

Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced With Aflatoxin B₁: Effects on Visceral Organ Weight and Organ Lesions Parameters

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Abstract: A study was conducted with an objective to compare the efficacy of bentonite (BT), *Spirulina platensis* (SP) and glucomannan mycotoxin binders (GMA) on aflatoxicosis in broiler breeders. Three levels of AF, three binders and combination of different levels of AF with binders were evaluated. The AF fed at the levels of 300, 400 and 500ppb for three periods, each with duration of three weeks in broiler breeders from 28 to 36 weeks of age. Inclusion of 500 AF in the diet significantly ($P \leq 0.05$) affected g organ lesion scores (liver, kidney, proventriculus and gizzard) and relative weights of heart as well as liver, when compared to that of control. The results indicated no significant ($P \geq 0.05$) effect of AF on relative weight of spleen when compared to that of control. The results showed dose dependent cumulative effects of AF on all the affected parameters. Among the binders, GMA showed better counteracting effect.

Key words: Bentonite • *Spirulina platensis* • Glucomannan • Broiler Breeders and Fertility • Hatchability

INTRODUCTION

Animal feed ingredients and compounded feed, by virtue of their high vital nutrients and moisture content provides conducive environment for fungi growth at all stages of food chain. Many of these molds produce toxic metabolites during their growth are called as mycotoxins. Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species [1]. Aflatoxin B₁ (AFB₁), the most toxic of all aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂), is produced by certain strains of fungi in greater quantities than in others. In poultry, aflatoxin ingestion leads to "Aflatoxicosis" syndrome [2]. The ill effects of mycotoxicosis is prevented by the addition of non-nutritive and natural adsorbent materials to contaminated feed in order to selectively bind the mycotoxins during the digestive process and make it harmless to the bird. It is postulated that the bentonite (BT) forms a complex with the toxin, thus preventing the absorption of aflatoxin across the intestinal epithelium. *Spirulina platensis* (SP), a blue - green algae, is known to be a rich source of important nutrients including several vitamins, minerals, essential amino acids, essential fatty acids, source of carotenoids and possess profound antioxidant property [3]. It is known that dietary inclusion of modified

mannanoligosaccharides (MOS), extracted from the cell wall of yeast, has some beneficial effects in preventing adverse effects of mycotoxins. Yegani *et al.* [4] reported that the feeding of mycotoxin contaminated grains decreased egg shell thickness. However, dietary supplementation with Glucomannan Mycotoxin Adsorbent (GMA) prevented this effect. Considering the above facts, an investigation was undertaken with the objective of studying the effects of graded levels of aflatoxin on production, reproduction of broiler breeders and to assess the efficacy of bentonite, *Spirulina platensis* and glucomannan as mycotoxin binders in counteracting the adverse effects of graded levels of aflatoxin in broiler breeders.

MATERIALS AND METHODS

The present study was carried out in the Department of Poultry Science, Veterinary College, Hebbal, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University with an objective of assessing the Biochemical and immunological parameters of broiler breeder hens fed with aflatoxin and also to evaluate the counteracting effects of bentonite, *Spirulina platensis* and glucomannan as mycotoxin binding agents.

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Experimental Design: One hundred and ninety two broiler breeder hens with uniform body weight at the age of 16 weeks were chosen and individually housed in Californian cages. They were fed with standard diets free from toxins till the start of experiment (28 weeks). The hens were randomly divided into 48 groups of four birds each. Three such groups were fed with one of the experimental diets for three periods of 21 days each starting from 28th week. Each hen was fed at the rate of 160g/day throughout the study with *ad libitum* water supply. The hens were inseminated twice a week with the semen from those cocks fed with the corresponding experimental breeder diet as hens.

