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# Effect of dietary supplementation of mannanoligosaccharides on growth performance, ileal microbial counts, and jejunal morphology in broiler chicks exposed to aflatoxins

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ABSTRACT

The present study was conducted to evaluate the effects of dietary inclusion of mannanoligosaccharides (MOS) on growth performance, ileal microbial population, and jejunal morphology in aflatoxin-challenged broiler chicks. A total of 336 seven-day-old Ross broiler chicks were randomly assigned into 7 experimental treatments with 4 replicates of 12 chicks each. Experimental treatments consisted of a control group (unchallenged group), and a $2 \times 3$ factorial arrangement of treatments, including 2 aflatoxin levels (0.5 and 2 ppm) and 3 supplemental MOS levels (0, 1, and 2 g/kg). Broiler chicks were challenged with a mix of aflatoxins during 7–28 d of age. Results showed that increasing aflatoxin level resulted in a marked decrease ($P < 0.01$) in average daily feed intake (ADFI) and subsequent average daily gain (ADG); consequently it impaired ($P < 0.001$) feed conversion ratio (FCR). Dietary MOS supplementation increased ADFI ($P < 0.01$) and ADG ($P < 0.001$) in aflatoxin-challenged chicks, resulted in the improvements in FCR values. The retarded ADG was ameliorated by inclusion of 2 g/kg of MOS into the diet of aflatoxin-challenged broiler chicks. Although incremental levels of aflatoxin decreased ($P < 0.05$) carcass yield, dietary supplementation of MOS up to 2 g/kg resulted in an increase in carcass yield. Contamination with 2 ppm aflatoxin resulted in increases ($P < 0.001$) in ileal enumerations of *Escherichia coli*, *Salmonella*, *Klebsiella*, and total negative bacteria at both 28 and 42 d of age. Although the lowest bacterial count was assigned to the control (unchallenged) group, supplemental MOS decreased ileal bacterial populations in aflatoxin-challenged broiler chicks. Dietary supplementation of 2 g/kg of MOS was more effective ($P < 0.05$) in depression of ileal microbial counts in broiler chicks challenged with 0.5 ppm aflatoxin. Incremental levels of aflatoxin resulted in considerable ($P < 0.001$) decreases in villi height, villi height to crypt depth ratio, villi absorptive area, and apparent villi absorptive area. Moreover, dietary aflatoxin contamination increased crypt depth, goblet cell counts, and lymphoid follicular diameter. These changes, however, were partly modulated by dietary MOS supplementation up to 2 g/kg. The present results indicated that although aflatoxicosis reduced growth performance, dietary inclusion of MOS ameliorated the retarded growth via suppressing ileal pathogenic bacteria and enhancing absorptive surface area in broiler chicks.



## 1. Introduction

Mycotoxin contamination is a worldwide concern due to unique nature of these fungi metabolites. It is difficult to eliminate mycotoxins from feed ingredients (Azarakhsh et al., 2011). Mycotoxins produced by certain fungi are estimated to contaminate 25% of world crop (Schatzmayr et al., 2006). Notably, the losses as a result of the presence of mycotoxins in contaminated crops have been evaluated as much as several million dollars per annum (CAST, 2003). Aflatoxins, as a main group of mycotoxins, contaminate various tropical and subtropical feeds (Galvano et al., 2005). Although there are 5 aflatoxin subfamilies, including B1, B2, G1, G2, and M1, aflatoxin B1 (mainly originated from *Aspergillus flavus* and *Aspergillus parasiticus*) is the most toxic and mutagenic aflatoxin existed in feed (Bennett and Klich, 2003; Sengstag, 1997). Aflatoxins have been demonstrated to induce digestive disorders, including diarrhea, vomiting, intestinal hemorrhage, and liver necrosis and fibrosis (Harriet, 2003). Deleterious effects of aflatoxins depend on dosage, length of exposure, animal species, age,

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and nutritional status (Whitlow and Hagler, 2005). Because aflatoxins have immunosuppressive activity, these compounds predispose animals to various infections resulted from bacteria, viruses, and other fungi (Robens and Richard, 1992).

Adsorbents could prevent mycotoxins from the absorption by gastrointestinal tract due to their strong binding capacity (Ramos and Hernández , 1996). Although several studies have examined inorganic adsorbents such as bentonite, aluminum silicates, and zeolites (Dvorska and Surai, 2001; Santin et al., 2002; Thieu et al., 2008), there is limited information in terms of organic adsorbents. It seems that yeast cell wall can be used as an alternative to inorganic adsorbing agents due to its higher adsorptive capacity. Organic mycotoxin binders are mostly derived from cell wall components of *Saccharomyces cerevisiae*. Yeast cell wall and its charge play an important role in the adsorption process of mycotoxins (Bejaoui et al., 2004). Besides adsorbent properties, the toxins' characteristics, including their polarity, solubility, and charge act as essential factors in adsorption process (Avantaggiato et al., 2005). Beta-glucan fraction of yeast cell wall increases its binding capacity (Shetty and Jespersen, 2006). Freimund et al. (2003) observed that yeast β-1,3-glucan had effective binding capacity for T-2 toxin. Aravind et al. (2003) observed that additionally-esterified glucomannan ameliorated the retarded growth in broilers fed diets containing mycotoxins. In contrast, Yiannikouris and Jouany (2002) reported that supplemental esterified glucomannan wasn't efficient in terms of mitigating the detrimental effects of combined mycotoxins. Similarly, Swamy et al. (2003) reported that supplemental glucomannan had no influence on average daily gain, feed intake, and feed efficiency in pigs challenged with high levels of *Fusarium* mycotoxins.

