

Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced with Aflatoxin B₁: Effects on Egg Quality 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 Aflatoxin B₁ (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 egg weight when compared to that of control. The results indicated no significant ($P \geq 0.05$) effect of AF on egg weight, shell thickness and Haugh unit 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 Egg Parameters

INTRODUCTION

Contamination of poultry feeds with mycotoxins is one of the major problems associated with feeding of poultry. Mycotoxins are the toxic metabolites synthesized by a certain naturally growing fungi on animal feed, feed ingredients and other agricultural crops. More than 350 mycotoxins have been identified so far in feedstuffs. Aflatoxin is the most commonly occurring mycotoxin in India. Aflatoxins are a group of secondary metabolites produced by a certain species of fungus of the genus *Aspergillus* (especially by *A. flavus* and *A. parasiticus*). These fungi are capable of growing and contaminating the grains and cereals at any time before and /or after the harvest, during storage, transportation and processing of feed ingredients and the formulated feeds after processing. Aflatoxin contamination of feedstuffs has been reported to be of a wide range from 1 to 900 $\mu\text{g}/\text{kg}$ in commonly used ingredients as well as mixed feed samples in developing countries [1] Poultry industry suffers greater economic losses due to the greater susceptibility of the species in comparison with other animals to the toxin apart from continuing intermittent occurrences in feeds [2]. Extensive research was conducted to counteract aflatoxicosis by physical, chemical, nutritional and

biological approaches. Chemical adsorbents such as bentonites, zeolites and aluminosilicates have been tested. Clay materials have the capability to bind molecules of certain size and configuration only. It is postulated that the bentonite forms a complex with the toxin, thus preventing the absorption of aflatoxin across the intestinal epithelium. *Spirulina platensis*, 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 [4,5] 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.

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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 egg production and egg quality characteristics 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.

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.

Egg Quality Characteristics Parameters

Egg Weight: The weight of eggs laid by all hens was recorded during the first five consecutive days at the

commencement of each period using electrical balance with 0.5g sensitivity. Then, the average weight of eggs was computed for each of the treatments.

Shell Thickness: For the assessment of shell thickness, two eggs at random from each replicate were collected daily for three days at the end of each period. Shell thickness was measured without the shell membrane at three different locations on the egg (air cell, equator and sharp end) using digital screw gauge (Ames 25M-5). The average for each treatment was obtained.

Haugh Unit: Eggs collected during the last two days of each period from each treatment were weighed individually. Three of those eggs per treatment were broken and the entire contents were carefully placed on a glass slab, then the height of albumen was recorded at two places (one near to yolk and the other at the end of dense albumen) using Ames Haugh unit meter.

Yolk Color Index: All the eggs broken to measure Haugh unit were only utilized to determine yolk color visually by matching with Roche Yolk Color Fan.

Statistical Analysis: The data were analyzed using the General Linear Model procedure of Statistical Analysis System (SAS®) software [6]. 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 [7].

RESULTS

Egg Weight: The average egg weight (g) recorded in AF fed breeder hens and those supplemented with bentonite, *spirulina platensis* and GMA during different periods is presented in Table 1. During the first period the egg weight values for 300 and 400 AF groups remained non significant ($P \geq 0.05$) but 500 AF fed group showed significantly ($P \leq 0.05$) lower than that of control group. There was no significant ($P \geq 0.05$) difference in egg weight among the groups of birds fed with binders alone when compared with the control group (57.20g). Upon BT and SP inclusion as binders, egg weight significantly ($P \geq 0.05$) decreased only in group fed with 400 AF. Upon inclusion of GMA as binder at all three levels of AF fed groups, the egg weight remained non significant ($P \geq 0.05$) when compared to their respective groups without binders.

Table 1: Effect of binders on egg weight (g) of broiler breeders fed with different levels of aflatoxin

