World Applied Sciences Journal 17 (3): 364-367, 2012
ISSN 1818-4952

Synergistic Effects of Two Commonly Contaminating Mycotoxins (Aflatoxin and T-2 Toxin) on Biochemical Parameters and Immune Status of Broiler Chickens

[1]M. Manafi, [2]B. Umakantha, [3]K. Mohan and [4]H.D. Narayana Swamy

[1]Department of Animal Science, Malayer University, Malayer, Iran
[2]Department of Poultry Science, Veterinary College, KVAFSU, Bangalore, India
[3]Department of Veterinary Pharmacology, Veterinary College, KVAFSU, Bangalore, India
[4]Department of Veterinary Pathology, Veterinary College, KVAFSU, Bangalore, India

Abstract: The synergistic effects of two contaminating mycotoxins aflatoxin (AF) and T-2 toxin (T-2) in the feed of poultry on the performance of broiler chickens were studied individually and in combination, by using one hundred and sixty eight day-old commercial broiler chicks obtained from a commercial hatchery and randomly separated 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 ($P \leq 0.05$) decrease in total serum protein, albumin and uric acid. The serum alanine amino transferase (ALT) levels were decreased and antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) were also decreased significantly ($P \leq 0.05$). These findings were more severe in the combined group of AF and T-2. Results indicated that the presence of AF and T-2 in the diet may have a very severe synergistic effect on these measured factors of the commercial chicks.

Key words: Aflatoxin · T-2 Toxin · Biochemical Parameters · Broilers

INTRODUCTION

The poultry feed and feed ingredients sometimes are prone for mould growth when ever the moisture content is high. Many of the 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 has been well documented [1]. 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* [1].

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 [2, 3]. Broilers fed diets containing 4ppm T-2 and 2.5ppm AF showed synergistic effect between T-2 and AF [4]. 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 [3, 4]. 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, where the weights of these organs increased more than those recorded in the groups receiving either of the toxins. Increased relative weights of liver, kidney, proventriculus, gizzard, spleen and pancreas were seen in broilers fed AF and T-2 combination [5] and a significant interaction of AF (0.3ppm) and T-2 (3ppm) for their additive effects on body weight and feed intake was also reported. Therefore, the aim of this study was initiated to characterize the interaction between AF and T-2 in young broiler chickens at lower levels.

Corresponding Author: M. Manafi, Department of Animal Science, Malayer University, Malayer, Iran.

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 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 was housed in an independent pen, conventional sided deep litter house. Chicks in all the replicates were reared up to five weeks 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 were 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 toxins produced on relevant Medias were extracted as described by Rukmini and Bhat [6] and quantified by thin layer chromatography (TLC) [7].

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 0.5ppm of AF B_1 and 2.0ppm of T-2. The given toxin levels were finally cross-checked by TLC method of analysis [7]. 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 was 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) [8]. 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 [8] 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) using automatic analyzer (Boehringer Mannhein Hitachi 704 automatic analyzer, Japan) and 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 [9]. Duncan multiple range test was employed for comparison of the means [10]. The results of this study were subjected to one way ANOVA test.

RESULTS AND DISCUSSION

The influence of mycotoxins supplementation in the diet on the antibody titers against ND and IBD, serum protein, serum albumin, uric acid, the activities of GGT and ALT were 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 immune suppressive effects of both AF and T-2 on humoral antibody response. These findings were well substantiated by previous reports [4,11,12]. 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 [13]. The reduced antibody titers in T-2 toxicity is in agreement with Ueno [14] 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 [15]. Lower serum albumin values in broilers receiving T-2 were also reported [4].

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 superscripts differed significantly at (p=0.05).

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

Reduction in concentration of serum protein and uric acid, when broilers were fed AF and combination of AF and T-2 was also reported [4, 15, 16]. The inconsistency of serum enzymes during T-2 toxicosis was also reported [17, 18]. These findings are in agreement with the findings of Arvind *et al.* [19] 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 are contrary to the findings of Salahi *et al.* [17] who reported no significant effects at 8mg/kg of T-2 on uric acid levels in broilers. Arvind *et al.* [19] and Ghahri *et al.* [20] 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 [21, 22]. Decreased uric acid levels presumably is due to decreased feed consumption leading to decreased protein utilization and metabolism as it is the end product of protein metabolism [23].

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 hepatocytes damage associated with T-2. On the contrary, Azarkhsh *et al.* [23] 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.

In the present experimental study, both AF and T-2 produced deleterious effects on the serum protein, albumin and uric acid of the birds. The serum ALT levels were decreased and antibody titers against ND and IBD were also 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.

