

## Effects of Addition of a Mycotoxin Detoxifier in Poultry Feed Containing Different Levels of Aflatoxins on the Performance of Broilers

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**ABSTRACT :** Effects of addition of a mycotoxin detoxifier in poultry feed were studied in broilers. Aflatoxins were present in the poultry feed as 28 ppb (normal feed), 78 ppb (contaminated feed) and 170 ppb (highly contaminated feed). The mycotoxin detoxifier was used in 3 concentrations i.e. 1, 3 and 5 kg/ton of feed. Aflatoxins reduced the body weight in broiler chicken and treatment of contaminated feed with low level of detoxifier improved the body weight equivalent to that of normal feed. Higher level of detoxifier proved better than lower level addition in alleviating the effects of highly contaminated feed. Addition of detoxifier also resulted in improvement of FCR to the level of normal feed. Antibody levels against Newcastle disease virus on day 28 of age were significantly lower in chicken fed on contaminated feed. Addition of detoxifier in feed improved the antibody levels in chicken. Mortality was highest in groups given contaminated feed throughout the study period of 7 weeks. Significant mortality was also observed in groups given highly contaminated feed for 2 weeks. Mortality in chicken given detoxifier added contaminated feed was lowest and similar to the group given normal feed. The study shows that mycotoxin detoxifier containing oxyquinol, dichloro-thymol and micronized yeast can effectively neutralize the ill-effects of aflatoxins in poultry feed. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 7 : 990-994)

**Key Words :** Mycotoxin Detoxifier, Aflatoxins, Performance, Broilers, Antibody Titers

### INTRODUCTION

Aflatoxins are the most important mycotoxins constituting a considerable health hazard for commercial poultry. Cases of acute aflatoxin poisoning have been reported worldwide (Heathcote and Hibbert, 1978). However, these cases represent a small percentage of losses caused by aflatoxins since majority of cases are sub-clinical. These sub-clinical levels of aflatoxins not only reduce production performance of birds (Howorth and Wyatt, 1976; Verma et al., 2003) but also cause immunosuppression resulting in susceptibility to infections (Sims et al., 1970; Sohane and Chaturvedi, 2001). Aflatoxins have been reported from various animal feedstuffs in Pakistan (Afzal et al., 1979; Chaudhry et al., 1981; Bhatti et al., 2001) and their involvement in disease and mortality among commercial poultry has also been documented (Siddique et al., 1987; Sabri et al., 1989; Anjum, 1990; Azim et al., 1990).

The best strategy to avoid the build-up of aflatoxins in feedstuffs is to avoid contamination with aflatoxin producing fungi both in the field and during harvesting and storage (Heathcote and Hibbert, 1978). However, it is a difficult proposition particularly in developing countries where harvesting and storage conditions are far below the required standards. There are many methods that can be used to neutralize ill-effects of aflatoxins in the poultry feeds. Addition of organic acids on inert support (e.g. vermiculite, zeolite, etc.) in feed results in fungistatic

actions and thus reducing the chances of aflatoxin production (Heathcote and Hibbert, 1978; Piva et al., 2000). Aflatoxins in feedstuffs can be degraded and destroyed by the use of oxidizing agents, chlorinating agents, organic and inorganic acids and alkalis (Heathcote and Hibbert, 1978; Sohane and Chaturvedi, 2001). However, most of these chemicals are unsafe to use and impractical. Detoxification of aflatoxins in the feed has been achieved commercially by ammonia treatment (Neal et al., 2001). However, this proposition is not suitable for the developing countries due to technology adoption problems and higher initial cost. Absorption of aflatoxins from feed can be effectively achieved by substances like bentonite, zeolite, hydrated calcium sodium aluminosilicate, kaolin, etc. or synthetic ion exchange resins (Piva et al., 2000; Rosa et al., 2001; Tozaki, 2001) but these substances show poor efficacy when contamination is large and also absorb vitamins and other nutrients from the feed resulting in poor feed conversion ratio. Enzymes and microorganisms can also degrade aflatoxins (Liu et al., 2001; Suman et al., 2001; Raju and Devegowda, 2002; Byun and Yoon, 2003) but cost is prohibitive and treatment methods have still not been developed.

Mycotox<sup>®</sup> is a medicated premix for the treatment of mycotoxicosis in poultry. It contains oxyquinol, dichloro-thymol and micronized yeast. All these ingredients are known to have antifungal and antimycotoxin properties. The present study was designed to determine the detoxifying effect of the mycotox on aflatoxins in poultry feed and its effects on the productive performance of the commercial broilers.