Description		Periods			
		Binder	I	II	III
Aflatoxin ppb	0	Nil	57.20±0.28 ^{ab}	57.73±0.34 ^{cde}	58.86±0.25 ^b
		BT	57.20±0.28 ^{ab}	57.73±0.34 ^{cde}	58.86±0.25 ^b
		SP	57.20±0.28 ^{ab}	57.73±0.34 ^{cde}	58.86±0.25 ^b
		GMA	57.20±0.28 ^{ab}	57.73±0.34 ^{cde}	58.86±0.25 ^b
	300	Nil	57.16±0.16 ^{ab}	58.09±0.28 ^{bcd}	58.11±0.29 ^{abc}
		BT	56.46±0.19 ^{bcd}	57.75±0.26 ^{cd}	58.75±0.23 ^b
		SP	57.20±0.28 ^{ab}	57.73±0.34 ^{cde}	58.86±0.25 ^b
		GMA	57.76±0.26 ^a	58.04±0.22 ^{bcd}	59.82±0.25 ^a
	400	Nil	56.98±0.09 ^{abc}	57.56±0.31 ^{cde}	58.53±0.30 ^{bc}
		BT	55.74±0.28 ^{de}	56.83±0.17 ^{ef}	58.00±0.29 ^{bcd}
		SP	55.95±0.55 ^{de}	57.35±0.30 ^{de}	58.17±0.28 ^{bc}
		GMA	56.68±0.32 ^{bcd}	58.41±0.21 ^{abc}	58.83±0.14 ^b
	500	Nil	56.08±0.28 ^{cde}	57.50±0.31 ^{cde}	57.73±0.28 ^{cd}
		BT	55.61±0.21 ^e	55.80±0.23 ^g	57.71±0.25 ^{cd}
		SP	55.74±0.19 ^{de}	56.44±0.26 ^{fg}	57.29±0.16 ^d
		GMA	55.96±0.29 ^{de}	56.82±0.11 ^{ef}	59.95±0.30 ^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: Glucmannan Mycotoxin Adsorbent (0.2%) Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.

Table 2: Effect of binders on shell thickness (mm) of broiler breeders fed with different levels of aflatoxin

Description		Periods			
		Binder	I	II	III
Aflatoxin Ppb	0	Nil	0.33±0.00 ^b	0.34±0.00 ^a	0.31±0.00 ^c
		BT	0.32±0.00 ^{bc}	0.34±0.00 ^a	0.32±0.00 ^{bc}
		SP	0.32±0.00 ^{bc}	0.34±0.00 ^a	0.32±0.00 ^{bc}
		GMA	0.34±0.01 ^a	0.34±0.00 ^a	0.32±0.00 ^{bc}
	300	Nil	0.33±0.00 ^b	0.33±0.00 ^{ab}	0.31±0.00 ^c
		BT	0.33±0.00 ^b	0.32±0.00 ^{bc}	0.32±0.00 ^{bc}
		SP	0.33±0.00 ^b	0.32±0.00 ^{bc}	0.31±0.00 ^c
		GMA	0.33±0.00 ^b	0.32±0.00 ^{bc}	0.31±0.00 ^c
	400	Nil	0.33±0.00 ^b	0.33±0.00 ^{ab}	0.31±0.00 ^c
		BT	0.33±0.00 ^b	0.33±0.00 ^b	0.31±0.00 ^c
		SP	0.33±0.00 ^b	0.33±0.00 ^b	0.31±0.00 ^c
		GMA	0.33±0.00 ^b	0.33±0.00 ^b	0.31±0.00 ^c
	500	Nil	0.32±0.00 ^{bc}	0.33±0.00 ^{ab}	0.31±0.00 ^c
		BT	0.32±0.00 ^{bc}	0.32±0.00 ^{bc}	0.31±0.00 ^c
		SP	0.33±0.00 ^b	0.33±0.00 ^b	0.31±0.00 ^c
		GMA	0.32±0.00 ^{bc}	0.32±0.00 ^{bc}	0.31±0.00 ^c

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: Glucmannan Mycotoxin Adsorbent (0.2%)

Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.

Table 3: Effect of binders on Haugh unit score of broiler breeders fed with different levels of aflatoxin

		Periods			
Description		Binder	I	II	III
Aflatoxin Ppb	0	Nil	73.17±0.24 ^{bc}	73.57±0.19 ^{abc}	73.35±0.36 ^{ab}
		BT	72.97±0.35 ^{bcd}	73.98±0.21 ^a	73.56±0.19 ^{ab}
		SP	72.86±0.19 ^{bcd}	73.30±0.36 ^{abcd}	73.26±0.23 ^{ab}
		GMA	73.14±0.28 ^{bcd}	73.22±0.24 ^{abcd}	74.20±0.35 ^a
	300	Nil	73.72±0.15 ^{abc}	73.25±0.26 ^{abcd}	73.45±0.25 ^{ab}
		BT	74.41±0.06 ^a	73.06±0.27 ^{bcd}	73.50±0.32 ^{ab}
		SP	73.82±0.14 ^{ab}	72.58±0.14 ^d	73.90±0.24 ^{ab}
		GMA	73.09±0.48 ^{bcd}	73.65±0.14 ^{abc}	72.98±0.16 ^b
	400	Nil	73.40±0.18 ^{bc}	73.15±0.25 ^{bcd}	73.33±0.39 ^{ab}
		BT	72.90±0.19 ^{bcd}	73.00±0.18 ^{bcd}	73.58±0.28 ^{ab}
		SP	72.78±0.21 ^{cde}	73.79±0.32 ^{ab}	73.58±0.30 ^{ab}
		GMA	73.46±0.26 ^{bc}	73.00±0.28 ^{bcd}	73.50±0.30 ^{ab}
	500	Nil	72.95±0.35 ^{bcd}	72.88±0.21 ^{cd}	72.98±0.28 ^b
		BT	73.34±0.26 ^{bc}	73.65±0.20 ^{abc}	73.70±0.24 ^{ab}
		SP	72.00±0.28 ^{de}	73.06±0.10 ^{bcd}	73.61±0.29 ^{ab}
		GMA	72.22±0.53 ^{de}	73.60±0.26 ^{abc}	73.46±0.28 ^{ab}

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%)

Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.

