Libyan Agriculture Research Center Journal International 1 (3): 177-181, 2010
ISSN 2219-4304

Studies on Tolerance Limit of Aflatoxin in Commercial Broilers

[1]G. Ananda Manegar, [2]B.E. Shambulingappa and [3]K.J. Ananda

[1]Department of Animal Sciences, University of Agricultural Sciences, G.K.V.K., Bangalore, India
[2]Department of Microbiology, Veterinary College, KVAFSU, Shimoga, Karnataka, India
[3]Department of Parasitology, Veterinary College, KVAFSU, Shimoga, Karnataka, India

Abstract: Totally sixty birds aged three days old and unsexed, unvaccinated broiler chicks (Hubbard) were used to study the tolerance limit of aflatoxin in the commercial broilers. The present study showed that the body weight significantly decreased in chickens receiving 400 and 600 ppb aflatoxin and feed consumption was significantly decreased in 400 and 600 ppb fed groups at 2nd, 4th and 6th weeks of age. A significant reduction in feed efficiency of bird was observed at 2nd, 4th and 6th week of age in 400 ppb and 600 ppb aflatoxin fed groups. The maximum mortality was encountered in 600 ppb aflatoxin fed group (21.66%) followed by 400 ppb (15%), 200 ppb (6.66%) and 100 ppb (3.33%). The relative weight of the liver was significantly increased at the aflatoxin treatment of 200, 400 and 600 ppb levels. A significant reduction in the relative size of bursa of fabracius recorded at 200, 400 and 600 ppb aflatoxin level as compared to 50 ppb and control. A progressive reduction in serum protein concentration was observed in broiler chickens fed diets containing 100, 200, 400 and 600 ppb of aflatoxin. The present study indicated that aflatoxin causes bursal regression and suppresses primary immune response for NCD and IBD as evident by fall in ELISA titres.

Key words: Broiler chicken % Tolerance limit of Aflatoxin

INTRODUCTION

Aflatoxins are a major concern in poultry production because of serious economic losses. Broiler production as showed a tremendous growth of 15-20% per annum. To meet the future projection, use of non-conventional feed resources, better utilization of available ingredients and judicious methods to counter act anti-nutritional factors become inevitable. The tropical climate with hot and humid condition prevailing in our country coupled with poor harvesting of crops during monsoon season, inadequate drying and storage facilities make feed stuff susceptible to fungal contamination [1] resulting in greater economic losses to poultry industry through Mycotoxicosis.

Mycotoxicosis is the condition associated with fungal contamination of feeds or feed ingredients. Among mycotoxins, Aflatoxins are predominant and are one of the major fungal toxins which are attributed for potential problem in livestock feeds. Aflatoxins are potent carcinogens and cause growth depression [2] and reduced disease resistance [3] in poultry, other livestocks and also human beings.

The Aflatoxin contamination of feed stuff has been reported to range from 10-1500 ppb in commercially used feed ingredients and 34-115 ppb in mixed feed samples [4]. The high level of contamination though not resulting in severe outbreaks of aflatoxicosis, causes heavy economic loss in terms of health and production. Field reports on the occurrence of aflatoxicosis in poultry in India are uncommon. So the present investigation aimed at the tolerance limit of aflatoxin in the present day fast growing commercial broilers.

MATERIALS AND METHODS

Birds and Diet: A total number of sixty unsexed, three days old unvaccinated broiler chicks (Hubbard) were obtained from a commercial hatchery and were individually weighed, wing banded, then randomly and equally distributed into 6 treatments of birds each. The chicks were housed in floor pens with continuous and

Corresponding Author: Dr. G. Ananda Manegar, Assistant Professor, Department of Animal Sciences, University of Agricultural Sciences, G.K.V.K., Bangalore, India.

subjected to light and were fed adlibitum with commercial feed from day one to 42 days of age. Chicks were vaccinated for Ranikhet disease on 5th day with F-strain and infectious bursal disease on 14th and 24th day with Georgia strain.

The basal diet (starter and finisher broiler diets) was formulated as per the bureau of Indian recommendation for all nutrients. (Maize and Soya bean meal based diet providing 22.25 percent protein, 2780 K cal per kg in broiler starter and 19.74% protein, 2869 K cal/kg in finisher diets) The basal diet was also tested for possible residual aflatoxin before feeding [5] and there was no detectable levels present (Detection limit 1mgm/Kg feed, recovery of the extraction method 95%).

