



## ORIGINAL ARTICLES

### Counteractive Effects of Mycotoxin Adsorbent and *Aspergillus parasiticus* on Broilers Performance Traits

Milad Manafi

Department of Animal Science, Chaloos Branch, Islamic Azad University, Chaloos, Iran.

---

#### ABSTRACT

Aflatoxin (AF) (0.5ppm) and a commercial Mycotoxin Adsorbent (0.5, 0.75 and 1%) were tested in an *in vivo* study forming 8 dietary treatments each with three replicates on a total of 336 on broiler chicks up to five weeks. Results showed that chicks receiving AF contaminated feed had suppressed body weight, which significantly improved with inclusion of Adsorbent. Supplementation of Adsorbent at 0.75 and 1% to the diets containing AF significantly (9.97 and 9.15%, respectively) improved feed consumption. Efficiency of feed utilization decreased significantly with addition of 0.5 pm AF, improved with inclusion of Adsorbent. The serum antibody titers against ND and IBD vaccination which were significantly depressed by AF, were restored with the inclusion of 1% Adsorbent. The serum concentration of total protein (38.37%) uric acid and albumin were not affected either in AF fed or Adsorbent supplemented groups. The activity of serum GGT significantly increased in AF fed group and the addition of Adsorbent did not show significant reduction in activity of serum GGT. Compared with control, activity of serum ALT was not affected either in AF, control or Adsorbent supplemented groups.

**Key words:** Aflatoxin, broilers, performance.

---

#### Introduction

Cereal grains and associated by-products constitute important sources of energy for poultry. There is increasing evidence that global supplies of cereal grains for animal feedstuffs are commonly contaminated with mycotoxins. Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species. Aflatoxin B1 (AFB1), the most toxic of all aflatoxins (AFB1, AFB2, AFG1 and AFG2), is produced by certain strains of fungi in greater quantities than in others. In poultry, aflatoxin ingestion leads to "Aflatoxicosis" syndrome which is characterized by retardation of growth, feed consumption, feed conversion efficiency, bruising, immunosuppression and mortality. Co-contamination of cereal grains with mycotoxins produced by different fungal genera, including *Fusarium* and *Aspergillus* has been reported to increase the toxicity symptoms in poultry (Hagler *et al.*, 1984).

At present, one of the more encouraging approaches is the addition of non-nutritive and natural adsorbent materials to contaminated feed in order to selectively bind the mycotoxin during the digestive process and make it harmless to the feed. The major advantages of these adsorbents include low cost, safety and the ease with which they can be added to animal feed. Layered amino silicates such as sodium bentonite found to be effective in counteracting mycotoxins (Unsworth *et al.*, 1989; Smith and Ross, 1991; Hagler *et al.*, 1992; Santurio *et al.*, 1999; Rosa *et al.*, 2001; Eralsan *et al.*, 2005). However, the ability of bentonite to bind mycotoxins depends on pH, molecular arrangements and its geographic region of origin (Vieira, 2003). Considering all these facts, the present study undertaken to investigate the ability of graded levels of Adsorbent to counteract the toxic effects of aflatoxin broilers.

#### Materials And Methods

##### *Experimental animals and design:*

Three hundred and thirty six, unsexed one-day old commercial broiler chicks were wing banded, weighed and assigned to a 4X2 factorial arrangement of two levels of Aflatoxin AF (0 and 0.5ppm) and four levels Adsorbent (0, 0.5, 0.75 and 1%) in a Completely Randomized Design manner, forming a total of 8 dietary treatments each with 3 replicates.

##### *Experimental housing, management and test diet:*

---

**Corresponding Author:** Milad Manafi, Department of Animal Science, Chaloos Branch, Islamic Azad University, Chaloos, Iran.  
E-mail: manafi\_milad@yahoo.com

Each replicate group of chicks was housed in an independent pen in an open sided deep litter conventional house. Chicks in all the replicate groups were reared up to five weeks of age under uniform standard conditions throughout the study. Brooding was done until three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided continuous light throughout the study.

Aflatoxin was produced using the pure culture of *Aspergillus parasiticus* MTCC 411 grown on potato dextrose agar. Then toxin produced on rice was then extracted as described by Romer (1975) and quantified by thin layer chromatography (TLC) as described by A.O.A.C. (1995).

The experimental diets were prepared by the addition of required quantities of rice containing aflatoxin to arrive at the levels of 0 and 0.5ppm of aflatoxin B<sub>1</sub>. To each of these diets, Adsorbent was added at 0, 0.5, 0.75 and 1% levels.

Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-3 wks) and finisher (4-5 wks) phases. Chicks were provided *ad libitum* supply of feed and water throughout the study. Feeding of test diets commenced at zero day of age and continued until the termination of the experiment at five weeks of age. Chicks were vaccinated against Newcastle Disease (ND) on the 7<sup>th</sup> day using F<sub>1</sub> strain (Ventri's Biologicals, Bangalore) and against Infectious Bursal Disease (IBD) on the 14<sup>th</sup> day using intermediate strain (Ventri's Biologicals, Bangalore). Both vaccines were given via the ocular route.

#### Data collection:

At the end of the trials, body weight, feed consumption and mortality, if any were recorded and gain in weight and feed efficiency were calculated. Six birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial. Blood was collected in non-heparinized tubes from six birds in each treatment (3 males and 3 females) by puncturing the brachial vein during the 5th week of age. Serum was separated after 8 to 10 hours as per the standard procedures (Calnek *et al.*, 1992) and was stored at -20 °C for subsequent analysis. The individual serum samples were analyzed for total protein, serum albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) using an automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan), antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) using ELISA technique.

#### Statistical analysis :

The experimental data were analyzed statistically by using the General Linear Model procedure of the Statistical Analysis System (SAS<sup>®</sup>) software (SAS Institute, USA, 2000). Overall data were analyzed by repeated measures design. The Duncan multiple range test was used to compare means (Duncan, 1955). The result of this study was subjected to one way ANOVA test.

### Results And Discussion

Body weight, feed consumption, feed conversion ratio and mortality data for broilers fed control and different experimental diets at fifth week of age are presented in Table1. Chicks receiving AF contaminated feed had significantly ( $P<0.05$ ) suppressed body weight, feed consumption and efficiency of feed utilization compared to chicks fed the control diet. Supplementary feeding of Adsorbent at 0.75 and 1.00 per cent to the diets containing AF significantly ( $P<0.05$ ) improved body weight and feed consumption when compared to the toxin control diet and it remained non significant with the control diet. Efficiency of feed utilization which was decreased significantly with addition of 0.5ppm AF, was improved with inclusion of 0.75 and 1.00 per cent Adsorbent. High mortality rate of 14.20 per cent was observed in the group fed with diet containing 0.5ppm AF. Mortality rate was reduced considerably in the groups supplemented with 0.5, 0.75 and no mortality in 1.00 per cent Adsorbent fed chicks.

The decreased body weight, feed consumption and increased feed conversion ratio due to AF are consistent with the findings of Swamy and Devegowda (1998); Raju and Devegowda (2000); Arvind *et al.* (2003); and Girish *et al.* (2004). The growth depression effects of AF may be due to their inhibitory action on protein synthesis and nutrient utilization (Marquardt and Frohlich, 1992). Addition of Adsorbent at graded levels (0.5, 0.75 and 1per cent) to control diet did not affect body weight and feed consumption in broilers. Feed conversion ratio was significantly ( $P<0.05$ ) superior in birds given either 0.75 or 1 per cent Adsorbent. The results indicated that the naturally occurring sorbent used in the study is inert and non toxic. Kurnick and Reid (1989) reported similar results. The results suggest a beneficial effect of addition of Adsorbent in the presence of AF on growth performance.

The effect of Adsorbent supplementation on the diets containing AF on the antibody titers against New Castle Disease (ND) and Infectious Bursal Disease (IBD), serum protein, serum albumin, uric acid, the activities

of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) are presented in Table 2. A significant ( $P < 0.05$ ) decrease in antibody titer values against ND and IBD vaccine was observed upon feeding AF. This depression in titer values is a clear indication of immunodepressing effects of AF on humoral antibody response. These findings agree with the previous reports (Umesh *et al.*, 1990; Devegowda *et al.*, 1994; Swamy and Devegowda, 1998; Ibrahim *et al.*, 2000; Kumar *et al.*, 2002; Gupta *et al.*, 2003). The reduction of antibody titers could be due to inhibition of DNA and protein synthesis by aflatoxin through impairment of amino acid transport and m-RNA transcription, resulting in lowered level of antibody production (Thaxton *et al.*, 1974).

Addition of graded levels of Adsorbent alone to control diets did not alter antibody titers against ND and IBD at five weeks of age as compared to control, whereas addition of Adsorbent to diets containing AF significantly ( $P < 0.05$ ) improved the antibody titers against ND and IBD vaccine when compared to their respective controls. The results demonstrated the protective effects of Adsorbent at 1.00 per cent inclusion to AF diet in chickens. These findings were comparable to the reports of Daoud (2002).

