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Protective effects of *Aloe vera* powder supplementation on some quantitative and qualitative characteristics of egg, histopathological changes and serum biochemistry of laying hens fed by Aflatoxin B1

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Abstract

In the recent years, the use of medicinal plants to reduce the effects of mycotoxins in foods and feeds has been considered. This study was conducted to investigate the effects of *Aloe vera* on performance, serum biochemical parameters and liver histopathology in laying hens fed on aflatoxin B1 (AFB1)-contaminated diet. Seventy-two White Leghorns (Hy-Line W-36) were randomly allocated to four treatments. 1) basal diet (control), 2) control plus 1.00 mg kg^{-1} AFB1, 3) control diet plus 1.00 mg kg^{-1} AFB1 + 100 ppm *Aloe vera* powder, and 4) control diet plus 1.00 mg kg^{-1} AFB1 + 300 ppm *Aloe vera* powder. Each treatment consisted of three replicates of 6 birds. Egg weight and Haugh units were not affected by AFB1. Egg production and eggshell thickness were lower for groups fed 1.00 mg kg^{-1} AFB1. Egg production, egg weight and eggshell thickness were improved by incorporation of *Aleo vera* in the AFB1 contaminated feed but were not significant. Chickens fed AFB1 had significantly lower aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid and higher cholesterol than other groups. *Aloe vera* powder improved levels of cholesterol, uric acid, AST, and ALT. AFB1 also caused histopathological changes in liver tissues, such as vacuolar degeneration, fatty infiltration, and necrosis. The addition of *Aloe vera* powder to the aflatoxin containing diet reduced the severity of lesions in liver. The data demonstrated the ability of *Aloe vera* to reduce the adverse effects of AFB1 exposure in laying hens.

Introduction

Aflatoxins (AFs) are mycotoxins produced by two species of Aspergillus (*Aspergillus flavus* and *Aspergillus parasiticus*) and can occur in foods and feedstuffs as a result of fungal contamination before and after harvest.[1] Many of the feed ingredients found in poultry rations can be contaminated by harmful mycotoxins that are ingested by birds and have a some of serious consequences such as: Reduced feed efficiency, decreased egg production and immune suppression.[2,3] In addition, residues of AFs can appear in edible poultry products for human consumption (e.g. meat and egg) which raise public health concerns due to their genotoxic and carcinogenic properties.[4,5]

Several types of aflatoxins (14 or more) occur in nature, however, four aflatoxins including B1, B2, G1 and G2 are particularly dangerous to humans and animals. Aflatoxin B1 (AFB1) is the most toxigenic aflatoxin (AF) subgroup.[2] The Food and Drug Administration (FDA) in the United States has established guidelines for the maximum toxin level that can be safely fed to the poultry from 20.00 ppb for chicks to 100 ppb in feed for mature chickens.[6] Thus, development of validated strategies for controlling AFs in poultry feed is very critical. There are several methods used to reduce mycotoxin concentration in poultry feeds. Detoxification of contaminated feed can be done by physical, chemical and biological treatments. Drying cereals before storage will reduce the high humidity content of the feed and is effective in reducing of moderate mycotoxins contamination.[7] Using chemical methods for detoxification is often expensive and inefficient. Also, utilization of some chemical methods is prohibited in some countries.[8] The most common way for protecting animals against mycotoxicosis is to use mineral

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adsorbents including bentonites, zeolites and alumino-silicates in the feed. On the other hand, it has been reported that many microorganisms including bacteria, yeast and molds can able to remove or reduce mycotoxins in food and feed.[9] Recently, the biological inhibitions of different natural substances such as medicinal plants have been investigated on fungal activities.[10] *Aloe vera* L. (*Aloe barbadensis* Miller) is a plant that belongs to the Liliaceae family, found in tropical and sub-tropical climates. *Aloe Vera* is one of the most important medicinal plants containing various components.[11] Many studies revealed the antibacterial, antiseptic, anti-inflammatory and immune-modulator effect of *Aloe vera*.[12,13] There were some studies showed that *Aloe vera* had antifungal effects in in-vitro conditions.[14,15] The objective of this study was to evaluate the efficacy of *Aloe vera* powder as dietary supplements for protection against toxicity of AFB1 in laying hens.

