Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B₁ in growing broilers
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ABSTRACT
Aflatoxins are a major problem in poultry production and are significant economic and public health burdens worldwide. Three commercial mycotoxin binders (TXB) were used to determine their efficacy in preventing the toxic effects of Aflatoxin B₁ (AFB₁) on broiler productivity, biochemical and hematological parameters, and liver function. A total of 600 day-old broilers were randomly allotted to 12 treatments in a 3 × 4 factorial arrangement with 3 levels of AFB₁ (0, 2, and 4 µg/g) and 4 TXB (no toxin binder, 1.0 g/kg Mycosorb, 1.0 g/kg Formycin, and 20.0 g/kg Anzymit) with 5 replicates of 10 chicks each per treatment. Results indicated that AFB₁, in comparison with the control, caused a significant decrease in feed intake, weight gain, feed efficiency, and hematological values. Serum proteins (globulin, albumin, and total protein) and serum Ca and P concentrations followed the same pattern. Relative weights of the heart, gizzard and liver, and AST activity in serum were increased in a dose-dependent manner, but ALT activity was not statistically affected by AFB₁. Adding TXB to the AFB₁ contaminated diet increased weight gain, feed efficiency, hematological values and serum proteins. The commercial mycotoxin binders decreased AST and ALT activities, but did not have a significant effect on the relative organs weight and serum Ca and P concentrations. Neither AFB₁ nor commercial toxin binders affected total cholesterol, LDL, or HDL in this study. It was concluded that the addition of the commercial toxin binders to the AFB₁ containing diets reduced the adverse effects of AFB₁, and could be helpful as a solution to the aflatoxicosis problem in young broiler chicks.

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Introduction
Globally, food and feeds have been seriously contaminated with mycotoxins among which aflatoxin, zearalenone, fumonisin, deoxynivalenol, and ochratoxin are the most commonly found (Qu et al. 2017). Furthermore, food and feeds are frequently co-contaminated with two or more mycotoxins, and their interactions may exert additive or synergistic effects (Resanovic and Sinovec 2006). The consumption of mycotoxin-contaminated food and feeds leads to reduced nutrient absorption (Verma et al. 2004), poor performance, immunosuppression (Richard 2007), residues in animal products (Qu et al. 2017), and increased susceptibility to infectious and parasitic diseases (Ma et al. 2015), and cause serious reproductive problems leading to economic losses in the poultry industry (Wade et al. 2017), resulting in a massive economic impact worldwide on human health, animal health, and agricultural trade. Mycotoxins have various acute and chronic effects on humans and animals (especially monogastrics) depending on species and susceptibility of an animal within a species. The health hazards of mycotoxins to humans or animals have been reviewed extensively in recent years (Qu et al. 2017; Barati et al. 2018).

Aflatoxin B₁ (AFB₁) is the most prevalent toxin in corn used in broiler feeds (Whitlow and Hagler 2005). The mutagenic, carcinogenic, and toxic effects of AFB₁ are well known, and the liver is the main target organ of AFB₁ (Ma et al. 2015). Metabolic changes associated with liver damage are decreased activity of digestive enzymes, and immune suppression. More recently, changes in gene expression of liver enzymes, and changes in intestinal morphology and function have also been reported (Ma et al. 2015; Qu et al. 2017).

Extensive research has been conducted to counter mycotoxicosis by nutritional, chemical, physical, or biological strategies (Murugesan et al. 2015; Rotimi et al. 2017; Barati et al. 2018). The best known method for mycotoxin deactivation is ‘binding’ with the use of binding agents, which are referred to as mycotoxin binders, adsorbents, or ‘enterosorbents’. They can be of organic (microbial) or inorganic (mainly clay minerals) nature. The inclusion of binding agents or enterosorbents in the diet has been given considerable attention as a strategy to reduce foodborne exposures to mycotoxins (Ye et al. 2009). There is wide interest in the use of biological products to decrease mycotoxin availability. Specifically, feed additives known as mycotoxin adsorbents or binding agents, are the most common approach to prevent and treat mycotoxicosis in birds (Siloto et al. 2013). The use of binding agents, which can adsorb the mycotoxin molecule and prevent their absorption by the gut, has gained considerable attention in recent years. The major advantages of adsorbents include cost,
safety and easy administration through addition to broiler feeds (Wan et al. 2013). A new approach to detoxify AFB₁ is to use an organic adsorbent in the diet of broilers, and one important organic adsorptive is Mycosorb. Mycosorb is a new product which acts by binding pathogens and mycotoxins without affecting gut bacteria (Wade et al. 2017). It was also reported that aluminosilicates (e.g. zeolite, Formycin, Anzymit, etc) have the ability to chemisorb aflatoxin from aqueous solutions (Liu et al. 2018).