Experimental Diets: Four levels of aflatoxin (0, 300, 400 and 500ppb) with two levels each of bentonite (0 and 1%), *Spirulina platensis* (0 and 0.1%) and Glucomannan mycotoxin adsorbent (0 and 0.2%) were incorporated into the basal diet in a 4 X 4 factorial manner, forming a total of 16 dietary treatment combinations. The basal diet was formulated using commonly available feed ingredients which were screened for AF prior to the formulation of diets. The experimental diets were prepared by adding required quantity of contaminated rice culture containing aflatoxin to arrive at the levels of 0, 300, 400 and 500ppb of AFB₁. Bentonite (1%), *Spirulina platensis* (0.1%) and Glucomannan mycotoxin adsorbent (0.2%) were used in the diets as sources of chemical, herbal and glucomannan extract mycotoxin binders, respectively. The formulated diets were analyzed for AF content to counter check the required levels. Basal diet was formulated and compounded to meet the nutrient requirements of broiler chicks during the starter (0-3 wks) and finisher (4-5 wks) phases without inclusion of either aflatoxin or binder.

Visceral Organ Weight and Organ Lesions: At the end of the trial, three hens from each treatment were sacrificed by cutting the jugular vein. Liver, kidney, gizzard, proventriculus, were extracted from the birds and weighed using digital top pan electronic balance (0.1g accuracy). The weights were adjusted to g/100 g of body weight and treatment means were calculated. Oral lesions were recorded when apparent. A score of one to four was allotted to evaluate the severity of the lesion.

Score 0: No oral lesions/ apparently normal

Score 1: organ slightly enlarged with slight paleness / mild oral lesions on inner aspect of the oral cavity.

Score 2: organ enlarged with pale streaks / apparent oral lesions at the angle of the mouth and inner oral cavity.

Score 3: Organ enlarged with pale streaks throughout

Score 4: organ enlarged with defused paleness, soft and fragile / prominent or severe oral lesions all over the mucosa or oral cavity and beak.

The total number of birds showing oral lesions in each treatment was also recorded.

Statistical Analysis: The data were analyzed using the General Linear Model procedure of Statistical Analysis System (SAS®) software [5]. Period wise data were analyzed by 4 x 4 factorial manner. Overall period data were analyzed by repeated measurement design. Duncan multiple range test at 0.05 probability level was employed for comparison of the means [6].

RESULTS

Organ Lesions (Liver, Kidney, Proventriculus and Gizzard): The data obtained on mean organ lesion scores of broiler breeder hens fed with varying levels of AF and toxin binders after slaughtering are presented in Table 1. All three groups fed with different levels of AF showed significantly ($P \leq 0.05$) higher liver lesions which ranged from 3.00 in group fed with 500 AF to 2.00 in group fed with 300 AF as compared to 0.00 in the control group. All groups which received binders alone did not show any liver lesions as with the case of control group. Among AF+Binder fed groups, AF+BT showed significantly ($P \leq 0.05$) less liver lesions compared to their control groups. Aflatoxin contaminated fed groups at different levels when treated with SP did not differ significantly ($P \geq 0.05$) when compared to their control group. Upon GMA inclusion as binder at all three levels of AF, the liver lesions significantly ($P \leq 0.05$) decreased in groups fed with 400 and 500 AF. All AF fed groups without binders showed significantly ($P \leq 0.05$) more kidney lesions than the control group. All groups when received binders alone showed no kidney lesions as that of control group. All three levels of AF fed groups along with BT showed a significant ($P \leq 0.05$) reduction in kidney lesions compared to their respective control groups. Aflatoxin contaminated fed groups at different levels when treated with SP did not show any reduction in kidney lesions. All three levels of AF fed groups along with GMA showed a significant ($P \leq 0.05$) reduction in kidney lesions as compared to their respective control groups. In case

Table 1: Effect of binders on oral lesion score of broiler breeders fed with different levels of aflatoxin