Materials and Methods

Animals, diets and experimental design. Seventy-two White Leghorns (Hy-Line W-36) in their first production cycle (42 weeks age) were purchased from a local layer farm. Birds were acclimatized for two weeks and then randomly divided into four groups (treatments) of which each experimental group consisted of 3 replications including 6 birds. No vaccination or drug administration was done in the experimental period (4 weeks). A standard diet was formulated to meet nutrient requirements of Hy-Line W-36 international recommendation.[16] Layer feed sample was tested for mycotoxin levels before feeding to birds. The hens were placed in cages in a room maintained at temperature of 20.00 ± 2.00 ˚C and a constant light-dark cycle (16L : 8D) was used. Egg production was calculated daily. Each experimental group of layer hens was fed a different diet. Group 1, the negative control group, received a basal diet (Table 1). Group 2, the positive control group, received a diet supplemented with AFB1 (1.00 mg kg^{-1} feed). Group 3 and 4 were administered with the same level of AFB1, also, 100 and 300 ppm *Aloe vera* powder mixed in their diet, respectively. Pure crystalline AFB1 (Faroogh life sciences research laboratory, Tehran, Iran) was added to the diets. First, AFB1 was dissolved in chloroform (1.00 mg 10.00 mL^{-1}) and then it was mixed with a suitable amount of feed. After solvent evaporation the premix was added into the basal diet to provide the desired level of AFB1 kg^{-1} of diet.[17] The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Iran (ir.ausmt.rec.1399.04.09).

Egg parameters. For egg assessment, a sample of two eggs per replicate was collected during the last three days of experimental period. The following parameters were determined: Egg weight, albumen height, Haugh units (HU), and shell thickness. A micrometer was used for albumen height measurement, then Haugh units was calculated employing the equation: HU = 100 log (H–1.70 W 0.37 + 7.60), where H = albumen height (mm), W = egg weight (g), 7.60 = correction factor for albumen height, and 1.70 = correction factor for egg weight.[18] Eggshell thickness was measured using a micrometer.

Table 1. Ingredient and chemical composition of the basal diet.[16]

Ingredient	Amount (g kg^{-1})
Corn	500
Wheat	181
Soybean meal (440 g kg $protein^{-1}$)	190
CaCo3	94.00
Oil	10.00
Di-calcium phosphate	15.00
Vitamin premix[1]	2.50
Mineral premix[2]	2.50
Salt	3.00
DL-methionine	2.00
Analysis	
Metabolizable energy (kcal kg^{-1})	2,750
Crude protein (g kg^{-1})	153
Calcium (g kg^{-1})	39.00
Available phosphorous (g kg^{-1})	4.00
Methionine (g kg^{-1})	3.40
Lysine (g kg^{-1})	6.60
Methionine + Cysteine (g kg^{-1})	6.00

[1] Supplied per kg of diet: Vitamin A, 10,000 IU; vitamin D3, 9,790 IU; vitamin E, 121 IU; vitamin K2, 2.00 mg; vitamin B12, 0.02 mg; thiamin, 4.00 mg; riboflavin, 4.40 mg; niacin, 22.00 mg; pyridoxine, 4.00 mg; biotin, 0.03 mg; folic acid, 1.00 mg; Ca-pantothenate, 40.00 mg; choline chloride, 840 mg; ethoxyquin, 0.125 mg.

[2] Supplied per kg of diet: Zn, 65.00 mg; Mn, 75.00 mg; Cu, 6.00 mg; Se, 0.20 mg; I, 1.00 mg; Fe, 75.00 mg.

