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Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced with Aflatoxin B_1: Effects on Fertility, Hatchability, Embryonic Mortality, Residues in Egg and Semen Quality

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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 Aflatoxin B1 (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 fertility, hatchability and embryonic mortality, when compared to that of control. The results indicated no significant ($P \geq 0.05$) effect of AF on residue in eggs, sperm count and per cent live sperm 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

Poultry production is one of the fastest growing sectors of Indian agriculture. Egg production is increasing at the rate of 6-8 per cent per annum while broiler production at the rate of 12-15 per cent. At present, India is the 3rd largest producer off eggs (only next to China and USA) and 5th largest producer of poultry meat (next to USA, China, Brazil and Mexico) in the world, thanks to a 16 fold increase for egg number and 14.7 fold increase for egg mass over 1961. Poultry meat production increased to 23.46 fold from 81 thousand tons in 1961 to 1900 thousand tons in 2005-06. Consequent to increased production, per capita consumption /availability has also increased from 7 eggs in 1961 to 42 eggs in 2008. Broilers are the major source of meat supply in the country. About 140 million broilers are produced every month. The per capita consumption of poultry meat has increased from 126g in 1961 to 1730g in 2005. This enormous growth and spurt in the poultry production has put a tremendous pressure on proper feeding of poultry in order to sustain the poultry industry in India [1, 2]. 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*). Aflatoxins are a group of secondary metabolites produced by a certain species of fungus of the genus *Aspergillus* (especially *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 900μg/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 [3]. Extensive research was conducted to counteract aflatoxicosis by physical, chemical, nutritional and biological approaches.

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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 [4]. 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. [5] reported that the feeding of mycotoxin contaminated grains decreased eggshell 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 Fertility, Hatchability, Embryonic Mortality, Residues in Egg and Semen Quality 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 16weeks were chosen and individually housed in Californian cages. They were fed with standard diets free from toxins till the start of experiment (28weeks). 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 AFB_1. 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.

Fertility, Hatchability and Embryonic Mortality: Hens were individually inseminated twice a week with 50µL of fresh, pooled semen from cocks fed corresponding diets. All the eggs laid by each treatment groups during the third week of each period were collected and stored for a week in the egg holding room. The eggs were incubated in a conventional forced-air incubator (Dayal Electric Automatic Incubation, New Delhi, India). The incubator was maintained at 37.5°C and 50% RH. All the fertile eggs were transferred on the 18th day of incubation to the Hatcher (Blue Star Poultry Equipments, Hyderabad, India) maintained at 36°C and 85% RH until 21st day of incubation. Hatchability was expressed as the percentage of chicks hatched out of the total number of fertile eggs set. Un-hatched eggs were broken to ascertain the percentage of embryonic mortality.

Residues in Egg: The aflatoxin B1 concentration was determined in the eggs produced by the hens from each of the experimental groups on during the last week of each of the periods for aflatoxin residue using HPLC - fluorescence method with detection limit of 5 ppb in accordance with the Association of Official Analytical Chemists (AOAC) [6] including modifications described by Girish *et al.* [7] and the averages for each replicate was calculated.

Semen Quality: Cocks were kept in individual cages during the experiment. At the end of each period, semen was collected individually by massage method (in graduated tubes) to assess its quality. In addition to actual milking for artificial insemination and laboratory assessments, cocks were being teased weekly throughout the experiment to prevent any possible negative effect on reproductive efficiency, due to the absence of natural floor mating. The density of spermatozoa or sperm concentration was determined by haemocytometric method. The differential count of live and dead spermatozoa for each replicate was done using the method described by Kubena *et al.* [8].

Statistical Analysis: The data were analyzed using the General Linear Model procedure of Statistical Analysis System (SAS®) software [9]. 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 [10].

