

Impact of Aflatoxin Contaminated Feed and Yeast Cell Wall Supplementation on Immune System in Broiler Chickens

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Abstract— Aflatoxins are a major problem in poultry industry and causes global economic losses. This experiment was conducted to determine the effect of aflatoxin and supplementing diets with yeast cell wall (esterified glucomannan) on the immune system in broiler chickens. A total of 256 one-day-old Ross 308 broiler chicks were randomly assigned to 4 treatments, with 4 pens per treatment and 16 birds for each experimental unit. Dietary treatments were: 1) control diet, 2) diet supplemented with 0.2% glucomannan; 3) diet supplemented with 1 ppm aflatoxin B1 and 4) diet supplemented with 1 ppm aflatoxin B1 and 0.2% glucomannan. Feed and water were provided *ad libitum* throughout the 35-d experiment. At 35 d of age, blood samples were taken from 8 chicks per treatment and the titers of antibody against Sheep Red Blood Cells (SRBC) were measured by haemagglutination inhibition test. The relative weights of the spleen, thymus and bursa of Fabricius were measured at the end of the experiment. The relative weights of the spleen and thymus were not affected by dietary treatments. Feeding contaminated diets significantly decreased relative weights of bursa of Fabricius and antibody levels against SRBC ($P < 0.05$). Glucomannan supplementation did not prevent or reduce the toxic effects of aflatoxin on relative weights of lymphatic organs and antibody titers against SRBC significantly. In conclusion, our results showed that aflatoxin impaired immune function in growing broiler and addition of yeast cell wall could not alleviate the adverse effects of 1ppm aflatoxin on immune systems.

Index Terms— aflatoxin, growth performance, yeast cell wall, broiler

I. INTRODUCTION

Aflatoxins are the most common mycotoxin produced by strain of *Aspergillus flavus*, and *Aspergillus parasiticus* [1]. This toxin has been categorized as a carcinogenic agent to humans by the International Agency for Research on Cancer [2]. For this reason, many countries have set legislation restricting the levels of aflatoxin B1 allowed in cereals for human consumption and animal products such as milk [3-4]. Aflatoxins contamination of poultry feed in some agricultural condition are unavoidable because of the ubiquitous nature of *Aspergillus* species in the environment

[5-4]. The presence of potential toxigenic strains of fungi (*Aspergillus*, *Fusarium*, *Penicillium*) and AFB1 in animal feeds has been extensively reported in previous studies [7-14]. Aflatoxicosis is characterized mainly by hepatic, kidney and spleen lesions, immune suppression, impaired productivity parameters and antioxidant functions, [15-17] as well as been carcinogenic, mutagenic, teratogenic, altered organ morphology, serum biochemistry and hematology in poultry and human [15, 18-19].

Various methods have been suggested to evaluation of immune status in chickens such as measurement of immune organs and antibody titers. Such immune related organs include thymus, bursa of fabricius, spleen, and liver. Development of these organs is vital for optimal antibody synthesis [20]. The thymus and bursa of Fabricius are the primary lymphoid and immune-important organs in broiler. Thymus and spleen plays an important role in producing lymphocytes cells and bursa of fabricius is the primary site of immunoglobulin synthesis [21].

Due to sensitivity of chickens to aflatoxin, consumption of aflatoxin can affect profitability and immune system of poultry. Aflatoxin B1 was shown to have adverse effects on immunity which decreased relative weight of bursa of Fabricius and contents of serum immunoglobulin, and increased percentage of apoptotic bursal cells and debris in the bursal lymphoid follicle in ducks [22]. The detrimental effects of aflatoxins vary according to the dose, length of exposure, natural or pure aflatoxin, and animal breed, species, age and nutritional status susceptibility [23]. There are various treatments and dietary approaches to reduce adverse effects AFB1 on animals in contaminated commodities, such as addition of mineral and biological adsorbents to the feed [24]. The present trial was conducted to evaluate the efficacy of a commercial yeast cell wall product as an aflatoxin binder on immune system in broiler chickens exposed to aflatoxin.

II. MATERIAL AND METHODS

A. Production of Aflatoxin B1

Aflatoxins were produced via fermentation of rice *in vitro* using *Aspergillus parasiticus* NRRL 2999. For this 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.