Serum biochemical parameters. At the end of experiment, 6 birds from each replicate were randomly selected and bled by wing vein with a 25G needle. Blood was centrifuged at 1,500 *g* for 10 min and serum was separated and stored at −20.00 ˚C for analyses. Cholesterol, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using an automatic analyzer (Elitech group, Puteaux, France) with commercial test kits (Pars-Azmoon Co., Tehran, Iran).

Histopathological examination. After taking blood samples, the birds were euthanized humanely and the livers were taken out and fixed in 10.00% buffered formalin.[19] Following fixation, the hepatic tissues were dehydrated by transferring through a series of alcohols with increasing concentrations, placed into xylol and embedded in paraffin. A microtome was used to make eight cuts that were 6.00 - 7.00 µm and they were stained with Hematoxylin and Eosin (H&E) for histopathological examination using optical microscopy (Olympus, Tokyo, Japan).

Statistical analysis. Data were subjected to statistical analysis using the one-way analysis of variance (ANOVA) according to SPSS Software (version 17.00; SPSS Inc., Chicago, USA). Duncan's multiple range test was used to separate means when the dietary treatment effect was significant ($p < 0.05$).

Results

Egg parameters. The effects of *Aloe vera* on egg parameters of hens are shown in Table 2. Egg production of birds fed the diet containing AFB1 (groups 2, 3, and 4) was lower compared to that of the control group ($p < 0.05$). As shown in Table 2, mean egg weight was not significantly different among treatments, however, the lowest weight was observed in positive control group (57.66 g) and negative control group had the highest egg weight (60.33 g). Eggshell thickness (Table 2) was lower in the eggs of birds fed AF compared to the control group ($p < 0.05$).

Serum biochemical parameters. The effects of AFB1 on blood chemistry of laying hens are shown in Table 3. Adding of AFB1 into the basal diet significantly ($p < 0.05$) decreased serum cholesterol (mg dL^{-1}) and uric acid (mg dL^{-1}) while a significant increase was found in AST (mg dL^{-1}) and ALT (mg dL^{-1}), compared to the control group.

Histopathological examination. Histopathological changes in the liver tissues of laying hens are presented in Figure 1 (A - F). In negative control group, liver showed normal hepatocytes and intact epithelium without any visible damage. No significant lesions were observed in liver tissue of control birds (Fig. 1A). The liver of birds fed diet containing AFB1 revealed severe lesions including: Hepatocellular degeneration and fatty vacuoles in hepatocytes, hepatocytes necrosis and slight congestion (Fig. 1B - 1D). The addition of *Aloe vera* powder to the aflatoxin containing diet reduced the severity of lesions. Mild to moderate congestion, vacuolar degeneration and partial necrosis were observed in liver of hens fed aflatoxin plus *Aloe vera* (Fig. 1E).