Experimental Design: The experimental design consists of six dietary treatments (1) Control: basal diet (2) 50 ppb of aflatoxin in diet (3) 100 ppb of aflatoxin in diet (4) 200 ppb of aflatoxin in diet (5) 400 ppb of aflatoxin in diet (6) 600 ppb of aflatoxin in diet.

Aflatoxin (AF): The aflatoxin was produced from the pure culture of *Aspergillus parasiticus* NRRL 2999. The culture was maintained on potato dextrose agar (PDA) for about 7 days at 25^0c before harvesting mould spores. The spore suspension was used for production of aflatoxin on rice as per the method by Shotwell *et al.* [6]. The toxin produced in the rice was extracted and analyzed by thin layer chromatography (TLC) as described by A.O.A.C [7]. The rice powder was incorporated into basal diet to provide the required, 50 ppb, 100 ppb, 200 ppb, 400 ppb and 600 ppb of aflatoxin/kg of feed and controlled group was not added aflatoxin.

Pathological Examination: When chicks reached 6 weeks of age, the feeding trial was terminated and 5 broilers from each group selected at random and killed for pathological examination, while the other chicks in the groups were used to measure growth parameters (biweekly) and serum bio-chemical and hematological variables were determined. A detailed necropsy was conducted. Liver tissue and bursa fabricius were collected in 10% neutral buffered formalin.

RESULTS AND DISCUSSION

The present study showed that the body weight significantly decreased in groups receiving 400 and 600 ppb AF (Table 1). However data shows that aflatoxin levels as low as 100 ppb can effect the growth at the end of 5th week in broilers. The depression in growth upon feeding aflatoxin could be attributed to reduced protein synthesis as reported by Smith *et al.* [8], impaired nutrient absorption reduced pancreatic digestive enzyme production by Osborne and Hamilton, [9] and reduced appetite by Sharline *et al.* [10].

Feed consumption in broilers was significantly decreased in 400 and 600 ppb of AF fed groups at 2nd, 4th and 6th weeks of age. This is suggestive of reduced appetite during aflatoxicosis due to impaired liver metabolism caused by the liver damage by the aflatoxins as reported by Sharline *et al.* [10]; Nandkumar *et al*. [11]; Rajasekhar Reddy *et al.* [12]; Johri and Majmudar, [13].

A significant reduction in feed efficiency of bird was observed at 2nd, 4th and 6th week of age in 400 ppb and 600 ppb AF fed groups. The poorer fed efficiency in broilers observed in the present study could be due to

Table 1: Effect of graded level of aflatoxin on Body weight, feed consumption and feed efficiency

		Body wt			Feed consumption			Feed efficiency		
		II Week	IV Week	VI Week	II Week	IV Week	VI Week	II Week	IV Week	VI Week
T1	0	255±8.65^{a}	793±16.65^{a}	1593±32.65^{a}	334±6.14^{a}	1322±38.17^{ab}	3087±10.42^{a}	1.57±0.03^{a}	1.76±0.03^{a}	1.99±0.02^{a}
T2	50	250±8.13^{a}	791±19.83^{a}	1550±33.94^{a}	335±4.35^{a}	1347±5.04^{a}	3146±94.14^{a}	1.62±0.03^{ab}	1.83±0.02^{a}	2.08±0.01^{a}
T3	100	256±7.53^{a}	746±18.59^{b}	1513±31.90^{b}	350±1.90^{a}	1308±14.51^{ab}	3098±73.11^{a}	1.64±0.03^{ab}	1.86±0.03^{a}	2.10±0.01^{a}
T4	200	243±7.93^{a}	621±26.31^{c}	1351±45.32^{c}	342±9.69^{a}	1120±17.18^{bc}	3032±62.04^{a}	1.71±0.02^{bc}	1.94±0.02^{a}	2.31±0.01^{b}
T5	400	214±8.52^{bc}	539±20.91^{d}	1110±51.52^{d}	301±5.91^{b}	965±16.35^{cd}	2579±27.78^{b}	1.76±0.02^{c}	2.02±0.01^{b}	2.41±0.01^{c}
T6	600	202±8.21^{c}	440±24.30^{e}	857±36.60^{e}	281±1.85^{b}	803±32.77^{d}	1999±96.17^{c}	1.76±0.02^{c}	2.03±0.01^{b}	2.43±0.02^{c}

Table 2: Effect of graded levels of aflatoxin on the mortality percentage of broilers

Treatment	Aflatoxin (ppb)	Mortality %
T1	0	0.00
T2	50	0.00
T3	100	3.33
T4	200	6.66
T5	400	15.00
T6	600	21.66

