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Comparative Study of Several Mycotoxin Binders During Aflatoxicosis in Body Weight, Feed Consumption, Feed Efficiency and Egg Production Parameters of Broiler Breeders

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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 (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 feed consumption and feed efficiency when compared to that of control. The results indicated no significant ($P \geq 0.05$) effect of 500 AF on body weight (2974gr) when compared to that of control group (2959gr). Inclusion of 500 AF in the diet significantly ($P \leq 0.05$) affected egg production (73.40%) when compared to that of control (80.97%). 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 Performance Parameters

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. The enormous growth and spurt in poultry production has put a tremendous pressure on proper feeding of poultry in order to sustain the poultry industry in India [1]. Contamination of poultry feeds with mycotoxins is one of the major problems associated with the feeding of poultry. 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 900 µg/kg in commonly used ingredients as well as mixed feed samples in developing countries [2]. 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. 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. Scientists [5] reported that the feeding of mycotoxin contaminated grains decreased eggshell thickness. However, dietary supplementation with Glucomannan Mycotoxin

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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 performance 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.

Performance Parameters

Changes in Body Weight: Hens were weighed individually at the start of the experiment (28th week) and at the end of the experiment (36th week).

Feed Consumption: Feed offered was 160g/bird/day. Total feed consumption for each of the three periods studied was recorded. The cocks were also fed with corresponding treatment diets *ad libitum* throughout the study as that of hens.

Feed Conversion Ratio: Based on the egg production and quantity of feed consumed, the average feed efficiency was computed as the unit feed consumed to produce a unit of egg mass (kg feed/kg egg).

Egg Production: Daily egg production record on each hen was maintained in all the groups of birds throughout the experimental period. The eggs were labeled and stored for estimating other parameters at a later stage. The per cent hen-day egg production was calculated for each experimental period using to the formula.

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

Changes in Body Weight: The data obtained on mean body weight of broiler breeder hens fed with different levels of AF and toxin binders in the beginning and at the termination of the experimental periods are presented in Table 1. Statistical analysis revealed that there is no significant ($P \geq 0.05$) difference between body weights of broiler breeder hens belonging to different treatment groups at 28 weeks of age. This indicated the uniformity of birds selected for the experiment. The range of highest and lowest body weight recorded were 3010g and 2959g for 300 AF+GMA and control groups, respectively. At the end of the experiment, the analysis of variance indicated

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

Description		Binder	Body weight (g) 28 weeks	36 weeks
Aflatoxin Ppb	0	Nil	2959.58±12.67	3394.58±14.85
		BT	2990.67±10.78	3445.25±11.87
		SP	2968.67±10.67	3401.83±21.29
		GMA	2991.42±13.89	3409.00±23.10
	300	Nil	2964.75±10.67	3340.25±27.79
		BT	2992.33±8.09	3397.08±21.79
		SP	2975.75±13.22	3373.58±14.03
		GMA	3010.00±14.61	3403.25±17.32
	400	Nil	2989.08±10.77	3345.67±18.61
		BT	2993.67±11.33	3432.58±15.93
		SP	2981.58±9.80	3390.50±17.52
		GMA	2991.83±14.64	3401.83±15.86
	500	Nil	2974.83±7.40	3403.75±24.19
		BT	2984.58±11.25	3412.08±14.85
		SP	2987.75±11.77	3414.25±19.64
		GMA	3003.75±15.25	3366.58±24.30

AF: Aflatoxin B_1; BT: Bentonite (1%); SP: *Spirulina platensis* (0.1%); GMA: Glucomannan Mycotoxin Adsorbent (0.2%)

a non significant ($P \geq 0.05$) difference in body weights of breeders at 36 weeks of age pertaining to different treatments. The maximum and minimum body weights recorded were control+BT (3445g) and 300 AF (3340g) groups, respectively.