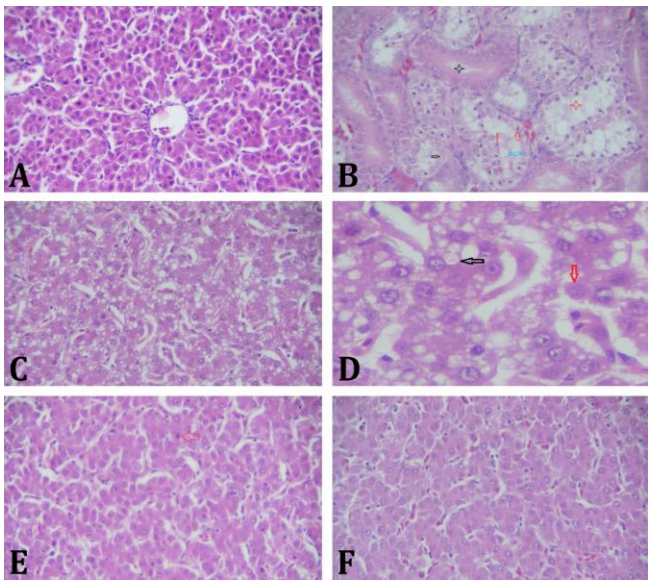

Fig. 1. Photomicrographs of liver sections from different treatments. **A)** Basal diet, **B)** Basal diet + AFB1. Nuclear and cytoplasmic changes in the stages of necrosis "pyknosis" (red arrow), "karyolysis" (blue arrow) and "nuclear disappearance" (black arrow); **C)** Basal diet + AFB1. Severe hepatocellular degeneration (fatty change) and mild hepatocytes necrosis; **D)** Basal diet + AFB1. Hepatocellular degeneration specified intercellular vacuoles "fatty change" (black arrow). Hepatocyte necrosis that is "karyolysis" stage (red arrow); **E)** Basal diet + AFB1 + *Aloe vera* (100 ppm), stages of necrosis "pyknosis" (red arrow); **F)** Basal diet + AFB1 + *Aloe vera* (300 ppm), (H&E, 40×).

Table 2. Effects of diets fed to laying hens on some egg parameters.

Parameter	Control	AFB1	AFB1 + ALO 100	AFB1 + ALO 300
Egg production (%)	85.97[a]	77.61[b]	80.17[b]	81.14[b]
Egg weight (g)	60.33	57.66	59.67	59.13
Eggshell thickness (µm)	390[a]	380[b]	380[b]	380[b]
Haugh units	87.41	86.23	87.48	86.53

AFB1 = diet supplemented with 1.00 mg kg^{-1} AFB1; AFB1 + ALO 100 = diet supplemented with 1.00 mg kg^{-1} AFB1 and 100 ppm *Aloe vera* powder; AFB1 + ALO 300 = diet supplemented with 1.00 mg kg^{-1} AFB1 and 300 ppm *Aloe vera* powder.
[ab] Means within the same row with different letters are significantly different ($p < 0.05$).

Table 3. Effects of AFB1 and *Aloe vera* on serum biochemical parameters of laying hen.

Treatment	Uric acid	Cholesterol	AST	ALT
Control	3.37[a]	171.16[a]	170.50[d]	30.66[c]
AFB1	1.97[c]	118.83[c]	278.66[a]	70.33[a]
AFB1 + ALO 100	2.75[b]	149.33[b]	237.16[b]	37.50[b]
AFB1 + ALO 300	3.66[a]	155.33[ab]	182.50[c]	28.83[c]
SEM	0.15	4.30	2.94	1.70
***p*-value**	< 0.0001	< 0.0001	< 0.0001	< 0.0001

AFB1 = diet supplemented with 1.00 mg kg^{-1} AFB1; AFB1 + ALO 100 = diet supplemented with 1.00 mg kg^{-1} AFB1 and 100 ppm *Aloe vera* powder; AFB1 + ALO 300 = diet supplemented with 1.00 mg kg^{-1} AFB1 and 300 ppm *Aloe vera* powder. AST = aspartate aminotransferase, and ALT = alanine aminotransferase.
[abcd] Means within the same column with different letters are significantly different ($p < 0.05$).

