Effects of ochratoxin and T-2 toxin combination on performance, biochemical and immune status of commercial broilers

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Abstract

Aim: To study the effects of Ochratoxin A (OA), T-2 toxin (T-2) and their combinations on the performance, biochemical and immune status of broiler chickens.

Materials and Methods: 168 day-old Cobb broiler chicks, obtained from a commercial hatchery were divided by Complete Randomized Design into four groups of three replicates and fourteen chicks per replicate, with dietary treatments of 0.0 (control), 1 ppm OA, 2 ppm T-2 and their combination (1 ppm OA + 2.0 ppm T-2). The chicks were housed in deep litter independent conventional system with feed and water *ad libitum* throughout the experimental study. Body weight and feed intake were recorded weekly. At the end of the trial, blood was collected and was analyzed for total protein, serum albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) and antibody titers against ND and IBD using ELISA technique.

Results: The toxin treated birds exhibited a significant (P<0.05) decrease in the body weights and weight of lymphoid organs. A significant (P<0.05) reduction in serum total protein, albumin and increase in serum uric acid levels were observed in toxin treated birds. The serum alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) levels were decreased and antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) were decreased significantly (P 0.05).

Conclusion: The presence of OA and T-2 in the diet showed depressing effects on performance, biochemical and immunological parameters indicating their adverse effects on the general health of broilers.

Key words: biochemical parameters, broilers, immune status, ochratoxin, T-2 toxin,

Introduction

Feed ingredients and compounded feeds used in poultry production by virtue of their moisture content are highly susceptible to the growth of mould, which produces toxic metabolites during their growth referred to as mycotoxins. The fungal contamination of feedstuffs occurs at various stages of production, harvesting, handling, processing and storage. Cocontamination of feedstuffs with multiple mycotoxins is a common scenario [1].

T-2 is a highly toxic trichothecene mycotoxin produced by different *Fusarium* species, mainly *Fusarium sporotrichoides* to a lesser extent by *F. poae* [2]. Similarly Ochratoxin A (OA), a nephrotoxic mycotoxin mainly produced by *Aspergillus ochraceus* and *Penicillium viridicatum*, has been shown to contaminate a wide variety of cereals and feed stuffs and is extremely toxic to domestic fowls. Ochratoxin A is classified as the second most important mycotoxin in terms of economic losses worldwide, aflatoxin being the first [3].

The effects of OA and T-2 toxins in poultry were

found to be quite pronounced in young broiler chicks resulting in poor growth rate and poor feed efficiency. In addition, these toxins have been implicated in causing immunosuppression in young birds thereby resulting in economic losses to the poultry farmers. Ochratoxin A and T-2 toxin affects immune function by decreasing the size of vital immune organs, such as thymus, spleen, and lymph nodes, and several immune cells within these tissues thereby causing immunosuppression [4, 5].

Hence, an attempt has been made in this study to evaluate the effect of Ochratoxin, T-2 toxin and their combination on performance, biochemical parameters and immune status in commercial broiler chicken.

Materials and Methods

Ethical approval: The birds were sacrificed as per the standard procedures and after due permission from the Institutional Animal Ethics committee (No. 03 PSC-LPM/IAEC/2004-2006 dt 14.07.2005) of Veterinary College, Bangalore.

Experimental animals and design: One hundred and sixty eight, unsexed one-day old commercial Cobb broiler chicks were wing banded, weighed and assigned to a 2X2 factorial arrangement with control (0.0), OA (1 ppm), T-2 (2.0ppm) and combination of 1

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Table-1. Effect of individual and combined toxicities of ochratoxin and T-2 toxin on body weight (g)) of broiler chicl	kens
from 0-5 wks of age (Mean <u>+</u> SE)		

Treatment	1 st week	2 nd week	3 rd week	4 th week	5 th week
Control	135.20+0.34ª	314.0+2.17 ^{ab}	615.6+2.22ª	1013 <u>+</u> 1.94 ^ª	1320+4.73°
OA	80.40+1.10 ^{bc}	111.9 + 3.28°	231.5+3.54°	446.4+3.72°	562.2+4.15°
T-2	131.0 + 1.36ª	304.3 ⁻ +0.74 ^₅	582.9 + 4.24 ^b	981.3 + 5.22⁵	1282 + 3.69⁵
OA +T-2	75.37 <u>+</u> 1.87°	98.33 <u>+</u> 3.66 ^d	193.6 <u>+</u> 2.25 ^d	321.5 <u>+</u> 2.28 ^d	491.2 <u>+</u> 2.28 ^d

Means within each column bearing common superscript do not differ significantly (P<0.05)

ppm OA +2.0 ppm T-2 (OA+T-2) in a Completely Randomized Design manner, forming a total of 4 dietary treatments with three replicates and fourteen chicks per replicate in each group. Each replicate group of chicks were housed in an independent pen of conventional deep litter house. Chicks in all the replicates were reared up to five week of age under uniform standard conditions throughout the study. Brooding was done till 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.

