Effects of Aflatoxin B1 and Yeast Cell Wall Supplementation on the Growth Performance of Broilers

Z. Nemati, A. Karimi, M. Besharati

Abstract**—** Aflatoxin is a worldwide problem in broiler production and cause great economic losses and health problem in animal and human. This study was conducted with the purpose of evaluating the effect of aflatoxin B_1 and supplementing diets with yeast cell wall (Esterified glucomannan) on the growth performance in broiler chickens. A total of two hundred fifty six 1-d-old 308 Ross chicks were randomly assigned to 4 treatments, with 4 pens per treatment and 16 birds for each experimental unit. Dietary treatments included control diet, diet supplemented with 0.2% glucomannan; diet supplemented with 1 ppm aflatoxin B1 and diet supplemented with 0.2% glucomannan and 1 ppm aflatoxin B1. Birds consumed the diets and water ad libitum. Body Weight (BW) gain and feed intake were recorded weekly. The results showed that compared with the control, supplementation with glucomannan had no significant effects on body weight gain and feed conversion ratio from d 1 to 35. Feeding contaminated diets significantly decreased BW gains and feed intake at the entire experimental period. Feed conversion rate (FCR) was lowest in the group consuming the aflatoxin diet ($P < 0.01$). Glucomannan supplementation did not prevent or reduce the toxic effects of aflatoxin on feed intake, BW gains and FCR significantly. Production index was significantly decreased after feeding contaminated diets compared with controls. The results indicated that glucomannan was not effective in preventing the adverse effects of 1ppm aflatoxin B1 on performance.

Index Terms—Aflatoxin, broiler, growth performance, yeast cell wall.

I. Introduction

Mycotoxins are secondary metabolites produced by fungi that contaminate food or feeds in the field or during storage [1]. It was estimated that over 25% of food/feed crops worldwide are contaminated annually with mycotoxin-producing fungi [2], More than 500 different mycotoxins are known. Aflatoxins, one of the most toxic groups of mycotoxins mainly produced by strain of *Aspergillus flavus*, and *Aspergillus parasiticus*, are a major concern in the poultry production [3]. Aflatoxin produce severe economic losses and health problems in the poultry industry because of their toxicity and frequency of occurrence in feedstuffs [4]. A survey from North West of Iran found that 27% of tested poultry diet contained more than 20 µg of aflatoxin/kg and 100% of feed ingredient contained aflatoxins [5]. Feeding aflatoxin contaminated feeds to poultry affects animal health and production and causes changes in biochemical and hematological parameters, changes in gene expression of liver enzymes, liver damage, kidney abnormalities, mortality, and immunosuppression, which may enhance susceptibility to infectious diseases [6-9]. The adverse effects of aflatoxins vary according to the dose, natural or pure aflatoxins, the duration of exposure, and animal factors such as age, sex, and level of stress [10-11].

There are a number of approaches that can be taken to protect of animal from toxic effect of aflatoxins and these involve prevention of fungal growth, and strategies to reduce or eliminate mycotoxins from contaminated feeds, such as physical, chemical, nutritional, and biological techniques [12]. Nonnutritive adsorbents are most practical approaches, which bind the mycotoxins and reduce their absorption from the gastrointestinal tract, thus minimizing the toxic effects in livestock [13]. Aluminosilicates, clay, bentonite, montmorillonite, zeolite, phyllosilicates, activated carbon, cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans and peptidoglycans, and synthetic polymers such as cholestryamine and polyvinylpyrrolidone have been extensively studied with varying results [14]. A polymeric glucomannan mycotoxin adsorbent derived from the cell wall of Saccharomyces cerevisiae has been used as a feed additive to binds aflatoxin and prevents their absorption by the gastrointestinal tract. Thus, the current experiment was conducted to investigate the effects of feeding diet naturally contaminated with 1 ppm B1 aflatoxin and the ameliorative efficacy of dietary adsorbent (modified glucomannas) on broilers.

II. Material And Methods

A. Aflatoxin B1 Production

Aflatoxins were produced via fermentation of rice *in vitro* using *Aspergilus parasiticus* NRRL 2999. The sterile rice, placed in Erlenmeyer flasks, was inoculated with 2 mL of the mold aqueous suspension containing 10^6 spores/ml. Cultures were allowed to grow for 7 d at 28°C in darkness. On the d 7 Erlenmeyer flasks were autoclaved, and culture material was dried at 40°C in an oven for 48 h. Aflatoxin B1 content in rice powder were measured using HPLC method. The ground rice was incorporated into the basal diet to provide 1 mg of aflatoxin B1/kg of diet.

