 500 µg kg^{-1} AFB_1, and (3) basal diet plus 500 µg kg^{-1} AFB_1+0.2% HMB. Compounded feed was analyzed for the presence of AFB_1 before incorporation of rice culture material, and then the diets (Table 1) were prepared by adding the required quantities of rice culture powder containing AFB_1 into the diet so as to obtain the levels of 0.0, and 500 µg kg^{-1} of AFB_1. The given toxin levels were finally cross-checked through HPLC. Basal diet was formulated to meet nutrient requirements of commercial broiler breeder (2690 Kcal kg^{-1} ME and 17.42% CP). All birds received the control diet prior to start of the experiment to be acclimated, and then fed the experimental diets. A restricted daily feeding regimen (135 and 145 g $bird^{-1}$ per day for cocks and hens, respectively) with unlimited access to water from channel drinkers provided throughout the experiment. Feed consumption of hens was

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Table 1. Nutrient composition of basal diet.

parameters	calculated value
Calculated values	
Metabolizable Energy (Kcal kg^{-1})	2690
Crude protein (%)	17.42
Crude fibre (%)	5.61
Crude fat (%)	2.63
Calcium (%)	2.87
Av. Phosphorous (%)	0.45
Lysine (%)	0.88
Methionine (%)	0.34
Analyzed values	
Metabolizable Energy (Kcal kg^{-1})	2685
Crude protein (%)	17.50
Crude fat (%)	2.5
Crude fibre (%)	5.4

restricted to 130 g bird^{-1} per day and increased as based upon the recommendation of the primary breeder to 160 g bird^{-1} per day by the end of the experiment.

Assessments

To evaluate the weight gain changes, the hens were weighed individually at the beginning (28th week of age) and at the end of the experiment (36th week). Feed intake was weekly assessed as feed provided minus the residual. The cocks were also fed with the corresponding diets throughout the study as the hens were. Based upon egg production and quantity of feed consumed, the average feed efficiency was computed as the unit feed consumed to a unit mass of egg produced (kg feed kg^{-1} egg). Daily egg production of each hen was recoded throughout the experimental period. The eggs were labeled and stored for further analyses. Egg production was calculated for each experimental period using the formula of North (1990). The weight of eggs laid by the hens was recorded during the first five consecutive days at the beginning of each period using electrical balance of 0.5 g accuracy. For shell thickness, two eggs from each replicate were randomly taken during

the last three days of each period. The average egg shell thickness with an exclusion of the shell membrane was estimated at three different locations on the egg (blunt end, equator, and pointed end) using digital screw gauge (Ames 25M-5). Eggs collected during the ending two days of each period were weighed individually. Three eggs per treatment were broken and the entire contents carefully placed in a petri dish. Albumin height was recorded at two spots (one near to yolk and the other at the end of the dense albumen) using Ames Haugh unit meter. Yolk color indexes were visually evaluated by being matched with Roche Yolk Color Fan as described by Roche Company (1969).

Statistical Analysis

The data were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000) in a completely randomized design of three treatments and 3 replicates each. The data of each period (PI, PII and PIII) were analyzed separately. Overall period data were also analyzed by proc mixed, taking consideration of each period as the repeated variable (Gill, 1985). Duncan Multiple Ranges Test at 0.05 probability level was employed for comparison of the means (Duncan, 1955).

RESULTS AND DISCUSSION

Feeding 500 μg kg^{-1} AFB$_1$ did not affect weight gain during the 8 weeks of experiment (Table 2). No mortality was observed. These findings are in agreement with Yegani *et al.* (2006) who reported no significant changes in body weight of broiler breeders fed naturally contaminated *Fusarium* Mycotoxins. Similar findings have been reported by other investigators (Iqbal *et al.*, 1983; Fernandez *et al.*, 1994; Zaghini *et al.,* 2005; Pandey and Chauhan, 2007; Thapa, 2008) in layer chicken fed with 1.00

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Table 2. Effect of dietary Aflatoxin and Herbal Mycotoxin Binder (HMB) on body weight in female broiler breeders (Mean±SE).

