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Immunotoxicity of Ochratoxin A and Role of *Trichosporon mycotoxinivorans* on the Humoral Response to Infectious Viral Disease Vaccines in Broilers

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Abstract.- The present study was planned to evaluate the effects of dietary ochratoxin A (OTA) in the presence and absence of a toxin deactivator on the organ weights, humoral immune response of broiler birds vaccinated against Newcastle disease (ND), hydropericardium syndrome (HPS) and infectious bursal disease (IBD). This was investigated in a 42 day completely randomized trial consisting of 9 dietary treatments with 1 negative control group. Birds were vaccinated against ND at 5 and 21, IBD at 11 and 24 and HPS at 15 days of age. The serum samples were collected at 14, 28 and 42 day of age were then assayed using haemagglutination inhibition (HI) for ND and indirect haemagglutination Inhibition test (IHA) for HPS and IBD, respectively. The exposure of broiler birds to two levels (500 and 1000ppb) of OTA reduced their humoral immune response against ND, HPS and IBD vaccines significantly (p<0.05) in dose dependent manner. At day 14, only titers for ND were significantly elevated in group supplemented with 2kg/ton of toxin deactivator. While on day 28 and 42 of age the supplementation with 2kg/ton toxin deactivator significantly (p<0.05) ameliorated the deleterious effects of OTA on humoral immune response of birds against ND, HPS and IBD as reflected by increase in the respective antibody titers. As far as the organ weights are concerned, the results of present study indicated a close interaction of OTA with infectious diseases which cause significant ($p<0.05$) suppression of humoral immune system, even if the birds adequately vaccinated and predisposed to ND, HPS and IBD on challenge.

Key words: Ochratoxin A, boiler chicken, infectious viral diseases, mycotoxin control.

INTRODUCTION

Poultry diseases are a serious impediment in the development of poultry enterprises and resulted in major worldwide economic losses. Among viral diseases, Newcastle disease (ND), hydropericardium syndrome (HPS) and infectious bursal disease (IBD) are the three most prevalent ones in Pakistan, inflicting heavy economic losses (Beenish *et al.,* 2013). These mass poultry diseases occur despite regular vaccination which may be ascribed to antigenic variant strains, interference by maternal antibodies and immunosuppressive agents such as mycotoxins in feed (Mahajan *et al*., 2002; Mustafa *et al*., 2005; Rashid *et al*., 2013). Mycotoxins have the potential to interfere with the native resistance mechanisms as well as development of active immunity. Aflatoxins, OTA and mycotoxins of the trichothecenes group are typically associated with interference of resistance to infectious diseases (Wyatt, 2005). However, it is difficult to discern their role, because the casual involvement of mycotoxins is often overshadowed by the infectious diseases and thus is not overtly evident in the overall syndrome (CAST, 2003). Ochratoxin A in particular, is a toxic product of the fungi *Aspergillus* and *Penicillium*, which affects several performance parameters as well as the immune system of the birds (Marquardt and Frohlich, 1992). It is approximately three times more toxic to young broiler chicks than aflatoxins (Leeson *et al.,* 2005) and has been implicated in significant field outbreaks of ochratoxicosis in poultry (Fink-Gremmel, 1999).

The addition of naturally occurring inert adsorbents to mycotoxin-contaminated feed has been a popular approach to decrease the toxicity of mycotoxins in animals and to offset the carry-over of mycotoxins from contaminated feed to animal finished and by-product. These adsorbents act primarily by decreasing bioavailability (by

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adsorption through animal's gastrointestinal tract) and their distribution to target organs (CAST, 2003). Several studies revealed that aluminosilicates and yeast *i.e.,* S*accharomyces cerevisiae* can alleviate the adverse effects of aflatoxins in poultry (Kubena *at al*., 1990; Stanley *et al*., 1993) but none of these ameliorate the deleterious effects of ochratoxins (Watts *et al*., 2003). Recent studies have shown that a specific yeast strain, *T. mycotoxinivorans* isolated from the hind gut of a lower termite (*Mastotermes darwiniensis*) has the ability to detoxify mycotoxins such as OTA and zearalenone (Politis *et al.,* 2005; Hanif *et al*., 2008, 2012). Isolated and skimpy attempts have been made for observing the modulating effects of mycotoxicosis on infectious viral diseases in broiler chicken. Most of this work is restricted to aflatoxicosis *vis-à-vis* infectious poultry diseases. Effects of the presence of OTA in feed on the humoral immunity, in particular against HPS have not been investigated thus far. In view of the foregoing, the present study was designed to determine the interaction of OTA with humoral immunity against ND, HPS and IBD along with determination of the efficacy of a mycotoxin deactivator (Mycofix Plus; MP) having *Trichosporon mycotoxinivorans* on immune response of broiler chicken to these vaccines.