Toxin production: OA and T-2 were produced using the pure culture of *Aspergillus ochraceus* MTCC 4643 and *Fusarium sporotrichoides* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India) grown on Czapek's agar and oatmeal agar, respectively. Then OA and T-2 toxin was extracted as described by [6, 7] and quantified by thin layer chromatography (TLC) as described by [8].

Diet formulation: Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-3 wks) (2895 Kcal/kg ME and 20.84% CP) and finisher (4-5 wks) (2994 Kcal/kg ME & 18.58% CP) feed. Chicks were provided ad libitum feed and water throughout the study. To this basal diet, required quantities of contaminated culture materials were added to attain dietary levels of 1.0 ppm of ochratoxin and 2.0 ppm of T-2 toxin to each of the toxin containing diets. Compounded experimental diets were analyzed for mycotoxin contents were finally cross-checked by TLC method of analysis. Feeding of test diets commenced at first day of age and continued till the termination of experiment at five weeks of age. Chicks were vaccinated against Newcastle Disease (ND) on 7th day using F1 strain and against Infectious Bursal Disease (IBD) on 14th day using intermediate strain (Ventri's Biologicals, Bangalore, India). Both the vaccines were given by oculonasal and ocular routes.

Data collection: Body weight and feed intake were recorded weekly. 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 5th week of age. Serum was separated after 8 to 10 hours as per the standard procedures [9] 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) were determined using automatic analyzer (Boehringer Mannhein Hitachi 704 automatic analyzer, Japan), antibody titers against ND and IBD using ELISA technique. The birds were sacrificed and liver, kidney, gizzard, spleen, bursa of Fabricius and thymus were extracted and weighed. The weights were adjusted to one kg live weight and the treatment means were calculated.

Statistical analysis: The experimental data were analyzed statistically by using the General Linear Model procedure of Statistical Analysis System [10]. Duncan multiple range test was employed for comparison of the means [11]. The result of this study was subjected to one way ANOVA test.

Results and Discussion

The data of effect of different dietary treatments on growth performance, organ weights, immune response and biochemical parameters are presented in Table 1, 2 and 3.

Growth performance: Body weights were significantly (P<0.05) lower beginning with first week for OA and OA plus T-2 treatments during third week for T-2 treatment and this reduction in body weights continued throughout the study. The lowest body weight was seen in group fed with combination of OA and T-2 toxin, whereas highest body weight was recorded in control diet. Our findings on reduction in body weights are consistent with earlier reports of feeding OA [12, 13], T-2 [14, 15] and OA plus T-2 combination [17]. Growth depression effects of OA and T-2 appears to have primarily resulted by their inhibitory action on protein synthesis and nutrient utilization [18].

Organ weights: Compared with those of controls, relative weights of liver, kidney and gizzard were significantly (P<0.05) increased in the groups receiving Ochratoxin A or T-2 toxin, singly and in combination, whereas relative weight of pancreas was not altered. Ochratoxin A plus T-2 combination fed group showed significant (P<0.05) increase in relative liver, kidney and gizzard weights followed by OA (and T-2 fed group, respectively). These findings agree with the results of [18, 19, 20] for OA, [21, 22] for T-2 and [18, 23] for OA plus T-2 combination treatments. The increase in relative weights of liver could be attributed to increased lipid metabolism in liver due to impaired

Table-2. Effect of individual and combined	toxicities of ochratoxin	and T-2 toxin on organ	1 weights (g/ kg live wt.) of
broiler chickens at 5 th wk of age (Mean <u>+</u> SE)		0	

Treatment	Liver	Kidney	Pancreas	Gizzard	Spleen [№]	Bursa	Thymus
Control	27.00+0.57°	8.00+0.56°	4.66+0.33	23.33+0.33 ^d	1.33+0.16	2.00+0.28ª	3.66+0.33ª
OA	30.67+0.66ª	11.33+0.66 ^{ab}	3.66+0.33	24.67+0.88 ^{ab}	1.83+0.16	1.16+0.16 ^{ab}	2.16+0.44 ^⁵
T-2	28.00+0.33 ^{ab}	10.00+0.57 ^{ab}	4.66+0.33	26.00+0.57 ^{abc}	1.83+0.16	1.33 + 0.16 ^{ab}	1.83 + 0.16 ^⁵
OA +T-2	30.67 <u>+</u> 0.66ª	11.17 <u>+</u> 0.16 ^{ab}	3.33 <u>+</u> 0.33	27.0 <u>+</u> 01.0 ^{ab}	1.83 <u>+</u> 0.16	0.83 <u>+</u> 0.16⁵	1.83 <u>+</u> 0.16⁵

Means within each column bearing common superscript do not differ significantly (P<0.05)

Table-3. Effect of individual and combined toxicities of ochratoxin and T-2 toxin on Immune and Biochemical parameters of broiler chickens at 5^{th} wk of age (Mean<u>+</u>SE)