RESULTS

Fertility: The fertility percentage recoded in various treatment groups pertaining to different periods is detailed in Table 1. During the first period the per cent fertility significantly ($P \leq 0.05$) reduced in all AF alone fed groups when compared to that of control group. Among the binder's alone fed groups, BT, SP and GMA showed a significantly ($P \leq 0.05$) higher fertility than that of control. Upon inclusion of binders at all three levels of AF in the diet, the fertility percentage significantly ($P \leq 0.05$) improved. During the second period all three groups fed with different levels of AF showed significantly ($P \leq 0.05$) lower fertility as compared to the control group. In the case binders alone fed groups significantly ($P \leq 0.05$) better fertility than that of control was noticed. All three levels of AF fed groups along with BT showed a significant ($P \leq 0.05$) increase in fertility percentage compared to their respective control groups. Upon GMA inclusion as binder at all three levels of AF in the diet, the fertility percentage significantly ($P \leq 0.05$) improved. During the third period all three groups fed with different levels of AF showed significantly ($P \leq 0.05$) lower fertility as compared to the control group. In the case binders alone fed groups significantly ($P \leq 0.05$) better fertility than that of control was noticed. Upon BT inclusion as binder at all three levels of AF groups, the fertility percentage showed an improvement. However, the improvement was significantly ($P \leq 0.05$) better in groups fed with 300 and 400 AF indicating the advantage of BT supplementation. Inclusion of both SP and GMA showed a significant ($P \leq 0.05$) improvement in fertility percentage irrespective of the level of AF.

Table 1: Effect of binders on per cent fertility of eggs from broiler breeders fed with different levels of aflatoxin

Description		Binder	Periods I	Periods II	Periods III
Aflatoxin Ppb	0	Nil	96.32 ± 0.13^{e}	94.79 ± 0.09^{c}	93.26 ± 0.17^{c}
		BT	97.26 ± 0.32^{bc}	97.52 ± 0.02^{a}	94.31 ± 0.21^{ab}
		SP	96.36 ± 0.24^{de}	96.74 ± 0.20^{b}	93.93 ± 0.09^{b}
		GMA	97.86 ± 0.15^{a}	97.19 ± 0.09^{a}	94.60 ± 0.28^{a}
	300	Nil	95.69 ± 0.02^{f}	93.35 ± 0.19^{e}	90.24 ± 0.27^{e}
		BT	96.36 ± 0.18^{de}	94.25 ± 0.09^{d}	91.31 ± 0.18^{d}
		SP	96.91 ± 0.07^{cd}	93.45 ± 0.21^{e}	89.39 ± 0.27^{f}
		GMA	97.74 ± 0.09^{ab}	94.35 ± 0.10^{d}	91.28 ± 0.11^{d}
	400	Nil	94.70 ± 0.31^{g}	90.30 ± 0.11^{g}	88.68 ± 0.25^{g}
		BT	97.52 ± 0.19^{ab}	91.84 ± 0.05^{f}	90.36 ± 0.32^{e}
		SP	95.68 ± 0.14^{f}	90.12 ± 0.21^{g}	89.49 ± 0.10^{f}
		GMA	96.32 ± 0.12^{e}	91.84 ± 0.16^{f}	89.29 ± 0.17^{f}
	500	Nil	93.90 ± 0.22^{h}	88.76 ± 0.17^{ij}	87.77 ± 0.24^{h}
		BT	95.67 ± 0.29^{f}	89.33 ± 0.12^{h}	87.85 ± 0.12^{h}
		SP	94.54 ± 0.14^{g}	88.78 ± 0.13^{i}	87.01 ± 0.18^{i}
		GMA	96.73 ± 0.13^{cde}	89.40 ± 0.22^{h}	87.03 ± 0.21^{i}

Means within each column bearing common superscript do not differ significantly ($P<0.05$) AF: Aflatoxin B_1; 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.

Hatchability: Hatchability on total egg set in AF fed breeder and supplemented with bentonite, *spirulina platensis* and GMA during different periods is presented in Table 2. During the first period the hatchability percentage values obtained for all AF fed groups showed a significant ($P \leq 0.05$) reduction when compared to that of control. Among the binders alone fed groups, the groups fed with BT showed significantly ($P \leq 0.05$) higher hatchability percentage when compared to that of control. Upon inclusion BT and GMA as binders, in all the AF fed groups significantly ($P \leq 0.05$) increased hatchability percentage was noticed when compared to that of control. The hatchability percentage values obtained during the second period for 300, 400 and 500 AF fed groups showed a significant ($P \leq 0.05$) reduction when compared to that of control. When the binders were either fed alone or with AF fed groups irrespective of the level of AF showed significantly ($P \leq 0.05$) better hatchability than the control. During the third period the hatchability percentage for 300, 400 and 500 AF fed groups showed a significant ($P \leq 0.05$) decrease compared to that of control group. All three levels of AF fed groups along with BT and GMA showed significant ($P \leq 0.05$) increase in hatchability percentage when compared to their respective control groups.