MATERIALS AND METHODS

A total of 270 day-old broiler chicks (Star Bro from Al-Noor Chicks, Faisalabad-Pakistan) were used in a completely randomized experimental design with one negative control group, two positive control groups for OTA (500ppb and 1000ppb) two positive control group for MP (1 and 2kg /ton of feed) and four MP test groups (500ppb OTA and 1kg/ton; 500ppb OTA and 2kg/ton; 1000ppb OTA and 1kg/ton; 1000ppb OTA and 2kg/ton) resulting in a total of 9 experimental dietary treatments. Each dietary treatment was replicated 3 times, with 10 birds per replicate (Table I).

Toxin deactivator

Toxin deactivator (Mycofix Plus$^{MTV\ INSIDE}$) containing yeast strain *T. mycotoxinivorans* was obtained from Biomin GmbH, Austria. It was in powdered form containing 6.0x 10^8 count/g of *T. mycotoxinivorans*.

Table I.- Levels of ochratoxin A (OTA) and toxin deactivator added to the diets of groups (in triplicates) of broiler chicks

Groups	OTA (ppb)	Toxin deactivator (kg/ton)	No. of birds
A	None	None	30
B	None	1	30
C	None	2	30
D	500	None	30
E	500	1	30
F	500	2	30
G	1000	None	30
H	1000	1	30
I	1000	2	30

Experimental diet

All groups of broiler chicks were fed a commercial mash, which consisted of standard feedstuffs and contained enough nutrients to meet all requirements (Protein 19.65%; metabolizable energy 2840kcal/kg). In the experimental groups D, E, F, G, H and I, OTA (OTA contaminated corn courtesy; Biomin, Austria) was added in an amount enough to provide 500 and 1000ppb OTA respectively. Feed and water were provided *ad libitum.* For the determination of interaction of OTA with humoral immunity against ND, HPS and IBD, birds were vaccinated against these diseases according to vaccination schedule as depicted in Table II. For serology, blood samples were collected from 6 randomly selected birds from each group at day 14, 28 and 42 of age and serum harvested by centrifugation and stored at -20° C till analysis. The immune response against ND was determined through haemagglutination inhibition (HI) test as recommended by Allen *et al*. (1978), while the response against HPS and IBD was assayed by indirect haemagglutination (IHA) tests as described by Rehman *et al*. (1989) and Rehman *et al*. (1994), respectively. The organs were collected during 28 and 42 day of experiment and absolute weight were calculated.

Statistical analysis

Data of serum titers were analyzed using ANOVA, SPSS software10.0. The experimental model included the effect of dietary treatments, antibody titers at sampling days, the interaction

Table II.- Vaccination schedule of experimental birds.

Vaccine type	Vaccine brand/strain	Route of vaccination	Age (days) at vaccination
Newcastle disease (ND)	TAD - ND Lasota clone	Eye drops	5
Infectious bursal disease (IBD)	TAD/Gumboro Vac-IBV CU1M	Eye drops	11
Hydropericardium syndrome (HPS)	Bio Angara/ hydropericardium local strain	Sub cut	15
Newcastle disease (ND)	TAD/ Lasota clone	Drinking water	21
Infectious bursal disease (IBD)	TAD/ Gumboro Forte – LC 75	Drinking water	24

between treatments and antibody titers. The Duncan Multiple range test was used to compare differences between means with significance at the $p<0.05$ level.

RESULTS AND DISCUSSION

Table III shows that significant reduction ($p<0.05$) in HI antibody titers was only observed only in birds fed diet containing 1000ppb OTA (group G). In the other groups, no significant influence/effect of OTA in the presence or absence of toxin deactivator was observed. At day 28, no significant influence on humoral response in broiler birds vaccinated against NDV in either presence or absence of OTA was observed. Albeit, at day 42, significant suppression in antibody titers against ND was recorded in groups receiving only OTA (group D and G) as compared to control group A, whereas significantly elevated titers were found in group F receiving both OTA and toxin deactivator.