The objective of this study was to determine the effect of exposure to AFB₁ on the growth performance, blood metabolites, liver function, and hematological parameters of broilers, and to investigate the efficacy of three mycotoxin binders, Mycosorb (MYC), Formycin (FMY), and Anzymit (ANZ) in reducing the deleterious effects of AFB₁.

Materials and methods

Experimental design and chick

Six hundred 1-d-old male chicks (Ross 308) were obtained from a commercial hatchery. The broilers were weighed and randomly allotted to 12 dietary treatments with 5 replicates of 10 birds each in a 3 × 4 factorial arrangement. The basal corn-soybean meal starter diet (containing no aflatoxin or adsorbent), was formulated to meet NRC (1994) requirements (3,100 kcal metabolizable energy/kg and 210 g crude protein/kg). The experimental treatments were as follows: T₁, basal diet (B); T₂, B + AFB₁ (2 µg/g); T₃, B + AFB₁ (4 µg/g); T₄, B + MYC (1.0 g/kg); T₅, B + AFB₁ + MYC (1.0 g/kg); T₆, B + AFB₁ (4 µg/g) + MYC (1.0 g/kg); T₇, B + FMY (1.0 g/kg); T₈, B + AFB₁ (2 µg/g) + FMY (1.0 g/kg); T₉, B + AFB₁ + FMY (1.0 g/kg); T₁₀, B + ANZ (20 g/kg); T₁₁, B + AFB₁ (2 µg/g) + ANZ (20 g/kg); T₁₂, B + AFB₁ (4 µg/g) + ANZ (20 g/kg). The basal diet was formulated with uncontaminated corn that had no detectable AFB₁.

Room temperature was kept at 31 °C with continuous lighting for the first week and was held at 27 °C for the remainder of the trial. Birds had ad libitum access to feed and water throughout the 21-d experiment. Mortality and clinical response were recorded daily, and chicks that died were examined by necropsy for cause of death. All procedures carried out in this experiment were reviewed and approved by the Animal Care and Use Committee of Isfahan University of Technology.

Aflatoxin production and binders

Aflatoxins were produced via fermentation of rice by A. parasiticus NRRL 2999 (USDA, Agricultural Research Service, Peoria, IL). The sterile substrate, placed in Erlenmeyer flasks, was inoculated with 2 mL of an aqueous suspension of the mould containing 106 spores/mL. Cultures were allowed to grow for 7 days at 25°C in darkness. On the seventh day, the Erlenmeyer flasks were autoclaved, and the culture material was dried for 48 h at 40°C in a forced-air oven and then ground to a fine powder. The AFB₁ levels in the rice powder were measured by thin-layer chromatography and HPLC following the method described by AOAC (2005) and Magnoli et al. (2011). The AFB₁ rice powder was added to the basal diet to achieve the targeted dietary AFB₁ concentrations of 2 and 4 µg/g. The commercial binders used in this study were obtained from a Brazilian company located in Araucaria, Parana. The levels of binders were selected according to the manufacturer’s recommendations. Mycosorb is the yeast cell wall-derived glucomannan product, whereas Formycin and Anzymit are composed of aluminosilicates. The mycotoxin binders were first mixed into a premix with corn flour before mixing into the corn-soy basal diet. The diets were analyzed again to confirm their AFB₁ content.

Data collection

Considering pen as the experimental unit, body weight (BW) and feed intake (FI) were recorded weekly. The data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (FCR) for 1 to 21 d.