B. Experimental design and birds

A total of 256 1-d-old 308 Ross chicks were obtained from a commercial hatchery and randomly distributed into four

Manuscript received August 9, 2014.

Nemati, Z., Depatrment of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Iran (e-mail: znemati@tabrizu.ac.ir).

Karimi, A. Depatrment of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Iran (e-mail: pekarimi@tabrizu.ac.ir).

Besharati, M. Depatrment of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Iran (e-mail: m_besharati@hotmail.com).

groups, with 4 pens per treatment and 16 birds for each experimental unit. During the 5 week experimental period, birds were fed the following diets: (1) control diet, (2) control diet supplemented with 0.2% glucomannan (Mycosorb™); (3) control diet supplemented with 1 ppm aflatoxin B1 and (4) control diet supplemented with 0.2% glucomannan and 1 ppm aflatoxin B1. A standard corn-soybean meal diet (basal diet) was formulated in accordance with the specifications of Ross 308 guidelines to meet the nutrient requirements of broilers. Diets were fed *ad libitum* from d 1 until the end of the experiment. A starter diet was given from d 1 to 14, and a grower diet was provided from d 15 to 35. Temperature and lighting regimen were in accordance with recommendation of the commercial broiler chickens. Feed Intake (FI), BW gain, and feed conversion rate (FCR) was measured weekly. The control diet was a typical commercial diet consisting of approximately 20.16% crude protein, and 3025 ME kcal/kg.

C. Statistical analysis

Data were subjected to analysis of variance by the GLM procedure of SAS (2002, SAS Institute Inc., Cary, NC). When the *F* test was significant, the means among treatments were compared by using Duncan's multiple range tests. Statements of statistical significance were based on $P<0.05$.

The effects of the dietary aflatoxin B1 and glucomannan on broiler chicks' feed intake, average BW gain, and FCR data in the periods of starter, and grower, and the whole trial are presented in Table 1. It can be seen that compared with the control, glucomannan had no significant effects on body weight gain and feed conversion rate from 1 to 35 d. Supplementation with glucomannan increased feed intake and BW Gain in grower period ($P< 0.05$). Aflatoxin in the diet with 1 mg of B1 per kilogram of feed showed a significantly lower ($P < 0.05$) BW gain and FI and a significantly higher FCR than the values observed for the control groups in grower and the whole experiment periods ($P<0.05$). FCR of broilers did not differ among treatments, during the first 14 d. however the inclusion of aflatoxin B1 decreased BW gain and FI by 34 and 31% respectively ($P<0.05$).

Addition of modified glucomannans into the aflatoxin contaminated diets not alleviated the growth depression effects in the chicken. Compared with the B1 group BW gain, FI and feed conversion rate was not affected by glucomannan supplementation in the periods of starter, grower, and the whole trial. As expected, the production index was decreased by the contaminated diets. Production index was significantly depressed in the B1 by 51% and GMB1 fed group by 44% ($P< 0.05$).

TABLE 1. EFFECTS OF AFLATOXIN B1 AND YEAST CELL WALL ON GROWTH PERFORMANCE OF BROILER CHICKENS

	Diet treatment[1]				
Item[2]	Control	GM	B1	GMB1	SEM
1 to 14 d					
FI[3] (g/bird per day)	358 [a]	361 [a]	247 [b]	250 [b]	8.63
BWG[3] (g/bird)	260 [a]	257 [a]	172 [b]	177 [b]	5.28
FCR[3] (g/g)	1.37	1.40	1.43	1.41	0.018
15 to 35 d					
FI (g/bird per day)	2415 [b]	2530 [a]	1796 [c]	1845 [c]	34.52
BWG (g/bird)	1287 [b]	1345 [a]	825 [c]	875 [c]	17.35
FCR (g/g)	1.87 [b]	1.88 [b]	2.17 [a]	2.11 [a]	0.01
1 to 35 d					
FI (g/bird per day)	2773 [b]	2892 [a]	2044 [c]	2095 [c]	37.80
BWG (g/bird)	1548 [a]	1602 [a]	998 [b]	1052 [b]	20.01
FCR (g/g)	1.80 [b]	1.78 [b]	2.05 [a]	1.99 [a]	0.034
Production index	241 [a]	247 [a]	117 [b]	134 [b]	9.30

[1]Control = basal diet; GM = basal diet + 2 g/kg Mycosorb; B1= basal diet + 1 mg/kg aflatoxin B1; GMB1= basal diet + 1 mg/kg aflatoxin B1+ 2 g/kg Mycosorb.
[2]Means within a row without a common superscript differ statistically ($P < 0.05$).
[3]BWG = BW gain; FI = feed intake; FCR = Feed conversion rate.