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**Table 1.** Composition of broiler starter and finisher feed used in the study

Description	Starter feed	Finisher feed
<b>Ingredients (%)</b>		
Corn	50.00	56.00
Rice polish	10.00	12.00
Corn gluten meal 60%	2.00	1.00
Rapeseed meal	3.00	2.00
Canola meal	8.00	6.00
Guar meal	3.00	3.00
Sunflower meal	3.00	2.00
Soybean meal	9.10	8.10
Fish meal	6.00	4.00
Molasses	3.00	3.00
Marble chips	1.00	1.00
Dicalcium phosphate	0.91	0.91
L-lysine	0.27	0.27
DL-methionine	0.08	0.08
Sodium bicarbonate	0.15	0.15
Vitamin mineral premix	0.50	0.50
<b>Chemical composition</b>		
Crude protein %	21.00	18.50
ME, kcal/kg	2,900	3,000
Calcium, %	0.90	0.90
Phosphorus, %	0.45	0.45
Lysine, %	1.00	1.00
Methionine, %	0.45	0.45
Fibre, %	4.50	4.50

Note: Different aflatoxin levels in diets were achieved by including batches of contaminated corn having various levels of aflatoxins

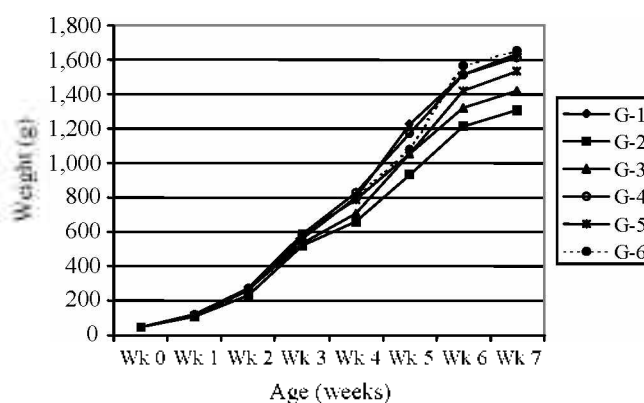
## MATERIALS AND METHODS

### Experimental birds

Three hundred hatched broiler chicks were purchased from a commercial hatchery (Islamabad Poultry Breeders, Rawalpindi). These birds were randomly divided into 6 groups of 50 each and kept in separate pens. The birds were reared under electric brooders. The birds were kept on floor and given starter feed for first 4 weeks and finisher ration for remaining 3 weeks. Feed and water were offered *ad lib* throughout 7 weeks of study period.

### Poultry feeds

Three poultry feeds were used in the experiment. All feeds were isonitrogenous (starter CP 21%, finisher CP 18.5%) and isocaloric (starter ME 2,900 kcal/kg, finisher ME 3,000 kcal/kg) and only varied in the aflatoxin contents. Aflatoxin contents in the feed were varied by incorporating different batches of corn having different levels of aflatoxins. Feed composition is shown in the Table 1. Feeds contained different levels of aflatoxins and were classified as normal feed (containing 28 ppb aflatoxin), contaminated feed (78 ppb of aflatoxins) and highly contaminated feed (containing 170 ppb aflatoxins).



**Figure 1.** Weight of chicken given various levels of aflatoxins and mycotoxin detoxifier. G-1 represents chicken given normal feed throughout 7 weeks; G-2 represents chicken given contaminated feed throughout 7 weeks; G-3 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7; G-4 represents chicken given contaminated feed added with Mycotox (1 kg/ton) throughout 7 weeks; G-5 represents chicken given normal feed for first 2 weeks, highly contaminated feed for week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (3 kg/ton), and G-6 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (5 kg/ton).

### Experimental protocol

Chickens of various groups were given the following feeding treatments:

- Group 1: Chicks were given normal feed throughout study period.
- Group 2: Chicks were given aflatoxin contaminated feed throughout 7 weeks study period.
- Group 3: Chicks were given normal feed for first 2 weeks, highly contaminated feed for next 2 weeks and then normal feed for the rest of study period.
- Group 4: Chicks were given mycotox<sup>®</sup> (1 kg/ton) mixed contaminated feed throughout 7 weeks of study period.
- Group 5: Chicks were given normal feed for first 2 weeks, highly contaminated feed for next 2 weeks and then mycotox mixed (3 kg/ton) normal feed for remaining 3 weeks.
- Group 6: Chicks were given normal feed for first 2 weeks, highly contaminated feed for next 2 weeks followed by mycotox mixed (5 kg/ton) normal feed for remaining 3 weeks.

Vaccination schedule was uniform for all groups and included Newcastle disease (ND) vaccine on day 5, infectious bursal disease vaccine on day 12, hydropericardium syndrome vaccine on day 18 and ND on day 21 of age.

