



ORIGINAL ARTICLES

The Additional Effects of Aflatoxin and T-2 Toxin Combination on Commercial Broilers: I: Effects on Performance Parameters and Internal Organs

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ABSTRACT

The effects of aflatoxin (AF) and T-2 toxin (T-2) on the performance of broiler chickens were studied individually and in combinations. One hundred and sixty eight day-old Cobb broiler chicks 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), 0.5 μ g/g AF, 2.0 μ g/g T-2 and their combination (0.5 μ g/g AF+2.0 μ g/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 ($P\leq 0.05$) decrease in body weight and feed consumption. The relative weights of pancreas, thymus and bursa of Fabricius were also reduced significantly ($P\leq 0.05$). These findings were more pronounced in the combined group. The data from the present study indicate that the presence of AF and T-2 in the diet can produce a synergistic affect on the performance of the birds.

Key words: aflatoxin, T-2 toxin, Performance, broilers.

Introduction

Cereal grains and associated by-products constitute important sources of energy for poultry. There is increasing evidence that global supplies of cereal grains for animal feedstuffs are commonly contaminated with mycotoxins. Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species. Aflatoxin B1 (AFB1), the most toxic of all aflatoxins (AFB1, AFB2, AFG1 and AFG2), is produced by certain strains of fungi in greater quantities than in others. In poultry, aflatoxin ingestion leads to "Aflatoxicosis" syndrome which is characterized by retardation of growth, feed consumption, feed conversion efficiency, bruising, immunosuppression and mortality. 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 (Hagler *et al.*, 1984).

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. Additive 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.

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 B₁ 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/wheat 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 7th day using F₁ strain (Ventri's Biologicals, Bangalore, India) and against Infectious Bursal Disease (IBD) on 14th day using intermediate strain (Ventri's Biologicals, Bangalore, India). Both the vaccines were given by ocular and ocular routes.

Data Collection:

At the end of the trial, body weight and feed consumption were recorded and gain in weight and feed efficiency were calculated. Six birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial and the weight of internal organs such as liver, kidney, gizzard, pancreas, spleen, thymus and bursa were recorded and expressed as grams per kilogram live body weight. 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 (Calnek *et al.* 1992) and was stored at -20 °C for subsequent analysis. 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

Dietary AF and T-2 produced lower body weight, feed consumption and higher feed conversion ratio significantly ($P \leq 0.05$) at the end of fifth week, when compared to controls (Table 1). In the combination group of AF+T-2, further suppression of body weight and feed consumption has been noticed during the experimental period. All the broilers were examined for oral lesions. However, oral lesions were found only in birds given the T-2. Growth depression and decreased feed consumption were recorded consistently in AF and T-2 toxicities by different scientists (Raju & Devegowda, 2000; Arvind *et al.* 2003; Swamy & Devegowda, 1998), which is in agreement with the findings of the present study. The growth depression could be attributed to the AF inhibitory action on protein synthesis as well as poor nutrient utilization (Marquardt & Frohlich, 1992). The T-2 is extremely caustic and produces radiomimetic action on dividing cells of organs like intestine (Uneo, 1977; Girish & Devegowda, 2004). The toxin also causes inhibition of protein synthesis by binding to ribosomes (Uneo, 1977; Kubena *et al.* 1990; Manafi *et al.* 2009). This property might be possibly responsible for growth depression and poor feed conversion in T-2 toxicosis. However, poor feed efficiency in AF and T-2 toxicosis was consistently observed by many researchers (Hoehler & Marquardt, 1996; Raju & Devegowda, 2002; Arvind *et al.* 2003; Manafi *et al.* 2008a). The relative weights of various organs were expressed as grams per kilogram live body weight are presented in Table 2. The AF showed significant ($P \leq 0.05$) increase in the size of liver,

kidney, gizzard and spleen and decrease weight of pancreas, bursa of fabricius and thymus. Inclusion of T-2 in the diet showed significant ($P \leq 0.05$) increase in the relative weights of the kidney, spleen, and decrease weight of pancreas, bursa of fabricius and thymus. In the combination treatment, significant ($P \leq 0.05$) increase in relative weights of liver, kidney, gizzard, spleen and decrease weight of pancreas, bursa of fabricius and thymus. The AF showed significant ($P \leq 0.05$) increase in the size of liver, kidney, gizzard and spleen and decrease weight of pancreas, bursa of fabricius and thymus which is in agreement with Shaline *et al.* (1980). Addition of T-2 toxin in the diet showed significant ($P \leq 0.05$) increase in the relative weights of the kidney, spleen, and decrease weight of pancreas, bursa of fabricius and thymus. In the combination treatment, significant ($P \leq 0.05$) increase in relative weights of liver, kidney, gizzard, spleen and decrease weight of pancreas, bursa of fabricius and thymus.

