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Research Paper:

Evaluation of the Effect of *Aloe vera* Extract on Aflatoxin B1 in Chicken Breast Muscle

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ABSTRACT

Background & Aims of the Study: Among aflatoxins, aflatoxin B1 (AFB1) is more toxic and dangerous. The presence of AFB1 in poultry feed is one of the most critical health and economic problems in the poultry industry. The critical concern caused by AFB1 contamination in the poultry industry is the transfer of toxins from the poultry feed to its products, such as meat, eggs, and edible parts. Thus, developing scientific strategies for controlling aflatoxin in poultry nutrition and protecting public health is very important. The present study aimed to evaluate the efficacy of *Aloe vera* extract in reducing the harmful effects of AFB1.

Materials & Methods: In this study, broilers were selected and divided into three groups. Group 1 is the negative control group, received a basal diet. Group 2 is the positive control group, received a diet supplemented with AFB1. Group 3 was administered AFB1 and 100 ppm *Aloe vera* powder mixed in their diet. At the end of the experiment (Day 28), the birds were humanely euthanized, and their breast meat samples were collected. The toxin residue in muscles was measured by a commercial AFB1 detection kit.

Results: No toxin was detected in the negative control group. In the third group, the residual amount of toxin was decreased by treating *Aloe vera*, but this difference was not significant. Significant differences are likely to be observed at higher doses.

Conclusion: Supplementation of the diet containing AFB1 with *Aloe vera* extract effectively reduced the adverse effects of AFB1 and could be a helpful solution for the aflatoxicosis problem.

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1. Introduction

Food safety is one of the most critical issues in the world. One of the food contaminants is mycotoxins, including aflatoxins. Of various types of mycotoxins, Aflatoxins (AFs) are highly toxic and cause food contamination worldwide. Aflatoxin B1 is the most toxic member of aflatoxins. Poultry feed contamination with AFs is a significant problem in poultry production since aflatoxicosis in chickens results in significant economic losses because of low feed intake, diminished feed conversion, weight loss, reduced egg production, and hatchability, increased susceptibility to various infections, and increased mortality [1, 2].

Moreover, AF residues can transfer to edible poultry products for human consumption (e.g., meat and egg), which raises public health concerns due to their mutagenicity, tumorigenicity, and carcinogenicity [3, 4]. Various methods are used to prevent and inhibit this toxicity in the world. However, due to the resistance of this toxin to chemical and physical methods, no definite method has been found to detoxify AFB1 so far. Besides, the available methods are costly and reduce the nutritional value of the feed [5]. Recently, the fungal inhibition of different natural substances, such as medicinal plants, has been investigated. The use of plant extracts to reduce or inhibit AFB1 toxicity is a safer method than chemical ones. The beneficial and practical effects of *Aloe vera* have been proven in medicine, cosmetics, and the food industry.

Aloe vera L. (*Aloe barbadensis* Mill) is a perennial plant belonging to the green Liliaceae family and has a gel with high viscosity. *Aloe vera* contains various compounds, including phenolic compounds, saponins, and enteroquinones [6]. Due to the nutritional and therapeutic properties of this plant, it is widely used, cultivated, and sold in the market [7]. Its compounds have antibacterial, antiviral, and antifungal activities [8, 9]. The target organ of AFB1 is the liver. One of the major human food resources is the chicken muscle, but liver tissue is less consumed, so it is necessary to study the effect of active compounds in reducing the accumulation of toxins in muscle. This study aimed to evaluate the inhibitory effect of *Aloe vera* on the AFB1 toxin in the muscles of chicken.

2. Materials and Methods

Experimental design

Thirty 1-day-old male chickens (Ross 308) were purchased from a local hatchery. The Mean±SD weight of chickens at the beginning of the experiment was 42 g [2]. The experimental diets were formulated according to the Ross 308 strain catalog [10]. The initial house temperature was set at 32°C and gradually decreased to 24°C on the 28th day. Average relative humidity was kept at 60% during the experimental period. A lighting schedule of 24 h incandescent illumination with approximately 20 lx was used for the entire period. The chicks were allowed to have free access to water and food along with the trial. The basal diet was tested for the presence of significant mycotoxins, including AFB1 and ochratoxin A, with ELISA. No mycotoxins were recorded. No vaccination or drug administration was done in the experimental period (4 weeks). The chicks were randomly assigned to three treatment groups: the first group was the negative control group fed by basal diet, the second group (positive control) received basal diet mixed by AFB1, and the third group received previous diet plus 100 ppm of *Aloe vera* extract (Barij essence, Tehran, Iran) [11]. In groups 2 and 3, pure crystalline AFB1 (Faroogh Life Sciences Research Laboratory, Tehran, Iran) was added to the diets. Firstly, AFB1 was dissolved in chloroform (1 mg/10 mL), and then it was mixed with a suitable amount of feed. For solvent evaporation, the prepared premix feed was left overnight at room temperature and then mixed with the basal diet to provide the intended level of AFB1/kg of diet (1 mg of AFB1/kg of feed) [12].

At the end of the experimental period (day 28), the chickens were beheaded following ethical principles, and their breast muscles were collected. The samples were stored in the refrigerator at -20°C until the day of the test. The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Amol City, Iran. The Ethics Committee Code was IR.AUSMT.REC.1399.05.12.

