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Impact of Mycotoxin Binders on Humoral Immunity, Lymphoid Organs and Growth Performance of Broilers

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ABSTRACT

This project was designed to evaluate the impact of two commercial mycotoxin binders (Mycotox® and Mycofix® Plus 3.0) on the humoral immunity, lymphoid organs and growth performance of broilers. In this study, a total of 118 day-old broiler chicks were used, of which 108 were randomly divided into 6 groups (A to F) on day 14th. Aflatoxins derived from *Aspergillus parasiticus* were mixed in the feed measuring 150 ppb and fed to various broiler groups. Mycotoxin binders were also added into the feed and offered to group E and F from 14th-42nd day of age. The impact of mycotoxin binders on antibody titer against New Castle disease virus, feed consumption, weight gain, relative weights of lymphoid organs and feed conversion ratio (FCR) of broilers were investigated. Chicks fed aflatoxins (group B) alone manifested significantly reduced feed consumption, body weight gain and feed conversion ratio. However, no variation in weight gain and FCR was found between the controls (A, B and C) and mycotoxin binders fed groups (experimental group E and F). Significantly reduced geometric mean titer (GMT) was recorded for birds fed aflatoxins alone. However, difference in the calculated values of GMT for control group and birds fed toxin binders was not significant. Treatment related changes in organ body weight ratio of thymus, spleen and Bursa of fabricius of aflatoxins binders administered groups (groups E and F) were also significant compared with ones fed aflatoxins mixed feed (groups B and C). In conclusion, mycotoxin binders counteracted the deleterious effects of aflatoxins on antibody profile, weight gain, feed consumption and visceral organs of broilers suggesting that these agents can be potential ameliorators against aflatoxicosis in broilers.

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Authors' Contribution
HN and IH conceived and designed the research. HN and SK conducted the experimental work. MA and AN wrote the manuscript. AS edited the manuscript. NK performed the statistical analysis.

Key words
Mycotoxin binders, Immunity, Feed consumption, Weight gain, Broilers.

INTRODUCTION

Commercial poultry production constitutes the second largest industry of Pakistan and its significant contribution towards the national economy and uplift of rural population has been considerably acknowledged. The tremendous momentum of this sector is attributable to technological innovation and ever-increasing demand of poultry products. However, despite its remarkable progressive pace, challenges in terms of disease outbreaks, scarcity of feed and contamination of feed with mycotoxins, also call for consideration. Aflatoxins are secondary metabolites produced by different fungal species such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Huwig *et al*., 2001; Tedesco *et al*., 2004; Manafi *et al*., 2011). Inappropriate harvesting and storage conditions of agricultural commodities and their by-products offer conducive environment for fungal growth and subsequent aflatoxins production (Valchev *et al*., 2013). Therefore, cereal grains and other agro-industrial by-products, forming the basis of compounded feeds, may contain substantial quantities of aflatoxins before incorporation into the feeds (Gowda *et al*., 2008). If ingested, these toxin-laden feeds would definitely exert negative effect on the health and productivity of birds. The direct and indirect implications of aflatoxicosis include increased mortality (Azzam and Gabal, 1998; Dafalla *et al*., 1987), decreased egg production (Bryden *et al*., 1980), carcass condemnation (Azzam and Gabal, 1998; Hegazy *et al*., 1991) and increased susceptibility to infectious diseases (Bryden *et al*., 1980). Mycotoxicoses represent the third most important health hazard affecting the broiler industry of Pakistan (Bhatti, 1989; Anjum, 1990) and mycotoxins, particularly the aflatoxins have been implicated in infecting animal feedstuffs thereby instigating morbidity and mortality in commercial poultry (Azim *et al*., 1990; Bhatti *et al*., 2001). Various pharmaceutical companies, both at national and international level, are involved in

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supplying a variety of natural or synthetic products to combat the problem of mycotoxins in livestock and poultry. Alternatively, herbs supplementation as feed additive has also mean shown to improve growth parameters in broilers (Rafeeq *et al*., 2017). Mycotox® (Ceva Sante Animale, Libourne, France) and Mycofix® Plus 3.0 (Biomin® GTI GmbH. Herzogenbeurg, Austria) have been specially formulated for detoxifying mycotoxins. This study was devised to investigate the impact of these commonly used commercial mycotoxin binders *i.e.,* Mycotox® (MT) and Mycofix® Plus 3.0 (MF) on humoral immunity, lymphoid organs and growth performance in broilers.