Table 3: Details of gross lesions of liver in birds fed with graded levels of aflatoxin in the diet

Treatments	T1	T2	T3	T4	T5	T6
Aflatoxin (ppb)	0	50	100	200	400	600
Size						
Normal	+	+	-	-	-	-
Enlarged	-	-	+	+	++	++
Consistency						
Normal	+	+	-	-	-	-
Enlarged	-	-	+	+	++	++
Colour						
Normal	+	+	-	-	-	-
Pale	-	-	+	+	++	++
Congested	-	-	+	+	++	++
Borders						
Normal	+	+	-	-	-	-
Rounded	-	-	+	+	++	++

+ = Present ++ = Present - = Negative

Table 4: Effect of supplementation of graded levels of aflatoxin on relative weights of liver and Bursa in Fabricius (% of body weight)

Treatment	Aflatoxin (ppb)	Liver	Bursa of Fabricius
T1	0	2.72 ± 0.23^{d}	0.103 ± 0.005^{a}
T2	50	2.89 ± 0.20^{cd}	0.103 ± 0.005^{a}
T3	100	3.30 ± 0.07^{bcd}	0.090 ± 0.009^{ab}
T4	200	3.42 ± 0.05^{bc}	0.060 ± 0.008^{bc}
T5	400	3.70 ± 0.11^{b}	0.066 ± 0.005^{bc}
T6	600	4.68 ± 0.17^{a}	0.056 ± 0.007^{c}

the reduced protein synthesis resulting in reduced digestive enzyme production necessary for utilization of nutrients as reported by (Lanza *et al.* [14].

The maximum mortality was encountered in 600 ppb AF fed group (21.66%) followed by 400 ppb (15%), 200 ppb (6.66%) and 100 ppb (3.33%) (Table 2). Increased death of the birds was observed with increased level of toxin indicating the dose related affected of AF. The mortality could be due to the production of 2, 3-dihydrodiol of AF B1 in the liver resulting in rapid onset of necrosis of the liver causing death [15] or due to interference of AF with immune system thereby reducing the resistance of the bird [16].

The liver is the main target for AF according to Dafalla, *et al.* [17]; Ortatatli *et al.* [18] so the weight and the toxin content of the liver were examined. The relative weight of the liver was significantly increased at the AF treatment of 200, 400 and 600 ppb levels (Table 3). The increase in the liver weight could be attributed to increased lipid deposits in the liver due to impaired fat metabolism [19]. The hepatic lipidosis is primarily mediated through inhibition of phospholipids synthesis and cholesterol. This in-turn affects the transportation of lipid from the liver.

A significant reduction in the relative size of bursa of fabracius recorded at 200, 400 and 600 ppb AF level as compared to 50 ppb and control (Table 4). AF is known to cause immune suppression in chicken and concomitantly reduce the relative size of the bursa of fabracius is responsible for immunological competence reported by Taxton *et al.* [16]; Kubena, *et al.* [20]. The immune suppressive effect of aflatoxin was further substantiated by increased mortality observed at highest level of toxin.

A progressive reduction in serum protein concentration was observed in broiler chickens fed diets containing 100, 200, 400 and 600 ppb of AF (Table 5). Dietary level of 50 ppb did not cause any change in the concentration of total serum protein. The reduction in the total serum protein in AF fed groups could due to impairment of amino acid transport, mRNA transcription thereby inhibiting DNA and protein synthesis as observed by Thaxton *et al.* [20], Campbell, [21], Kuben *et al.* [22].

Liver is the prime target organ during aflatoxicosis. Wherever there is Liver disorder like inflammation of liver, a space occupying lesion or obstruction of biliary tract, the activity of AST and ALT found to be altered. Alanine amino transferase activity showed a non significant difference among treatment groups. This suggests that ALT may not suggest the extent of the liver damage or could be true indicator of liver damage. The present study revealed a significant decrease in AST activities upon feeding 200, 400 and 600 ppb AF as compated to control group. The results are contradictory to findings of Dalvi

Table 5: Effect of graded levels of aflatoxins on serum proteins, AST and ALT

Treatment	Aflatoxin (ppb)	Total Serum Protein g/dl	AST IU/dl	ALT IU/dl
T1	0	2.76 ± 0.02^{a}	243 ± 8.39^{ab}	24.00 ± 2.94
T2	50	2.53 ± 0.02^{a}	256 ± 1526^{a}	22.00 ± 3.68
T3	100	2.14 ± 0.12^{bc}	240 ± 11.33^{abc}	13.00 ± 1.88
T4	200	1.95 ± 0.18^{cd}	188 ± 14.28^{d}	12.33 ± 1.96
T5	400	1.59 ± 0.07^{dc}	208 ± 4.95^{bcd}	12.33 ± 1.51
T6	600	1.36 ± 0.06^{c}	198 ± 7.78^{cd}	14.66 ± 3.84