Treatment Description		Body weight (g)	
		28 weeks	36 weeks
Aflatoxin (mg kg^{-1})	0	2959.5±12.67	3394.5±14.85
	500	2974.8±7.40	3403.7±24.19
	500+0.2% HMB	3003.7±15.25	3366.5±24.30

to 5.00 mg kg^{-1} of AFB$_1$ for 4 to 40 weeks. However, Sims *et al.* (1970) found significant (P< 0.05) decrease in body weight in laying hens that received 2.0 to 8.00 mg kg^{-1} of AFB$_1$ for 29 days. Similarly, feeding AFB$_1$ (500 μg kg^{-1}) in layer chicken from 15 to 67 weeks of age reduced body weight (Kim *et al.* 2003). Discrepancy found in the results of the experiments on the effect of dietary AFB$_1$ on live weight of layer birds could be due to such factors as the strain of the bird, age, dose and the period of exposure.

Feed consumption (g/day) was decreased in birds fed 500 μg kg^{-1} AFB1 as compared to the control group for all the three periods. Incorporation of HMB in the diet partially restored feed consumption (P<0.05). This reduction in cumulative feed consumption in AFB$_1$ fed birds might have been due to impaired hepatic metabolism, interference of AFB$_1$ with phosphenolpyruvate carboxylase, inhibition of elongation and/or termination of the translational process of protein synthesis and interference of AFB$_1$ in consecutive steps in mitochondrial respiratory chain. Further, AFB$_1$ accumulation in the liver and high content of microsomal cytochrome P-450 enzymes of hepatocytes favors the formation of DNA-AFB$_1$ adducts (Jay *et al.,* 2007). The improvement in feed consumption for the birds fed on diets containing AFB$_1$ plus HMB could be due to binding of AFB$_1$ with HMB and subsequent prevention of its absorption in the gut. This might be due to the presence of diatomaceous earth minerals in the binder. Diatomaceous earth mineral is a powerful natural adsorbent and which might effectively adsorb the toxins through its polar ends of the toxin (Gowda *et al.,*

2008). Moreover, presence of curcuminoids and enzymes in the binder used might have acted as antioxidants and detoxified epoxides, respectively. Curcumin induces drug metabolizing enzymes like gluthathione-s-transferase which results in efficient detoxification of toxin effects (Srinivas *et al.,* 1992; Soni *et al.,* 1992). While vitamins E and C are the main natural antioxidants that inhibit free radical damage in biological systems, vitamin E per se curcumin acts as antioxidant and removes these free radicals (Fanelli *et al.* 1985; Amani *et al.,* 2010). The other such commonly used binding agents as aluminosilicates, activated charcoal, bentonite, mannnanoligosaccharides etc. have been reported to have different mechanisms of binding the aflatoxin (Gowda *et al.,* 2008), ochratoxin (Irina *et al.,* 2007), *Fusarium* toxins (Yegani *et al.,* 2006) and T-2 toxin (Raju and Devegowda, 2002). However, the ability of the toxin binder to bind mycotoxins depends on such factors as pH, molecular arrangement and its geographic region of origin (Vieira, 2003).

Feed efficiency was significantly suppressed (P< 0.05) in 500 μg kg^{-1} AF fed group during all the three periods. Supplementation with 0.2% HMB resulted in no improvement in feed efficiency (Table 3). Poor feed conversion noted with the AFB$_1$ seems to have mediated decreased nutrient utilization. Yegani *et al.* (2006) with broiler breeders and Iqbal *et al.* (1983), Muthiah *et al.* (1998), and Pandey and Chauhan (2007) with commercial layers showed that chickens are sensitive to the feeding of mycotoxins with respect to feed efficiency.

Contamination of feed with 500 μg kg^{-1} AFB$_1$ resulted in significant decrease (2%)

Table 3. Effect of different dietary treatments on performance parameters of female broiler breeders (Mean±SE).