Table 2: Effect of binders on per cent hatchability of eggs from broiler breeders fed with different levels of aflatoxin

Description		Binder	Periods I	II	III
Aflatoxin Ppb	0	Nil	86.14±0.06[def]	85.22±0.06[b]	83.47±0.18[ab]
		BT	88.93±0.44[a]	86.79±0.10[a]	83.78±0.22[a]
		SP	86.03±0.13[defg]	85.24±0.09[b]	83.15±0.09[bc]
		GMA	86.31±0.51[cde]	86.87±0.05[a]	83.10±0.06[bc]
	300	Nil	86.84±0.12[c]	80.20±0.05[e]	78.32±0.15[fg]
		BT	87.55±0.15[b]	81.04±0.06[c]	79.43±0.06[d]
		SP	86.70±0.11[cd]	80.40±0.07[de]	78.32±0.14[fg]
		GMA	87.43±0.03[b]	80.65±0.21[d]	79.35±0.12[d]
	400	Nil	85.42±0.10[ghi]	76.82±0.11[g]	73.00±0.05[jk]
		BT	86.85±0.09[c]	78.00±0.05[f]	73.74±0.11[i]
		SP	85.69±0.15[efgh]	76.51±0.15[g]	73.37±0.10[ij]
		GMA	86.84±0.14[c]	77.82±0.08[f]	76.76±0.08[h]
	500	Nil	83.75±0.33[j]	72.46±0.14[j]	70.57±0.17[m]
		BT	85.03±0.20[hi]	73.98±0.02[i]	72.05±0.12[l]
		SP	83.83±0.16[ij]	72.28±0.12[j]	70.57±0.19[m]
		GMA	85.48±0.07[fghi]	74.31±0.18[h]	72.86±0.19[k]

Means within each column bearing common superscript do not differ significantly (P<0.05) AF: Aflatoxin B_1; 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 3: Effect of binders on per cent embryonic mortality in eggs from broiler breeders fed with different levels of aflatoxin

Description		Binder	Periods I	II	III
Aflatoxin Ppb	0	Nil	2.50±0.05[c]	3.46±0.20[b]	4.43±0.06[c]
		BT	1.06±0.10[b]	1.52±0.16[a]	1.25±0.11[b]
		SP	2.50±0.05[c]	3.42±0.06[b]	4.43±0.06[c]
		GMA	0.00±0.00[a]	1.47±0.05[a]	0.55±0.05[a]
	300	Nil	9.16±0.22[e]	8.43±0.18[d]	8.46±0.25[e]
		BT	7.10±0.10[d]	6.13±0.57[c]	6.18±0.55[d]
		SP	9.15±0.15[e]	8.10±0.39[d]	8.15±0.18[e]
		GMA	7.10±0.10[d]	6.55±0.50[c]	6.23±0.50[d]
	400	Nil	11.43±0.17[f]	10.93±0.10[e]	13.06±0.08[g]
		BT	9.16±0.22[e]	8.36±0.13[d]	10.20±0.23[f]
		SP	11.43±0.17[f]	10.33±0.34[e]	13.48±0.12[g]
		GMA	9.16±0.22[e]	8.83±1.05[d]	10.09±0.14[f]
	500	Nil	19.00±0.57[h]	17.03±0.15[g]	16.10±0.18[i]
		BT	16.96±0.15[g]	14.70±0.39[f]	14.30±0.20[h]
		SP	19.96±0.59[h]	17.46±0.44[g]	16.14±0.12[i]
		GMA	16.96±0.15[g]	14.80±0.35[f]	14.36±0.28[h]

Means within each column bearing common superscript do not differ significantly (P<0.05) AF: Aflatoxin B_1; 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 sperm count (million/ml) in breeder cocks fed with different levels of aflatoxin

Description		Binder	Periods I	II	III
Aflatoxin Ppb	0	Nil	2426.00±0.28	2362.00±0.91	2299.00±0.91
		BT	2426.00±0.28	2366.00±0.91	2293.00±0.91
		SP	2425.00±0.91	2362.00±0.91	2298.00±0.91
		GMA	2424.00±0.91	2363.00±0.91	2293.00±0.91
	300	Nil	2425.67±0.40	2369.00±0.91	2293.00±0.91
		BT	2428.00±0.91	2363.00±0.91	2293.00±0.91
		SP	2425.00±0.91	2362.00±0.91	2296.00±0.91
		GMA	2426.00±0.91	2366.00±0.91	2293.00±0.91
	400	Nil	2428.00±0.28	2369.00±0.91	2292.00±0.91
		BT	2427.00±0.91	2364.00±0.91	2293.00±0.91
		SP	2425.00±0.91	2368.00±0.91	2292.00±0.91
		GMA	2425.00±0.91	2361.00±0.91	2299.00±0.91
	500	Nil	2429.00±0.28	2362.00±0.91	2292.00±0.91
		BT	2427.00±0.91	2368.00±0.91	2292.00±0.91
		SP	2426.00±0.91	2369.00±0.91	2298.00±0.91
		GMA	2426.00±0.91	2363.00±0.91	2393.00±0.91