Description		Organ lesion score				
		Binder	Liver	Kidney	Proventriculus	Gizzard
Aflatoxin ppb	0	Nil	0.00±0.00 ^e	0.00±0.00 ^h	0.00±0.00 ^b	0.00±0.00 ^c
		BT	0.00±0.00 ^e	0.00±0.00 ^h	0.00±0.00 ^b	0.00±0.00 ^c
		SP	0.00±0.00 ^e	0.00±0.00 ^h	0.00±0.00 ^b	0.00±0.00 ^c
		GMA	0.00±0.00 ^e	0.00±0.00 ^h	0.00±0.00 ^b	0.00±0.00 ^c
	300	Nil	1.00±0.00 ^c	1.00±0.00 ^f	0.00±0.00 ^b	0.00±0.00 ^c
		BT	1.00±0.00 ^c	1.00±0.00 ^f	0.00±0.00 ^b	0.00±0.00 ^c
		SP	1.00±0.00 ^c	1.00±0.00 ^f	0.00±0.00 ^b	0.00±0.00 ^c
		GMA	0.66±0.16 ^f	0.66±0.16 ^e	0.00±0.00 ^b	0.00±0.00 ^c
	400	Nil	2.00±0.00 ^c	2.00±0.00 ^d	1.00±0.00 ^a	1.00±0.00 ^a
		BT	2.00±0.00 ^c	2.00±0.00 ^d	1.00±0.00 ^a	1.00±0.00 ^a
		SP	2.00±0.00 ^c	2.00±0.00 ^d	1.00±0.00 ^a	1.00±0.00 ^a
		GMA	1.66±0.16 ^d	1.66±0.16 ^c	1.00±0.00 ^a	1.00±0.16 ^a
500	Nil	3.00±0.00 ^a	3.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	
	BT	3.00±0.00 ^a	3.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	
	SP	3.00±0.00 ^a	3.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	
	GMA	2.33±0.16 ^b	2.33±0.16 ^c	1.00±0.00 ^a	1.00±0.16 ^a	

Means within each column bearing common superscript do not differ significantly (P<0.05)

AF: Aflatoxin B₁; BT: Bentonite (1%); SP: *spirulina platensis* (0.1%); GMA: Glucomannan Mycotoxin Adsorbent (0.2%)

Table 2: Effect of binders on organ weight (g/100 g of BW) of broiler breeders fed with different levels of aflatoxin

Description		Organ weight			
		Binder	Spleen	Heart	Liver
Aflatoxin ppb	0	Nil	0.12±0.00 ^a	0.43±0.0 ^a	2.30±0.11 ^a
		BT	0.13±0.00 ^b	0.44±0.02 ^b	2.30±0.02 ^a
		SP	0.13±0.00 ^b	0.45±0.01 ^c	2.30±0.14 ^a
		GMA	0.13±0.00 ^b	0.46±0.17 ^d	2.30±0.07 ^a
	300	Nil	0.12±0.00 ^a	0.43±0.00 ^a	2.28±0.10 ^b
		BT	0.12±0.00 ^a	0.43±0.01 ^a	2.28±0.05 ^b
		SP	0.12±0.00 ^a	0.43±0.01 ^a	2.29±0.01 ^{ab}
		GMA	0.13±0.00 ^b	0.43±0.01 ^a	2.29±0.03 ^{ab}
	400	Nil	0.12±0.00 ^a	0.45±0.01 ^c	2.27±0.06 ^{bc}
		BT	0.12±0.00 ^a	0.45±0.02 ^c	2.27±0.02 ^{bc}
		SP	0.12±0.00 ^a	0.45±0.00 ^c	2.28±0.04 ^b
		GMA	0.13±0.00 ^b	0.45±0.01 ^c	2.28±0.26 ^b
500	Nil	0.12±0.00 ^a	0.46±0.00 ^d	2.26±0.08 ^c	
	BT	0.12±0.00 ^a	0.46±0.00 ^d	2.26±0.02 ^c	
	SP	0.12±0.00 ^a	0.46±0.01 ^d	2.27±0.03 ^{bc}	
	GMA	0.13±0.00 ^b	0.46±0.01 ^d	2.27±0.06 ^{bc}	

AF: Aflatoxin B₁; BT: Bentonite (1%); SP: *spirulina platensis* (0.1%); GMA: Glucomannan Mycotoxin Adsorbent (0.2%)

of proventriculus and gizzard, the lesion values for 400 and 500 AF fed group showed significantly (P≤0.05) higher scores compared to that of control group. All the groups when received binders alone had no proventriculus and gizzard lesions were noticed. When BT, SP and GMA were used as binders at all three levels of AF fed groups, no change in lesion scores of proventriculus and gizzard was observed.