	P I	P II	P III
feed consumption (g day^{-1})			
Control	157.0±0.28[a]	158.0±0.28[a]	158.6±0.16[a]
500 μg kg^{-1} AFB$_1$	154.3±0.44[b]	154.6±0.33[b]	156.1±0.35[b]
500 μg kg^{-1} AFB$_1$+HMB	156.6±0.28[a]	158.0±0.28[a]	158.6±0.16[a]
feed efficiency (kg feed kg^{-1} egg mass)			
Control	3.3±0.04[b]	3.4±0.03[b]	4.0±0.02[b]
500 μg kg^{-1} AFB$_1$	3.9±0.01[a]	4.0±0.01[a]	4.3±0.04[a]
500 μg kg^{-1} AFB$_1$+HMB	3.9±0.01[a]	4.0±0.01[a]	4.3±0.04[a]
Egg production (%)			
Control	80.9±1.01[a]	79.7±0.85[a]	68.6±0.69[a]
500 μg kg^{-1} AFB$_1$	71.8±1.09[c]	69.8±0.88[c]	63.8±1.09[c]
500 μg kg^{-1} AFB$_1$+HMB	73.4±0.92[b]	71.8±0.70[b]	65.8±0.79[b]
Egg weight (g)			
Control	57.2±0.28[a]	57.7±0.34[a]	58.8±0.25[a]
500 μg kg^{-1} AFB$_1$	56.0±0.28[b]	56.5±0.31[b]	57.7±0.28[b]
500 μg kg^{-1} AFB$_1$+HMB	56.0±0.29[b]	56.8±0.11[b]	56.9±0.30[b]

Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.
AFB$_1$= Aflatoxin B$_1$, HMB= Herbal Mycotoxin Binder.
[a-b] Means with the same superscript in the same column for each variable showing no significant difference at ($P< 0.05$).

in egg production ($P< 0.05$) in all the three periods as compared with the control birds. Egg production was significantly improved by incorporation of HMB in the feed (Table 3). Mean egg weight was found to be significantly lower ($P< 0.05$) for the birds fed 500 μg kg^{-1} AFB$_1$; and while there was no influence of HMB supplementation in the diet on egg weight (Table 3). These results are in agreement with Hamilton and Garlich (1971), Huff *et al.* (1975) and Washburn *et al.* (1985) who recorded a significant reduction in egg weight with AFB$_1$ in commercial layers. Lack of significant effect of dietary AFB$_1$ on egg weight was reported by Stephen *et al.* (1991) in layer chickens fed with 5.00 and 10.00 mg kg^{-1} AFB$_1$ for three weeks. Also Yegani *et al.* (2006) reported no effect of *Fusarium* mycotoxin on egg weight of layers and on broiler breeder hens. The decrease in egg production coincided with a transient decrease in feed consumption and was probably associated with reduced-feed consumption. However, the greater metabolic reserves and lower egg production

rate of broiler breeders could explain the lack of the effect on egg production. A cumulative depletion of metabolic reserves due to slightly reduced feed intake might be due to decreased yolk weight being the major contributing factor to the reduced egg weight. The same trend is being reported by Iqbal *et al.* 1983.

Diet contamination with 500 μg kg^{-1} AFB$_1$ in broiler breeders resulted in no alteration in shell thickness (Table 4) and this could be because of toxin level in the diets which may not be sufficient enough to alter shell thickness. This result is in agreement with the known phenomenon of inverse relationship between age of bird and egg shell thickness (McDaniel *et al.*, 1979). Garlich *et al.* (1973) reported a significant reduction in plasma calcium of WL layers fed with AFB$_1$ which may impair the normal egg shell calcification and thereby lowered shell thickness. Several earlier trials also indicated lack of significant effect on egg shell thickness by dietary AFB$_1$ (Hamilton and Garlich, 1971; Iqbal *et al.*, 1983). Chowdhury and Smith (2004) reported lack
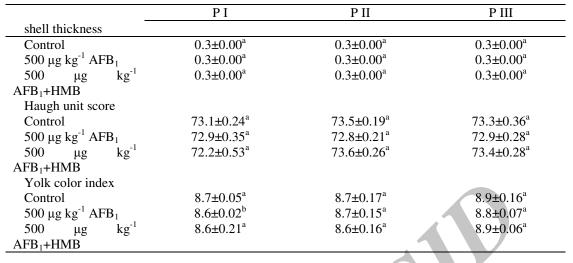
Table 4. Effect of different dietary treatments on egg quality parameters (Mean±SE).

