ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Anomaly in the Levels of Protein and DNA of the Lymphoid Organs of Two Weeks Old Broilers Intubated with AFB1 Suspension

Lalitha Kumari S, Nathanael PJR, and Viveka Vardhani V

Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar – 522 510, Andhra Pradesh, India.

ABSTRACT

Poultry industry has the largest economic scenario in India providing vital food products engrossed with high levels of proteins, low fat and all essential amino acids. This sector is threatened with mycotoxic upheaval particularly Aflotoxin B1 which brings about heavy mortality to edible fowls. AFB1 causes immunosupression in animals effecting the cell mediated immunity causing reduced thymus and spleen which are the primary and secondary lymphoid organs respectively. Aflotoxins are responsible for carcinogenic, mutagenic and terrotogenic effects in edible fowls. The present study is focused on the study of biochemical aspects like proteins and DNA in bursa fabricus and cecal tonsils of Plymouthrock broilers orally intubated with AFB1. The organs are taken and the respective tissues are well homogenized, and analyzed for the evaluation for the levels of proteins and DNA for marking the changes in the molecular level and to interpret them in physiological and immunological dimensions.
Keywords: Aflotoxicosis, AFB1, *Aspergillus flavus*, thymus, cecal tonsils, oral intubation

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Poultry rearing with regard to domestic fowls and table egg production is the largest agriculturally based food enterprise in third world countries, especially in India. Production of eggs, broilers, commercial layers and poultry meats enhance an unique and positive development Mohan Bajikar [1,2].The incidence of contamination of the poultry feed by mycotoxic fungi like *Aspergillus flavus* brings about complete annihilation of the flock. The aflotoxins are a group of mold metabolites produced by *Aspergillus flavus* these toxins are highly toxic as well as carcinogenic [3], in these AFB1 is prominent among these toxins. AFB1 causes mortality, listlelessness, anorexia, opisthotonous condition, decreased egg production in layer flocks, fatty liver syndrome, negative feed conversions, broodiness in layers, and makes the birds highly immune- suppression to various hazardous diseases [4, 5]. The breakdown of immunocompetence alters the physiology causing severity of cecal coccidosis, decreased total serum proteins, severe congestion, hydro pericardial syndrome, severe odema, multiple blood clots and petcheial feathers [6,7]. AFB1 after entering in to the alimentary canal of birds exert systemic effects and cause specific damage to certain vital organs like liver (principal target organ and centre for detoxification), thymus and bursa of fabricus (primary lymphoid organs), kidney (principal organ of excretion) likewise. AFB1 causes induced DNA adduction and mutagenesis [8,9]. Aflotoxins are immunosuppressive as they are shown to interact with DNA and polymerases responsible for DNA and RNA synthesis mechanisms [10]. Thymus and bursa of fabricus play a vital role for the production of cellular and humoral antibodies ([11,12], and AFB1 brings the alteration in cellular integrity in these tissues. With regard to the biochemical changes caused by aflotoxins the most important initial event appears to be the interaction of AFB1 with DNA and protein metabolism resulting in the inhibition of cell mitosis. The suppression of DNA synthesis may result in the production of cells in a non-dividing stage and this may lead to the abnormal production of cells [2,13]. High levels of AFB1 exposure clearly manifested altered physiological responses associated with altered gene expression in liver and thymus [14]. Therefore, a new vista has been opened to study the level of protein and DNA in thymus and bursa fabricus of two weeks old broilers infected with two varied doses of AFB1.

MATERIALS AND METHODS

Two weeks old broilers belonging to *Plymouthrock* strain (with average body weight 220-230gms) were procured and kept in open litter system, and acclimatized to laboratory environment. All the animals were feed with the standard balanced diet and water was given *ad libitum.* 0.5ml of AFB1 suspension at a dose of 0.25 ng/bird (group A- 20 birds), and 0.5ng/bird (group B- 20 birds), was intubated orally by using a 16 gauze oral feeding needle. These doses were administered after a preliminary study. Control birds (Group C) which are kept for comparison received 0.5. ml of distilled water only. Infected and control birds are separately kept throughout the study. The two experimental groups were sacrificed at day 1,4,8 and 11 of infection. Five animals from the control group were also sacrificed on the same designated days. Tissues of bursa fabricus and cecal tonsils were processed for protein and DNA evaluation. Estimation of proteins and DNA was done following respectively [15]and Diphenylamine methods [16].