MATERIALS AND METHODS

Collection and culturing of fungal samples for mycotoxins production

Naturally growing fungal samples from different sources such as bread, rice and maize were collected from various locations around the University of Veterinary and Animal Sciences, Lahore (Pakistan) and subsequently cultured on Sabouraud agar. After inoculation of samples on the agar, petri dishes were kept at a temperature of 28±2ºC for 10 days in the incubator to allow the fungal spores grow in the form of colonies. Green colored, likely *Aspergillus* colonies were transferred using a sterile needle and sub-cultured on Sabouraud agar at 28ºC for another 5-6 days. After incubation, single blue green colonies were again sub-cultured on Sabouraud agar until a single blue green fluorescing colony was isolated. Fungal identification was performed by cultural and morphological characteristics as suggested by the taxonomic schemes of Raper and Fennel (1973) for the genus, *Aspergillus*. For microscopic identification, fungal growth was placed on glass slides, stained with lactic acid and lacto-phenol blue and examined at 100-400X magnifications. On the other hand, toxigenic nature of the fungus was confirmed by its aflatoxin producing potential.

Aflatoxins were produced using the method described by Shotwell *et al*. (1966). This study involved the production of AF from a toxigenic strain of *A. parasiticus,* derived from maize and purified on Sabouraud agar through repeated subculture technique. The purified sample of the fungus was harvested and inoculated on autoclaved broken rice (Shotwell *et al*., 1966). On 3rd day of incubation, the rice color turned deep brown indicating fungal growth and toxin production. After 7 days incubation, the rice were crushed in a blender to yield powder and the level of aflatoxins was estimated through Enzyme Linked Immunosorbent Assay (ELISA). The toxin concentration was found adequate as per the requirement of the study.

Estimation of aflatoxins level in powdered rice and commercially available feed

Aflatoxins containing material was ground in pulverized form and estimated quantitatively by direct competitive ELISA as described by Barabolak (1977) using RIDA SCREEN® FAST Aflatoxin kit.

Experimental birds

A total of 118, day-old broiler chicks were procured from a local hatchery and housed in experimental poultry sheds, Department of Microbiology, University of Veterinary and Animal Sciences, Lahore. Of these, 10 birds were randomly selected and slaughtered to obtain blood samples for the determination of maternally derived antibodies titre on day 1. The remaining 108 birds were acclimatized by rearing them as a single group and providing standard feed. After two weeks, these birds were divided into six groups (A, B, C, D, E and F), allocating 18 birds to each group. Each group was replicated three times comprising 6 birds per replicate. These birds were offered controlled quantity of feed from day 14th of age according to the following scheme: Group A, only feed offered (Negative control for AF); Group B, Aflatoxins (AF) given @ 150 µg/Kg of feed (Positive control for AF); Group C, Mycotox (MT) @ 1gm/Kg of feed (Positive control for MT); Group D, Mycofix (MF) @ 2.5gm/Kg of feed (Positive control for MF); Group E, AF @ 150 µg/Kg and MT @ 1gm/Kg of Feed (Experimental group for MT) and Group F, AF @ 150 µg/Kg and MF @ 1gm/Kg of Feed (Experimental group for MF).