Randomly selected 10 birds from each group were

weighed weekly. At the end of the experiment all birds were weighed. Blood samples were collected from 7 birds from each group at the age of day 5, 15 and 28 of age for determining the antibody titres against ND. Serological titres against ND were determined using a commercial ELISA Kit (IDDEX) and titers were expressed as geomeans.

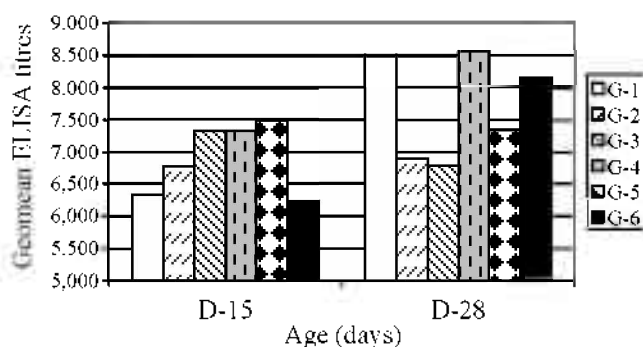
The data were analyzed statistically using computer package SPSS.

## RESULTS

Body weight of the birds was measured weekly and is shown in Figure 1. Chickens given contaminated feed throughout the experiment (group 2) had the lowest weight gain followed by chicken belonging to group 3 which were given highly contaminated feed during 3<sup>rd</sup> and 4<sup>th</sup> week. Treatment of contaminated feed with mycotoxin detoxifier resulted in the improvement of weight gain. Body weight of chicken given detoxifier mixed contaminated feed (group 4) for 7 weeks was similar to chicks given normal feed (group 1). Higher levels of mycotoxin detoxifier (5 kg/ton) proved better than lower level (3 kg/ton) in alleviating the effects of highly contaminated feed.

FCR was similar in groups 1, 4 and 6 showing that mycotoxin detoxifier did not increase the FCR (Table 2). FCR was higher in groups given contaminated feeds but no detoxifier when compared with group given normal feed. Dressing percentage (without skin and giblets) did not differ among various groups and ranged from 56.73 to 58.77%.

The broiler chicks had good maternal antibody titres as on day 5 geomean ELISA titre against ND was 6,491. At 15 days of age, aflatoxins at lower level did not seem to depress immune response to ND and birds of all six groups had good immune response and geomean ELISA titres varied from 6,235 in group 6 to 7,484 in group 5. However, at 28 days of age antibody levels against ND were significantly lower in chicken given aflatoxin contaminated feed than those given detoxifier treated contaminated feed



**Figure 2.** Antibody titres of chicken given various levels of aflatoxins and mycotoxin detoxifier against Newcastle Disease virus. The figure shows antibody titres on day 15 and day 28 of the age of chicken. Each bar shows geomean titre of 7 chicken. G-1 represents chicken given normal feed throughout 7 weeks; G-2 represents chicken given contaminated feed throughout 7 weeks, G-3 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7; G-4 represents chicken given contaminated feed added with Mycotox (1 kg/ton) throughout 7 weeks; G-5 represents chicken given normal feed for first 2 weeks, highly contaminated feed for week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (3 kg/ton), and G-6 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (5 kg/ton).

or normal feed (Figure 2). Detoxifier given in higher dose (5 kg/ton) after feeding highly contaminated feed for 2 weeks (group 6) resulted in better humoral antibody titre than those given feed containing medium level (3 kg/ton) of detoxifier (group 5).

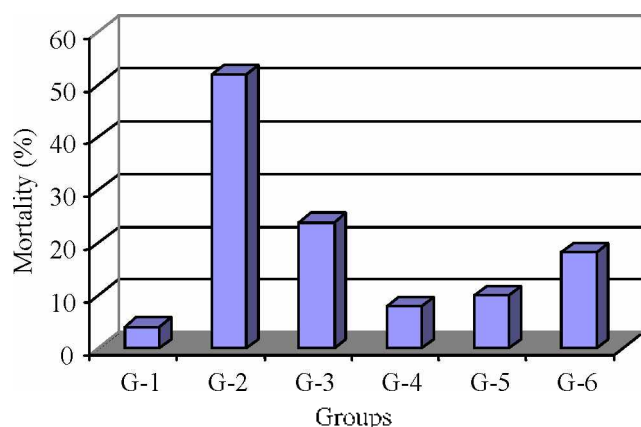
Mortality was highest in group 2 which was given contaminated feed throughout 7 weeks and it reached 52% by the end of the experiment (Figure 3). In fact in this group an outbreak of infectious bursal disease was seen in 4<sup>th</sup> week of age which resulted in heavy mortality in 5<sup>th</sup> week. This outbreak spread to all other groups also. Significant mortality was also recorded in group 3 which was given highly contaminated feed for 2 weeks. Mortality was lowest in group given normal feed. Mortality did not differ