Feed Consumption: The data on feed consumption (g/day) 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 feed consumption (g/day) in all AF fed groups showed a significant ($P \leq 0.05$) decrease compared to the control group. Among the binders fed groups without AF, the group GMA only showed a significant ($P \leq 0.05$) increase in feed consumption compared to the control group. All the three levels of AF fed groups along with BT and GMA showed significant ($P \leq 0.05$) increase in feed consumption compared to their respective control groups. All three levels of AF fed groups along with SP showed non significant ($P \geq 0.05$) differences in feed consumption compared to their respective control groups. During the second period the feed consumption was significantly ($P \geq 0.05$) lower in all AF fed groups compared to that of control. Among the binders fed groups without AF, there was no significant ($P \leq 0.05$) difference when compared to that of the control. All three levels of AF fed groups along with BT and GMA showed significant ($P \leq 0.05$) increase in feed consumption, compared to their respective control groups. All three levels of AF fed groups along with SP showed a no significant ($P \geq 0.05$) difference in feed consumption values when compared to their respective control groups. During the third period feed consumption values for 500 AF fed group showed a significant ($P \leq 0.05$) decrease when compared to that of control. Among the binders alone fed groups, the groups fed with BT only showed a significant ($P \leq 0.05$) decrease in feed consumption compared to that of control. All three levels of AF fed groups along with BT and SP showed non significant ($P \geq 0.05$) difference in feed consumption values compared to their respective control groups. Values ranged from 156g to 158g. The 500 AF+GMA group showed significantly ($P \leq 0.05$) higher feed consumption, compared to their respective control group.

Feed Efficiency: The feed efficiency measured as kg of feed consumed to produce a kg of egg mass in breeder hens subjected to different dietary treatments is presented in Table 3. During the first period all three AF fed groups (300, 400 and 500ppb) showed significantly ($P \leq 0.05$) poor feed efficiency values of 3.60, 3.75 and 3.92, respectively when compared with 3.33 of control group and they differed significantly ($P \leq 0.05$) with each other. Similar feed efficiency as that of control group was observed when binders alone were included in the diets of breeder hens. The groups fed with AF at different levels when treated with BT, SP and GMA showed no significant ($P \geq 0.05$) improvement in their feed efficiency. During the second period the same trend as that of first period continued. The values ranged from 3.42 to 4.03 in this period. During the third period all three AF fed groups (300, 400 and 500ppb) showed significantly ($P \leq 0.05$) poor feed efficiency compared to the control group. The groups

Table 2: Effect of binders on feed consumption (g/day) of broiler breeders fed with different levels of aflatoxin

			Periods		
Description		Binder	I	II	III
Aflatoxin Ppb	0	Nil	157.00±0.28^{b}	158.00±0.28^{a}	158.66±0.16^{a}
		BT	157.33±0.16^{ab}	157.33±0.16^{abc}	157.33±0.16^{b}
		SP	157.66±0.16^{ab}	157.66±0.16^{ab}	158.66±0.16^{a}
		GMA	158.00±0.28^{a}	158.00±0.28^{a}	158.00±0.28^{ab}
	300	Nil	155.00±0.28^{c}	156.66±0.16^{c}	158.00±0.28^{ab}
		BT	157.00±0.28^{b}	158.00±0.28^{a}	158.00±0.28^{ab}
		SP	155.00±0.28^{c}	156.66±0.16^{c}	158.00±0.28^{ab}
		GMA	157.00±0.28^{b}	158.00±0.28^{a}	158.66±0.16^{a}
	400	Nil	154.66±0.16^{c}	157.00±0.28^{bc}	158.00±0.28^{ab}
		BT	157.00±0.28^{b}	158.00±0.28^{a}	158.00±0.28^{ab}
		SP	154.66±0.16^{c}	157.00±0.28^{bc}	158.00±0.28^{ab}
		GMA	157.00±0.28^{b}	158.00±0.28^{a}	158.66±0.16^{a}
	500	Nil	154.33±0.44^{c}	154.66±0.33^{d}	156.11±0.35^{c}
		BT	157.00±0.28^{b}	158.00±0.28^{a}	156.11±0.35^{c}
		SP	154.33±0.44^{c}	154.66±0.33^{d}	156.11±0.35^{c}
		GMA	157.00±0.28^{b}	158.00±0.28^{a}	158.66±0.16^{a}