As can be seen in Table III on days 28 and 42, antibody titers of birds receiving 1000ppb OTA alone (group G) were significantly ($p<0.05$) reduced. Supplementation of 2kg toxin deactivator per ton of feed in the presence of 500ppb OTA (group F) resulted in significantly higher titers against IBD at day 28 and 42 than the titers observed in the absence of the toxin deactivator (group D). Similarly, significantly ($p<0.05$) higher titers were recorded at days 28 and 42 in groups H and I (1000ppb OTA and 1kg/ton toxin deactivator; 1000ppb OTA and 2kg/ton toxin deactivator) compared to titers observed in group G (receiving 1000ppb OTA alone). At days 28 and 42, the humoral response to HPS vaccine was significantly lower ($p<0.05$) in group D (500ppb OTA alone) than in groups E and F (500ppb OTA and toxin deactivator at 1 and 2 kg/ton of feed, respectively). At these sampling time points, significantly lower in group G (1000ppb OTA alone) than in groups H and I, respectively (Table III).

Determinations of absolute weights of lymphoid organs *i.e.*, bursa of Fabricius and spleen of birds at day 28 and 42 of age are shown in Table IV. During days 28 and 42 of experiment, bursal absolute weights were significantly decreased with level 1000ppb of OTA (group F) when compared with the control group (A). Similarly, the groups fed on higher level of OTA (1000ppb) in the presence and/or absence of toxin deactivator were found decreased significantly ($P<0.05$) from control group (A) but did not different from group D. Whereas, at day 42 as can be observed in Table IV, no pronounced effect of OTA or toxin deactivator at either levels were observed when compared with the control group (A). No significant effect of OTA and toxin deactivator on absolute weights of spleen was observed at day 28 of age. While at day 42, the absolute weights of spleen were non-significantly decreased only in group D (fed 500ppb OTA). In groups fed on toxin deactivator no pronounced effect was observed at either day 28 or 42.

Lower weight of spleen and bursa of Fabricius may account for reduction in the antibody titers reported with the presence of mycotoxins in feed. This reduction is likely a consequence of degenerative changes and decreased lymphoid tissues in these organs in OTA treated groups. The mechanism of immunosuppression is not well elucidated, but it is supposed that OTA as an inhibitor of protein synthesis delays the division of the cells of the immune system. Impaired protein synthesis in lymphocytes could lead to the impairment in their activation, differentiation and proliferation. OTA is known to cause regression

Table III.- Antibody titers (Mean±SD) against Newcastle disease (ND), Infectious bursal disease (IBD) and hydropericardium syndrome vaccines in broilers

Groups	OTA levels (ppb)	Toxin deactivator (kg/ton)	ND antibody titers (Mean ±SD)			IBD antibody titers (Mean ±SD)		HPS antibody titers (Mean ±SD)	
			Day 14	Day 28	Day 42	Day 28	Day 42	Day 28	Day 42
A	0	0	7.43±0.58 ^{b}	6.67±1.53 ^{a}	6.67±1.53 ^{bc}	6.65±2.08 ^{bc}	8.33±0.56 ^{e}	7.67±1.53 ^{bc}	8.67±0.57 ^{d}
B	0	1	7.00±1.15 ^{b}	6.20±0.23 ^{a}	7.00±1.10 ^{c}	6.67±0.58 ^{bc}	6.67±0.50 ^{cd}	8.11±0.37 ^{bc}	8.00±0.23 ^{cd}
C	0	2	6.01 ±0.57 ^{b}	7.33±1.53 ^{a}	7.00±1.08 ^{c}	6.12±1.25 ^{ab}	7.33±0.58 ^{de}	7.67±0.50^{bc}	8.00±1.11 ^{cd}
D	500	0	6.00 ±1.20 ^{b}	6.67±1.15 ^{a}	5.00±0.11 ^{a}	5.67±0.44 ^{ab}	5.33±0.66 ^{ab}	5.33±0.21 ^{a}	5.67±0.45 ^{ab}
E	500	1	6.13±1.15 ^{b}	6.67±0.57^{a}	5.33±0.57 ^{ab}	6.33±0.57 ^{ab}	6.00±0.67 ^{bc}	7.33±0.77 ^{bc}	7.67±1.15 ^{cd}
F	500	2	8.00±1.52 ^{c}	7.67±0.63 ^{a}	8.67±0.87 ^{d}	8.10±0.58 ^{c}	8.00±0.22 ^{e}	8.68±0.17 ^{c}	8.67±0.50 ^{d}
G	1000	0	4.22 ±2.00 ^{a}	6.33±0.57 ^{a}	5.00±0.19 ^{a}	5.23±0.89 ^{a}	4.33±0.47 ^{a}	4.67±0.23 ^{a}	5.00±0.21 ^{a}
H	1000	1	5.09±0.58 ^{b}	7.10±1.00 ^{a}	5.33±0.57 ^{ab}	7.29±0.57 ^{bc}	5.67±0.24 ^{bc}	7.21±0.34 ^{b}	6.67±0.62 ^{bc}
I	1000	2	6.11±2.08 ^{b}	7.23±1.07^{a}	6.00±0.10 ^{abc}	7.13±0.99 ^{bc}	6.33±0.98 ^{bcd}	8.67±0.57 ^{c}	7.67±1.15 ^{cd}

