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ORIGINAL ARTICLES

The Additional Effects of Aflatoxin and T-2 Toxin Combination on Commercial Broilers: II: Effects on Biochemical Parameters and Immune Status

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ABSTRACT

The combination effects of two mycotoxins (aflatoxin (AF) and T-2 toxin (T-2)) on the performance of broiler chickens were tested individually and in combinations using one hundred and sixty eight day-old Cobb broiler chicks which were obtained from a commercial hatchery and divided into four groups in 2X2 Complete Randomized Design of three replicates and fourteen chicks per replicate, with dietary treatments of 0.0 (control), 0.5µg/g AF, 2.0µg/g T-2 and their combination (0.5 µg/g AF+2.0 µg/g T-2). The chicks were housed in deep litter independent conventional system with feed and water *ad libitum* throughout the experimental study. The toxin treated birds exhibited a significant (P≤0.05) decrease in total serum protein, albumin and uric acid. 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). These findings were more pronounced in the combined group. Results showed that the presence of AF and T-2 in the diet may have a synergistic impact on the studied parameters of the broilers.

Key words: aflatoxin, T-2 toxin, Biochemical Parameters, broilers.

Introduction

The poultry feed and feed ingredients sometimes prone for mould growth when ever the moisture content is high. Many of these molds produce toxic metabolites during their growth called as mycotoxins. Aflatoxins are toxic metabolites and the most frequent contaminants of feed or feed ingredients produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The toxicity of AF in young broiler chickens have been well documented (Duarte & Smith, 2005). The T-2 is a highly toxic type A trichothecene mycotoxin produced by different *Fusarium* species, mainly *F. sporotrichoides* and to a lesser extent by *F. poae*. Both AF and T-2 are important to the poultry industry due to their synergistic toxicity and occurrence in the feeds. 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 (Huff *et al.* 1988; Hagler *et al.* 1992; Manafi *et al.* 2009). Broilers fed diets containing 4ppm T-2 and 2.5ppm AF showed synergistic effect between T-2 and AF (Huff *et al*. 1988). Both of these mycotoxins in combination produced a significant interaction effect on body weight. Additives effects of dietary T-2 and AF were also observed in broilers receiving 8ppm T-2 and 3.5ppm AF (Kubena *et al*. 1990). Combination of both the toxins decreased the body weight gain to a greater level than did either of the toxins. Synergistic toxic effects between T-2 (4ppm) and AF (2.5ppm) on relative weights of kidney, gizzard and heart was also reported by them, where the weights of these organs increased more than those recorded in the groups, received either of the toxin. Increased relative weights of liver, kidney, proventriculus, gizzard, spleen and pancreas were seen in broilers by feeding AF and T-2 combination (Kubena *et al*. 1990). Raju & Devegowda (2000) reported significant interaction of AF (0.3ppm) and T-2 (3ppm) for their additive effects on body weight and feed intake. Therefore, the aim of this study was initiated to characterize the interaction between AF and T-2 in young broiler chickens at lower levels.

Materials and Methods

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), two levels of AF (0.0 & 0.5ppm), two levels of T-2 (0.0 & 2.0ppm) and combination of 0.5ppm AF +2.0ppm T-2 (AF+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.

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Experimental Housing, Management and Test Diet:

Each replicate group of chicks housed in an independent pen, conventional sided 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.

AF and T-2 produced using the pure culture of *Aspergillus parasiticus* MTCC 1894 and *Fusarium sporotrichoides* MTCC 1894 (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160 036, India) grown on potato dextrose agar. Then AF and T-2 produced on rice and wheat respectively and toxin was extracted as described by Rukmini & Bhat (1978) and Romer (1975) and quantified by thin layer chromatography (TLC) as described by AOAC (1995).

Compounded feed was analyzed for the presence of AF and T-2 before including the rice and wheat culture materials, then the diets were prepared by incorporating required quantities of rice/wheat culture powder containing AF/T-2 into the diet so as to give the levels of 0.0 & 0.5ppm of AF B_1 and 2.0ppm of T-2. The compounded feed was analyzed for the presence of AF and T-2, after ascertaining the quality, the required quantities of rice/what culture powders were incorporated into the feed and so as to provide the levels of 0.5 & 2.0ppm of AF and T-2 respectively. The given toxin levels were finally cross-checked by TLC method of analysis.

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. 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 7^{th} day using F_1 strain (Ventri's Biologicals, Bangalore, India) and against Infectious Bursal Disease (IBD) on 14^{th} day using intermediate strain (Ventri's Biologicals, Bangalore, India). Both the vaccines were given by oculonasal and ocular routes.

Data Collection:

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 5^{th} 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 ºC 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 automatic analyzer (Boehringer Mannhein Hitachi 704 automatic analyzer, Japan), antibody titers against ND and IBD using ELISA technique.

The experimental data were analyzed statistically by using the General Linear Model procedure of Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Duncan multiple range test was employed for comparison of the means (Duncan, 1955). The result of this study was subjected to one way ANOVA test.