Means within each column bearing common superscript do not differ significantly ($P \leq 0.05$)

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 feed efficiency (kgs feed/kg egg mass) of broiler breeders fed with different levels of aflatoxin

			Periods		
Description		Binder	I	II	III
Aflatoxin Ppb	0	Nil	3.33±0.04^{d}	3.42±0.03^{d}	4.08±0.02^{b}
		BT	3.33±0.04^{d}	3.42±0.03^{d}	4.08±0.02^{b}
		SP	3.33±0.04^{d}	3.42±0.03^{d}	4.08±0.02^{b}
		GMA	3.33±0.04^{d}	3.42±0.03^{d}	4.08±0.02^{b}
	300	Nil	3.60±0.03^{c}	3.71±0.03^{c}	4.15±0.03^{b}
		BT	3.60±0.03^{c}	3.71±0.03^{c}	4.15±0.03^{b}
		SP	3.60±0.03^{c}	3.71±0.03^{c}	4.15±0.03^{b}
		GMA	3.60±0.03^{c}	3.71±0.03^{c}	4.15±0.03^{b}
	400	Nil	3.75±0.01^{b}	3.88±0.01^{b}	4.17±0.01^{b}
		BT	3.75±0.01^{b}	3.88±0.01^{b}	4.17±0.01^{b}
		SP	3.75±0.01^{b}	3.88±0.01^{b}	4.17±0.01^{b}
		GMA	3.75±0.01^{b}	3.88±0.01^{b}	4.17±0.01^{b}
	500	Nil	3.92±0.01^{a}	4.03±0.01^{a}	4.36±0.04^{a}
		BT	3.92±0.01^{a}	4.03±0.01^{a}	4.36±0.04^{a}
		SP	3.92±0.01^{a}	4.03±.0.01^{a}	4.36±0.04^{a}
		GMA	3.92±0.01^{a}	4.03±0.01^{a}	4.36±0.04^{a}

Means within each column bearing common superscript do not differ significantly ($P \leq 0.05$)

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

which received binders alone in their diets had similar feed efficiency as that of control group, which in turn did not differ significantly ($P \geq 0.05$) from 300 and 400 AF fed groups with and without binders. Further, AF fed groups (at different levels) when treated with BT, SP and GMA showed no significant ($P \geq 0.05$) difference in their respective feed efficiency levels. The values ranged from 4.15 to 4.36 in this period.

Egg Production: Per cent hen day egg production recorded in AF fed breeder hens and those supplemented with bentonite, *spirulina platensis* and GMA during different periods is presented in Table 4. During the first period all the three groups fed with different levels of AF showed significantly ($P \leq 0.05$) lower percentage of egg production which ranged from 71.81 per cent in group fed with 500 AF to 77.00 per cent in group fed with 300 AF as

Table 4: Effect of binders on per cent egg production of broiler breeders fed with different levels of aflatoxin