At the end of the experiment, birds were fasted for 6 h and 10 birds (2 per replicate) were randomly selected from each treatment. A 5-ml blood sample was collected from the jugular vein of each bird into a test tube without anticoagulant. Blood samples were incubated at 37°C for 2 h, centrifuged at 1,500 × g for 10 min, and serum was separated and stored in 1.5 mL centrifuge tubes at −20°C until analysis. After blood collection, the same birds were weighed individually and anaesthetized with carbon dioxide and killed by cervical dislocation. Broiler organs, including heart, liver, and gizzard were weighed and expressed as a percentage of body weight. The serum samples were analyzed for total protein (TP), albumin (ALB), globulin (GLO), Ca and P concentrations, and total cholesterol (CHO), low density lipoprotein (LDL), high density lipoprotein (HDL) levels, activity of aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) using the Express Plus (Ciba-Corning Diagnostics Corp., Medfield, MA) automated clinical chemistry analyzer. This analyzer employs enzymatic procedures using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Zone Industrielle, 61500, SEES, France) with two replicates, at 25 °C, that have been described by Elliott (1984).

The hematological analysis was performed using a Hemavet 950 (Drew Scientific Inc., Waterbury, CT) immediately after collection of the blood. The hematological analysis included hematocrit and differential WBC composition (lymphocytes, monocytes, eosinophils). The analysis uses electronic impedance and laser light-scattering to estimate hematocrit and the percentage of lymphocytes, monocytes, and eosinophils. Hematologic parameters for all the samples were determined within 4 h of collection.

Statistical analyses

All data were analyzed as a completely randomized design with 3 × 4 factorial treatment arrangements using the general linear model (GLM) procedures of SAS (SAS 2003). No interaction was observed between mycotoxin and toxin binders; therefore, the one-way ANOVA was used to separate differences among main effect means.
The ADG and ADFI were significantly improved in Table 2. Compared with the control birds, a greater feed conversion ratio was recorded in chicks receiving Formycin. Additionally, the chickens receiving Mycosorb and Mycosorb decreased significantly the serum AST activity but did not affect CA and P concentrations.

Experimental diets contaminated with AFB1 significantly decreased (P < 0.01) haematocrit values and monocyte, lymphocyte, and eosinophil counts (Table 3). The addition of TXB to the AFB1-containing diet significantly increased eosinophil, monocyte (except for Formycin), and lymphocyte counts and haematocrit values compared with the control group (P < 0.01).

The main effect means of AFB1 and TXB and their interactions on the blood serum biochemical parameters in young broiler chicks are shown in Table 4. Serum TP, ALB, and GLO concentrations were significantly lower (P < 0.01) in the broilers fed the AFB1-contaminated diet, whereas the addition of TXB to the AFB1-containing diets significantly increased these serum proteins (P < 0.05). No effect of AFB1 or TXB was found on serum levels of CHO, LDL, and HDL of broilers.

Data on the levels of blood serum enzymes (AST and ALT) and mineral concentrations (Ca and P) are presented in Table 5. Experimental diets containing AFB1 caused a significant increase in AST activity (P < 0.05), but decreased Ca and P concentrations (P < 0.05) compared with the control diet. TXB significantly reduced serum ALT activity (P < 0.05). In addition, Mycosorb and Anzymit decreased significantly the serum AST activity but did not affect Ca and P concentrations.

Discussion
This study evaluated the efficacy of three commercial toxin binders to partially or totally eliminate the toxic effects of AFB1 on growth performance, organ weights, haematological values, and serum components of young broiler chicks. Aflatoxins are the most important mycotoxin that concern poultry farmers. Aflatoxins are considered extremely toxic due to their fast absorption in the gut (Whitlow and Hagler 2005), and the signs of aflatoxicosis in poultry have been clearly established (Wan et al. 2013). The metabolism of these compounds in the liver generates toxic metabolites that cause liver injury and inhibition of protein synthesis, culminating in anorexia (Yunus et al. 2011). The results indicated that the concentrations of AFB1 used in this study decreased the effects of the ADFI in growing broilers. This result is in agreement with that from Barati et al. (2018), who reported reduced feed intake in broilers fed on 1 mg AFB1/kg diet. In contrast, Siloto et al. (2013) observed no differences in ADFI in layers fed AFB1-contaminated diet (1 mg/kg of feed). Most studies were carried out in the initial rearing phase, because birds at this stage present an intense amount of protein synthesis that is critical for their growth.
development process, and the harmful effects are expressed more (Ma et al. 2015). A considerable number of studies were conducted under simulated acute aflatoxicosis and have shown important changes in both productivity and biochemical parameters (Aravind et al. 2003, Yunus et al. 2011, Siloto et al. 2013). However, taking into account the natural levels of AFB1 contamination in feeds, the importance of performing studies under simulated chronic aflatoxicosis conditions in growing broiler chicks is self-evident.

