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Study of performance broiler chickens fed of contaminated wheat by aflatoxin and ammoniac

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ABSTRACT

Aflatoxins are fungal metabolites by strains of *Aspergillus* (*A.parasiticus* and *A.flavus*) are produced. The clinical signs of aflatoxin poisoning include surgery autopsy, histological lesions and also create effects on the production of poultry flocks in experimental and natural occurrence have been reported in broiler chickens worldwide. The main signs of aflatoxin poisoning, loss of appetite, delayed growth, decreased body weight and food intake are reduced. Aim of this article is Study of performance broiler chickens fed of contaminated wheat by aflatoxin and neutralized by ammonia method. Thus, 280 male broiler chickens of Ross breed were randomly divided into 16 cages and feeding of recommended NRC and All of the experimental diets were same, except of wheat that was considered as fallow: Diet 1(control diet): contains Iranian healthy wheat (no aflatoxin), diet 2: contains ammoniated wheat, diet 3: contains wheat contaminated with aflatoxin (1 ppm) and diet 4: contains ammoniated wheat and contaminated with aflatoxin. According to the results increase in body weight in chickens fed the control diet (without aflatoxin) with ammoniated wheat and contaminated with aflatoxin (diet 4) was not significantly different ($p<0.05$) but body weight on diets contaminated with aflatoxin (diet 3) was significantly reduced ($p<0.05$). By the results, feed intake in broilers fed diets 3 (1 ppm aflatoxin) is lower than other groups ($p<0.05$).While, not significant differences between diets 2, diets 4 and control diet were observed ($p<0.05$). Lower feed intake and growth rate due to decreased activity of important enzymes in the digestion of carbohydrates, lipids, proteins and nucleic acids, and impaired and defects in some of the nutrient. Also The results obtained in this study were showed that aflatoxin in broiler diets due to increase relative kidney weight compared with chicks fed the diet has no aflatoxin ($p<0.05$). Ammoniate of wheat due to reduction and neutralization aflatoxin in wheat and ammoniated wheat that contaminate with aflatoxin has no effect on the relative weight of kidney ($p<0.05$).

Keywords: Aflatoxin, Wheat, Broiler chicken, Ammoniac

INTRODUCTION

Aflatoxins are toxic secondary metabolites produced by two species of the genus *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin due to spread it on some food grains, especially wheat. 50 to 60 percent of poultry diets to make have made the biggest concerns associated with mycotoxins (Stanley,

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V. *et al.*, 1993). Therefore, exposure through food should be kept as low as possible. Maximum levels of aflatoxins (aflatoxins B1, B2, G1, G2 and M1) in food stuffs are laid down in Commission Regulation (EC) No 1881/20064 as amended by Commission Regulation (EU) No 165/20105 and Commission Regulation (EU) No 1058/20126. Maximum levels in legislation are specified for aflatoxin B1, aflatoxin M1, and for the sum of aflatoxins B1, B2, G1 and G2.

Data providers can report aflatoxin levels to EFSA in different manners (i.e. as "Aflatoxins", as "Aflatoxin (sum of B1, B2, G1, and G2)" or as levels of individual aflatoxins B1, B2, G1, G2, etc...). Clinical signs of aflatoxin poisoning include surgery autopsy, histological lesions and also create effects on the production of poultry flocks in experimental and natural occurrence have been reported in broiler chickens worldwide. The main signs of aflatoxin poisoning, loss of appetite, delayed growth, decreased body weight and food intake are reduced (Bakshi, CS. *et al.*, 1998). Aflatoxins are fungal metabolites by strains of *Aspergillus* (*A.parasiticus* and *A.flavus*) are produced. The mycotoxins that may pre-harvest food between harvest and drying in warehouse or manufacturing process of the favorable conditions of temperature and humidity are generated, weakened immune system, liver toxicity, mutation, carcinogenesis, malformations, and bleeding (Baily, R.B. *et al.*, 1998). Aflatoxins are known from a variety of aflatoxins (B1, B2, G1, G2), which inhibits synthesis of DNA, RNA and protein synthesis and decreases susceptibility to microbial and environmental stress (Stanley, V. *et al.*, 1993). The main target of aflatoxin is liver and in the histopathologic experiment, changes of fatty acids in hepatocytes, fibrous tissue in the portal vein area and excessive proliferation of bile duct cell can be observed in some animal species (Obido, O. 1986). Remove aflatoxins from contaminated food as a fundamental problem in livestock and poultry feed and removal methods based on a decomposition of contamination, damage, disable or remove aflatoxins or remove aflatoxins through biological, chemical or physical concentrates (Brekke, O.L. *et al.*, 1977) and (Leeson, S. *et al.*, 1995). Today, cost-effective and non-toxic for foods contaminated by aflatoxins aren't available, thus the use of aflatoxin-contaminated food as a problem with considers able economic losses caused by the remaining (Ledoux, D.R. *et al.*, 1999). Aim of this article is Study of performance broiler chickens fed of contaminated wheat by aflatoxin and neutralized by ammonia method.