The powdered material containing AF was mixed according to the calculation to get the required level of AF (150 µg/Kg) in the feed. The prepared experimental diets were analyzed again through ELISA to ensure the AF levels in the feed. All the birds were fed ad-libitum. On day 6, all the birds had been vaccinated via ocular route with "Nobilis ND LaSota" (Intervet International, B.V. Boxmeer, Holland) and boosted on 24th day of age via drinking water. Other vaccines were administered according to the recommended vaccination schedule.

Blood collection and determination of specific antibodies level against Newcastle disease virus

Blood samples of about 2.5 ml were collected on day 1 directly in test tubes by slaughtering, and then at 7, 14, 21, 28, 35 and 42nd day of age from the jugular vein of the birds using disposable syringes. On each blood collection, 10 birds from each group were randomly selected. The sera were harvested from blood samples and stored at -40^{o}C till further processing.

Each serum sample was processed for determining the specific antibodies titre against ND virus. Commercially available "Nobilis ND LaSota" vaccine was used as a

source of virus. Antibody titre of each serum sample was determined by haemagglutination inhibition (HI) test as described by Allan *et al*. (1978). Geometric mean titer (GMT) for various groups was calculated using Tube number and Table (Modified Log_2) method as described by Brugh (1978).

Lymphoid organs weight

At the end of experiment, 3 birds from each group were randomly selected for recording weight of thymus, spleen, and Bursa of fabricius. Organ weights were expressed as relative organ weights (g of organ/100g of body weight).

Statistical analysis

The data thus obtained for all the variables were subjected to repeated measures ANOVA using completely randomized design and Two Way Analysis of Variance test and Least Significant Difference (LSD) using SPSS-21.

RESULTS

Geometric mean titer against ND virus on day 1 were calculated as 64 which declined to 26 and then 7.5 on days 7 and 14, respectively (Table I). Overall, the antibody titers declined from 1st to 14th days of age in all groups followed by an increase on day 21. Another drop in antibody titer became evident on 28th day of age. This was again followed by a gradual rise from 35th day onwards continuing till 42nd day. Exposure to AF without the presence of any toxin binder remarkably diminished the antibody titer response in the challenged birds (group B) as compared to other groups. Additionally, these low titers persisted throughout the monitoring period. Supplementation of MT and MF did not instigate any deleterious effects on antibody titer of exposed birds (groups C and D). Both mycotoxin binders exerted comparable restorative effects on the antibody profile of birds. These results showed that aflatoxins adversely affected the immune response of birds, thereby; suppressing the antibody titer against ND vaccine. However, concurrent administration of mycotoxins and mycotoxin binders ameliorated the immunosuppressive effect of AF (groups E and F).

Table I.- Relative geometric mean titre (GMT) of various groups against ND vaccine.

Age (days)	Groups					
	A	B	C	D	E	F
1	64.0^{a}	64.0^{a}	64.0^{a}	64.0^{a}	64.0^{a}	64.0^{a}
7	26.0^{b}	26.0^{b}	26.0^{b}	26.0^{b}	26.0^{b}	26.0^{b}
14	7.5^{e}	7.5^{e}	7.5^{e}	7.5^{e}	7.5^{e}	7.5^{e}
21	64.0^{d}	34.4^{f}	59.7^{e}	64.0^{d}	59.7^{e}	64.0^{d}
28	42.2^{g}	17.1^{h}	45.3^{i}	42.4^{g}	45.3^{i}	42.2^{g}
35	90.5^{g}	39.4^{e}	84.4^{f}	90.5^{g}	90.5^{g}	90.5^{g}
42	111.4^{h}	59.7^{j}	111.4^{h}	111.4^{h}	104.0^{k}	111.4^{h}

Superscripts with similar letters in the same row show non-significant (P>0.05) difference among different groups.

Table II.- Weekly feed intake, weight gain and FCR of various groups.

