

EFFICACY OF TURMERIC (*CURCUMA LONGA*) AND GARLIC (*ALLIUM SATIVUM*) POWDER TO PROTECT BROILER CHICKEN RECEIVING DIET CONTAINING EXOGENOUS AFLATOXIN

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Abstract

Aflatoxin (AF), a secondary metabolite of *Aspergillus* species, is carcinogenic and mutagenic for chicken. Chickens are highly sensitive to the adverse effect of AF and it is causing economic loss in poultry industry. The aim of the current study was to evaluate the efficacy of turmeric powder (TP) and garlic powder (GP) on broiler receiving diet containing exogenous AF. Randomly selected 14 days old Cobb-500 broiler chicks (N=84) were equally divided into four treatments. Four isonitrogenous and isocaloric experimental diets were prepared viz; diet-A: 1.5 mg of AF; Diet-B: 1.5 mg of AF and 1g of TP; Diet-C: 1.5 mg of AF and 1 g of GP contained per kg feed and control diet had no AF, TP and GP. Each dietary treatment had 3 replications (having 7 birds in each) and respective feed was offered to the broilers according to the entire duration (35 days) of experiment. All experimental broilers were reared following same management practices. At 36 days of age all experimental broiler were slaughtered for examining the necroscopic changes in internal organs such as liver, kidney and spleen to estimate AF residues. Growth of the broiler chicken was monitored and feed conversion ratio (FCR) was calculated. The study demonstrated significant ($P<0.01$) effect of turmeric and garlic powder on final body weight of birds and so also the FCR. Liver and kidney weight use showed significantly ($P<0.01$) the highest in diet-A, followed by diet-B, diet-C and the control diet, respectively but, spleen weight did not differ significantly ($p>0.05$) with the treatments. The residue of AF in different internal organs was also significantly ($P<0.05$) different between the treatments; being higher in diet-A as compared to the rest of the dietary treatment groups. Evidently, the present study substantiated the potentiality of TP in broiler chicken to overcome the adverse effect of AF, a common mycotoxin.

Keywords: Aflatoxin, Broiler diet, Deactivating, Garlic, Turmeric

Introduction

Aflatoxins (AF) are the most commonly known mycotoxin resulting adverse effects in poultry. Aflatoxins are produced by certain molds of the genus *Aspergillus*, particularly *A. flavus* and *A. parasiticus* which grow in soil, decaying vegetation, hay, and grains. The molds can colonize and contaminate food before harvest or during storage, especially following prolonged exposure to a high-humidity environment, or to stressful

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conditions such as drought. In addition, the accumulation of toxin residues in tissues such as liver muscle and milk above the permissible limit are considered to represent a human health hazard to consumers of such animal products (Gareis *et al.*, 2000; Hussain *et al.*, 2010). So, prevention of mold growth and AF contamination in feed and foodstuffs is utmost important in view of public health, food safety and safe food production. Although 18 different aflatoxins have been identified, only aflatoxin B1, B2, G1 and G2 have been detected as natural contaminants of feed and feedstuffs (Leeson *et al.*, 1995). Aflatoxicosis in chickens causes mortality, listlessness, anorexia, decreased growth rates, negative feed conversions, fatty liver, decreased egg production, poor pigmentation, and increased susceptibility to other diseases (Tedesco *et al.*, 2004; Rangasaz *et al.*, 2011; Gholami- Ahangaran *et al.*, 2013). Aflatoxin B1 (AFB1) is the most biologically active form of AF (Boutrif, 1998) and causes poor performance, liver lesions and immune-suppression in poultry (Ledoux *et al.*, 1999; Rosa *et al.*, 2001; Magnoli *et al.*, 2011). Many scientists applied different chemicals during storage and prior to feeding of feed for control of fungi and detoxification of mycotoxins (Phillips *et al.*, 1988; Smith *et al.*, 1994; Harper *et al.*, 2010).

Herbal components like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and green algae (*Spirulina platensis*) counteract mycotoxins, improve growth performance and also act as good antioxidants (Sawarkar *et al.*, 2012). Garlic extract was found to be the most effective in the prevention of aflatoxin-induced toxicity and free radical generation in rats (Mosaad *et al.*, 2003) and turmeric provide protection against the toxic effects of aflatoxin on liver and kidney (Majid *et al.*, 2015). In view of the above perspective, the current research was aimed to evaluate the efficacy of turmeric powder (TP) and garlic powder (GP) to overcome the adverse effect of mycotoxin in broiler maintained on diet containing exogenous AF. The study also encompassed assay of AF residues in vital organs of birds.