III. RESULT

Considerable research has been directed in animal studies to investigate toxicity of mycotoxin. Commercially available purified mycotoxins unusually are expensive for use in animal feeding trials and do not represent the natural condition of mycotoxins in the field and during storage. In this study, the effects of natural aflatoxins were determined. Aflatoxins were produced by fermentation of rice by the NRLL 2999 strain of *Aspergillus parasiticus*, under stirring and controlled temperature in our laboratory. Aflatoxin analyses revealed that total of 481 mg of aflatoxin/kg of rice substrate containing 81.4% aflatoxin B1, 2.2% aflatoxin B_2, 0% aflatoxin G_1, and 16.2% aflatoxin G_2.

IV. DISCUSSION

In this study aflatoxin was chosen because it is commonly found in Iran poultry diets [5] and when these toxins ingested by animal can cause impair metabolism and immune suppression which may result in increased disease incidence and decreased production index. The presence of aflatoxins in the diet induced a negative effect on all performance parameters. In the present study, the toxic effect of 1 ppm AFB1 and the ameliorative efficacy of modified glucomanan on broilers were evaluated.

Feeding contaminated diets depressed growth and feed intake compared to the control groups. These results agree with those reported by Cravens et al. 2013 [15], who reported that BW gain and FI decreased when levels of 0.75 to 1.5 mg of AFB1/kg of feed were provided. Similar results

were also observed by other researchers [13, 16, 17] who reported that 1 to 5 mg/kg of dietary aflatoxin B1 induced negative changes in both productivity and biochemical parameters of broilers. The adverse effects of aflatoxin on growth performance may be due to anorexia, listlessness, impaired liver and intestinal functions, and inhibition of protein synthesis and impaired lipogenesis, and protein/lipid utilization mechanisms [18-20]. These results are in disagreement with Chowdhury et al., 2005 [21] who reported that the diet containing blends of grains naturally contaminated with Fusarium mycotoxins failed to affect body weight gains, feed consumption, and feed efficiency in ducks.

The addition of adsorbents to contaminated feed is considered the most promising and economical approach of reducing aflatoxicosis in animals [17, 22]. However, the efficacy of these adsorbents is not well understood. The glucan-based binders have been reported to prevent the toxic effects of mycotoxins by binding to mycotoxins during digestion and preventing their absorption from the gastrointestinal tract [21, 23-24]. The beneficial effects of Polymeric glucomannan on preventing aflatoxicosis have been shown in turkeys [25], laying hens [26], and broiler breeders [27]. In the current study, addition of Mycosorb, containing yeast cell wall, at 0.2% of the diet did not ameliorate the adverse effects of AFB1. These results are consistent with previous studies reported by Karaman et al., 2005 [23] and Devegowda and Murthy, 2005 [24]. The lack of efficacy of Glucomannan at the 1 mg/kg level of AFB1 is may be due to saturation or limited binding capacity of yeast cell walls that was used in this experiment. Similar results were reported in dairy cows by Kutz et al. 2009 [29], with yeast cell wall product at 0.5% of diets containing 170 µg of AFB1 /kg of feed, not effective in reducing milk aflatoxin M_1 concentrations. The overall growth rate was statistically similar but numerically higher in the group fed GMB1 compaired with B1 group. The results of this experiment suggest that dietary glucomannan may need to be supplemented at levels higher than the 0.2% used in the current study to achieve protective effects against 1 mg/kg.

V. Conclusion

In conclusion, our study clearly revealed that AFB1 in the diet at levels of 1 mg/kg resulted in a reduced growth performance. Addition of 0.2% Mycosorb (Glucomannan) to the basal diet was not effective in preventing the adverse effects of aflatoxin in broiler which warrant further investigation.

References

[1] A. Yiannikouris and J. P. Jouany, "Mycotoxins in feeds and their fate in animals: A review," Anim. Res. Vol. 51, pp. 81–99, 2002.