Parameters	Groups	Age in weeks					
		1	2	3	4	5	6
Feed intake (g)	A	145.30	556.50	1223.04	2148.79	3333.75	4582.19
	B	148.40	550.20	1076.88	1799.63	2724.75	3699.43
	C	149.30	560.20	1204.14	2103.64	3255.00	4468.04
	D	147.52	559.90	1210.44	2118.69	3281.25	4506.09
	E	150.20	550.30	1197.84	2088.59	3228.75	4374.99
	F	149.21	561.40	1201.62	2097.62	3244.50	4552.82
Weight gain (g)	A	107.23	366.12	706.96	1125.02	1522.26	1975.08
	B	109.12	358.40	598.27	844.90	1164.42	1445.09
	C	109.38	367.80	688.08	1084.35	1466.22	1909.42
	D	108.30	365.20	695.66	1097.77	1484.73	1933.94
	E	110.20	360.20	676.75	1071.07	1441.41	1899.16
	F	108.38	370.20	686.64	1081.25	1461.49	1911.08
FCR	A	1.35	1.52	1.73	1.91	2.19	2.32
	B	1.36	1.54	1.80	2.13	2.34	2.56
	C	1.37	1.52	1.75	1.94	2.22	2.34
	D	1.36	1.53	1.74	1.93	2.21	2.33
	E	1.36	1.53	1.77	1.95	2.24	2.31
	F	1.37	1.51	1.75	1.94	2.22	2.38

The average feed consumption amongst all the groups was 148.3 g and 556.4 g during 1st and 2nd weeks, respectively. Compared to control (A) group, AF-fed birds (group B) exhibited a significant ($P<0.05$) and consistent diminution in feed consumption from 3rd week till completion of the trial (Table II). This showed that administration of AF drastically reduced the feed intake by the challenged birds. While, the addition of MT and MF did not induce any adverse effects on feed consumption of treated birds (groups C and D). Nevertheless, the concomitant use of mycotoxin binders improved the feed intake of birds (groups E and F) as to nearly normal level.

The average weight gain was 108.8 g and 364.7 g per bird during 1st and 2nd week, respectively. However, at 14th day of age post-treatment, AF-fed birds demonstrated relatively less weekly weight gain than rest of the groups (Table II). Furthermore, this reduced weight gain was consistently evident throughout the observational period. Unlike AF, toxin binders had no detrimental impact on weight gain of targeted birds (groups C and D). Moreover, the use of MT and MF neutralized the negative consequences associated with the consumption of AF in exposed birds (groups E and F).

The values of FCR determined were 1.35 and 1.52 during 1st and 2nd week, respectively. Relatively high FCR was recorded for birds consuming AF (group B) than their other counterparts (Table II). Utilization of AF deleteriously affected the bird's performances and resulted in high FCR (group B). However, these ill effects were abolished through incorporating toxin binders in the feed (group E and F).

At the end of trial, 3 birds from each group were weighed and subsequently slaughtered. The weight of thymus, spleen and bursa of fabricius were recorded and relative organ body weight ratio (organ weight/100g of live body weight) was calculated. A significant increase ($P<0.05$) in the weight of spleen (splenomegaly) while reduction in the weights of thymus and bursa of fabricius in AF-treated birds was recorded (Table III; Fig. 1). Analogous values of relative organ body weight ratio were also noted for birds of control group (group A) and for groups E and F (AF+ toxin binders). This study revealed that aflatoxins negatively affected the thymus and bursa of targeted birds. While, mycotoxins binders counteracted the deleterious effects of aflatoxins on visceral organs of broilers as indicated by the organ weights of the birds in groups E and F.