Table 4: Effect of binders on yolk color index of broiler breeders fed with different levels of aflatoxin

		Periods			
Description		Binder	I	II	III
Aflatoxin ppb	0	Nil	8.70±0.05 ^{ef}	8.76±0.17 ^{ab}	8.63±0.16 ^{ab}
		BT	8.50±0.13 ^{efgh}	8.78±0.09 ^{ab}	8.67±0.17 ^{ab}
		SP	11.16±0.08 ^b	8.68±0.13 ^{abc}	8.70±0.17 ^{ab}
		GMA	8.22±0.14 ^{fghi}	8.81±0.04 ^a	8.48±0.17 ^{ab}
	300	Nil	8.81±0.08 ^e	8.74±0.11 ^{ab}	8.60±0.16 ^{ab}
		BT	8.68±0.13 ^{ef}	8.83±0.09 ^a	8.56±0.15 ^{ab}
		SP	10.50±0.02 ^c	8.47±0.17 ^{abc}	8.78±0.13 ^{ab}
		GMA	8.37±0.09 ^{efghi}	8.74±0.13 ^{ab}	8.45±0.16 ^{ab}
	400	Nil	8.76±0.07 ^e	8.74±0.13 ^{ab}	8.68±0.06 ^{ab}
		BT	8.53±0.14 ^{efg}	8.32±0.16 ^{bc}	8.60±0.16 ^{ab}
		SP	12.02±0.03 ^a	8.56±0.16 ^{abc}	8.65±0.14 ^{ab}
		GMA	8.63±0.18 ^{ef}	8.65±0.14 ^{abc}	8.72±0.07 ^{ab}
	500	Nil	8.15±0.02 ^{hi}	8.36±0.15 ^{abc}	8.92±0.07 ^a
		BT	8.14±0.06 ⁱ	8.51±0.10 ^{abc}	8.68±0.06 ^{ab}
		SP	9.80±0.02 ^d	8.66±0.14 ^{abc}	8.58±0.19 ^{ab}
		GMA	8.50±0.21 ^{efgh}	8.56±0.16 ^{abc}	8.91±0.06 ^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%)

Periods: I: 28-30 weeks; II: 31-33 weeks; III: 34-36 weeks.

During the second period the egg weight values in all AF alone fed groups showed a non significant ($P \geq 0.05$) difference compared to that of control. There was no significant ($P \geq 0.05$) difference in egg weight values among the groups of birds fed with binders alone when compared with control group and was 57.33g. The 500 AF fed groups along with BT, SP and GMA showed significant ($P \leq 0.05$) variation in egg weight compared to

their respective control groups. The values ranged from 55.80g to 58.82g. During the third period the egg weight values for 300 and 400 AF groups remained non significant ($P \geq 0.05$) but 500 AF fed group showed a significant ($P \geq 0.05$) decrease when compared to that of the control group. There was no significant ($P \leq 0.05$) difference in egg weight values among the groups of birds fed with binders alone compared with the control group

(58.86g). When BT and SP were used as binders at all three levels of AF fed groups, the egg weight remained same when compared to their respective groups without binders and ranged from 57.29g to 58.86g. The 500 AF fed groups along with GMA showed a significant ($P \leq 0.05$) increase in egg weight (59.95g) compared to that of control.

Shell Thickness: The average shell thickness (mm) measured in AF fed breeder hens and supplemented with bentonite, *spirulina platensis* and GMA during different periods is presented in Table 2. During the first period the average shell thickness did not differ significantly ($P \geq 0.05$) either between all AF alone fed groups or AF+binders fed groups when compared to that of control. The group fed with GMA alone as binder differed significantly ($P \leq 0.05$) in shell thickness compared to that of control. During the second and third period, the shell thickness did not differ significantly ($P \geq 0.05$) between any of the treatments including the control group.

Haugh Unit: The means for Haugh unit score computed with respect to AF fed breeder hens and supplemented with bentonite, *spirulina platensis* and GMA during different periods is presented in Table 3. During the first period all the values of Haugh unit pertaining to all AF alone fed groups, either binders alone or AF+binders fed groups and the control did not differ significantly ($P \geq 0.05$). During the second as well as the third period, the same trend as that of the first period was observed with respect to Haugh unit values.