Description		Binder	Periods I	II	III
Aflatoxin Ppb	0	Nil	80.97±1.01^{ab}	79.79±0.85^{a}	68.65±0.69^{cd}
		BT	81.76±0.98^{ab}	80.58±0.91^{a}	71.81±1.09^{ab}
		SP	81.76±0.98^{ab}	80.18±0.98^{a}	70.61±0.52^{bc}
		GMA	82.16±0.84^{a}	80.98±0.82^{a}	74.20±1.09^{a}
	300	Nil	77.00±0.99^{cd}	73.40±0.92^{cde}	67.86±0.84^{cd}
		BT	77.79±1.22^{cd}	75.80±0.92^{bc}	68.65±0.69^{cd}
		SP	77.00±0.99^{cd}	73.40±0.92^{cde}	67.86±0.84^{cd}
		GMA	79.00±0.92^{bc}	77.00±0.99^{bc}	69.84±0.88^{bc}
	400	Nil	73.40±0.92^{ef}	71.80±0.70^{def}	65.89±0.79^{de}
		BT	75.80±0.92^{de}	71.81±1.09^{def}	66.67±1.01^{de}
		SP	73.40±0.92^{ef}	71.80±0.70^{def}	65.89±0.79^{de}
		GMA	77.00±0.99^{cde}	74.20±1.09^{cd}	66.67±1.01^{de}
	500	Nil	71.81±1.09^{f}	69.84±0.88^{f}	63.89±1.09^{e}
		BT	73.80±0.92^{ef}	70.61±0.52^{ef}	64.69±0.92^{e}
		SP	71.81±1.09^{f}	69.84±0.88^{f}	63.89±1.09^{e}
		GMA	73.40±0.92^{ef}	71.80±0.70^{def}	65.89±0.79^{de}

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

compared to 80.97 per cent in the control group. There was no significant (P≥0.05) difference in per cent egg production among the groups of birds fed with binders alone when compared with hen's production in control. When BT and SP were used as binders at all three levels of AF fed groups, the per cent egg production remained unchanged compared to their respective groups without binders. Upon GMA inclusion as binder at all AF levels has increased per cent egg production in general when compared to that of control group but significantly (P≤0.05) increased in the group fed with 400 AF. During the second period all three groups fed with different levels of AF showed significantly (P≥0.05) lower percentage of egg production ranging from 69.84 per cent in group fed with 500 AF to 73.40 per cent in group fed with 300 AF as compared to 79.79 per cent in the control group. There was no significant (P≥0.05) difference in per cent egg production among the groups of birds fed with binders alone when compared with control group. When BT, SP and GMA were used as binders at all three levels of AF fed groups, the per cent egg production was remained unchanged compared to their respective groups without binders. The values ranged from 69.84 per cent to 77.00 per cent. During the third period the per cent egg production was same in 300 and 400 AF fed groups but 500 AF fed group showed significantly (P≤0.05) lower production when compared to that of control group. Among the binders fed groups without AF the groups fed with BT and GMA showed significantly (P≤0.05) higher egg production compared to that of control. When BT, SP and GMA were used as binders, at all the three levels of AF feeding, the per cent egg production did not alter when compared to their respective groups without binders.

DISCUSSION

Changes in Body Weight: The AF at levels of 300, 400 and 500ppb fed to broiler breeders from 28 to 36 weeks of age did not significantly (P≥0.05) affect the body weight at 36 weeks of age. This is in agreement with the findings of [5] who reported no significant change in BW of broiler breeders fed with mycotoxin. Similar observation on body weight was also reported by many investigators [8, 9] in layer chicken fed with 1.00 to 5.00ppm of AF from 4 to 40 weeks. However, [10] found significant (P≤0.05) decrease in body weight in laying hens fed with 2.0 to 8.00ppm of AF for 29 days. Similarly, feeding AF (500ppb) to layer chicken from 15 to 67 weeks of age reduced the body weight [11].

The possible reasons for the non-significant (P≥0.05) effect of AF on body weight would be due to the dose at which it is fed in the diet may not be sufficient to reduce the body weight or the birds (colored broiler breeders) are more tolerant to AF at these levels.