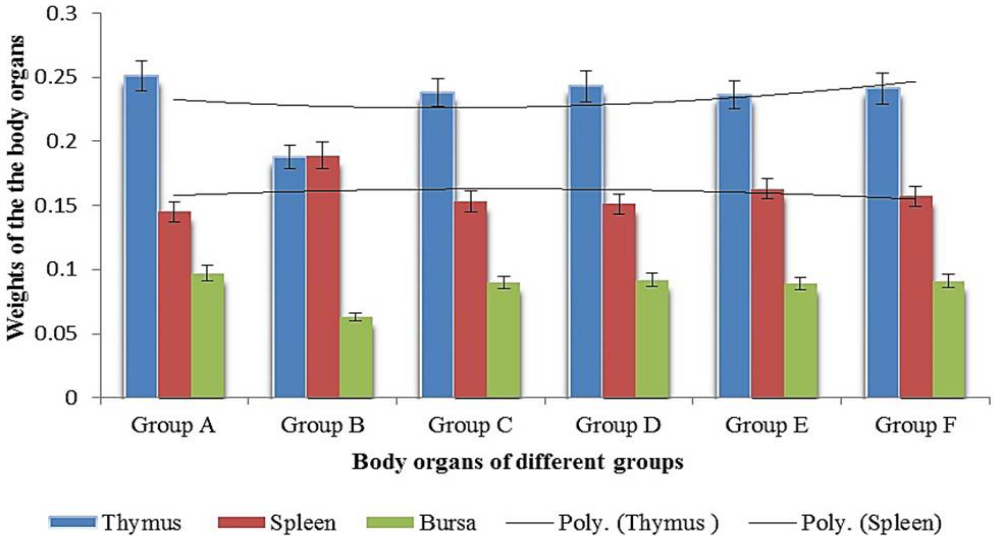

Fig. 1. Relative body organ weights of the birds on day 42 compared with controls showing polynominal trend-line increase in the weight of Spleen and decrease in the weightsof Thymus and Bursa in various groups.

Table III.- Relative organ body weight ratio (gm/100gm of body weight ± Standard Error) of various groups on 42nd day.

Organs	Groups					
	A	B	C	D	E	F
Thymus	0.251 ± 0.012	0.188 ± 0.009	0.238 ± 0.011	0.243 ± 0.012	0.236 ± 0.011	0.241 ± 0.012
Spleen	0.145 ± 0.008	0.189 ± 0.010	0.153 ± 0.008	0.151 ± 0.008	0.163 ± 0.008	0.157 ± 0.008
Bursa	0.097 ± 0.006	0.063 ± 0.003	0.090 ± 0.005	0.092 ± 0.005	0.089 ± 0.005	0.091 ± 0.005

DISCUSSION

Asperfillus flavus and *Aspergillus parasitcus* survive in a wide range of environments and can inhabit soil, plants, animal remains, grains and seeds such as maize and peanuts (Pitt, 2000). The toxicity profile of aflatoxins has been extensively analyzed in terms of their carcinogenic, mutagenic, teratogenic (Wild *et al*., 2000; Sur and Celik, 2003) and growth suppressing (Oguz and Kurtoglu, 2000) effects in broilers. Aflatoxins impede cellular protein synthesis and primarily affect rapidly multiplying cells and tissues with high protein turnover such as liver, immune system and gut epithelium. Mycotoxin binders have been commercially introduced to reduce the oral bioavailability AF and subsequently prevent their systemic absorption from the feed t acting as sequestering agents (Davidson *et al*., 1987; Phillips *et al*., 1990; Araba and Wyatt, 1991). The current study investigated the impact of AF on antibody titer, feed consumption, body weight gain, relative weights of lymphoid organs (thymus, spleen and bursa of Fabricius) and FCR of broilers. Furthermore, the detoxification potential of two commercially available mycotoxin binders (MT and MF) was also evaluated.

It was observed that utilization of AF in the feed by the birds deteriorated their immune response against ND vaccine. This outcome of our study is in conformity with findings of earlier studies reporting a positive correlation between the outbreaks of ND in broilers and the consumption of AF-contaminated ration (Verma *et al*., 2004; Yunus *et al*., 2011). Suppressed development of thymus and bursa of Fabricius may be due to impaired macrophage functions, altered cytokine expression and apoptosis of splenic lymphocytes triggering AF-mediated immunosuppression in exposed birds as reported previously (Celik *et al*., 2000; Meissonnier *et al*., 2008; Chen *et al*., 2013). Mycotoxin binders restored the immune response in AF-treated birds in the current study, thus reinforcing the findings of an earlier study (Afzal and Saleem, 2004).