Results and Discussion

The influence of mycotoxin supplementation in the diet on the antibody titers against ND and IBD, serum protein, serum albumin, uric acid, the activities of GGT and ALT are presented in Table 1.

Significant ($P \leq 0.05$) increase in GGT and decrease in antibody titer values against ND and IBD, serum protein, serum albumin, uric acid and ALT were noticed during AF and T-2 feeding in the diets, individually as well as in combination. However, in the combined treatment group, these findings were more severe, which is attributed to synergistic effects between AF and T-2.

The depression in titer values are clear indication of immuno suppressive effects of both AF and T-2 on humoral antibody response. These findings were well substantiated by the previous reports (Umesh *et al*. 1990; Devegowda *et al*. 1994; Swamy & Devegowda, 1998; Ibrahim *et al*. 2000; Kumar *et al.* 2002; Gupta & Amarjit, 2003; Singh *et al.* 2003). The reduction of antibody titers could be due to inhibition of DNA and protein synthesis by AF through impairment of amino acid transport and mRNA transcription resulting in lowered level of antibody production (Thaxton *et al*. 1974). The reduced antibody titers in T-2 toxicosis is in agreement with the reports of Raju & Devegowda (2002) who reported significant reduction of antibody titers against ND and IBD values in commercial broilers fed 3ppm T-2.

The depression in serum albumin concentration resulting from feeding T-2 was clear indication of impairment in protein synthesis by inactivation of initiation and termination, possibly through its binding to ribosomes (Uneo, 1977). The finding of lower serum albumin values in broilers receiving T-2 were also reported by Kubena *et al*. (1998) and Bailey *et al*. (1998).

Reduction in concentration of serum protein and uric acid, when fed AF and combination of AF and T-2 were also reported by Kubena *et al.* (1998) and Huff *et al.* (1992). The inconsistency of serum enzymes during T-2 toxicosis was also reported by Chi & Mirocha (1978); Kubena *et al.* (1998) and Raju & Devegowda, (2002); Denli & Okan, (2006). These findings were in agreement with the findings of Kubena *et al.* (1998) who reported a similar reduction in uric acid and albumin values of broilers fed T-2.

Values for serum uric acid levels were decreased significantly ($P \leq 0.05$) during T-2 toxicosis of this study. The results were contrary to the findings of Huff *et al.* (1988) and Bailey *et al.* (1998) who reported no significant effects at 8mg/kg of T-2 on uric acid levels in broilers. Bailey *et al*. (1998) and Garcia *et al*. (2003) reported similar reduction in uric acid and albumin values in broilers receiving diets containing 5ppm T-2. Reduction in serum uric acid levels due to T-2 was also reported by Huff *et al.* (1988). Decreased uric acid levels presumely is due to decreased feed consumption leading to decreased protein utilization and metabolism as it is the end product of protein metabolism.

In the current study, GGT activity was significantly ($P \leq 0.05$) increased in broilers fed T-2 containing diets as compared to control. This indicates hepatocyte damage associated with T-2. On the contrary, Kubena *et al*. (1990) did not notice any increase in GGT activity on feeding 8ppm T-2 in broilers.

The AF and T-2s have been known to occur concomitantly in grain samples and the toxicololgical consequences of this interaction appear to be significant. The interaction between AF 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 concern to the poultry industry due to its synergistic toxicity as they coexist in feeds and feed ingredients.

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Table 1: Effects of aflatoxin and T-2 toxin 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.

Aflatoxin (µg/g)	T-2 Toxin (µg/g)	ND titer	IBD titer	Serum protein (g%)	Serum albumin (g%)	Uric acid (µg/dl)	GGT (IU/L)	ALT (IU/L)
0	0	4298±17.05^{ab}	4281±8.083^{a}	2.71±0.17^{a}	1.28±0.17^{a}	647.9±7.54^{a}	9.53±1.15^{d}	28.17±0.60^{a}
0.5	0	3204±106.3^{e}	3149±69.72^{d}	1.67±0.15^{bc}	1.10±0.18^{b}	600.4±6.73^{b}	17.8±1.72^{ab}	25.83±1.36^{b}
0	2.0	3255.0±75.92^{cd}	3713.3±67.51^{c}	2.60±0.12^{bc}	1.18±0.06^{bc}	506.60±5.29^{bc}	13.93±1.89^{ab}	25.50±1.30^{b}
0.5	2.0	3529.0±54.60^{c}	3513.3±71.02^{d}	2.33±0.9^{cd}	1.13±0.09^{bc}	581.10±1.08^{d}	16.40±1.27^{a}	18.93±1.23^{c}

$^{a-e}$ Means in column with different superscript differ significantly at ($p \leq 0.05$).
AF: 0.5ppm and T-2: 2ppm.

Conclusion:

In the present experimental study, the AF and T-2 were fed to the broiler chickens. Both the toxins were produced deleterious effects on the serum protein, albumin and uric acid of the birds. The serum ALT and GGT levels were decreased and antibody titers against ND and IBD were decreased significantly. The abnormalities are more pronounced in the combined toxicity of AF and T-2. Based on these findings, it can be concluded that AF and T-2 act synergistically at low level and hamper the production in the birds.

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