ISSN: 0975-8585

RESULTS AND DISCUSSION

All the orally intubated broilers(groups A and B) showed lethargy, restlessness, reluctance to feed, loss of appetite, asthenia, anorexia, closing of eyes and a particular condition where the broilers exhibited deep arched neck, convulsions and twisting of legs(known as opisthotonous condition). Continuous exudation of mucous whitish diarrhea, exhaustation, dehydration, gasping, and reluctance to movement are also noticed. The feathers become pale and ruffled. Persistent spleenomegaly, fatty liver syndrome with multiple hemorrhages and molted texture of hepatic organ was observed in all the experimental fowls at peak days of infection. Autopsies performed clearly gives an insight of liver necrosis, odematous bursa, and other in vital organs, hydro pericardium syndrome and severe inflammation. Enlargement of cecal tonsils and atrophy of bursa was evident in chicks which received higher dose (0.5ng/bird) of AFB1. Smith and Hamilton (1970), Sivachandra et.al,(2004) and Zadrozny et.al,(2010) [6,8,17] , also reported odematous bursa (in low doses), hydropericardium syndrome and atrophy of vital organs respectively during chronic aflotoxicosis. Microscopic lesions and inflammation are observed in cecal tonsils as well as intestine on day 8 to 11 of infection in groups A and B.

Protein content in bursa fabricus: In comparison with the untreated ones, the broilers of groups A (AFB1 @ 0.25ng/bird) showed a rapid increase of protein level on the initial day, but drastically decreased from day 1 to day 3, and further decreased to below normal value on day 8 and 11. Broilers of group B which received an exact the double amount of dosage of AFB1 i.e. 0.5ng/bird showed a different pattern, the protein value was found to be below normal on day 1 and 3 , and enhanced on day 8 and reached its zenith on day 11 of infection. The aflotoxin activity is evident by disturbed activity of DNA in bursal cells, which may directly influence the protein constitution of this organ(Yarru et.al,2009). (Figure 1)

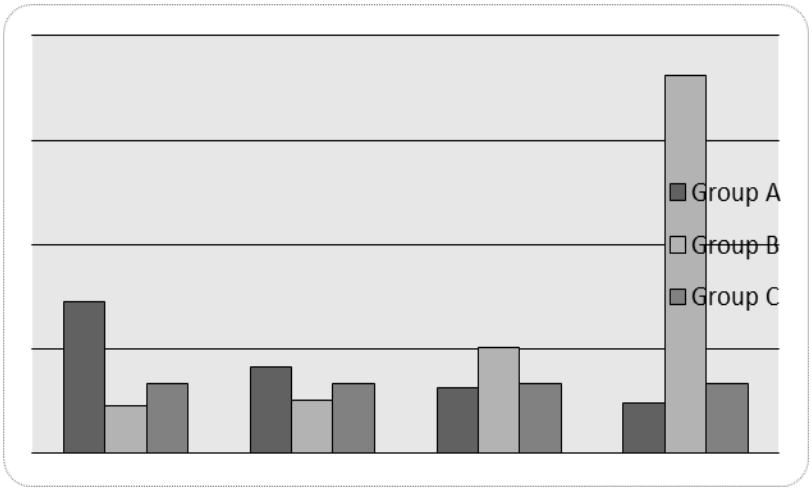

Figure 1: Comparison of protein activity in the bursa fabricus of experimental (treated with two different doses of AFB1 in group A, 0.25ng/bird and group B 0.5ng/bird and controls (untreated) (group c)broilers of two weeks old.