MATERIALS AND METHODS

280 male broiler chickens of Ross breed were randomly divided into 16 cages and feeding of recommended NRC (1994) (Table 1).

All of the experimental diets were same, except of wheat that was considered as fallow:

A- Diet 1(control diet): contains Iranian healthy wheat (no aflatoxin).
B- Diet 2: contains ammoniated wheat
C- Diet 3: contains wheat contaminated with aflatoxin (1 ppm).
D- Diet 4: contains ammoniated wheat and contaminated with aflatoxin.

Investigative chickens had free access to food and water. Used a NRRL-2999 *A.parasiticus* standard vial for producing aflatoxin and sabro dextrose agar was used for primary fungi culture. Thus, for producing of more culture medium of fungi, 1 liter flask was used. Each flask contains 150 gram rice with 150 ml autoclave water, then added 6.5×10^6 fungal suspension per ml under sterile condition.

After 5 days in 28 C, 40 kg of healthy wheat mixed in two flasks to producing contaminated wheat and after 7 days incubated in room temperature, we measured aflatoxin of rice and wheat by TLC and HPLC method (Shotwell, O. L. *et al.*, 1966) and (Trucksess, M.W. *et al.*, 1983).

Table 1: materials of experimental diet in starter and grower period (0-21 and 21 42).

Diet components	Starter period %	Grower period %
Wheat	59.89	65
Soybean meal	31.7	29.53
Fish Meal	3	-
Soybean oil	1.81	1.88
Shellpowder	1.41	1.58
DiCalciumPhosphate	1.16	1.09
VitaminSupplements	0.25	0.025
Complete mineral	0.25	0.025
D-L -methionine	0.14	0.07
Amprulyum	0.05	0.05
Salt	0.38	0.31
Dietary energy	2950	3000
Crudeprotein(%)	21.22	18.75
Lysine (%)	1.16	0.96
Methionine(%)	0.5	0.36
(%) Methionine+cysteine	0.83	0.67
(%) ca	0.92	0.84
Available phosphorus (%)	0.41	0.32

Contaminated wheat contains AFB1 (7.80), AFB2 (4.90), AFG1 (7.90) and AFG2 (2.00). aflatoxin was isolated from rice powder for contaminated wheat. To ammoniated contaminated wheat, dry matter percent was reached to 18%. Ammoniated and neutralization reaction of contaminated wheat done with adding 1% ammoniac to neutralization tank with 200 kg capacity and dried after 48 hours (Bakshi, CS. *et al.*, 1998). At the end of the weeks 3 and weeks 6 taken 3 chickens from each cage with average weight, then weight of liver and kidney (gram/100 gram B.W.) was recorded. Liver tissue samples in 10% neutral buffered formalin were fixed and then select pieces of tissue that were fixed and cut out and passing tissue preparation. So the preparation of paraffin sections 5 microns in diameter were prepared at the end were stained with Hematoxylin and Eosin for histopathological examination. Feed intake and weight gain were recorded weekly. For data analysis SAS statistical software method and linear model analysis was performed.

RESULTS AND DISCUSION

Effects of feeding diets on weight gain, feed intake and feed conversion at 3 and 6 weeks with the effects of ammoniated on their aflatoxin was showed on table (2). Based on these results, increase in body weight in chickens fed the control diet (without aflatoxin) with ammoniated wheat and contaminated with aflatoxin (diet 4) was not significantly different ($p<0.05$) but body weight on diets contaminated with aflatoxin (diet 3) was significantly reduced ($p<0.05$). By the results, feed intake in broilers fed diets 3 (1 ppm aflatoxin) is lower than other groups ($p<0.05$).While, not significant differences between diets 2, diets 4 and control diet were observed ($p<0.05$). Lower feed intake and growth rate due to decreased activity of important enzymes in the digestion of carbohydrates, lipids, proteins and nucleic acids, and impaired and defects in some of the nutrient. The reduction in protein synthesis affected by aflatoxin may due to disruption of transcription mRNA and transport amino acid that thus protein synthesis and DNA were prevented (Thaxton, J.P. *et al.*, 1974).