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B. Animal and diets

A total of 256 1-d-old 308 Ross chicks were obtained from a commercial hatchery and randomly distributed into four 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 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. The control diet was a typical commercial diet consisting of approximately 20.16% crude protein, and 3025 ME kcal/kg. Birds were maintained under standard conditions of temperature, humidity and lighting regimen.

At the end of the experiment, 4 birds from each treatment were killed by cervical dislocation and lymphoid organs, Spleen, thymus and bursa of Fabricius, were removed and weighed. Lymphoid organ weights were expressed on a relative BW basis. At 28 day of age, 4 chicks per treatment were randomly chosen and 0.1 mL/kg of BW of 7% SRBC was injected into the brachial vein of chick. Eight days after injection, blood was collected in tubes by puncturing the brachial vein. Blood plasma was obtained by centrifuging at $1500 \times g$ for 10 min at home temperature, and stored at -20°C until analysis. Blood plasma tested for total SRBC antibody by the procedure of Wegmann and Smithies (1966). Antibody titers were expressed as the \log_2 of the reciprocal of the highest dilution in which agglutination was observed macroscopically.

C. Statistical analysis

Data were analyzed by analysis of variance using GLM procedure of SAS (2002, SAS Institute Inc., Cary, NC).

the dietary aflatoxin B1 and yeast cell wall product (Mycosorb) on immune systems of broiler chicks are shown in Table 1. In the current study, relative weights of the bursa of Fabricius and total SRBC antibody titers decreased in birds fed the AFB1-contaminated diets. Contaminated diets numerically reduced the relative weights of thymus, but the reductions were not statistically significant. The relative weight of spleen was not altered by 1 mg/kg of aflatoxin B1. The current results support the earlier work of Chen et al 2013 [25] for decreasing of the bursa of Fabricius and thymus weights. The reduction in relative weight of the bursa of Fabricius might have been due to necrosis and cellular depletion by the aflatoxins. Similarly Chen et al. 2014, [22] reported that 0.3 mg/kg of aflatoxin decreased relative weight of bursa of Fabricius and contents of serum immunoglobulin, more vacuoles and debris in the bursal lymphoid follicle, and increased percentage of apoptotic bursal cells in broilers.

In this study antibody titers against SRBC were significantly decreased in aflatoxin fed groups. Similarly Girish and Devegowda, 2006 and He et al., 2013, [26-27] reported the reduction in antibody titers during aflatoxocoses. Aflatoxin B1 cause hepatocytotoxicity by the formation of AFB1-DNA adducts [28] and can also impaired the thymocytes and B cells and induced immunosuppression. Supplementation of contaminated diets with Mycosorb did not alter antibody titers to SRBC and relative weight of spleen, thymus and bursa of Fabricius. The results are in contrast with finding by Raju and Devegowda, 2002 [29] and Girish and Devegowda, 2006, [26] who reported improvement in the weight of lymphoid organs and antibody titers against both Newcastle disease and infectious bursal disease with the supplementation of glucomannan-containing yeast product. 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.

TABLE 1. EFFECTS OF AFLATOXIN B1 (AFB1) AND YEAST CELL WALL ON IMMUNE ORGAN WEIGHTS AND SRBC ANTIBODY TITERS IN BROILER CHICKENS

Treatment		SRBC (\log_2)	Organ weight (g/100 g of BW)		
AFB1 (mg/kg)	Glucomannan (g/kg)		Spleen	Thymus	Bursa of Fabricius
0	0	5.50 a	0.15	0.75	0.18 a
0	2	4.25 a	0.13	0.78	0.16 a
1	0	3.25 b	0.18	0.57	0.10 b
1	2	3.75 ab	0.16	0.71	0.12 b
SEM		0.58	0.02	0.09	0.01
P-value		*	NS	NS	*

²Means within a column without a common superscript differ statistically ($P < 0.05$).

* $P < 0.05$

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 $P < 0.05$.

III. RESULT AND DISCUSSION

The present study was conducted to determine the effect of feeding diet naturally contaminated with aflatoxins on commercial broilers and the efficacy of yeast cell wall product to counteract the toxic effects in Iran. The effects of

IV. CONCLUSION

In conclusion, feeding aflatoxin B1 contaminated diet at levels of 1 mg/kg from d 1 to 35 had negative effect on immune systems and addition of 0.2% Glucomannan to the basal diet was not effective in preventing the adverse effects of aflatoxin.

