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ORIGINAL ARTICLES

Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced with Aflatoxin B1: Effects on Biochemical and Immunological 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 gamma glutamyl transferase (GGT), alanine amino transferase (ALT) and antibody titers against Newcastle and Infectious bursal diseases levels in the serum 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 (Mohapatra and Misra, 2008, Manafi *et al.,* 2011). 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 (Mohanamba *et al.,* 2007). 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 (Fraga *et al.,* 2007 and Thapa, 2008). 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 (Verma *et al.,* 2004). 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 (Chandrashekharan, 2000). Yegani *et al.* (2006) reported that the feeding of mycotoxin contaminated grains decreased eggshell thickness. However, dietary supplementation with

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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.

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 28^{th} 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 as per BIS (1997) 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.

Biochemical And Immunological Parametersl

Serum levels of GGT and ALT:

Blood was collected in non-heparinized tubes from six hens of each treatment by puncturing the brachial vein on the last day of each period. Serum was separated after 8 to 10 hours as per the standard procedures (Calnek *et al*., 1992) and was stored at –20° C for subsequent analysis. The individual serum samples were analyzed for total proteins, serum albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) using automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). The methodology and the set of reagents used in respect of each parameter were as per the recommendations of the manufacturer of the analyzer system. Treatment wise means were computed.

Serum antibody titers for ND and IBD:

The serum samples were assayed for antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) using ELISA technique on the last day of each period. Treatment-wise means of titers were computed.

Statistical analysis:

The data were analyzed using the General Linear Model procedure of Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Period wise data were analyzed by 4 x 4 factorial manner. Overall period data were analyzed by repeated measurement design (Gill, 1985). Duncan multiple range test at 0.05 probability level was employed for comparison of the means (Duncan, 1955).

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Results:

Gamma glutamyl transferase (GGT) levels in serum:

The influence of dietary AF and binders viz., bentonite, *spirulina platensis* and GMA on serum GGT activity during different periods are presented in Table 4.28 and in Fig. 4.14. The mean squares from analysis of variance are shown in Table 1. During the first period all three groups fed with different levels of AF showed significantly (P≤0.05) higher serum GGT levels as compared to that of control group. When binders included to all three levels of AF, the serum GGT levels showed significant (P≤0.05) reduction only in the groups fed with BT and GMA, when compared to their respective control groups. During the second as well as third period, the results were of similar trend to that of first period.

Alanine amino transferase (ALT) levels in serum:

The influences of dietary AF and bentonite, spirulina platensis and GMA on serum ALT activity during different periods are presented in Table 4.30 and Fig. 4.15. The mean squares from analysis of variance are given in Table 2. During the first period all three groups fed with different levels of AF showed significantly (P≤0.05) higher serum ALT levels as compared to that of control group. When binders were supplemented to diets with AF, the serum ALT levels showed significantly (P≤0.05) lowered values only in the groups fed with BT and GMA when compared to their respective control groups. During the second as well as third period, the results were of similar trend to that of first period.

Serum antibody titers for ND and IBD:

The influence of dietary AF and binders on antibody titers against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) during different periods is presented in Table 3&4. During the first period all three groups fed with different levels of AF showed significantly (P≤0.05) lower titers against ND and IBD as compared to those of control groups. The groups fed with binders alone showed significantly (P≤0.05) higher titers against ND and IBD when compared to that of control groups. When binders were included in the diet with AF at different levels, the ND and IBD titers improved significantly (P≤0.05) compared to their respective control groups. During the second and third periods, the results with respect to antibody titers against ND and IBD were same as that observed in the first period.

Discussion:

Gamma Glutamyl Transferase (GGT) And Alanine Amino Transferase (ALT) Levels In Serum:

Aflatoxin in the diet had increased the activity of serum GGT and ALT in breeder hens in a dose dependent manner. BT and GMA significantly (P≤0.05) decreased the serum GGT and ALT activities during all the three periods of study. This indicated the positive effect of BT and GMA in counteracting the adverse effects of aflatoxicosis on enzymeactivities. The elevation of serum GGT and ALT activities during aflatoxicosis could be attributed to hepatocyte degeneration and subsequent leakage of enzymes into the circulation. Increased GGT and ALT activities is the most sensitive indicator of liver damage during aflatoxicosis (Kubena *et al*., 1990). Aflatoxin B1 is especially likely to cause dose-dependent induction or inhibition of liver mixed-function-oxygenase activities, which may affect the liver's metabolism of endogenous and exogenous substrates (Zaghini *et al*., 2005). The results of the present investigation are in agreement with those reported by Johri *et al*. (1990) in Japanese quail layers and DaFalla *et al*. (1987), Kubena *et al*. (1990) and Manafi, M. (2006) in commercial broilers. On the contrary, Fernandes *et al*. (1994) reported no alteration in the activity of these enzymes in broilers fed with AF at 2.50 to 5.00ppm for 35 days.

Serum antibody titers for ND and IBD:

The antibody titers against ND and IBD were reduced significantly (P<0.05) upon inclusion of different levels of AF in the diet during all the three periods. The data revealed a dose dependent decrease in antibody titers as the dietary level of AF increased. Compared to their respective controls, inclusion of BT, SP and GMA significantly (P≤0.05) increased the antibody titers against ND and IBD during all three periods of study. Boulton *et al*. (1981) also recorded a significant reduction in HI titers in layer breeders at 500ppb levels of AF. Further, such a depression in antibody titers were reported by Manafi, M. (2006) in broilers. It is presumed that reduced plasma proteins for antibody synthesis might be responsible for this lowered ND and IBD titers

observed during aflatoxicosis (Verma *et al*., 2004). The positive effect of BT with AF observed in the present study is in agreement with the findings of Ibrahim *et al*. (2000) and Pasha *et al*. (2007). The advantage of feeding *spirulina* platensis as binder in the present study is in agreement with that of Raju *et al*. (2005) who reported that *spirulina* at 0.02 per cent level in the diet improved cellular immune response while no effect was seen in broilers fed with 300 AF. The reports of *spirulina platensis* on effectiveness on immune status of AF fed birds are plenty. *Spirulina* is a powerful tonic for the immune system and has immune regulatory role. The blue-green natural protein pigment, phycocyanin present in animal studies was showed to increase lymphocyte activity and stimulate the production of blood. Phycocyanin probably prevents a host of degenerative organ diseases by increasing immunity. Complex sugars from *spirulina* have been shown to increase antibody production and infection fighting T-cells. Mice have shown increased immunity, bone marrow reproduction, growth of thymus and spleen when fed complex sugars and phycocyanin from *spirulina*. GMA was also effective against the AF effects on antibody responses. The findings of the present study are in contrary with the findings of Raju *et al*., 2004, who reported that Modified-MOS significantly ($P \leq 0.05$) improved the antibody titers against both the antibody responses. The exact mechanism by which the response is affected by GMA is unclear. It is possible that the binder included in the diet may bind the mycotoxins present in the feed and thereby prevent their absorption from the GIT tract, thus avoiding the possible immunosuppressant effects of mycotoxins which would have occurred otherwise.

Table 1: Effect of binders on GGT levels in broiler breeders fed with different levels of aflatoxin

Description		Periods			
		Binder	I	II	III
Aflatoxin ppb	0	Nil	9.60±0.02^{a}	11.16±0.04^{a}	13.00±0.02^{a}
		BT	9.60±0.02^{a}	11.16±0.02^{a}	13.00±0.04^{a}
		SP	9.60±0.02^{a}	11.16±0.02^{a}	13. 00±0.04^{a}
		GMA	9.60±0.02^{a}	11.16±0.02^{a}	13. 00±0.04^{a}
	300	Nil	10.30±0.02^{c}	12.90±0.02^{b}	14.70±0.02^{b}
		BT	10.03±0.01^{b}	12.30±0.02^{c}	13.90±0.02^{a}
		SP	10.30±0.02^{c}	12.90±0.02^{b}	14.70±0.04^{b}
		GMA	9.90±0.02^{a}	12.03±0.01^{d}	13.50±0.02^{a}
	400	Nil	11.90±0.02^{d}	13.20±0.02^{e}	15.10±0.05^{c}
		BT	10.60±0.02^{c}	12.70±0.02^{f}	14.13±0.04^{b}
		SP	11.90±0.02^{d}	13.20±0.02^{e}	15.10±0.04^{c}
		GMA	10.16±0.04^{c}	12.16±0.04^{g}	13.36±0.04^{a}
	500	Nil	12.60±0.02^{e}	14.40±0.02^{h}	16.66±0.04^{e}
		BT	10.80±0.02^{c}	13.30±0.02^{i}	15.16±0.04^{d}
		SP	12.60±0.02^{e}	14.40±0.02^{h}	16.66±0.02^{e}
		GMA	10.40±0.09^{c}	12.80±0.02^{j}	14.80±0.02^{b}