Table 6: Effect of graded levels of aflatoxin on ELISA titres against New Castle disease and Infectious bursal disease in broilers

		ELISA titres	
Treatment	Aflatoxin (ppb)	NCD	IBD
T1	0	5341 ± 49.12^{a}	4318 ± 139.54^{a}
T2	50	4806 ± 141.69^{b}	3085 ± 312.75^{b}
T3	100	3943 ± 88.84^{c}	2600 ± 140.05^{b}
T4	200	2920 ± 103.16^{d}	2332 ± 99.09^{c}
T5	400	2006 ± 106.41^{c}	2006 ± 87.34^{cd}
T6	600	300 ± 245.24^{f}	1644 ± 60.19^{d}

and Adenoyern, [23] who reported an increase in AST activity upon feeding 10 ppm of aflatoxin. On the contrary Dalvi and Mac Gowan [24] and Giroir *et al.* [25] could not record any significant alteration in enzyme profile upon feeding birds with 10 ppm and 2.5 ppm AF respectively. The variation in enzyme profile as reported by several researchers indicate that AST level may not suggest the extent of liver damage or could be a true indicator during aflatoxicosis.

ELISA titres against NCD and IBD were observed upon feeding graded levels of AF in the diet. The observation indicate that AF was a potent immunosuppressant being able to suppress the primary immune response for NCD and IBD as evidenced by fall in ELISA titre with simultaneous reduction in the size of bursa was observed. This immunosuppressive ability of AF demonstrated to inhibit RNA polymerase in-vivo and subsequently to limit the protein synthesis, [26] inhibit the reticulo endothelial system in a dose related fashion, inhibition of immunological tissues in an equally attractive, the efferent limb of the immunological system in chicken dependent on the bursa of fabricius and on antibody according to Co-oper *et al.* [27] enhanced degradation of antibodies because of AF increases the specific activity of lysosomal enzymes in the liver and muscles observed by Tung *et al.* [28].

The present study indicated that AF causes bursal regression and suppresses primary immune response for NCD and IBD as evident by fall in ELISA titres. The analysis of liver tissue for AF content revealed highest concentration in 600 ppb AF fed group (16.32 ppb) followed by 400 ppb (9.10 ppb) and 200 ppb (3.68 ppb) (Table 6). The results of the present investigation is in accordance with the findings of Vanzytreld *et al.* [29] who reported such transfer of toxin into the liver tissue when AF was fed directly into the crop at a very high doses.

REFERENCES

1. Devegowda, G., 1989. Aflatoxin, aflatoxicosis and animal production. Proc. National Symposium on Aflatoxins. Compound Livestock Feed Manufacturers Association of Inida (CLFMA), pp: 14-25.
2. Umesh, D., G. Devegowda and B.S. Barmase, 1990. Reducing the adverse effects of aflatoxin through nutrition in broiler chickens XIII Annual Poultry Conference and Symposium, Abst. PMHE L., pp: 50-136.
3. Giambrone, J.J., M. Partadivedja, C.S. Edison and S.H. Klevan, 1978. Interaction of aflatoxin with infectious bursal disease virus infection in young chickens. Avian Dis., 22: 431-439.
4. Devegowda, G. and B.I.R. Aravind, 1993. Survey of aflatoxin B1, contamination of feedstuffs. Proc. 6th Animal Nutrition Research Workers Conference, Bhubaneshwar, pp: 194.
5. Howel, M.V. and P.W. Taylor, 1981. Determination of aflatoxins,Ochratoxins A,and Zearalenone in mixed feeds,with detection by thin layer chromatography or high performance liquid chromatography, J. Association of Official Analytical Chem., 64: 1356-1363.

6. Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield and W.G. Sorenson, 1966. Production of aflatoxin on rice. Appl. Microbiol., 14: 425-428.
7. A.O.A.C., 1990. Official Methods of Analysis, 14th edn. Association of Official (Analytical) Chemists, Washington. D.C.
8. Smith, J.W., C.J. Hill, and P.B. Hamilton, 1971. The effect of dietary modifications on aflatoxicosis in broiler chicken. Poult. Sci., 50: 768-774.
9. Osborne, D.J. and P.B. Hamilton, 1981. Decreased pancreatic digestive enzymes during aflatoxicosis. Poult. Sci., 60: 1818-1821.
10. Sharline, K.S.B., B.J. Howarth and R.D. Wyatt, 1980. Effect of dietary aflatoxin on reproductive performance of matureWhite Leghorn. Poult. Sci., 59: 1311-1315.
11. Nandakumar Reddy, D., P.V. Rao, V.R. Reddy and B. Yadgiri, 1984. Effect of selected levels of dietary aflatoxin on the performance of broiler chicken. Indian J. Anim. Sci., 54(1): 68-73.
12. Rajashekhara Reddy, A., V.R. Reddy, P.V. Rao and B. Yadgiri, 1982. Effect of experimentally induced aflatoxicosis on the performance of commercial chicks. Indian J. Anim. Sci., 52: 405-410.
13. Johri, T.S. and S. Majumdar, 1990. Effect of methionine, choline, BHT, supplemented aflatoxic diets, proc. XIII National Symp. Indian Poultry Science., Assoc, held at Bombay on 20-22 Dec., 1990.
14. Lanza, G.M., K.W. Washburn and R.D. Wyatt, 1980. Variation with age in response of broilers to aflatoxin. Poult. Sci., 59(2): 282-288.
15. T.D.R.I., 1984. Mycotoxin training Manual, Tropical Development and Research Institute, London.
16. Thaxton, J.P., H.T. Tung and P.B. Hamilton, 1974. Immunosuppression in chickens by aflatoxin. Poult. Sci., 53: 721-725.
17. Dafalla, R., A.I. Yagi and S.E.I. Adam, 1987. Experimental aflatoxicosis in hybro-type chicks: Sequential changes in growth and serum constituents and histopathological changes,Vet. Hu. toxicol., 29: 222-226.
18. Ortatatli, M., H. Oguz, F. Hatipoglu and M. Karaman, 2005. Evaluation of pathological changes in broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Res. vet. Sci., 78: 61-68.
19. Smith, J.W. and P.B. Hamilton, 1970. Aflatoxicosis in the broiler chicken. Poult. Sci., 49: 207-215.
20. Kubena, L.F., R.B. Harvey, T.D. Phillips, D.E. Corrier and W.E. Huff, 1990. Diminution of aflatoxicosis by hydrated sodium calcium aluminosilicate. Poult. Sci., 69: 727-735.
21. Campbell, J.R.M.L., J.D. May, W.E. Huff and J.A. Doerr, 1983. Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and Ochratoxicosis. Poult. Sci., 62: 2138-2144.
22. Kubena, L.F., R.B. Harvey, T.D. Phillips and B.A. Clement, 1993. Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poult. Sci., 72: 651-657.
23. Dalvi, R.R. and A.A. Ademoyeru, 1984. Toxic effects of aflatoxin B1 and its reversal by activated charcoal, Phenobarbital and some chemical agents. Avian Dis., 28: 61-69.
24. Dalvi, R.R. and C. Mac Gowan, 1984. Experimental induction of chronic aflatoxicosis in chickens by purified aflatoxin B1 and its reversal by activated charcoal, phenobarbitol and reduced glutathione. Poult. Sci., 63: 485-491.
25. Girior, L.E., W.E. Huff, L.F. Kubena, R.B. Harvey, M.H. Elissalda, D.A. Witzel, A.G. Yersin and G.W. Ivie, 1991. The individual and combined toxicity of kojic acid and aflatoxin in broiler chickens. Pout.Sci., 70: 1351-1356.
26. Lafarge, C. and C. Frayssinet, 1970. The reversibility of inhibition of RNA and DNA synthesis induced by aflatoxin in rat liver. A tentative explanation for carcinogenic mechanism. Int. J. Cancer., 6: 74-83.
27. Co-oper, M.D., R.D.A. Peterson, M.A. South and R.A. Good, 1965. The functions of the thymus system and bursa system in the chicken. J. Exp. Med., 123: 75-123.
28. Tung, H.T., R.D. Wyatt, P. Thaxton and P.B. Hamilton, 1975. Concetrations of serum proteins during aflatoxicosis. Toxicol. Appl. Pharmacol., 34: 320-326.
29. Vanzytveld, W.A. D.C. Kelley and S.M. Dennis, 1970. Aflatoxicosis. The presence of aflatoxins or their metabolites in liver and skeletal muscles of chicken. Poult. Sci., 48: 1350-56.