REFERENCES

- [1] N. Chand, D. Muhammad, F.R. Durrani, M.S. Qureshi, S.S. Ullah, "Protective effects of Milk Thistle (*Silybummarianum*) against aflatoxin B in broiler chicks," *Asian-Aust. J. Anim. Sci.* vol. 24, pp. 1011-1018, 2011.
- [2] IARC, Monographs on the evaluation of carcinogenic risk to human, vol. 82. "Some Traditional Medicines, Some Mycotoxins, Naphthalene and Styrene," *International Agency for Research on Cancer, Lyon*, pp. 169-366, 2002
- [3] European Commission. 2010. Commission Regulation (EU) No. 165/2010 of 26 February 2010, amending Regulation (EC) No. 1881/2006, "setting maximum levels for certain contaminants in foodstuffs as regards aflatoxin" *Off. J. Eur. Union L* Vol. 50, pp. 8-12.
- [4] FAO, "Food safety and quality. Mycotoxins," <http://www.fao.org/food/foodsafety-quality/a-z-index/mycotoxins/en/>.2012
- [5] J. Wilkinson, D. Rood, D. Miniour, K. Guillard, M. Darre, and L. K. Silbart. "Immune response to a mucosally administered aflatoxin B1 vaccine," *Poult. Sci.* vol. 82, pp. 1565-1572, 2003 <http://dx.doi.org/10.1093/ps/82.10.1565>
- [6] E. Matur, E. Ergul, I. Akyazi, E. Eraslan, and Z. Cirakli, "The effects of *Saccharomyces cerevisiae* extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins" *Poult. Sci.* vol. 8, pp. 2213-2220, 2010 <http://dx.doi.org/10.3382/ps.2010-00821>
- [7] Kh. AliReza, M. Dakhili and H. Shokri, "A mycological survey on feed ingredients and mixed animal feeds in Ghom province, Iran," *Pakistan Journal of Nutrition* vol. 7 pp. 31-34, 2008 <http://dx.doi.org/10.3923/pjn.2008.31.34>
- [8] Magnoli C, Chiacchiera S, Miazio R, Palacio G, Angeletti A, Hallak C, Dalcerro AM, "The mycoflora and toxicity of feedstuffs from a production plant in Co'rdoba, Argentina," *Mycotoxin Res* vol. 18, pp. 7-22, 2002 <http://dx.doi.org/10.1007/BF02946135>
- [9] Z. Nematii, H. Janmohammadi, A.Taghizadeh, H. MalekiNejad, 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.
- [10] C. A. F. Oliveira, R. R. Ogido, D. Ledoux, E. George, B. C. Rottinghaus, T. A. Reis, and G. Edlayne, "The quality of eggs Japanese quail, *Coturnix japonica*, fed rations containing aflatoxin B1 and fumonisin B1," *Poult. Sci.* vol. 44, pp. 29-33, 2007 <http://dx.doi.org/10.2141/jpsa.44.29>
- [11] M. E. Fraga, F. Curvello, M. J. Gatti, L. R. Cavaglieri, A. M. Dalcerro, and C. A. da Rocha Rosa, "Potential aflatoxin and ochratoxin A production by *Aspergillus* species in poultry feed processing." *Vet. Res. Commun.* Vol. 31, pp. 343-353, 2007 <http://dx.doi.org/10.1007/s11259-006-3434-x>
- [12] D., Naicker, G. J. Marais, H. Van den Berg, and M. G. Masango, "Some fungi, zearalenone and other mycotoxins in chicken rations, stock feedstuffs, lucere and pasture grasses in the communal farming area of Rhenosterkop in South Africa." *J. S. Afr. Vet. Assoc.* vol. 78, pp. 69-74, 2007 <http://dx.doi.org/10.4102/jsava.v78i2.293>
- [13] A, Zinedine JM. Soriano, JC. Molto, J. Manes "Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An estrogenic mycotoxin," *Food Chem Toxicol* vol. 45, pp.1-18, 2007 <http://dx.doi.org/10.1016/j.fct.2006.07.030>
- [14] Y. Azarakhsh, "Incidence of the Most Common Toxicogenic *Aspergillus* Species in Broiler Feeds in Kermanshah Province, West of Iran." *Global Veterinaria* vol. 6, pp. 73-77, 2011
- [15] 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. <http://dx.doi.org/10.1053/rvsc.2000.0395>