**Table 2.** Body weight, feed conversion ratio (FCR) and dressing percentage of broilers given various treatments of aflatoxins and mycotox

Group	Treatment (ad)	Final body weight (kg)	FCR	Dressing*, % age
1	Normal feed	1,633 <sup>ab</sup>	2.47 <sup>c</sup>	58.53
2	Contaminated feed for 7 weeks	1,306 <sup>c</sup>	2.72 <sup>a</sup>	57.90
3	Normal feed for 2 weeks, highly contaminated feed for week 3 and 4 and normal feed for week 5, 6 and 7	1,421 <sup>d</sup>	2.77 <sup>a</sup>	57.70
4	Contaminated feed with Mycotox (1 kg/ton) for 7 weeks	1,611 <sup>b</sup>	2.47 <sup>c</sup>	56.73
5	Normal feed for 2 weeks, highly contaminated feed for week 3 and 4 and normal feed with Mycotox (3 kg/ton) for week 5, 6 and 7	1,532 <sup>c</sup>	2.60 <sup>b</sup>	58.77
6	Normal feed for 2 weeks, highly contaminated feed for week 3 and 4 and normal feed with mycotox (5 kg/ton) for week 5, 6 and 7	1,654 <sup>a</sup>	2.39 <sup>c</sup>	58.40

Different letters in the column show that figures are statistically different ( $p < 0.01$ ).

\* Dressing weight was taken after removing the skin and without giblets i.e. gizzard, liver, spleen and heart.



**Figure 3.** Mortality in chicken given various levels of aflatoxins and mycotoxin detoxifier. The graph shows cumulative mortality for 7 weeks. G-1 represents chicken given normal feed throughout 7 weeks; G-2 represents chicken given contaminated feed throughout 7 weeks; G-3 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7; G-4 represents chicken given contaminated feed added with Mycotox (1 kg/ton) throughout 7 weeks; G-5 represents chicken given normal feed for first 2 weeks, highly contaminated feed for week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (3 kg/ton), and G-6 represents chicken given normal feed for first 2 weeks, highly contaminated feed during week 3 and 4 followed by normal feed for week 5, 6 and 7 added with Mycotox (5 kg/ton).

significantly between groups given normal feed and that given detoxifier mixed contaminated feed throughout 7 weeks.

The study shows that mycotoxin detoxifier containing oxyquinol, dichloro-thymol and micronized yeast can effectively neutralize ill effects of aflatoxins (the most important mycotoxin) in poultry feed. This detoxifier can also be used to off-set the ill effects of poultry feed containing higher levels of aflatoxins.

## DISCUSSION

Mycotoxins in general, and aflatoxins in particular are of great economic concern in commercial poultry production in all developing countries. This is particularly important in Pakistan where mycotoxicosis has been ranked as the third most important disease factor in broiler poultry (Bhatti, 1989; Sabri et al., 1989; Anjum, 1990). The problem has also been reported in layers resulting in up to 37.8 per cent reduction in egg production (Siddique et al., 1987). The aflatoxins have been reported from almost all commonly used poultry feedstuffs and commercial poultry rations with wide range of concentration (Afzal et al., 1979; Bhatti et al., 2001). The aflatoxins have also been detected in different organs of poultry (Azim et al., 1990; Begum et al., 2001).

Although a large number of methods have been

researched to detoxify aflatoxins in poultry feedstuffs (Heathcote and Hibbert, 1978; Piva et al., 2000; Sohane and Chaturvedi, 2001), a few have found commercial application and those too only in industrialized countries. This study evaluates the effects of a mycotoxin detoxifier on the performance of broiler chicken. The study results showed that this detoxifier can alleviate the negative effects of aflatoxins in broilers as indicated by improved weight gain, better humoral immune titres and lower mortality. This detoxifier has antifungal activity that can check further production of aflatoxins but exact mechanism of action for detoxification of already present aflatoxins has still not been elucidated.

The study indicates that the mycotoxin detoxifier containing oxyquinol, dichloro-thymol and micronized yeast can be used by the poultry farmers or the feed manufacturer for improving the quality of aflatoxin containing feed without any ill-effects.