[2] Food and Agriculture Organization, "Food safety and quality. Mycotoxins," http://www.fao.org/food/foodsafety-quality/a-z-index/mycotoxins/en/.2012

[3] N. Chand, D. Muhammad, F.R. Durrani, M.S. Qureshi, S.S. Ullah, "Protective effects of Milk Thistle (Silybum marianum) against aflatoxin B in broiler chicks," Asian-Aust. *J. Anim. Sci.* vol. 24, pp. 1011–1018, 2011.

[4] L. F. Kubena, R. B. Harvey, T. D. Phillips, and B. A. Clement."Effect of hydrated calcium aluminosilicates on aflatoxicosis in broiler chicks," *Poult. Sci.* vol. 72, pp.651–657, 1993.

[5] Z. Nemati, H. Janmohammadi, A.Taghizadeh, H. Maleki Nejad, Gh. Mogaddam and M. Arzanlou, "Occurrence of Aflatoxins in poultry feed and feed ingredients from northwestern Iran" *European Journal of Zoological* Research, vol. 3, pp. 56-60, 2014.

[6] L. P. Yarru, R. S. Settivari, E. Antoniou, D. R. Ledoux, and G. E. Rottinghaus, "Toxicological and gene expression analysis of the impact of aflatoxin B on hepatic function of male broiler chicks" *Poult. Sci.* vol. 88, pp. 360–371, 2009.

[7] X. Y. Han, Q. C. Huang, W. F. Li, J. F. Jiang, and Z. R. Xu. "Changes in growth performance, digestive enzyme activities and nutrient digestibility of Cherry Valley ducks in response to aflatoxin B1 levels" *Livest. Sci.* vol. 119, pp. 216–220, 2008.

[8] B J. He, K.Y. Zhang , D.W. Chen , X.M. Ding, G.D. Feng ,X. Ao, "Effects of vitamin E and selenium yeast on growth performance and immune function in ducks fed maize naturally contaminated with aflatoxin" *livest. Sci*.,vol. 152, pp. 200-207, 2013.

[9] Z. Nemati, H. Janmohammadi, A. Taghizadeh, Gh. Moghaddam, H. Maleki Nejad, "Effect of Bentonite supplementation to the contaminated diets with aflatoxin B1 on broiler performance," presented at the *6th Iranian congress on animal science*, Tabriz, Iran, Aug, 2014.

[10] G. D. Osweiler, "Mycotoxins and livestock: What role do fungal toxins play in illness and production losses?," *Vet. Med*. Vol. 85, pp. 89–94, 1990.

[11] L. W. Whitlow, and W. M. Hagler, "Mycotoxins in feeds". *Feedstuffs*, vol. 77 pp. 69–79, 2005.

[12] L.B. Wayne, "Mycotoxin contamination of the feed supply chain: Implications for animal productivity and feed security," *Anim. Feed Sci. Technol.* 2012, doi:10.1016/j.anifeedsci.2011.12.014

[13] R. Miazzo, M. F. Peralta, C. Magnoli, M. Salvano, S. Ferrero, S. M. Chiacchiera, E. C. Q. Carvalho, C. A. Rosa, and A. Dalcero," Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin," *Poult. Sci.* 84:1–8. 2005.

[14] A. Huwig, S. Freimund, O. Käppeli, and H. Dutler, "Mycotoxin Detoxication of animal feed by different adsorbents," *Toxicol. Lett.* Vol. 122, pp.179–188, 2001.

[15] R. L. Cravens, G. R. Goss, F. Chi, E. D. De Boer, S. W. Davis, S. M. Hendrix, J. A. Richardson, and S. L. Johnston, "The effects of necrotic enteritis, aflatoxin B1 and virginiamycin on growth performance, necrotic enteritis lesion scores, and mortality in young broilers," *Poult. Sci.* vol. 92, PP. 1997–2004, 2013.

[16] J. Dixon, B. I. Kannewischer, M. G. Tenorio Arvide, and A. L. Barrientos Velazquez, "Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan," *Appl. Clay Sci.* vol. 40, pp. 201–208, 2007.

[17] R. P. Pizzolitto, M. R. Armando, M. A. Salvano , A. M. Dalcero , and C. A., "Rosa Evaluation of Saccharomyces cerevisiae as an antiaflatoxicogenic agent in broiler feedstuffs," *Poult. Sci.* Vol. 92, pp. 1655–1663, 2013

[18] H. Oguz, T. Kececi, Y. O. Birdane, F. Onder, and V. Kurtoglu, " Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis," Res.Vet. Sci. vol. 69, pp. 89–93, 2000.