Discussion

In the present study we evaluated the potential effect of *Aloe vera* powder on egg characteristics, serological and histopathological parameters in layer hens fed AFB1-contaminated diets. Some researchers showed AFB1 could decrease egg production. Bintvihok *et al*. reported decreased quail egg production with exposure to 50.00 µg AFB1 kg^{-1}.[20] In another research, an insignificant decrease in egg production for the laying hens fed AFB1 contaminated diets was observed.[21] Siloto *et al*. noted that birds fed AF presented the lowest percentage of lay.[22] On the other hand, Oliveira *et al*. reported that egg production was not affected by using AF in dietary treatments.[23] Some reports indicated that high levels of AF (higher than 600 µg kg^{-1}) could reduce egg production in layers.[24] In our study, a high level of AFB1 (1.00 mg kg^{-1}) was added in basal diets of treated groups, and this might have reduced egg production. Aflatoxin cause liver damage and influence on fat metabolism in the liver, thus, aflatoxicosis can lead to reduced egg production. Furthermore, some studies described that daily feed intake reduction was observed in poultry that consumed diets containing AFB1. This may be an important factor to reduce egg production.[23,24] Egg production was improved by incorporation of *Aleo vera* in the feed but not significantly. It was reported that *Aloe vera* had protective effect on experimental aflatoxicosis in the rat models. This could be due to its anti-inflammatory and anti-oxidative properties.[25] Also, it was shown that *Aloe vera* powder could act as a biosorbent and could be used as an alternative for removal of AFB1.[26] Utilization of *Aloe vera* in AFB1 contaminated diets slightly increased egg weight. Similar results were reported in previous studies.[22,27] In contrast, significant reduction in egg weight with AFB1 in layers was recorded in some studies.[28,29] Some reports show aflatoxicosis can cause poor availability of calcium and phosphorus and interferes with absorption of vitamin D3.[30,31] Contrary to this, Chowdhury and Smith reported a lack of effect of Fusarium on shell thickness in 45 week-old layers.[32] It was shown that feeding 2.00 mg kg^{-1} of ochratoxin A had no effect on the shell thickness in 47 week-old layers.[33] Difference was found in the results of the experiments on the effect of dietary AFB1 on eggshell thickness of layers that could be due to factors such as: Age, type of toxin, dose and the period of exposure. In the present study, application of *Aloe vera* powder in the diet improved eggshell thickness, however, this effect was not significant. Result of this study indicated no significant differences among the various treatments on the Haugh units of eggs (Table 2). Lack of significant effect of dietary aflatoxin on HU of eggs has been previously reported.[27,33] The decreased serum cholesterol and uric acid values and increased AST and ALT activity observed in the present study were due to the hepatotoxic effects of AFB1 characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism.[34] Following absorption, aflatoxins are mainly metabolized and accumulated in the liver. A similar increase in AST and ALT related to hepatic injury in birds during aflatoxicosis has been reported.[17,35,36] In the present study, serum uric acid concentration was decreased in AF-contaminated diet. This finding was in agreement with the results of Harvey *et al*., Ledoux *et al*. and Saki *et al*.[37-39] Some other studies reported that uric acid was increased by aflatoxicosis.[36,40,41] Aflatoxin is an oxidant and uric acid is an antioxidant substance,[42] therefore, it seems that a portion of uric acid was used for modulating free radicals which are caused by aflatoxin.

As seen in Table 3, the addition of *Aloe vera* powder to AFB1 containing diet significantly ameliorated the adverse effects of AFB1 on some biochemical parameters. Adding 300 ppm *Aloe vera* powder to the contaminated diet improved and restored the elevated activity of cholesterol, uric acid, AST, and ALT and this treatment had no significant difference with control treatment ($p > 0.05$). It is well established that the liver is the target organ for AFB1 and most aflatoxins are bioactivated here to the reactive form which is known to bind DNA and proteins, and damaging the liver structures.[43] Similar to our findings, degenerative damages and necrosis in the liver following aflatoxicosis has been reported in previous studies.[34,36,44] According to the results of the present study, *Aloe vera* inclusion in the diet had a protective effect against aflatoxin exposure. It seems that application of *Aloe vera* powder in the diet reduced the adverse effects of AFB1 exposure. Lower severity of lesions in chickens receiving aflatoxin plus *Aloe vera* powder could be related to antioxidant property of *Aloe vera*. Cui *et al*. described strong capacity of antioxidation and radical scavenging of *Aloe vera* polysaccharides which reduced the AFB1-induced oxidative stress damage.[25]

In conclusion, our study showed that AFB1 in the laying hen diet at levels of 1 mg/kg resulted in impaired productivity parameters, an alteration of the serum biochemical and significant histopathology changes in liver. The addition of *Aloe vera* powder can ameliorate some toxic effects of AFB1. This work showed that *Aloe vera* could be a good additive for birds underexposure to AFB1 in poultry farms.

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Conflict of interest

There was not any conflict of interest in this paper.

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