AF: Aflatoxin B_1; 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 5: Effect of binders on per cent live sperm in breeder's cocks fed with different levels of aflatoxin

Description		Binder	Periods I	II	III
Aflatoxin Ppb	0	Nil	89.36±0.19	88.48±0.09	87.66±0.05[a]
		BT	90.07±0.21	88.71±0.11	87.29±0.02
		SP	89.55±0.13	89.03±0.19	87.46±0.11
		GMA	89.95±0.15	88.78±0.07	87.41±0.05
	300	Nil	90.00±0.28	88.41±0.08	87.80±0.08
		BT	89.58±0.22	88.81±0.21	87.67±0.10
		SP	89.61±0.22	88.81±0.08	87.51±0.07
		GMA	89.98±0.23	89.29±0.14	87.42±0.07
	400	Nil	90.09±0.26	88.57±0.09	87.82±0.14
		BT	89.41±0.14	89.33±0.21	87.45±0.09
		SP	89.73±0.21	88.85±0.11	87.62±0.07
		GMA	90.01±0.23	89.32±0.14	87.41±0.06
	500	Nil	89.59±0.24	88.72±0.06	87.74±0.07
		BT	89.94±0.15	88.79±0.11	87.35±0.04
		SP	89.45±0.11	88.99±0.21	87.55±0.08
		GMA	89.61±0.14	88.66±0.09	87.46±0.10

AF: Aflatoxin B_1; 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.

Embryonic Mortality: The embryonic mortality percentage recorded in breeders subjected to different dietary treatments (AF fed alone, supplemented with binders alone and AF+binders Viz., bentonite, *spirulina platensis* and GMA) for different durations is presented in Table 3. During the first period all those groups fed with different levels of AF alone showed significantly ($P \leq 0.05$) higher embryonic mortality percentage as compared to that of control. Among binders alone fed groups, BT and GMA fed groups had significantly

($P<0.05$) lower embryonic mortality than that of control. When different binders were included with different levels of AF, the embryonic mortality percentage showed significant ($P\leq0.05$) reduction only in the groups fed with BT and GMA than their respective control groups. During the second as well as the third period, the same trend as that of the first period was observed with respect to embryonic mortality.

Residues in Egg: The eggs collected on the last day of each period when subjected to HPLC analysis indicated no detectable levels of AF residue in any of the treatments and/or periods.

Semen Quality - Sperm Count and Live Sperm Percentage: The sperm counts (million/ml) as well as live sperm percentage recorded in the case of cocks fed with different dietary treatments (AF alone, binders alone and AF+binders) are presented in Tables 4&5. The results showed no significant ($P\geq0.05$) changes either in sperm counts or live sperm percentage between any of the dietary treatments and during any period.

DISCUSSION

Fertility: Fertility values during all the periods of study showed a significant ($P\leq0.05$) reduction as the dietary AF level increased indicating a dose dependent response. In the present study although the binders were effective in improving fertility in general, BT and GMA showed significantly ($P\leq0.05$) positive effects in improving fertility throughout the period. The increase in fertility values in bentonite and GMA groups could be due to binding of AF and subsequent prevention of hepatic damage. It is hypothesized that the glucomannan matrix of modified-MOS preparations traps the mycotoxins in an irreversible way [11]. The mechanism of bentonite action is assumed to be through binding the AF molecule and excreting it in the faeces. Detrimental effects of dietary AF on fertility have been reported by Calnek *et al.* [12] in duck and [13] in Japanese quail. [11] also reported a significant improvement in fertility values of broiler breeder hens fed with AF employing modified-MOS in broiler breeders is supportive of the results obtained in the present study. On the contrary, Santurio *et al.* [14] recorded no influence of dietary AF on fertility percentage in breeder hens.