Since aflatoxin B1 in vivo inhibits the enzyme RNA polymerase and subsequent protein synthesis is impaired. As a result, reducing the synthesis of albumin and globulin and the immunoglobulin may be encountered in the body (Devegowda, G. *et al.*, 1994). Significant reduction in body weight and growth rate compared with the control group could be compensated with ammonia aflatoxin-contaminated wheat. Thus ammoniated due to reduce aflatoxin (Table 2) and weight gain is improved. Galvanva *et al.* stated, liquid or gaseous ammonia effectively reduces aflatoxin levels up to 99% (Galvano, F. *et al.*, 2001) and Vasan *et al.* to broiler diets containing aflatoxin B1 (0, 0.25, 0.5, 1 mg/kg) were fed for four weeks (Vasan, P. *et al.*, 1998). Loss and weight gain, feed intake and feed efficiency in groups fed diets containing aflatoxin B1 was dose dependent to aflatoxin. Burke et al (Brekke, O.L. *et al.*,1977) reported that liquid ammonia Effectively Acute toxicity of aflatoxin in naturally contaminated wheat had to be removed and no adverse effects when consumed in ducks (4 days), broiler chickens (3 weeks) and Salmon (4 months).

Table 2: The total amount of aflatoxins (ppb) in wheat diets used

Diet	Rate of aflatoxin in wheat	Ammoniated (w/w)	Last concentrations after ammoniated and mixed with diet
1	0	-	0
2	0	1%	0
3	1000	-	650 (80.7% AFB)
4	1000	1%	3

Considering that in the present study consumed aflatoxin-contaminated diet resulted in a significant reduction in food intake compared with chickens fed with aflatoxin-free diet and Ammoniated due to reduction of aflatoxins in aflatoxin-contaminated wheat (table 2), It can be said that due to the lowered intake in chickens suffering from anorexia is aflatoxicosis and This reduction results in defects and impaired liver metabolism due to liver damage caused by aflatoxin occurs. The subjects of this study correspond with the results obtained by histopathological examination. In the present study, feeding diets containing ammoniated wheat and ammoniated wheat that contaminate with aflatoxin in feed consumption is improved that indicate that removal of aflatoxin-contaminated wheat (table 2).

The liver is the target organ toxic effects of aflatoxins and Low levels of aflatoxin in the relative weight faster than other organs are affected (Jones,T.C. *et al.,* 1997). Aflatoxin can cause liver and kidney toxication and will change obligations and general appearance of them (Tung, H.T. *et al.,* 1972). In this study, the relative weight of liver and kidney increased significantly affected by aflatoxin ($p<0.05$) (table 3). Merkley *et al.* increased relative liver weight during the aflatoxicosis due to accumulation of neutral lipids, mainly triglycerides in the liver were compared and in this study, fatty liver syndrome in laying hens was observed (Merkley, J.W. *et al.,* 1987). Exposure to low doses of aflatoxin but in long-term may reduce growth rates and increase the size of the liver. Liver enlargement related to hypertrophy of smooth endoplasmic reticulum in hepatocytes and changes in fat is stored.

Table 3: effect of the diet ammoniated wheat that contaminate with aflatoxin on relative weight of the liver and kidney of broilers

t	Liver (gram/100 gram B.W.)		Kidney (gram/100 gram B.W.)	
	21 days	42 days	21 days	42 days
	5.9± 0.60	3.2± 0.22	1.5± 0.27	0.80± 0.02
	5.5± 0.67	3.45± 0.23	1.67± 0.27	0.94± 0.02
	8.6± 0.70	5.97± 0.26	2.8± 0.27	1.24± 0.03
	5.9± 0.72	3.28± 0.26	1.8± 0.24	0.83± 0.28

Neutralization of wheat contaminated with aflatoxin due to improve relative liver and kidney weights and this represents the elimination of the toxic effects of aflatoxin in broilers fed diets containing ammoniated wheat and contaminated with aflatoxin (table 2 and 3).

The results obtained in this study were showed that Aflatoxin in broiler diets due to increase relative kidney weight compared with chicks fed the diet has no aflatoxin ($p<0.05$). Ammoniate of wheat due to reduction and neutralization Aflatoxin in wheat and ammoniated wheat that contaminate with aflatoxin has no effect on the relative weight of kidney ($p<0.05$).The results findings in this paper corresponded with of Fernandez et al (1993).

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