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 2: Effect of binders on ALT levels in broiler breeders fed with different levels of aflatoxin

Description		Periods			
		Binder	I	II	III
Aflatoxin ppb	0	Nil	17.85±0.21^{a}	19.88±0.10^{a}	20.48±0.34^{b}
		BT	17.69±0.11^{a}	19.06±0.04^{a}	20.38±0.21^{b}
		SP	17.99±0.11^{a}	19.26±0.27^{a}	20.52±0.19^{b}
		GMA	17.36±0.17^{a}	19.44±0.32^{a}	20.84±0.06^{b}
	300	Nil	19.86±0.20^{c}	21.79±0.07^{c}	21.81±0.18^{c}
		BT	18.41±0.17^{b}	20.91±0.23^{b}	20.86±0.08^{b}
		SP	19.86±0.05^{c}	21.79±0.11^{c}	21.81±0.32^{c}
		GMA	17.79±0.05^{a}	20.11±0.28^{b}	19.61±0.11^{a}
	400	Nil	22.13±0.17^{f}	23.35±0.17^{e}	23.87±0.08^{e}
		BT	21.48±0.25^{e}	22.45±0.07^{d}	22.26±0.10^{d}
		SP	22.13±0.13^{f}	23.35±0.03^{e}	23.87±0.14^{e}
		GMA	20.98±0.18^{d}	21.88±0.17^{c}	21.90±0.10^{c}
	500	Nil	25.73±0.11^{i}	26.94±0.09^{g}	25.22±0.19^{f}
		BT	24.20±0.18^{h}	25.93±0.22^{f}	23.98±0.15^{e}
		SP	25.73±0.09^{i}	26.94±0.13^{g}	25.22±0.14^{f}
		GMA	23.85±0.09^{g}	23.50±0.12^{e}	22.00±0.25^{d}

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 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}

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 ND titers of broiler breeders fed with different levels of aflatoxin

Description		Periods			
		Binder	I	II	III
Aflatoxin ppb	0	Nil	3697.53±6.95^{m}	3744.63±12.46^{m}	3178.47±23.87^{m}
		BT	3696.53±6.62^{m}	3749.73±18.74^{m}	3180.27±3.04^{m}
		SP	3696.10±1.86^{m}	3746.40±12.56^{m}	3177.70±9.00^{m}
		GMA	3697.33±1.83^{m}	3748.80±3.40^{m}	3178.13±18.90^{m}
	300	Nil	2866.31±15.55^{i}	2872.67±3.01^{i}	2674.70±12.72^{i}
		BT	2902.66±4.25^{j}	2907.97±3.98^{j}	2760.70±6.89^{j}
		SP	2933.53±11.05^{k}	2938.87±17.24^{k}	2806.63±10.25^{k}
		GMA	2961.57±8.44^{l}	2967.93±5.08^{l}	2848.27±9.24^{l}
	400	Nil	2404.80±16.03^{e}	2148.87±12.78^{e}	1964.00±11.73^{e}
		BT	2460.69±15.47^{f}	2231.57±13.57^{f}	2026.80±14.97^{f}
		SP	2493.37±12.74^{g}	2251.67±10.04^{g}	2089.37±23.12^{g}
		GMA	2538.93±11.45^{h}	2286.13±17.64^{h}	2188.30±16.19^{h}
	500	Nil	2078.93±15.26^{a}	1581.47±3.88^{a}	1230.50±13.00^{a}
		BT	2148.83±17.57^{b}	1690.33±4.12^{b}	1364.07±11.37^{b}
		SP	2192.67±13.82^{d}	1723.83±5.84^{c}	1371.13±12.18^{c}
		GMA	2282.27±6.64^{d}	1712.07±4.46^{d}	1392.03±31.29^{d}

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.

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