ISSN: 0975-8585

DNA content in bursa fabricus : A gradual enlargement of cecal tonsils and progressive atrophy of bursa were noticed in both the groups of broilers which received AFB1 at 0.25ng/bird and 0.5ng/bird respectively. Broilers (group A) which received 0.25 ng of AFB1 showed a gradual increase of DNA from day 1 to 11. Even the same pattern is manifested with the broilers (group B) which we treated with a higher dose of AFB1 (0.5ng/bird). The DNA content is increased throughout the period of experimentation and reached its peak on the final day (day 11). It has been observed that birds, which received 0.25ng and 0.5 ng of AFB1 showed an adverse effect of toxin in the synthesis of DNA from day 1 to 11 when compared with the contrls. In the present investigations, AFB1 might have inhibited the DNA synthesis within 24 hours of infection and this infection led to survival and abnormal production of cells. These adverse changes brought out abnormal content of DNA in this infected organs. This clearly shows the mutagenic property of AFB1. These results are similar to those of Panda and Johri (1983) who explained the initial inhibition of DNA synthesis and abnormal production of cells at later periods. (Figure 2)

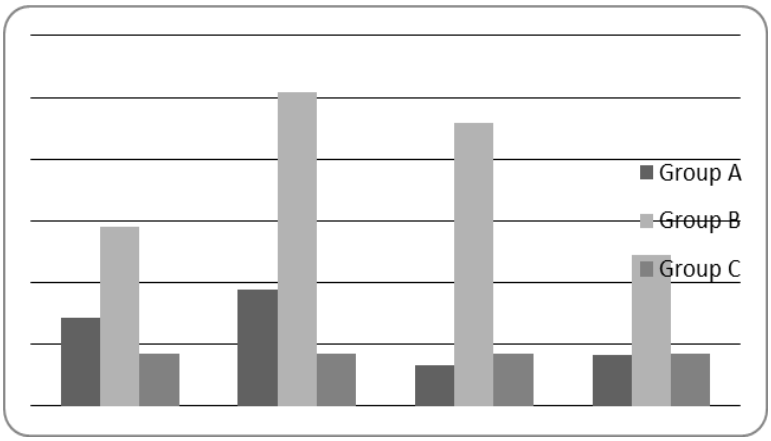

Figure 2: Comparison of DNA activity in the bursa fabricus of experimental (treated with two different doses of AFB1 in group A, 0.25ng/bird and group B, 0.5ng/bird and controls (untreated) (group C) broilers of two weeks old.

Protein content in cecal tonsils: Broilers which received 0.25ng of AFB1 showed a very slight increase of proteins on the initial day of infection than the normal value, on day 3 the protein value declined reaching the normal level , again it was decreased to below normal value on day 8 and 11. On the other hand broilers (group B) which received an higher dose (AFB1@ 0.5ng/bird) showed low protein values on initial day of experimental period, but showed drastic enhancement on the latter period of experimentation even reaching its peak value on day 11. These results clearly gives a strong evidence of interaction of AFB1 with DNA synthesis to interfere with protein metabolism. The interference of cellular activity of cecal tonsils is also evident with the decreased and increased level of protein in an irregular fashion (Figure 3)

ISSN: 0975-8585

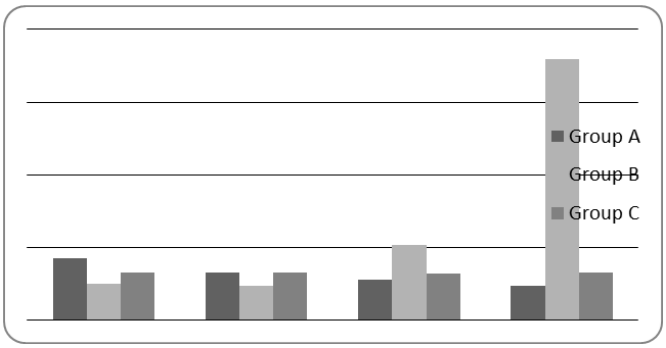

Figure 3: Comparison of protein activity in the cecal tonsils of experimental (treated with different doses of AFB1 in group A, 0.25ng/bird and group B, 0.5ng/bird) (untreated broilers) (group C) of two weeks old.