$^{a-d}$Means within a columns with different superscript differ significantly ($p<0.05$).

and cellular depletion (lymphocytes) of major lymphoid organs, significantly affecting cellular immunity in poultry. OTA cause lesser secondary suppression of humoral immunity with lower circulation immunoglobulin resulting in depletion of immune system effector cells especially macrophages. The reduction of phagocytic activity of natural killer cells and T-killer cells is probably due to reduction in basal interferons (Harvey *et al.*, 1992).

Mycotoxins have been implicated as potent inhibitors of the avian immune response by interfering with protein synthesis and also impose significant effects on bird health because of their interference with vaccination programs (Frederie, 2010). Ochratoxin A has increasingly been coming into focus in recent years due to its ubiquitous occurrence in human food and animal feed and associated toxic effects. Furthermore, the main target organs of OTA are kidneys, liver, lymphoid organs, hemopoietic tissues and skeletal system (Xue *et al.*, 2010). In a mouse model, it is found that subchronic oral exposure to OTA affects certain immune functions at exposure levels that may be found in contaminated food products. The results of the present study substantiated these findings since two dietary levels of OTA (500 and 1000ppb) significantly suppressed the antibody response of broiler birds to ND, HPS and IBD vaccines at day 28 and 42 of age.

The titers of haemagglutination inhibiting antibodies induced by the Lasota vaccine strain of ND were significantly reduced by the dietary inclusion of OTA. This indicates the negative influence of ochratoxin A on the humoral immune response of broilers vaccinated against ND. These findings are in line with those of Balachandran (2006) who observed significant reduction in HI titers to ND in the vaccinated mycotoxin-fed (Aflatoxin, 100ppb; OTA, 250ppb and T-2 toxin, 500ppb) groups and lymphoid depletion and lymphocytosis in all lymphoid organs of ND challenged birds. Similarly, Gounalan *et al*. (2006) found significant reduction in antibody titers to ND vaccinated layer chicken fed 250ppb of OTA from 0 to 14 weeks of age. Singh *et al*. (1990) documented that OTA fed to broilers at dietary concentration of 2ppm reduced either humoral or cellular immune

Table IV.- Mean (±SEM) values of absolute weight of lymphoid organs at days 28 in broiler birds fed two levels of Ochratoxin A and toxin deactivator.

Treatment Groups	Day 28		Day 42	
	Bursa of Fabricius	Spleen	Bursa of Fabricius	Spleen
A	2.10±0.15 [c]	1.38±0.25 [a]	0.90±0.06 [ab]	2.27±0.61 [ab]
B	1.57±0.43 [bc]	1.13±0.12 [a]	0.76±0.20 [ab]	2.20±0.26 [ab]
C	1.40±0.35 [b]	1.10±0.12 [a]	1.26±0.29 [b]	2.67±0.39 [b]
D	1.0±0.06 [ab]	1.07±0.14 [a]	0.90±0.15 [ab]	1.13±0.23 [a]
E	1.10±0.12 [ab]	1.10±0.36 [a]	1.0±0.15 [ab]	2.10±0.78 [ab]
F	1.0±0.15 [ab]	0.80±0.25 [a]	1.17±0.06[b]	1.67±0.58 [ab]
G	0.53±0.09[a]	0.90±0.15 [a]	0.50±0.11[a]	1.76±0.20 [ab]
H	0.67±0.12 [a]	0.86±0.14 [a]	0.73±0.23[ab]	2.46±0.22 [ab]
I	1.03±0.15 [ab]	0.85±0.08 [a]	0.87±0.27 [ab]	1.73±0.32 [ab]