Although aflatoxicosis has been characterized by many studies, little information is available regarding the effects of supplemental mannanoligosaccharides (MOS) on gut microflora and histological indices in aflatoxin-challenged broiler chicks. The present study was conducted to investigate the effects of dietary MOS supplementation on growth performance, ileal microbial counts, and jejunal morphology in broiler chicks exposed to marginal or toxic levels of aflatoxins.

## 2. Materials and methods

### 2.1. Experimental design and dietary treatments

This study was conducted in the Poultry Research Station of Isfahan University of Technology and all protocols were approved by Isfahan University of Technology Animal Care and Use Committee. A total of 336 seven-day-old Ross 308 broiler chicks were randomly assigned into 7 experimental treatments with 4 replicates of 12 chicks each. Experimental treatments consisted of a control group (unchallenged group), and a 2 × 3 factorial arrangement, consisting 2 aflatoxin levels (0.5 and 2 ppm) and 3 MOS levels (0, 1, and 2 g/kg), Aflatoxin was fed to the chicks from 7 to 28 d of age. The experimental diets (Table 1) were formulated to provide all of the nutritional considerations of broiler chicks throughout the experimental periods according to Ross 308 Guidelines (Ross Broiler Management Manual, 2009). Light was on continually for the first week; after that, a 23 L: 1 D lighting program was used for the remaining trial period. Broilers had free access to feed and water throughout the trial period. Temperature was adjusted at 33 °C during the first week and was decreased by 3 °C/week.

### 2.2. Aflatoxin production

To prepare aflatoxin, *Aspergillus parasiticus* PTCC-5286 was obtained from the Iranian Research Organization for Science and Technology (Tehran, Iran). Then, *Aspergillus parasiticus* was cultured on sterile potato dextrose agar and kept at 28 °C for 5 d. After preparing a uniform fungus spore suspension, the number of spores per mL of distilled water was counted using Hemocytometer (Model 521-10, Funakoshi Co., Ltd., Tokyo, Japan). To produce a high quantity of fungus, amount of 150 g of rice with 150 mL of water were thoroughly mixed in the flask and autoclaved. Afterwards, $7 \times 10^6$ to $7.5 \times 10^6$ spores/mL of suspension were inoculated to the flask and incubated at 28 °C for 5 d.

**Table 1**
Ingredients and chemical composition of basal diets.

| Items | Starter (1–14 d of age) | Grower (15–28 d of age) | Finisher (29–42 d of age) |
|---|---|---|---|
| Ingredients (g/kg) | | | |
| Corn | 526.1 | 567.5 | 636.8 |
| Soybean meal | 401.7 | 362.2 | 293.6 |
| Soybean oil | 22.3 | 23.8 | 24.7 |
| Dicalcium phosphate | 19.7 | 18.0 | 17.0 |
| Calcium carbonate | 12.1 | 11.3 | 11.0 |
| Common salt | 2.4 | 2.4 | 2.4 |
| DL-Met | 1.8 | 1.8 | 1.6 |
| L-Lys · HCl | 0.8 | 0.2 | 0.4 |
| L-Thr | 0.6 | 0.3 | – |
| Na bicarbonate | 1.5 | 1.5 | 1.5 |
| Vitamin premix[a] | 3.0 | 3.0 | 3.0 |
| Mineral premix[b] | 3.0 | 3.0 | 3.0 |
| Inert filler[c] | 5.0 | 5.0 | 5.0 |
| Composition | | | |
| Metabolizable energy (MJ/kg) | 12.1 | 12.4 | 12.7 |
| Crude protein (g/kg) | 220.0 | 205.0 | 180.0 |
| Ca (g/kg) | 10.3 | 9.5 | 9.0 |
| Available P (g/kg) | 5.0 | 4.5 | 4.2 |
| Na (g/kg) | 1.5 | 1.5 | 1.5 |
| Lys (g/kg) | 13.0 | 11.6 | 10.0 |
| Met (g/kg) | 5.4 | 5.2 | 4.8 |
| Met + Cys (g/kg) | 9.0 | 8.6 | 7.8 |
| Thr (g/kg) | 8.7 | 7.8 | 6.6 |

[a] Vitamin premix provided the following per kilogram of diet: vitamin A, 11,500 IU; cholecalciferol, 2,100 IU; vitamin E, 22 IU; vitamin $K_3$, 1.5 mg; thiamine, 3 mg; riboflavin, 4.4 mg; pantothenic acid, 25 mg; niacin, 40 mg; choline chloride, 560 mg; biotin, 0.1 mg; folic acid, 0.8 mg; pyridoxine 10 mg; vitamin $B_{12}$, 0.06 mg.
[b] Mineral premix provided the following per kilogram of diet: iron, 50 mg; zinc, 55 mg; manganese, 75 mg; iodine, 1.8 mg; copper, 8 mg; selenium, 0.3 mg; cobalt, 0.2 mg.
[c] Variable amounts of an inert filler (washed sand) or mannanoligosaccharides.

### 2.3. Aflatoxin measurement

The inoculated rice powder was used to detect the concentration of produced aflatoxins using high performance liquid chromatography (LC-10, Shimadzu, Kyoto, Japan; AOAC, 2000; Method 994.08) according to Buttinger (2010). The determined aflatoxins concentrations were shown in Table 2. Finally, the contaminated rice powder was added to the experimental diets to obtain the final concentrations of 0.5 and 2 mg of aflatoxins/kg of feed.

**Table 2**
Final aflatoxin concentrations in aflatoxin-contaminated rice powder.

| Aflatoxins | Concentration (μg/kg) |
|---|---|
| B1 | 17,760 |
| B2 | 1,500 |
| G1 | 3,180 |
| G2 | 120 |
| Total aflatoxins | 22,560 |