Materials and Methods

Production of aflatoxins

Aflatoxins producing mold (*A. parasiticus* NRRL 2999) was obtained from the Toxicology Laboratory of Huazhong Agricultural University, Wuhan, P. R. China and was grown on rice after following the standard protocol (West *et al.*, 1973). The AF containing rice was autoclaved and ground to powder. Thereafter, the concentration of AF was determined applying indirect method of quantitative Enzyme-Linked Immunosorbent Assay (ELISA).

Preparation of turmeric and garlic powder

Fresh garlic bulbs (*Allium sativum*) and turmeric rhizomes (*Curcuma longa*) were obtained from local market. Then, they were washed, dried and milled (Fig.1). The powder was prepared according to the method as previously described by Ahmadi *et al.*, (2007).



Fig. 1. Preparation of garlic and turmeric powder

Experimental diets

Four isonitrogenous and isocaloric experimental diets were prepared, such as in diet-A: 1.5 mg of AF; in Diet-B 1.5 mg of AF and 1g of TP; in Diet-C 1.5 mg of AF and 1 g of GP contained per kg of feed and control diet had no AF, TP and GP (Table 1).

Table 1. Ingredients and calculated composition of diets

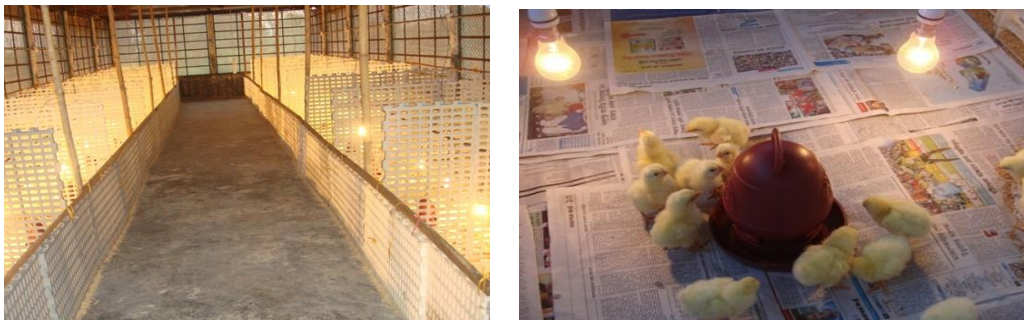
Ingredients	Diet A	Diet B	Diet C	Control Diet
Maize/ Corn (kg/100kg)	53	52.5	50	50
Rice polish (kg/100kg)	14	14	13	11
Soybean meal (kg/100kg)	23	23.5	27	29
Protein concentrates (kg/100kg)	8	8	8	8
Aflatoxins (mg/kg)	1.5	1.5	1.5	0
Turmeric powder (TP, g/kg)	0	1.0	0	0
Garlic powder (GP, g/kg)	0	0	1.0	0
Others (2% of the total)*	2	2	2	2
ME (kcal/kg)	2898	2893	2882	2881
CP (%)	22.12	22.14	22.07	22.04

*Calcium carbonate, Dicalcium phosphate, Vitamin.-mineral premix, Salt, Lysine and Methionine

Experimental birds and design

A total of 100 day old Cobb 500 broiler chicks were purchased from a commercial hatchery, weighted, wing banded and reared with proper brooding up to 14 days. Afterwards, randomly 84 chicks were equally divided into four dietary groups with 3 replications and assigned to 12 pens, where each pen having 7 chicks. Respective feed was offered to the broilers according to the strain standard for the duration (35 days) of experiment (Fig. 2). All experimental broilers were reared following same management practices. On 36 days of age, all experimental broiler chickens were slaughtered for

examining the necroscopic changes in internal organs. Liver and muscle tissues were also collected to estimate AF residues. Weekly growth rate and FCR were monitored during the experimental period.



Experimental house

Brooding

Fig. 2. Experimental house and brooding of birds

Determination of aflatoxin residues

Detection of AF residues was performed according to Tavcar-Kalcher *et al.*, (2007). Briefly, 1g ground sample was mixed thoroughly with an aqueous solution of citric acid and diatomaceous earth. The mixture was extracted with dichloromethane. The filtered extract was dried, filtered again, and an aliquot was evaporated near to dryness. The residue was dissolved in methanol and mixed with buffer and applied into an immune affinity column. Aflatoxin was eluted from the column and its concentration of AF in the final solution was determined by an HPLC method with fluorescence detection.

Results and Discussion

The results revealed that there were significantly different ($P < 0.01$) in body weight gain and FCR among the dietary treatment. Body weight gain and FCR use showed highest in diet-B, followed by diet-C as well as control diet and the lowest in diet-A. But there was no significant ($p > 0.05$) difference in feed intake among the dietary treatments (Table 2). The findings of the current research indicated positive effects of TP and GP on body weight gain and FCR in broiler chicken. Evidently, a usage of TP and GP as a feed additive in the diet of broiler chicken was successful to minimize the loss of weight gain and FCR caused by exogenous source of AF.