- [16] N. K. S. Gowda, D. R. Ledoux, G. E. Rottinghaus, A. J. Bermudez, and Y. C. Chen. "Efficacy of turmeric (*Curcuma longa*), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks" *Poult. Sci.* Vol. 87, pp. 1125-1130, 2008. <http://dx.doi.org/10.3382/ps.2007-00313>
- [17] H. N., Bilgic, and T. Yesildere, "Renal lesions on experimental aflatoxicosis in chickens." *I. U. Vet. Fak. Derg.* 18:102-108, 1992
- [18] T.W. Kensler, B.D. Roebuck, G.N. Wogan, J.D., Groopman, "Aflatoxin: a 50- year odyssey of mechanistic and translational toxicology," *Toxicol. Sci.* vol. 120, pp. 28-48, 2011. <http://dx.doi.org/10.1093/toxsci/kfq283>
- [19] Bintvihok , A., and S. Kositcharoenkul, "Effect of dietary calcium propionate on performance, hepatic enzyme activities and flatoxin residues in broilers fed a diet containing low levels of aflatoxin B1," *Toxicol* vol. 47, pp. 41-46, 2006 <http://dx.doi.org/10.1016/j.toxicol.2005.09.009>
- [20] Glick, B., The bursa of fabricius and immunoglobulin synthesis. *Int. Rev. Cytol.* Vol. 48, pp. 345, 1977 [http://dx.doi.org/10.1016/S0074-7696\(08\)61749-0](http://dx.doi.org/10.1016/S0074-7696(08)61749-0)
- [21] W.L. Welles, J.R., Battisto, "The function of the spleen in immune responses and its influence on other lymphoid organs. In: Gershwin, M.E., Cooper, E.L. (Eds.), *Animal Models of Comparative and Developmental Aspects of Immunity and Disease*," Pergamon Press, New York., pp. 236-249, 1978
- [22] X. Chen, N. Horn, P. F. Cotter and T. J. Applegate, "Growth, serum biochemistry, complement activity, and liver gene expression responses of Pekin ducklings to graded levels of cultured aflatoxin B1," *Poult. Sci.* 2014 <http://dx.doi.org/10.3382/ps.2014-03904>
- [23] L. W. Whitlow, and W. M. Hagler, "Mycotoxins in feeds," *Feedstuffs* vol. 77 pp.69-79, 2005
- [24] E. Kotan, L. Alpsoy, M. Anar, A. Aslan, G. Aga, "Protective role of methanol extract of *Cetraria islandica* (L.) against oxidative stress and genotoxic effects of AFB1 in human lymphocytes in vitro," *Toxicol Ind. Health* vol. 27, pp. 599-60, 2011. <http://dx.doi.org/10.1177/0748233710394234>
- [25] K. Chen, Gang Shu, Xi Peng, Jing Fang, Hengmin Cui, Jin Chen, Fengyuan Wang, Zhengli Chen, Zhicai Zuo, Junliang Deng, Yi Geng, Weimin Lai Protective role of sodium selenite on histopathological lesions, decreased T-cell subsets and increased apoptosis of thymus in broilers intoxicated with aflatoxin B1" *Food and Chemical Toxicology* 59 (2013) 446-454 <http://dx.doi.org/10.1016/j.fct.2013.06.032>
- [26] C. K. Girish and G. Devegowda, "Efficacy of Glucomannan containing Yeast Product (Mycosorb) and Hydrated Sodium Calcium Aluminosilicate in Preventing the Individual and Combined Toxicity of Aflatoxin and T-2 Toxin in Commercial Broilers." *Asian-Aust. J. Anim. Sci.* Vol. 19, pp. 877-883, 2006
- [27] J. He, K.Y. Zhang, D.W. Peng, Jing Fang, 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 B1," *Livestock Science I*, vol. 52, pp. 200-207, 2013
- [28] A. Yiannikourisand, J. P. Jouany, "Mycotoxins in feeds and their fate in animals: A review," *Anim. Res.* Vol. 51, pp. 81-99,2002. <http://dx.doi.org/10.1051/animres:2002012>
- [29] M. V. L. N. Raju, and G. Devegowda, "Esterified glucomannan in broiler chicken diets contaminated with aflatoxin, ochratoxin and T-2 toxin: Evaluation of its binding ability (in vitro) and Efficacy as immunomodulator," *AsianAust. J. Anim.Sci.* vol.15 pp.1051-1056, 2002