The serum concentration of total protein which was significantly ( $P < 0.05$ ) decreased by AF, was elevated to normal level with the inclusion of 1.00 per cent Adsorbent. Compared with the control treatment, serum concentrations of uric acid and albumin were not affected either in AF fed group or Adsorbent supplemented groups.

**Table 1:** Effect of Adsorbent on fifth week body weight, feed consumption, feed conversion ratio and mortality of broilers fed aflatoxin.

AF (ppm)	Adsorbent (%)	Body weight (g)	Feed consumption (g/bird)	Feed Conversion Ratio	Mortality (%)
0	0	1313.4±0.88 <sup>a</sup>	2513.1±6.07 <sup>a</sup>	1.91±0.00 <sup>c</sup>	4.70
0.5	0	1180±7.49 <sup>c</sup>	2306±2.92 <sup>b</sup>	2.09±0.005 <sup>a</sup>	14.20
0	0.5	1314±11.11 <sup>a</sup>	2505±10.3 <sup>a</sup>	1.90±0.008 <sup>d</sup>	0
0	0.75	1326±4.91 <sup>a</sup>	2501±8.13 <sup>a</sup>	1.88±0.006 <sup>de</sup>	0
0	1.0	1339±10.07 <sup>a</sup>	2495±14.5 <sup>a</sup>	1.86±0.003 <sup>e</sup>	0
0.5	0.5	1202±8.81 <sup>c</sup>	2305±2.89 <sup>b</sup>	2.05±0.006 <sup>b</sup>	7.10
0.5	0.75	1240±6.35 <sup>b</sup>	2517±14.63 <sup>a</sup>	2.03±0.005 <sup>b</sup>	4.70
0.5	1.0	1274±0.73 <sup>b</sup>	2536±7.77 <sup>a</sup>	1.99±0.005 <sup>a</sup>	0

Means bearing at least one common superscript in a column do not differ significantly ( $P < 0.05$ )

**Table 2:** Effect of Adsorbent on the antibody titers against New Castle Disease (ND) and Infectious Bursal Disease (IBD), serum protein, serum albumin, uric acid, the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) in broilers fed aflatoxin.

AF (ppm)	Adsorbent (%)	ND titer	IBD titer	Serum protein (g%)	Serum Albumin (g%)	Uric acid (µg/dl)	GGT (IU/L)	ALT (IU/L)
0	0	4297.7±17.05 <sup>ab</sup>	4281.0±8.08 <sup>a</sup>	2.72±0.18 <sup>a</sup>	1.28±0.17 <sup>a</sup>	647.9±7.54 <sup>a</sup>	9.53±1.15 <sup>d</sup>	28.17±0.60 <sup>a</sup>
0.5	0	3204±106.3 <sup>c</sup>	3149±69.72 <sup>d</sup>	1.67±0.15 <sup>bc</sup>	1.10±0.18 <sup>a</sup>	600.4±6.73 <sup>a</sup>	17.8±1.72 <sup>ab</sup>	25.83±1.36 <sup>e</sup>
0	0.5	4018±119.2 <sup>bc</sup>	4252±21.79 <sup>a</sup>	2.43±0.23 <sup>abc</sup>	1.23±0.06 <sup>a</sup>	610.6±0.69 <sup>a</sup>	11.65±0.37 <sup>bcd</sup>	25.07±1.47 <sup>a</sup>
0	0.75	4305±93.19 <sup>ab</sup>	4329±25.48 <sup>a</sup>	2.51±0.20 <sup>ab</sup>	1.26±0.07 <sup>a</sup>	629.0±2.02 <sup>a</sup>	11.65±0.14 <sup>bcd</sup>	28.6±1.62 <sup>a</sup>
0	1.0	4418±56.72 <sup>a</sup>	4378±26.74 <sup>a</sup>	2.72±0.15 <sup>a</sup>	1.36±0.06 <sup>a</sup>	653.6±3.01 <sup>a</sup>	10.6±0.96 <sup>cd</sup>	29.67±2.34 <sup>a</sup>
0.5	0.5	3582±30.19 <sup>d</sup>	3352±73.59 <sup>cd</sup>	1.66±0.11 <sup>bc</sup>	1.14±0.17 <sup>a</sup>	614±34.09 <sup>a</sup>	22.57±2.16 <sup>a</sup>	27.73±0.34 <sup>a</sup>
0.5	0.75	3797±10.73 <sup>cd</sup>	3694±73.64 <sup>bc</sup>	1.61±0.15 <sup>c</sup>	1.19±0.17 <sup>a</sup>	610.6±3.00 <sup>a</sup>	17.47±2.25 <sup>ab</sup>	28.67±0.14 <sup>a</sup>
0.5	1.0	4225±78.83 <sup>ab</sup>	4046±182.3 <sup>ab</sup>	2.57±0.22 <sup>a</sup>	1.27±0.37 <sup>a</sup>	636.3±6.98 <sup>a</sup>	13.7±1.01 <sup>bcd</sup>	28.87±0.49 <sup>a</sup>