The addition of TXB to diets free of mycotoxins produced no adverse effects on growth performance when compared with the control, indicating that even with some loss of nutrient by association with the adsorbent, as described by Qu et al. (2017), it did not alter performance characteristics. The results of the present study indicate that Mycosorb and Anzymit reduced the adverse effects on ADG and FCR caused by AFB1 in broiler chicks. A previous study reported that the addition of Mycosorb (1.0 g/kg of feed) to an AFB1-containing diet (0.3 µg/kg of feed) reduced the growth inhibitory effects in broiler chicks fed diets for 21 days (Wade et al. 2017). The adverse effects of AFB1 on ADG are due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (Rotimi et al. 2017). Using an experimental model with a diet naturally contaminated with aflatoxin, ochratoxin, and zearalenone, Aravind et al. (2003) suggested that esterified glucomannan might counter the effect of mycotoxins. Although the precise mode of action of Mycosorb is not known, it is hypothesized that Mycosorb might trap the mycotoxin molecule in its glucomannan matrix and prevent toxin absorption from the gastrointestinal tract (Diaz et al. 2005). Thus the beneficial effects of Mycosorb might be attributed to its ability to trap the mycotoxins in the gastrointestinal tract (Wade et al. 2017).

It has been suggested that clinical, hematological-biochemical, and histopathological changes occur in broilers fed 50–200 µg/kg of dietary AFB1 (Whitlow and Hagler 2005, Resanovic and Sinovec 2006). The observed differences in response to chronic aflatoxicosis are probably due to differences in AFB1 sensitivity of the particular bird population assayed in each experiment.

A healthy immune system is important for young broilers, especially in an industry setting where birds are often challenged by many different pathogens. It is known that the immune system is very sensitive to AFB1 (Verma et al. 2004). Ma et al. (2015) reported that 74 µg/kg of AFB1 depressed growth and altered many aspects of humoral and cellular immunity in broilers. Application of AFB1 to mice demonstrated a decrease of leukocytes and neutrophils (Magnoli et al. 2011). Significant changes in serum proteins and immune response in Oreochromis mossambicus (tilapia) fed diets containing 1.5, 5.0, and 15.0 mg/kg AFB1 were also observed by Zacharia et al. (2003).

Aflatoxin has been shown to cause inhibition of protein synthesis (Rotimi et al. 2017). Consistent with the reduced serum protein contents observed with AFB1-contaminated diets in this study, earlier trials also recorded reductions in protein content of serum when AFB1 was fed to broilers (Zacharia et al. 2003). The elevation of AST may be due to disruption of hepatic cells as a result of necrosis or a consequence of altered membrane permeability (Barati et al. 2018). In our study the decreases in the mean values of haematocrit, percentage of eosinophil, monocyte, and lymphocyte counts in AFB1-fed chicks indicated the depressing effect of AFB1 on haemopoietic tissue and immune responses as reported by others. Immune suppressive effects associated with AFB1 feeding in chicks include reduced T lymphocyte counts (Qu et al. 2017), suppression of cell-mediated immunity and reduced immunoglobulin production (Ma et al. 2015). In addition, AFB1 exposure has been shown to reduce resistance to various bacterial, viral, and protozoan diseases in poultry (Aravind et al. 2003).

### Conclusions

The present study demonstrated that feeding AFB1-contaminated diets (2-4 µg/g) from 1 to 21 d of age induced deleterious effects on growth performance, hematological parameters, plasma minerals concentration, and serum proteins. The addition of Mycosorb, Anzymit or Formycin to the AFB1-containing diet significantly decreased the adverse effect of AFB1 on...
performance and biochemical-haematological values of the growing broiler chicks. There was apparent protection noted for some of the organ, hematological, and serum biochemical changes associated with aflatoxin toxicity. The data suggests that these TXB may alleviate some of the toxic effects of aflatoxin in growing broilers, and might prove to be beneficial in the management of aflatoxin-contaminated feedstuffs for young broiler chicks.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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