Feed Consumption: The results of the present investigation showed that AF at all levels in the diet decreased significantly ($P \leq 0.05$) feed consumption of breeder hens during the first two periods (28-30 and 31-33 weeks) whereas only AF 500 significantly ($P \leq 0.05$) decreased feed consumption in the third period (34-36 weeks). This could be due to the fact that the birds get used to the toxic effect of the lower doses by the time the experiment entered the third period. Further, feeding of 500 AF significantly ($P \leq 0.05$) reduced feed consumption clearly indicating its toxic effect on the breeders. Upon incorporation of binders alone in the diet, significant ($P \leq 0.05$) improvement in feed consumption was noticed only in GMA group in the first period while BT and SP in the third period. Feeding of AF+BT diet significantly ($P \leq 0.05$) increased the feed consumption of breeder hens during the first two periods only while in AF+GMA increased the feed consumption of breeder hens during the third period only. Bentonite, a layered aluminosilicate being a nonnutritive sorptive material, reduces absorption of the fungal metabolites in GIT by forming an inert, stable and insoluble complex with AF which is assumed to be responsible for preventing toxin absorption and is excreted in the faeces [12]. This property of bentonite triggered indirectly the hens to consume more feed even in the presence of AF. The increased feed consumption in AF+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. This finding supports the results of the present experiment. Similar result was also reported by Ghahri *et al.* [13] on broilers. Further, many investigators opined that significant drop in feed consumption with AF feeding is a constant observation in commercial broilers [1]. In contrast, a non significant effect of *Fusarium* mycotoxins contaminated diets on feed consumption was observed in commercial layers and broiler breeders, respectively by Yegani *et al*., Zaghini *et al*. [5, 9]. The probable reason for the variation in results reported could be attributed to differences in the source of contamination (natural and purified), using a single source of mycotoxin compared with a blend of contaminated grains and the level and duration of exposure in different experiments.

Feed Efficiency: The results of the present investigation on feed consumption among all AF fed hens (300, 400 and 500ppb) showed significant ($P \leq 0.05$) reduction in feed efficiency in the first two periods. In the third period, only hens fed with 500 AF showed significantly ($P \leq 0.05$) reduced feed efficiency with respect to egg production. Feeding of AF+binders in the diets of breeders did not influence their feed efficiency positively. Zaghini *et al.* [9] reported a numerical improvement of feed efficiency in broiler breeder hens fed with AF and modified MOS for 12 weeks. The current study indicated that binders are not effective in improving feed efficiency of egg production in broiler breeders. This is similar to the findings of Hagler *et al.* [8] and Miazzo *et al*. [14] in commercial layers [7] reported that feed efficiency decreased when layers were fed *Fusarium* mycotoxins contaminated diets compared with controls and supplementation of GMA increased the feed efficiency values.

Egg Production: The results of the present investigation showed significantly ($P \leq 0.05$) decreased hen day egg production in broiler breeder hens fed with AF alone as well as in combination with bentonite, *spirulina platensis* and GMA during first and second periods as compared to only AF at 500 in third period. As in the case of body weight, lower level of AF did not affect egg production when fed for longer duration also. Therefore, in the present study only 500 AF fed for a longer duration reduced egg production significantly ($P \leq 0.05$) when compared to the control. In support of the present findings, [8] reported significant ($P \leq 0.05$) drop in egg production after three weeks when fed 5.00 to 10.00ppm AF to broiler breeder hens. Zaghini *et al*. [9] recorded complete cessation of egg production with follicular atresia in egg type breeders which were fed with AFB1 for three weeks. Hagler *et al*. [8] also reported a significant drop in egg production in egg type breeder hens fed with 0.50, 1.00 and 1.50ppm AF for six weeks. Similarly in layers, many scientists reported significant reduction in egg production due to aflatoxicosis [9, 10] According to Sims *et al*. [11] drop in egg production with AF feeding appears to be a result of reduced liver synthesis and transport of yolk precursors and inhibition of maturation of ova. Further, they stated that reduced plasma levels of protein, triglycerides and calcium with AF feeding, might have also contributed in part to the detrimental effect of AF on egg production. The possible reason for reduced egg production is decreased feed consumption due to the feeding of breeder hens with diets containing AF.

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