DNA content in cecal tonsils: The broilers of group A (AFB1 @ 0.25ng/bird) showed slight increase in DNA level on day 1 and 3, and decreased below the normal value on day 8 and 11. But those broilers(group B) which treated with higher dose (AFB1 @ 0.5ng/bird) showed higher levels of DNA through out the experimental period. Interestingly they are at peak in the middle of the experimental period (Day 3). These results are similar to that of Panda and Johri (1983) who also suggested that in sub lethal doses the toxin acts in a cumulative fashion bringing adverse changes in DNA content. (Figure 4)

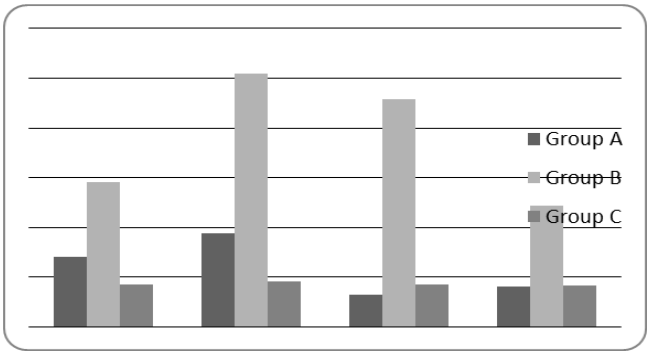

Figure 4: Comparison of DNA activity in the cecal tonsils of experimental (treated with different doses of AFB1 in group A, 0.25ng/bird and group B,0.5ng/bird (untreated broilers)s (group C) of two weeks old.

The bursa of fabricus is indispensible for the normal development of humoral immune response and cecal tonsils are involved in the production of anti-bodies in birds. Though these organs are playing a key role bestowing immunity, these are also affected by aflotoxin bringing about adverse changes and disturbed levels of proteins and DNA, leaving the broilers susceptible to various secondary opportunistic infections. Alterations in the metabolism of proteins and DNA in bursa of fabricus and cecal tonsils of broilers treated with AFB1 are the important biochemical effects of AFB1.

ACKNOWLEDGEMENTS

Two of us , S. Lalitha kumari and P. J. R. Nathanael are soberly thankful to Prof. V. Viveka Vardhani who is then Head , Department of Zoology , whose benevolence and earnest upholding made our work successful

ISSN: 0975-8585

REFERENCES

[1] Mohan Bajikar R. Poultry Punch 1999;15:79 - 84.
[2] Panda PC, and Johri TS. Indian Poultry Rev 1983;14 : 11-14.
[3] Buchi G, and Ian D Rae. The structure and chemistry of the aflatoxins. In "Aflatoxin" edited by L.A. Goldblatt Academic Press, New York, 1969;PP 55-73.
[4] Qureshi AA. Poultry Int 1988;27: 48-49.
[5] Toro H, Gonzabez C, Cerdal, Hess M Reyes E and Geisse C. Avian Dis 2000;44: 51-58.
[6] Smith JW and Hamilton PB. Poultry Sci 1970;49: 207-215.
[7] Anjum AD, Sabbri MA, and Iqbal Z. Veterinary Record 1989;124: 247-248.
[8] Shivachandra SB1 Singh SD, Kataria JM and Manimaran K. Indian J Animal Sci 2004;74(6) : 600-604.
[9] Besaratinia A, Kim SI, Hainaut P, Pfeifer GP. Gastroenterol 2009;137(3):1127-37.
[10] Schroeder EC, Nair KPC and Cardeihac PS. Poultry Sci 1972;51: 1552-1556.
[11] Glick B. Bioscience, 1970;20:10-13.
[12] Cooper MD, Peterson RD and Good RA. Nature (London) 1965;205:143.
[13] Ranchal I, et al. Int J Cancer 2009;(11): 125(10):2270-2280
[14] Yarru LP, Settivari RS, Antoniou E, Ledoux DR, Rottinghaus GE. Poultry Sci 2009;88(2):360-371.
[15] Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. J Biol Chem 1951;177 : 751 – 766 .
[16] Burton. K. A study of the conditions and mechanism of the Diphenylamine reaction for the calorimetric estimation of Deoxyribonucleic acid. Medical research council, cell metabolism research unit, Department of Biochemistry, University of Oxford. Vol 1976;62: 315-323.
[17] Zadrozny LM, Williams CV, Remick AK, Cullen JM. Vet Pathol 2010;47(2):306-311.