[a-e]Means with in a column with no common superscript differ significantly (P< 0.05)
A (control group), B (1kg/ton of Mycofix Plus[MTV INSIDE]), C (2kg/ton of Mycofix Plus[MTV INSIDE]), D (500ppb OTA), E (500ppb OTA and 1 kg/ton of Mycofix Plus[MTV INSIDE]), F (1000ppb OTA and 2 kg/ton of Mycofix Plus[MTV INSIDE]), G (1000ppb OTA), H (1000ppb OTA and 1 kg/ton of Mycofix Plus[MTV INSIDE-]), I (1000ppb OTA and 2 kg/ton of Mycofix Plus[MTV INSIDE-])

response or both, and inhibited phagocytosis. The findings of the present study with respect to effect of OTA on the haemagglutination inhibition titers against ND vaccine are congruent with those reported by Stoev *et al. (*2000).

IBD is known to have a tremendous impact on the poultry industry causing high mortalities, lowered productivity among infected chicks, predisposition to other infectious diseases and reduced response to vaccines. In the present study, the birds were vaccinated against IBD at day 11 with a booster dose at day 24 of age. At day 14 no titers could be detected as the titers developed were below the levels detectable by IHA test. This was further supported by detrimental effects of OTA on humoral immune response was observed even after booster vaccination. Mohiuddin (1993) documented a marked decline in antibody titers and phagocytic activity in birds fed an aflatoxins dose as low as 200ppb. Similarly, Anjum (1994) reported a severe IBD outbreak with a mortality rate of 35.80% in grower pullets that were vaccinated with IBD vaccine, but were immunodepressed owing to aflatoxicosis.

HPS is an immunosuppressive disease of 3-6 weeks old broilers characterized by sudden onset, high mortality, typical hydropericardium and enlarged mottled and friable liver, with intranuclear inclusion bodies in the hepatocytes (Balamurugan and Kataria, 2006). It was originally reported from Angara Goth near Karachi, Pakistan, during 1987 (Khawaja *et al*., 1988), and it has been reported to be particularly important in Asia and America (Abe e*t al*., 1998). As far as could be ascertained, reports concerning the interaction of OTA with IBD and HPS are nonexistent heretofore. The present study documented for the first time that incorporation of OTA in the feed led to significantly lower antibody titers at day 28 and 42 of inoculation of IBD and HPS vaccines. The significantly lower antibody titers in birds receiving OTA supplemented feed compared to those of the control may be ascribed to the potent immunosuppressive effect of OTA (Frederie, 2010).

Recent studies showed that a yeast strain isolated from a termite (*Mastotermes darwiniensis*) can transform certain mycotoxins into non-toxic metabolites (Molnar *et al.*, 2004; Hanif *et al.*, 2012). A toxin deactivator, having *T. mycotoxinivorans* was used in the present study. This toxin deactivator is especially designed to counteract the toxic effects of mycotoxins based on 3 strategies: biotransformation of mycotoxins, elimination of the toxins (adsorption) and elimination of the toxic effects. In the present study, the deleterious effects on immunity caused by OTA were ameliorated by dietary inclusion of toxin deactivator 2kg/ton of feed. These findings also support previous studies showing that Mycofix Plus is capable of counteracting the deleterious effects of

trichothecenes, aflatoxins and OTA (Diaz *et al*., 2005; Xue *et al*., 2010).

Evidence in the literature together with the results of the present study suggest that mycotoxins of various types are a contributing factor in reducing the immunity and thus are likely to increase the susceptibility of birds to infectious diseases by reducing their responses to vaccines (Ragland *et al*., 1998). Mycofix Plus is capable of counteracting the negative impact of ochratoxicosis at an inclusion level of 2kg/ton of feed. Further studies are required to evaluate the interaction of OTA with other infectious diseases and their vaccines.

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Conflict of interest

Authors declare that they have no conflict of interest.

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