	P I	P II	P III
shell thickness			
Control	0.3 ± 0.00^a	0.3 ± 0.00^a	0.3 ± 0.00^a
500 µg kg^{-1} AFB$_1$	0.3 ± 0.00^a	0.3 ± 0.00^a	0.3 ± 0.00^a
500 µg kg^{-1} AFB$_1$+HMB	0.3 ± 0.00^a	0.3 ± 0.00^a	0.3 ± 0.00^a
Haugh unit score			
Control	73.1 ± 0.24^a	73.5 ± 0.19^a	73.3 ± 0.36^a
500 µg kg^{-1} AFB$_1$	72.9 ± 0.35^a	72.8 ± 0.21^a	72.9 ± 0.28^a
500 µg kg^{-1} AFB$_1$+HMB	72.2 ± 0.53^a	73.6 ± 0.26^a	73.4 ± 0.28^a
Yolk color index			
Control	8.7 ± 0.05^a	8.7 ± 0.17^a	8.9 ± 0.16^a
500 µg kg^{-1} AFB$_1$	8.6 ± 0.02^b	8.7 ± 0.15^a	8.8 ± 0.07^a
500 µg kg^{-1} AFB$_1$+HMB	8.6 ± 0.21^a	8.6 ± 0.16^a	8.9 ± 0.06^a

Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.
AFB$_1$= Aflatoxin B$_1$, HMB= Herbal Mycotoxin Binder.
[a-b] Means with the same superscript in the same column for each variable showing no significant difference at ($P< 0.05$).

of *Fusarium* mycotoxins' effect on shell thickness in 45 week-old layers. Denli *et al.* (2008) reported feeding 2 mg kg^{-1} of ochratoxin A had no effect on shell thickness parameter in 47 week old layers. Aflatoxin contamination in boiler breeder diets did not significantly ($P\geq 0.05$) alter the Haugh unit scores and yolk color index of eggs. These findings are compatible with those of Chowdhury and Smith (2004) who reported that there was no effect of *Fusarium* mycotoxin contaminated diets on Haugh units or eggshell deformation in 45 week old layers. Denli *et al.* (2008) also reported that feeding 2 mg kg^{-1} of ochratoxin A did not show any significant difference on Haugh unit score of 47 week old layers. It was concluded that inclusion of Herbal Mycotoxin Binder could counteract with dietary AFB$_1$ and decline its inverse effect on egg production and as well as feed intake. Thus it could be construed that the binder used in the present study has got broad-spectrum of activity against aflatoxin as it contains diatomaceous earth minerals, curcuminoids as well as enzymes.

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تأثیر آفلاتوکسین بر روی عملکرد مرغان مادر گوشتی و کاهش اثرات آن به وسیله جاذب گیاهی مایکوتوکسین

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چکیده

این تحقیق به منظور بررسی اثر آفلاتوکسین (AFB_1) بر روی عملکرد و کیفیت تخم مرغ در مرغان مادر گوشتی و کاهش اثرات مضر آن به وسیله جاذب گیاهی مایکوتوکسین (HMB) انجام گرفت. ۳۶ قطعه مرغ مادر گوشتی ۲۸ هفته ای انتخاب و به ۳ تیمار زیر تقسیم گردیدند: ۱) جیره پایه (کنترل)؛ ۲) تیمار کنترل به همراه AFB_1 ۵۰۰ μg/kg ؛ ۳) تیمار کنترل به همراه 0.2%HMB + AFB_1 ۵۰۰ μg/kg. هر تیمار شامل ۳ دوره و هر دوره شامل ۳ هفته بود. سن مرغان مورد آزمایش از ۲۸ تا ۳۶ هفتگی را در بر داشت. نتایج نشان داد که تیمار ۲ مصرف غذا، راندمان غذایی، تولید و وزن تخم مرغ را به طور معنی داری کاهش داده است. اضافه نمودن HMB به طور نسبی مصرف غذا و تولید تخم مرغ را جبران نمود و اثرات جانبی AFB_1 را کاهش داد.