Table 2. AF deactivating effect of TP and GP on feed intake, body weight and FCR in broiler

Parameters	Diet-A	Diet-B	Diet-C	Control Diet	P-value
Feed intake (g)	4333±189	4138±88	4019±86	4095±170	NS
Body weight (g)	1705±11.31 ^c	2066±14.5 ^a	1907±20.29 ^b	1711±21.98 ^c	*
FCR	2.54±0.12 ^a	2.0±0.04 ^b	2.11±0.5 ^c	2.4±0.12 ^c	*

Mean ± SEM; *Significant at $P < 0.01$; ^{NS} Not-significant

Relative liver and kidney weight showed significantly ($P < 0.01$) the highest in diet-A followed by diet-B, diet-C, respectively and the control diet but relative spleen weight was not significantly difference ($P > 0.05$) among the diets (Table 3). A gross observation of internal organs of broiler chicken was showed in Fig. 3. Control group of broilers did not found any changes (Fig. 3A). Same lesions were found in liver of broilers (Fig. 3B). But, aflatoxin uncontaminated diet (control diet) fed group of broilers were showed no gross lesions in kidneys (Fig. 3C). The relative kidneys weights from the aflatoxin fed group of broilers were enlarged, pale or congested with a few petechial (Fig. 3D).

Table 3. Relative organ weight of broilers comparing different dietary treatments

Parameters	Diet-A	Diet-B	Diet-C	Control Diet	P-value
Liver wt. (g/100 g of BW)	2.56±0.065 ^b	2.23±0.024 ^a	2.21±0.023 ^a	2.09±0.056 ^c	*
Kidney wt. (g/100 g of BW)	1.13±0.025 ^b	0.85±0.016 ^a	0.93±0.016 ^a	0.74±0.031 ^c	*
Spleen wt. (g/100 g of BW)	0.183±0.002	0.181±0.003	0.184±0.002	0.180±0.003	NS

*Significant at $P < 0.01$



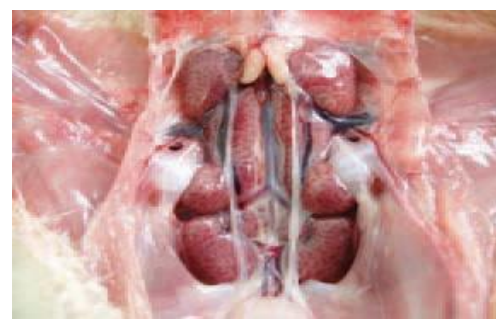
A



B



C



D

Fig. 3. Gross observations of internal organs of broiler chicken; A: Liver of control diet, B: Livers of AF fed diet, C: Kidney of control diet and D: Kidney of AF fed diet

These changes in liver and kidney in broilers receiving AF plus TP or AF plus GP were much less than broilers fed AF alone. There was no gross lesion on spleen and the weight of spleen was not significantly difference ($P>0.05$) among the diets. The residues of AF found in muscle and liver was also significantly ($P<0.05$) higher in broiler diet-A as compared to the chicken diet-B and diet-C, respectively but in the control diet group had no detectable concentration of AF residues in liver and muscles of broilers, where the detection limit of analytical method were $0.05 \mu\text{g}/\text{kg}$ and threshold permissible value were $> 2.0 \text{ ng}/\text{g}$ (Table 4)

Table 4. The Concentration of AFB1 in liver and muscle of broiler at 35 days old

Parameters	Diet-A	Diet-B	Diet-C	Control Diet	¹ P-value
AF residues in liver (ng/g)	3.06±0.25 ^a	2.03±0.29 ^b	2.13±0.25 ^c	ND	**
AF residues in muscle (ng/g)	1.99±0.207 ^a	1.03±0.292 ^b	1.46±0.169 ^c	ND	**

**Significant at $P < 0.05$; ND Not detected

Conclusion

It was confirmed that dietary aflatoxin has negative effects on the body gain, FCR, and organ function of broilers. The current study demonstrated the effects of dietary inclusion of turmeric and garlic powder as a feed additive in overcoming the detrimental effect of AF in broiler chicken. Therefore, it may be concluded that turmeric powder and also garlic powder can decrease adverse effect of aflatoxin on liver and kidney and can be used as a supportive treatment against aflatoxicosis in broiler rearing. Practical application of this research is supplementation of turmeric powder and garlic powder in the diet of broiler to prevent or reduce the adverse effects of feeding AF contaminated diets.

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