[19] M. Ortatatli, and H. Oguz., "Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis," Res. Vet. Sci. vol. 71, pp. 59–66, 2001

[20] T. J. Applegate, G. Schatzmayr, K. Prickel, C. Troche, and Z. Jiang.. "Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens". *Poult. Sci.* vol. 88, pp.1235–1241, 2009.

[21] Chowdhury, S.R., Smith, T.K., Boermans, H.J., Sefton, A.E., Downey, R., B. Woodward, "Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on performance, metabolism, hematology, and immunocompetence of ducklings," *Poult. Sci*., vol. 84, pp. 1179–1185. 2005.

[22] S.R. Chowdhury, and T.K., Smith, "Effects of feeding grains naturally contaminated with Fusarium mycotoxins on hepatic fractional protein synthesis rates of laying hens and the efficacy of a polymeric glucomannan mycotoxin adsorbent," *Poult. Sci*., vol. 84, pp. 1671–1674, 2005.

[23] G. Devegowda, M.V.L.N. Raju, and H.V.L.N. Swamy, "Mycotoxins: novel solutions for their counteraction," *Feedstuffs*, 70, 12–15, 1998.

[24] M. Karaman, H. Basmacioglu, M. Ortatatli, H. Oguz, "Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopa thology," Br. Poult. Sci. vol. 46, pp.394–400, 2005

[25] Chowdhury, S. R., and T. K. Smith, "Effects of feed-borne Fusarium mycotoxins on performance, plasma chemistry and hepatic fractional protein synthesis rates of turkeys," *Can. J. Anim. Sci*. 87:543–551. 2007

[26] E. V. Siloto, E. F. A. Oliveira, J. R. Sartori, V. B. Fascina, B. A. B. Martins, D. R. Ledoux, G. E. Rottinghaus, and D. R. S. Sartori

"Lipid metabolism of commercial layers fed diets containing aflatoxin, fumonisin, and a binder" *Poult. Sci.* vol. 92, pp. 2077–2083, 2013

[27] Yegani, M., T. K. Smith, S. Leeson, and H. J. Boermans. 2006b. "Effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance and metabolism of broiler breeders" *Poult. Sci*. 85:1541–1549.

[28] G. Devegowda, and T.N.K. Murthy, "Mycotoxins: their effects in poultry and some practical solutions," In: Diaz, D. (Ed.), Mycotoxin Blue Book. Nottingham University Press, Nottingham, pp. 25–56, 2005.

[29] Kutz, R. E., J. D. Sampson, L. B. Pompeu, D. R. Ledoux, J. N. Spain, M. Vazquez-Anon, and G. E. Rottinghaus, "Efficacy of Solis, NovasilPlus, and MTB-100 to reduce aflatoxin M levels in milk of early to midlactation dairy cows fed aflatoxin B1," *J. Dairy Sci*. vol. 92, pp. 3959–3963, 2009

Z. Nemati was born in 22/07/1977, Ardabil city, Iran.
Educations
B.Sc, 2003, Animal Science, University of Ilam, Iran.
M.Sc, 2006, Animal Science- Poultry Nutrition, University of Tabriz, Iran.
Ph.D, 2014, Animal Science- poultry Nutrition, University of Tabriz, Iran

Ph.D in poultry Nutrition, Assistant Professor, University of Tabriz, Department of Animal Science.

Publications:

Nemati, Z. H. Janmohammadi, A. Taghizadeh, H. Maleki Nejad, Gh. Moghaddam, M. Arzanlou. Occurrence of Aflatoxins in poultry feed and feed ingredients from northwestern Iran, European Journal of Zoological Research, 2014, Mar, 56-60

Barzegari A., S. Atashpaz, K. Ghabili, Z. Nemati, M. Rustaei and R. Azarbaijani. Polymorphisms in GDF9 and BMP15 Associated with Fertility and Ovulation Rate in Moghani and Ghezel Sheep in Iran. Reproduction in domestic animal journal. 2009.

Taghizadeh, A. N. Zabihollah.. Degradability Characteristics of Treated and Untreated Barley Grain UsingIn situ Technique American Journal of Animal and Veterinary Sciences. 2008 (2), 53

Taghizadeh, A. Z Nemati, H Janmohamadi, N Pirani .. The Determination of Fermentation Characteristics of Treated and Untreated Barley Grain Using Gas Production Technique Journal of Animal and Veterinary Advances 2007 (12), 1395-1397