Means bearing at least one common superscript in a column do not differ significantly ( $P < 0.05$ )

The activity of serum GGT significantly ( $P < 0.05$ ) increased in AF fed group. The addition of Adsorbent to AF containing diet did not show significant reduction in the activity of serum GGT. Compared with the control, activity of serum ALT was not affected either in AF fed group or control, Adsorbent supplemented groups.

It maybe concluded that Adsorbent is partially effective in counteracting the adverse effects of aflatoxin in broilers. Among the various levels of Adsorbent, 1.00 per cent showed the best level against aflatoxicosis in broilers.

## References

- AOAC, 1995. Official Methods of Analysis. 16<sup>th</sup> Ed., Association of Official Analytical Chemists, Washington, D.C.
- Arvind, K.L., V.S. Patil, G. Devegowda, B. Umakantha, S.P. Ganpule, 2003. Efficacy of Esterified glucomannan to counteract Mycotoxicosis in naturally contaminated feed on performance, serum biochemical and hematological parameters in broilers. *Poult. Sci.*, 82: 571-576.
- Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid, H.W. yoder Jr. 1992, Diseases of Poultry, 9<sup>th</sup> Edn., Wolfe Publishing, Ltd., USA.

- Chaturvedi, V.B., K.S. Singh, 2004. Effect of Aflatoxin and hydrated sodium bentonite on nutrient utilization in chickens. *Animal Nutrition and Feed Technology*, 4: 187-195.
- Daoud, A.Z., 2002. Amelioration of Na-bentonite of Aflatoxin depressive effect on the antibody protection after new castle disease vaccination in broilers. *Iraqi Jour.of Vety.Science*, 16: 161-165.
- Devegowda, G., B.I.R. Arvind, K. Rajendra, M.G. Morton, A. Baburathna, C. undareshan, 1994. A biological approach to counteract aflatoxicosis in broiler chickens and ducklings by the use of *Saccharomyces cerevisiae* cultures added to feed. In: *Biotechnology in the Feed Industry*. Eds. T.P. Lyons and K.A. Jacques. Proc. Altech's 10<sup>th</sup> Ann. Symp., Ky, USA, pp: 235-245.
- Duncan, D.B., 1955. Multiple range and multiple F tests. *Biometrics.*, 11: 1-42.
- Eralsan, G., D. Essz, M. Akdogan, F. Sahindokuyucu, L. Altrintas, 2005. The effects of aflatoxin and sodium bentonite and alone on some blood electrolyte levels in broiler chickens. *Turk Veterinerlik ve Hayvanclık Dergisi.*, 29: 601-605.
- Girish, C.K., G. Devegowda, 2004. Evaluation of modifies glucomannan (Mycosorb) and HSCAS to ameliorate the individual and combined toxicity of aflatoxin and T-2 toxin in broiler chickens. *Aust. Poult. Sci. Symp.* 16: 126-129. Sydney, Australia.
- Gupta, K., Amarjit Singh, 2003. Experimental studies on aflatoxicosis, infectious bursal disease and their interaction in broiler chicks. *Ind. J. Vety. Pathology*, 27: 5-7.
- Hagler, W.M., K. Tyczkowska, P.B. Hamilton, 1984. Simultaneous occurrence of deoxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from Midwestern United States. *Appl. Env. Microbiol.*, 47: 151-154.
- Hagler, W.M. Jr, J.L. Grimes, Fairchild, 1992. Effects of Astra-Ben 20<sup>®</sup> on broiler chicks exposed to AFB<sub>1</sub> or T-2 toxin. *North Carolina State University. (poult. Abstr., 16: 123).*
- Ibrahim, I.K., A.M. Shareef, K.M.T. Joubory, 2000. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. *Research in Vety.Science*, 69(2): 119-122; 35 ref.
- Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Carrier, T.D. Phillips and G.E. Rottinghaus, 1990. Efficacy of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. *Poult. Sci.*, 69: 1078-1086.