Hatchability: The results on hatchability followed the same trend as fertility since hatchability is very much dependent on fertility. Hatchability values during all the periods of study showed significant ($P\leq0.05$) reduction as the dietary AF level increased, indicating a dose dependent response. [14] also noticed a significant drop in hatchability in broiler breeder hens by feeding AF in a level equal to or higher than 5.00ppm. [13] also recorded significant reduction in hatchability in breeding Japanese quails with AF feeding. On the contrary, reports with WL breeder hens indicated no effects on hatchability [15-18]. In the present study although the binders were effective in improving hatchability in general, BT and GMA showed significantly ($P\leq0.05$) positive effects in improving hatchability throughout the period. The mechanism of action of binders viz., BT and GMA are already explained under fertility and the same mechanism holds good for hatchability as evident from literature. [19] also reported similar reduction in hatchability with AF feeding in broiler breeder and egg type breeder hens. Aflatoxin feeding has been known to have severe detrimental effect on the developing chicken embryo. [20] recorded embryonic abnormalities like everted viscera, exposed brain, crossed beak, under developed eyes and heads and twisted limbs with single dose exposure of six day old embryonic malformations in egg type breeders with AF feeding at graded levels. [20] recorded similar response in ducks. [21] while comparing the hatchability of AF containing eggs and AF free eggs, noticed low hatchability in the former category.

Embryonic Mortality: The embryonic mortality of breeder hens was significantly ($P\leq0.05$) higher in all AF alone treatments in all the three periods. The frequency of embryonic mortality arising due to feeding of AF contaminated diets to broiler breeders has not been previously described. The reduction of embryonic mortality observed in all periods of the current experiment is in accordance with studies of laying hens by [16] and [18]. The possible reason for significant ($P\leq0.05$) differences in embryonic mortality rates observed in the present study could be attributed to changes in the composition and availability of nutrients in the eggs set for incubation and differences in magnitude of eggshell thickness between AF fed and other groups. [22] also reported that eggshell thickness can affect moisture loss during incubation, thus hatchability can be reduced as shell quality deteriorates, resulting in embryonic mortality.). Although Kubena *et al.* [18] reported chick developmental anomalies when laying hens were fed diets containing 4.90mg of DON/kg of feed for 10 weeks. Further, it was shown that only trace amounts of *Fusarium* mycotoxins are transferred into the eggs of laying hens, which are unlikely to be of significance with respect to embryonic mortality [23]. Inclusion of binders alone viz., BT and GMA significantly ($P \leq 0.05$) reduced embryonic mortality during all the three periods. Upon inclusion of BT, SP and GMA as binders along with different levels of AF significantly ($P \leq 0.05$) reduced embryonic mortality in all the three periods of study. The decrease in embryonic mortality in bentonite and GMA fed groups could be due to binding of AF and subsequent prevention of hepatic damage. The findings of present experiment are in agreement with those of Arvind *et al.* [11] on broiler breeders and Salahi *et al.* [24] on broilers.

Residues in Egg: The residue of AF in eggs collected from breeder females fed varying levels of AF independently as well as in combinations with binders did not significantly ($P \geq 0.05$) show any effect as reported by Raju *et al.*, Raju *et al.*, Ghahri *et al.* and Raju *et al.* [25-28]. Mycotoxin residues in eggs have the potential to post a significant effect on progeny chicks as well as human health hazard. Aflatoxins and some of their metabolites in the liver of hens generates a variety of toxin hydroylated metabolites that can be carried over to eggs in ratios ranging from 5,000:1 to 66,200:1 and even to 125,000:1 [29, 30].

Semen Quality - Sperm Count and Live Sperm Percentage: The present study revealed that feeding of AF and binders independently or in combinations in the diet of breeder cocks failed to influence either sperm count or live sperm percentage in any of periods. The results of this study is in conformity with that of Yegani *et al.* [5] who reported a non significant difference in semen volume, sperm concentration, viability and motility in broiler breeder males fed with DON. Therefore, it can be presumed that that the feeding of low levels of AF may not affect the sperm quality and live sperm percentage in the breeder males. [30, 31] in their study observed drastic deterioration in semen quality of breeder cocks fed with AF. The traits affected were semen volume, semen concentration, motility and abnormalities. [30, 31] demonstrated that feeding diets contaminated with 10.00 and 20.00mg of DON per kg of feed decreased the fertility in broiler breeder males, though there was no difference in the volume of semen produced.

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