Treatment	ND titer	IBD titer	Serum protein (g %)	Serum Albumin (g %)	Uric acid (µg/dl)
Control	4756.0 <u>+</u> 90.22 ^ª	4404.0 <u>+</u> 45.82 ^ª	2.93 <u>+</u> 0.13 ^{ab}	1.25 <u>+</u> 0.08ª	675.6 <u>+</u> 12.72 ^d
OA	3438.0 <u>+</u> 49.2 [°]	3024.0 <u>+</u> 73.04 ^d	1.88 <u>+</u> 0.02 ^{cd}	0.76 <u>+</u> 0.01⁵	1712.0 <u>+</u> 11.38ª
T-2	3255.0 <u>+</u> 75.92 ^b	3713.0 <u>+</u> 67.51 [°]	2.60 <u>+</u> 0.06 ^{bc}	1.17 <u>+</u> 0.06ª	1507.0 <u>+</u> 5.28 ^b
OA +T-2	3411.0 <u>+</u> 57.45 ^c	2993.0 <u>+</u> 63.25 ^d	1.84 <u>+</u> 0.03 ^d	0.78 <u>+</u> 0.01⁵	1664.0 <u>+</u> 58.69 ^{ab}

Means within each column bearing common superscript do not differ significantly (P<0.05)

fat metabolism which brings appreciable changes in the general functioning and gross appearance of liver. The effects on gizzard are believed to be due to severe inflammation and the resultant thickening of the gastric mucosa [19]. Increase in kidney weight was more likely to be due to enlargement and hypertrophy of proximal convoluted tubules and infiltration of lymphoid cells as suggested by [24].

Lymphoid organs: The relative weight of thymus was significantly (P<0.05) decreased in the groups receiving OA or T-2 toxin singly and in combination, whereas relative weights of bursa and spleen were not altered compared to that of control (Table-2). The relative weight of bursa and thymus were significantly (P<0.05) depressed in the OA plus T-2 combination fed group, followed by OA and T-2 alone fed groups as compared to control group at fifth week of age. Mycotoxins are known to cause immunosuppression in chickens and concomitantly reduce the relative sizes of bursa of Fabricius and thymus responsible for immunological competence [25]. The reduction in size of lymphoid organs (bursa and thymus) might have been due to necrosis and cellular depletion by the mycotoxins. The findings of the study were comparable to the reports of [19, 26].

Immune response: The titer values against ND and antibody titres for IBD were significantly (P<0.05) decreased by OA, T-2 toxin alone and OA plus T-2 toxin combination treatments (Table-2). The highest depression was recorded in the group fed combined OA plus T-2 toxin followed by OA and T-2 toxin alone fed groups. This depression in titer values are clear indication of immunodepressing effects of mycotoxins on humoral antibody response. The reduction of antibody titers could be due to mycotoxins inhibiting DNA and protein synthesis through impairment of amino acid transport and m-RNA transcription, resulting in lowered level of antibody production [27, 28, 29]. The reduced antibody titers in T-2 toxicosis is in agreement with the reports of [30] who reported significant reduction of antibody titers against ND and IBD values in commercial broilers fed 3ppm T-2 toxin.

Serum biochemistry: A significant (P<0.05) reduction in serum total protein, albumin and increase in serum uric acid levels were observed in OA alone and OA plus T-2 toxin combination fed groups. In T-2 toxin alone treated group, serum uric acid values significantly (P<0.05) reduced from control values at fifth week of age (Table-3). The highest depression of serum total protein, albumin and highest increased levels of serum uric acid was recorded in the group fed OA plus T-2 toxin combination, followed by OA and T-2 toxin alone fed groups. The mechanism by which these toxins cause decrease in serum protein levels is thought to be due to the impairment of protein synthesis by inhibiting m-RNA transportation by binding to DNA. The findings of the present study were in agreement with [18, 22, 31].

Serum enzyme activity: Serum GGT activity was significantly (P<0.05) increased by OA and OA plus T-2 toxin combination as compared to control group, but the level of GGT was not altered by T-2 toxin alone treatment (Table-3). Highest level of serum GGT activity was recorded in OA plus T-2 combination group followed by OA and T-2 alone fed groups and lowest level of activity was observed in group fed control diet. The activity of serum ALT was significantly (P<0.05) decreased in chicks fed OA alone and OA plus T-2 toxin combination as compared to control group at fifth week of age. The lowest level of serum ALT activity was seen in OA plus T-2 combination group followed by OA and T-2 fed alone groups and the highest level of serum ALT activity was recorded in control diet. The increased serum GGT levels noted could be due to the sequalae of hepatocyte degeneration and subsequent leakage of enzymes into the circulation. The results of altered levels of serum GGT and serum ALT by feeding OA were in agreement with reports of [32-35].

Conclusion

The OA and T-2 toxins have been known to occur concomitantly in grain samples and the toxicological consequences of this interaction appear to be significant. The interaction between OA and T-2 for many parameters measured was significant and clearly indicates synergistic effect. Although understanding the mechanism of this interaction is beyond the scope of this study, this mycotoxin combination should be of major concern to the poultry industry due to its synergistic toxicity as they coexist in feeds and feed ingredients.

Authors' contributions

HCI carried out the experiment and drafted the final manuscript. BU designed the experiment, guided during the experiment and helped in analysis of the data. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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