Visceral Organ Weight: The data obtained on mean organ weights (g/100 g of BW) viz., spleen, heart and liver of slaughtered broiler breeder hens fed with varying levels of AF and binders on the last day of the experiment are presented in Table 2. The relative weight of spleen was not altered by feeding AF at 300,400 and 500ppb while feeding of binders significantly (P≤0.05) increased the spleen weight as compared to the control. When the

binders were fed along with different levels of AF, inclusion of only GMA showed significantly ($P \leq 0.05$) increased spleen weight than the respective control, Significantly ($P \leq 0.05$) heavier heart weights were recorded in breeder hens fed with diet containing 400 and 500 AF compared to control. Feeding binders alone resulted in significantly ($P \leq 0.05$) lower weight hearts than those of control. When binders were fed along with AF also did not alter the heart weight when compared with the respective controls.

DISCUSSION

Organ Lesions (Liver, Kidney, Proventriculus and Gizzard): The liver lesion scores for breeders fed with AF showed an increasing trend as the dose of AF increased from 300 to 500ppb in the diet and differences observed in the lesion scores were significant ($P \leq 0.05$). Inclusion of BT in the diet significantly ($P \leq 0.05$) reduced the lesion scores at all AF levels while GMA inclusion showed a positive contracting effect only at 400 and 500 AF levels. Similar lesions were reported in layer chicken treated with 120ppb onwards for varying periods [7- 9]. Higher dose level and prolonged feeding as that of Salahi *et al.* [10] who fed 2.50 to 3.91ppm at 1 to 40 weeks of age to the WL layers caused more severe changes in liver weight. The kidney lesion scores for breeders fed with AF also showed an increasing trend as the dose of AF increased from 300 to 500ppb in the diet and differences observed in the lesion scores were significant ($P \leq 0.05$). Inclusion of BT and GMA in the diets significantly ($P \leq 0.05$) reduced the lesion scores at all AF levels. These findings agreed with those of other scientists [8, 11] at higher levels i.e. 2.50 to 3.91ppm. In the case of proventriculus and gizzard, the lesion scores were significantly ($P \leq 0.05$) more in groups fed with 400 and 500 AF groups than those fed with 300 and control. None of the binders used in the study were effective in reducing the lesions in proventriculus and gizzard. The gizzard lesion scores of breeder hens fed with mycotoxin has not been previously reported.

Visceral Organ Weight: The relative weight of spleen was not altered by feeding AF at 300, 400 and 500ppb while feeding of binders significantly ($P \leq 0.05$) increased the spleen weight as compared to the control. When the binders were fed along with different levels of AF, inclusion of only GMA showed significantly ($P \leq 0.05$) increased spleen weight than its respective control, Significantly ($P \leq 0.05$) heavier heart weights were recorded in breeder hens fed with diet containing 400 and 500ppb

AF compared to control. Feeding binders alone resulted in significantly ($P \leq 0.05$) lower weight hearts than those of control. When binders were fed along with AF also did not alter the heart weight when compared with its respective controls. The present study also revealed significantly ($P \leq 0.05$) higher liver weights in breeder hens when fed with all three levels of AF. The inclusion of binders did not alter the liver weights either fed alone or in combination with different levels of AF. The present study revealed that the levels of AF fed and the duration for which it was fed were not sufficient enough to adversely alter weights of spleen, liver and heart. Therefore the inclusion of binders did not yield significant ($P \geq 0.05$) differences from respective controls except in the case of liver when GMA was fed with all levels of AF. This is clearly evident by the report of a study [12, 13] who observed increase in liver weights of White Leghorn hens fed with 8.00ppm AF from seventh day onwards. Pandey and Chauhan [14] reported an increase in relative weights of kidney and they attribute it for elevation in blood uric acid concentration in laying hens fed with diets containing combination of mycotoxins for 12 weeks. Pasha *et al.* and Moran *et al.* [15, 16] reported that relative weights of liver, spleen, kidney and testes were not significantly affected by dietary feeding of grains naturally contaminated with *Fusarium* mycotoxins in broiler breeders. There are many reports with contradictory results concerning the effects of feeding mycotoxins on poultry organ weights stating no effect of diet was found on relative weights of the spleen, heart or liver [11, 17, 18].

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