- Kumar, M.R., H.S.A. Kumar, B. Kalakumar, K.S. Reddy, 2002. Effect of Zeetress on Aflatoxin induced humoral immunosuppression in broiler chicks. *Ind.Journ.of Comparative Microbiology and Infectious Disease*, 23: 203-204.
- Kurnick, A.A., B.K. Reid, 1989. Poultry nutrition studies with bentonite. *Feedstuffs*, 32: 34.
- Marquardt, D.R., A. Frohlich, 1992. A review of recent advances in understanding ochratoxicosis. *J.Anim. Sci.*, 70: 3968-3988.
- Miazzo, R., M.F. Pevalta, C. Magnoli, M. Salvano, S. Ferrero, S.M. Chiacchiera, E.C.Q. Carralno, C.A.R. Rosa, A. Dalcerro, 2005. Efficacy of sodium bentonite as a detoxifier of broiler fed contaminated with aflatoxin and fumonisin. *Poult. Sci.*, 84:1-8.
- Perozo, F., S. Rivera, 2003. Effect of Aflatoxin B<sub>1</sub> exposure and selenium supplementation on immune response in broilers. *Ind. Vety. Journal.*, 80: 1218-1221.
- Pier, A.C., McLaughlin, 1985. Mycotoxic suppression of immunity. In: *Trichothecenes and Other Mycotocins*. Ed. J. Lacey, Wiley and Sons, Chichester, pp: 507-519.
- Raju, M.V.L.N. and G. Devegowda, 2000. Influence of esterified glucomannan on performance and organic morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). *Br. Poult. Sci.*, 41: 640-650.
- Raju, M.V.L.N., G. Devegowda, 2002. 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. *Asian-Aust.J.Anim. Sci.*, 15: 1051-1056.
- Romer, T.R., 1975. Screening method for the detection of aflatoxins in mixed feeds and other agricultural commodities with subsequent confirmation and quantitative measurement of aflatoxins in positive samples. *J.A.O.A.C.*, 58: 500-506.
- Rosa, C.A.R., R. Miazoo, C. Magnoli, M. Salvano, S.M. Chicchiera, S. Ferrero, M. Saenz, E.C.Q. Carvalho, A. Dalcerro, 2001. Evaluation of the efficacy of bentonite from the south Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Janerio Institute de Veterrinaria, Brazil.*, 2001.
- Santurio, J.M., C.A. Mallmann, A.P. Rosa, G. Appel, A. Heer, S. Dageforde, M. Bottcher, 1999. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxin. *Br. Poult. Sci.*, 40: 115-119.
- SAS Institute, 2000. SAS<sup>®</sup> User's Guide: Statistics. Version & Edition. SAS Institute Inc., Cary, NC, USA.
- Shaline KSB, Howrth Jr B, Wyatt RD (1980). Effect of dietary aflatoxin in reproductive performance on mature white leghorn. *Poult. Sci.*, 59: 1311-1315.
- Smith, J.E., K. Ross, 1991. The toxigenic *Aspergilli*. In: *Mycotoxins and Animal Foods*, Eds: J.E. Smith and Henderson, CRC Press, Boca Raton, pp: 101-118.

- Swamy, H.V.L.N., G. Devegowda, 1998. Ability of mycosorb to counteract aflatoxicosis in commercial broilers. *Indian J. Poul. Sci.*, 33: 273-278.
- Thaxton, J.P., J.T. Timb, B. Jaotpm, 1974. Immunosuppression in chicken by aflatoxin. *Poult. Sci.*, 53: 721-725.
- Unsworth, E.F., J. Pearce, C.H. McMurray, B.W. Moss, F.J. Gordon, D. Rice, 1989. Investigations of the use of clay minerals and Prussian Blue in reducing the transfer of dietary radiocesium to milk. *Sci Total Environ*, 85: 339-347.
- Umesh, D., G. Devegowda, B.S. Barmase, 1990. Reducing adverse effects of aflatoxin through nutrition in broiler chickens. *Proc. 13<sup>th</sup> Annual Conf. Symp. Indian Poul. Sci. Assn., Bombay, PHME 50 (Abstr.)*.
- Vieira, S.L., 2003. Nutritional implication of mould development in feed stuffs and alternatives to reduce the mycotoxin problem in poultry feeds. *World's Poultry Science Journal*, 59: 111-122.