Yolk Color Index: The yolk color index documented in respect of AF fed breeder hens and supplemented with bentonite, *spirulina platensis* and GMA pertaining to different periods is presented in Table 4. During the first period among the AF alone fed groups, only 500 AF fed group showed a significantly ($P \leq 0.05$) lower index for yolk colour than that of control. Among the binders alone fed groups, the group fed with SP only showed significantly ($P \geq 0.05$) higher yolk index value when compared to that of control group. All three levels of AF fed groups along with BT and GMA showed non significant ($P \leq 0.05$) differences in yolk color index values compared to their respective control groups. All three levels of AF fed groups along with SP showed significantly ($P \leq 0.05$) higher yolk color index when compared to its control group. During the second and third periods, no significant ($P \geq 0.05$) difference was observed between any of the treatment groups.

DISCUSSION

Egg Weight: The results of the present investigation showed that feeding AF at 300 and 400ppb levels did not affect the weight of eggs obtained from breeders in all the three periods. However, 500 AF fed hens showed significantly ($P \leq 0.05$) reduced egg weight during first and third periods only. There was no clear-cut trend visible in the effect of binders on the AF diet on improving egg weight. Lack of significant effect of dietary AF on egg weight was reported by Girish and Devegowda [11] in WL layers, [12] in Japanese quail, [13] in layer chickens fed with 5.00 and 10.00ppm AF for three weeks, [12, 14] by feeding 2.00ppm AF for 50 days in 42 week old laying hens and Yegani *et al.* [5] who reported that there was no effect of *Fusarium* mycotoxins contaminated diets on egg weight of layers and broiler breeders. On the contrary, [12, 13] recorded a significant reduction in egg weight with AF feeding in commercial layers. The probable reason for these conflicting reports on this aspect might be due to differences in levels of AF tested by these workers.

Shell Thickness: The results of the present investigation showed that feeding of AF or binders in the diets did not significantly ($P \geq 0.05$) alter shell thickness of eggs obtained from breeders during the whole experimental period. The study indicated that the level of toxin included in the diets may not be sufficient enough to alter shell thickness. This trend is in agreement with the known phenomenon of inverse relationship between age of bird and egg shell thickness [15]. Kim *et al.* [16] reported a significant reduction in plasma calcium in WL layers fed with AF. Reduced availability of plasma calcium for egg shell calcification during aflatoxicosis may impair the normal egg shell calcification and thereby lowered shell thickness. Several earlier trials also indicated lack of significant effect of dietary AF on egg shell thickness [15] reported lack of effect of *Fusarium* mycotoxins contaminated diets on shell thickness of 45 week-old layers. [14] reported lack of effect of feeding 2 mg/kg of ochratoxin A on shell thickness parameter of 47 week old layers.

Haugh Unit: The results of the present investigation showed that feeding of either AF or inclusion of binders alone or in combination in the diets of broiler breeder hens did not significantly ($P \geq 0.05$) alter the Haugh unit scores of eggs. These findings are comparable with those of Yegani *et al.* [5] who reported that there was no effect of *Fusarium* mycotoxins contaminated diets on Haugh units

or eggshell deformation in 45 week old layers. Zaghini *et al.* and Ahmadi [9, 13] also reported that feeding 2mg/kg of ochratoxin A did not show any significant differences on Haugh unit score of 47 week old layers.

Yolk Color Index: The results of the present investigation showed that when SP was fed alone significant ($P \leq 0.05$) increase in yolk color index only in first period of study. Further, SP fed in combination with all AF levels also significantly ($P \leq 0.05$) increased yolk color index only in first period. From the literature, it is gathered that *spirulina platensis* is a good source of pigments i.e. phycocyanin (15g/100g), Chlorophyll (1.05g/100g) and Carotenoids (0.38g/100g). Presence of pigments in the daily diet could be the reason for improving the yolk color index in *spirulina platensis* fed breeder eggs. Zeaxanthin content in the yolk tended to increase significantly with the dosage of *spirulina* [10, 16]. Hence, it is expected to improve yolk color index when included in the diet. In support of this study, [11, 14] reported that only the yolk color in the group fed on 5 per cent of algae was 2.4 times darker compared to the control of laying hens. Miazzo *et al.* and Raju *et al.* [17, 18] reported that when adding 2 per cent and 10 per cent of dry biomass from fresh water algae of *Chlorella* genus in the combined forages for laying hens, the yolk pigmentation became significantly more intensive by 2.5 units by the Roche's scale. [19, 20] found that yolk color index was significantly improved by the addition of *spirulina* in the chicken feed.

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