## REFERENCES

- Afzal, M., R. A. Cheema and R. A. Chaudhry. 1979. Incidence of aflatoxins and aflatoxin producing fungi in animal feedstuffs. *Mycopath.* 69:149-151.
- Anjum, A. D. 1990. Weather and Disease: 1. Prevalence of the poultry diseases in and around Faisalabad and their relationship to weather. *Pakistan Vet. J.* 10:42-45.
- Azim, A., K. N. M. Khan, A. H. Cheema, F. A. Mir and M. Afzal. 1990. Occurrence of aflatoxins in poultry liver and associated pathological changes. *Pakistan Vet. J.* 10:51-54.
- Begum, F., A. Rehan, G. Maliha and J. Nuzhat. 2001. Distribution of aflatoxin B1 from poultry feed to different body tissues of broilers. *Pakistan Vet. J.* 21:121-123.
- Bhatti, B. M. 1989. Incidence of poultry diseases and their implications in poultry production in Pakistan. *Pakistan Vet. J.* 9:194-197.
- Bhatti, B. M., T. Talat and R. Sardar. 2001. Estimation of aflatoxin B1 in feed ingredients and compound poultry feeds. *Pakistan Vet. J.* 21:57-60.
- Byun, J. R. and Y. H. Yoon. 2003. Binding of aflatoxin G<sub>1</sub>, G<sub>2</sub> and B<sub>2</sub> by probiotic *Lactobacillus spp.* *Asian-Aust. J. Anim. Sci.* 16:1686-1689.
- Chaudhry, R. A., H. Afzal and M. Afzal. 1981. Incidence of aflatoxins in cottonseed cake and corn gluten feed at various storage conditions. *Pakistan Vet. J.* 1:111-113.
- Heathcote, J. G. and J. R. Hibbert. 1978. Aflatoxins: Chemical and Biological Aspects. *Developments in Food Science I*, Elsevier Scientific Publishing Company, Amsterdam.
- Howorth, B. Jr. and R. D. Wyatt. 1976. Effect of dietary aflatoxin on fertility, hatchability and progeny performance of broiler breeder hens. *Appl. Environ. Microbiol.* 31:680-684.
- Liu, D. L., D. S. Yao, Y. Q. Liang, T. H. Zhou, Y. P. Song, L. Zhao, and L. Ma. 2001. Production, purification and characterization of an intracellular aflatoxifizyme from *Armillariella tabescens* (E-20). *Food Chem. Toxic.* 39:461-466.
- Neal, G. E., D. J. Judah, P. Carthew, A. Verma, I. Latour, L. Weir, R. D. Coker, M. J. Nagler and L. A. P. Hoogenboom. 2001.

- Differences detected *in vivo* between samples of aflatoxin-contaminated peanut meal, following by two ammonia based processes. *Food Add. Contam.* 18:137-149.
- Piva, G., F. Galvano, A. Pietri and A. Piva. 2000. Detoxification methods of aflatoxins. *Zootec. Intern.* 23:7-8.
- Raju, M. V. L. N. and 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.
- Rosa, C. A. R., R. Miazzi, C. Magnoli, M. Salvano, S. M. Chiacchiera, S. Ferrero, M. Saenz, E. C. Q. Carvalho and A. Dalcero. 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Poult. Sci.* 80:139-144.
- Sabri, M. A., M. Siddique, M. Z. Khan and H. A. Samad. 1989. Prevalence and pathology of mycotoxicosis in young broiler chicks in and around Faisalabad. *Pakistan Vet. J.* 9:106-108.
- Siddique, M., T. Javed and M. A. Sabri. 1987. Incidence and pathology of various poultry diseases prevalent in Faisalabad and surrounding districts. *Pakistan Vet. J.* 7:148-154.
- Sims, W. M., D. C. Kelly and P. E. Sanford. 1970. A study of aflatoxicosis in laying hens. *Poult. Sci.* 49:1082-1084.
- Sohane, R. K. and V. B. Chaturvedi. 2001. Detoxification of aflatoxin in feed. *Pashudhan.* 16:3-8.
- Suman, M., K. Singh, S. More and K. Singh. 2001. Degradation of preformed aflatoxins in compound animal feed and groundnut cake by detoxifying organisms. *Ind. J. Anim. Sci.*, 71:169-173.
- Tozaki, T. 2001. Adsorption and reduction of aflatoxin into feed by using clay (hydrated sodium calcium aluminosilicate, HSCAS). *Mycotoxins.* 51:123-125.
- Verma, J., T. S. Johri and B. K. Swain. 2003. Effect of varying levels of aflatoxin, ochratoxin and their combinations on the performance and egg quality characteristics in laying hens. *Asian-Aust. J. Anim. Sci.* 16:1015-1019.