Table 1: Effects of aflatoxin and T-2 toxin on fifth week body weight, feed consumption and feed conversion ratio of broilers.

Aflatoxin ($\mu\text{g/g}$)	T-2 Toxin ($\mu\text{g/g}$)	Body weight (g)	Feed consumption (g/bird)	Feed conversion ratio
0	0	2513 \pm 6.07 ^a	4799.8 \pm 6.07 ^a	1.91 \pm 0.003 ^a
0.5	0	2306 \pm 2.92 ^b	4819.5 \pm 2.92 ^b	2.09 \pm 0.005 ^c
0	2.0	2282.0 \pm 3.70 ^c	4586.8 \pm 15.56 ^c	2.01 \pm 0.00 ^b
0.5	2.0	949.37 \pm 4.48 ^d	4421.5 \pm 7.24 ^d	2.16 \pm 0.01 ^d

^{a-d} Means in column with different superscript differ significantly at ($P \leq 0.05$).

AF: 0.5ppm and T-2: 2ppm.

Table 2: Effects of aflatoxin and T-2 toxin on relative weights of organs (grams per kg body weight) in broilers.

Aflatoxin ($\mu\text{g/g}$)	T-2 Toxin ($\mu\text{g/g}$)	Liver	kidney	Gizzard	Pancreas	Spleen	Bursa	Thymus
0	0	27.60 \pm 0.76 ^c	8.16 \pm 0.16 ^a	24.53 \pm 0.60 ^a	5.10 \pm 0.17 ^b	1.57 \pm 0.28 ^b	1.69 \pm 0.02 ^a	4.36 \pm 0.21 ^a
0.5	0	33.00 \pm 0.57 ^a	9.66 \pm 0.16 ^b	25.67 \pm 1.20 ^b	4.66 \pm 0.33 ^a	1.66 \pm 0.16 ^a	1.16 \pm 0.16 ^b	2.66 \pm 0.33 ^b
0	2.0	28.00 \pm 0.58 ^{cd}	8.67 \pm 0.33 ^{bc}	24.33 \pm 0.67 ^{abc}	4.67 \pm 0.33 ^a	1.83 \pm 0.17 ^a	1.33 \pm 0.17 ^a	3.33 \pm 0.33 ^{bc}
0.5	2.0	32.33 \pm 0.88 ^a	10.67 \pm 0.33 ^d	25.33 \pm 0.33 ^{bc}	4.33 \pm 0.33 ^a	1.83 \pm 0.17 ^a	1.00 \pm 0.29 ^c	2.33 \pm 0.33 ^b

^{a-d} Means in column with different superscript differ significantly at ($p \leq 0.05$).

AF: 0.5ppm and T-2: 2ppm.

The increase in liver and kidney weights were in accordance with the findings of Raju & Devegowda (2000); Perozo & Rivera, (2003); Miazzo *et al.* (2005); Manafi *et al.* (2008b)

The increase in the weights of liver and kidney signifies the accumulation of lipid in these organs because of fat metabolism primarily occurs in the liver, while lipidemia with subsequent fat deposition might contribute for increased kidney weights as well as severe inflammation. The increase in organ weights in AF+T-2 was reported by Kubena *et al.* (1998); Raju & Devegowda, (2002); Girish & Devegowda (2004). They opined that the increased weights could be attributed to increased lipid deposition in the liver due to impaired fat metabolism which brings appreciable changes in the general functioning and gross appearance of liver. The effects on gizzard were believed to be as a result of severe inflammation and the resultant thickening of the mucosa (Kubena *et al.* 1997).

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 performance, decrease in body weight, feed consumption, of the birds. The relative weights of pancreas, thymus and bursa of Fabricius were also reduced. 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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