Measurement of ABF1 toxin in breast muscle by ELISA kit method

After weighing, we poured 5 g of the homogenized sample into a suitable container and added 25 mL of 70% methanol, then mix it with a shaker for 10 minutes. In the next step, 1 mL of the obtained solution was diluted with 1 mL of distilled water. Then, 50 µL was poured into special wells. The procedure was continued according to the kit instructions (RIDASCREEN® Aflatoxin B1, R-Biopharm, Germany). Analytical quality was determined by calculating LOD (limit of detection) and LOQ (limit of quantity) by R-Biopharm Company, which is the manufacturer of the kit.

Statistical analysis

Statistical analyzes and Mean±SD of the data was calculated in SPSS version 20.0 (SPSS Inc., Chicago, IL). The 1-way analysis of variance was used for the statistical evaluation. Bar and column diagrams were drawn by Prism.

3. Results

The effects of dietary treatment on toxin residues in the breast muscle are shown in Figure 1. The used ELISA kit could detect toxin levels below 0.01 mg/kg feed. The toxin was not isolated in the negative control group (NC). The Mean±SD concentration of toxin in the Positive Control group (PC) was 0.047 µg/kg, (0.01) and the Mean±SD concentration of toxin in the treated group (TA) was 0.045 µg/kg (0.01). Results showed that *Aloe vera* supplementation could decrease the amount of AFB1 in chicken muscle, but this decrease was not significant (P>0.05).

4. Discussion

Consumption of chicken meat and eggs is prevalent due to its availability. Natural cooking processes do not destroy the contaminated chicken meat and eggs with mycotoxins. In addition to health threats to consumers, the toxin in chickens leads to weight loss, growth and reduced egg production, and economic losses. Therefore, necessary measures should be taken to monitor and control various toxins, including AFB1 in poultry feed. AFB1 is a major health threat for humans because it is a carcinogenic substance and causes liver cancer [2]. Since the carcinogenicity of AFB1 has been approved by the International Association of Cancer Research (IACR) [13], effective and efficient measures to control this toxin in poultry feed are necessary for the poultry industry. Furthermore, high levels of AFB1 in poultry feed samples led to high toxin levels in liver and muscle samples. Previous studies were reported residues of AFB1 in the liver and muscle of poultry [14, 15].

Aloe vera has a protective effect against liver toxicity by AFB1. The protection could be due to its antioxidant and anti-inflammatory properties, as well as the ability of the *Aloe vera* to regulate the production and excretion of this toxin [16]. Therefore, the present study was designed to evaluate the efficacy of *Aloe vera* extract on the reduction of AFB1 residues in broiler muscle. This study showed that *Aloe vera* at a level of 100 ppm has no significant effects on the levels of AFB1 in the breast meat of broilers (Figure 1). The higher doses of *Aloe vera* are likely to reduce toxin accumulation in muscle. According to previous studies, the toxin concentration in muscle is usually lower than its concentration in the liver. Denli et al. reported the detection of 0.166 µg/kg AFB1 in the liver tissue of chickens after adding 1000 µg/kg of toxin [12]. Bintvihok et al. [17] studied aflatoxin B1 residues in poultry muscle, liver, and eggs at a dose of 3 ppm for 7 days. Their results showed that the residual amount of AFB1 in the liver is 10 times more than muscle.

Furthermore, Herzallah et al. [18] conducted a study to examine the levels of aflatoxin residues in eggs, muscles, and liver of laying hens over 6 weeks. Their results showed the highest and lowest levels of toxins in the liver (2.12 ppb) and muscle (0.6 ppb), respectively. In another study, broiler chickens were fed contaminated feed at a dose of 8µg/kg for 7 days. Their results showed that AFB1 was not observed in broiler muscle [19]. In the present study, the amount of toxin accumulation in muscle following administration of 1 mg/kg of toxin during 28 days of exposure was negligible (mean=0.047 µg/kg). The differences in the results of the experiments on the effect of dietary AFB1 on the eggshell thickness of layers could be due to factors such as type of birds, age, type of toxin, dose, and the period of exposure.

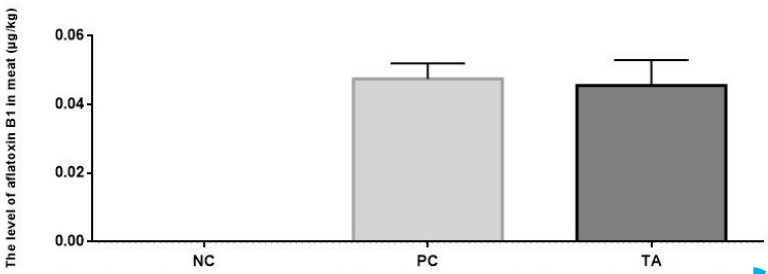

Figure 1. Comparing mean levels of aflatoxin B1 in broiler muscle

NC: Negative Control; PC: Positive Control; TA: Treated group (100 ppm *Aloe vera*).

The P-value between PC and TA was more than 0.05.

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5. Conclusion

Considering that the main target organ and accumulation of AFB1 is the liver and kidney, it is likely that the effectiveness of this plant can be seen in the target tissue because the accumulation of this toxin in muscles is much less than that in the target tissue. Therefore, to study the effect of *Aloe vera* in muscle, it is necessary to increase both the dose of toxin and the dose of *Aloe vera*.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Amol University of Special Modern Technologies (Code: IR.AUSMT.REC.1398.04).

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Authors' contributions

All authors equally contributed in preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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