REFERENCES

1. Hagler, W.M., K. Tyczkowska and P.B. Hamilton, 1984. Simultaneous occurrence of deoxynivalenol, zearalenone and aflatoxin in 1982 scabby wheat from Midwestern United States. Appl. Environmen. Microbiol., 47: 151-154.
2. Kubena, L.F., R.B. Harvey, W.E. Huff, D.E. Carier, T.D. Phillips and G.E. Rottinghaus, 1990. Efficacy of hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. Poult. Sci., 69: 1078-1086.
3. Huff, W.E., R.B. Harvey, L.F. Kubena and G.E. Rottinghaus, 1988. Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. Poult. Sci., 67: 1418-1423.
4. Manafi, M., K. Mohan and M. Noor Ali, 2011. Effect of Ochratoxin A on Coccidiosis-Challenged Broiler Chicks. World Mycotoxin J., 4: 177-181.
5. Rukmini, C. and R.V. Bhat, 1978. Occurrence of T-2 toxin in Fusarium infested sorghum from India. J. Agri. Food Chemist., 26: 647-649.
6. Fraga, M.E., F. Curvello, M.J. Gatti, L.R. Cavaglieri, A.M. Dalcero and C.A. Darocha Rosa, 2007. Protemtial aflatoxin and ochratoxin A production by Aspergillus species in poultry feed processing. Vet. Res. Communications, 31: 345-353.
7. A.O.A.C. 1995. Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists, Washington, D.C.
8. Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid and Jr. H.W. Yoder, 1992. Diseases of Poultry, 9th ed. Wolfe Publishing, Ltd. USA.
9. S.A.S. 2000. Statistical Analysis Systems User's Guide: Statistics. SAS Institute Inc. Cary, NC, USA,

10. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
11. Verma, J., T.S. Johri, B.K. Swan and S. Ameena, 2004. Effect of graded levels of aflatoxin and their combination on the performance and immune response of broilers. British Poult. Sci., 45: 512-518.
12. Raju, M.V.L.N. and G. Devegowda, 2000. Influence of esterified glucomannan on performance and organic morphology, serum biochemistry and hematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). British Poult. Sci., 41: 640-650.
13. Ahmadi, M., 2010. Effect of Turmeric (Curcumin longa) Powder on Performance, Oxidative Strees State and Some of Blood Parameters in Broilers Fed on Diet Containing Aflatoxin B1. Global Veterinaria, 5: 312-317.
14. Ueno, Y., 1997. Mode of action of trichothecenes. Pure and Applied Chemistry, 49: 1737-3745.
15. Shaline. K.S.B., B. Jr. Howrth and R.D. Wyatt, 1980. Effect of dietary aflatoxin in reproductive performance on mature white leghorn. Poult. Sci., 59: 1311-1315.
16. Marquardt, D.R. and A. Frohlich, 1992. A review of recent advances in understanding ochratoxicosis. J. Anim. Sci., 70: 3968-3988.
17. Salahi, A., S.N. Mousavi, F. Foroudi, M.M. Khabisi and M. Norozi, 2011. Effects of in ovo Injection of Butyric Acid on Broiler Breeder Eggs on Hatching Parameters, Chick Quality and Performance. Global Veterinaria, 7: 468-477.
18. Girish, C.K. and G. Devegowda, 2004. Evaluation of modifies glucomannan (Mycosorb) and HSCAS to ameliorate the individual and combined toxicity of aflatoxin and T-2 toxin in broiler chickens. Aust. Poult. Sci. Symp., 16: 126-129. Sydney, Australia.
19. Arvind, K.L., V.S. Patil, G. Devegowda, B. Umakantha and S.P Ganpule, 2003. Efficacy of Esterified glucomannan to counteract Mycotoxicosis in naturally contaminated feed on performance, serum biochemical and hematological parameters in broilers. Poult. Sci., 82: 571-576.
20. Ghahri, H., R. Habibian and M. Abdolah Fam, 2010. Effect of Sodium Bentonite, Mannan Oligosaccharide and Humate on Performance and Serum Biochemical Parameters During Aflatoxicosis in Broiler Chickens. Global Veterinaria, 5: 129-134
21. Miazzo, R., M.F. Pevalta, C. Magnoli, M. Salvano, S. Ferrero, S.M. Chiacchiera, E.C.Q. Carralno, C.A.R. Rosa and A. Dalcero, 2005. Efficacy of sodium bentonite as a detoxifier of broiler fed contaminated with aflatoxin and fumonisin. Poult. Sci., 84: 1-8.
22. Thaxton, J.P., J.T. Timb and B. Jaotpm, 1974. Immunosuppression in chicken by aflatoxin. Poult. Sci., 53: 721-725.
23. Azarakhsh, Y., A. Sabokbar and M. Bayat, 2011. Incidence of the Most Common Toxigenic Aspergillus Species in Broiler Feeds in Kermanshah Province, West of Iran. Global Veterinaria 6: 73-77.