Decreased feed consumption, diminished weight gain and relatively high FCR observed in mycotoxin administered birds were consistent with previous findings reporting similar performance depressing effects of AF (Edds and Bortel, 1983; Kubena *et al*., 1990). A plethora of studies support the reduction of body weight in broilers associated with aflatoxicosis (Bintvihok, 2002; Basmacioglu *et al*., 2005; Girish and Devegowda, 2006; Abousadi *et al*., 2007; Yildirim *et al*., 2011; Kaki *et al*., 2012; Valchev *et al*., 2013) which is in line with our findings. The observed decline in body weight gain could be attributed to disruption of protein synthesis, impaired hepatic metabolism, suppressed appetite and malabsorption of nutrients (Verma *et al*., 2004; Safameher, 2008). The addition of mycotoxin binders counteracted the growth-suppressing effects of AF. Administration of MT attenuated the detrimental effects of mycotoxins on weight gain and FCR of broilers as suggested by the previous studies also (Afzal and Saleem, 2004; Hanif *et al*., 2008). Addition of MF to mycotoxin-contaminated feed has been reported to neutralize the deleterious effects of mycotoxins on the performance of broilers (Hofstetter *et al*., 2005; Ghareeb *et al*., 2011; Nesic *et al*., 2012).

Thymus, spleen and bursa of Fabricius constitute the principal immune organs of birds, responsible for humoral as well as cell-mediated immunity (Sakhare *et al*., 2007). There was significant ($P<0.05$) increase in relative weight of spleen while weights of thymus and bursa of Fabricius decreased in broilers exposed to aflatoxins. These changes in relative weights of lymphoid organs were consistent with previous reports (Verma *et al*., 2004; Valchev *et al*., 2013). Lower weights of thymus and bursa of Fabricius in AF-treated birds could possibly be caused by atrophy, necrosis and diminution in lymphoid cell count (Ortatatli and Oguz, 2001; Sakhare *et al*., 2007). Whereas, the higher weight of spleen could be elucidated as a compensatory mechanism to the weights of thymus and bursa of Fabricius (Teleb *et al*., 2004; Khadem *et al*., 2012). Mycotoxin binders offset the deleterious effects of AF on visceral organs of broilers in the experimental groups.

Several studies have demonstrated the effectiveness of MT and MF in ameliorating the broilers health and performance (Diaz, 2002; Afzal and Saleem, 2004; Diaz *et al*., 2005; Jameel and Sharif, 2007; Hanif *et al*., 2008; Xue *et al*., 2010). De-epoxydase and esterase enzymes represent the integral components of MF and carry out detoxification of the mycotoxins (Jameel and Sharif, 2007). While, Terpenoides, saponins and flavolignans exhibit anti-inflammatory, anticancer and hepatoprotective actions, respectively (Garcia *et al*., 2003; Jameel and Sharif, 2007). MT consists of dichlorothymol, oxyquinol and micronized yeast imparting to it substantial detoxification potential (Afzal and Saleem, 2004). There was no significant ($P>0.05$) difference between the control and the chicks fed mycotoxin binders alone for the various parameters evaluated, indicating the absence of any deleterious effects on broiler health and performance.

CONCLUSION

In conclusion, the use of commercially available toxin binders (MT and MF) is beneficial for broiler farming in terms of mitigating the injurious effects of mycotoxins on the overall growth performance of broilers. Economic gains (body weight) not only outweigh the cost of such binders, the very use of toxin binders can also result in

the production of superior quality chicken meat for human consumption.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.5.1611.1618

Statement of conflict of interest

The authors have no conflict of interest with any of the research work done by other researchers.

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