Biolife 2(3):845-849
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

AFB1 INDUCED CHANGES IN LIVER AND SERUM OF COMMERCIAL BROILERS PERTAINING TO CERTAIN BIOCHEMICAL ASPECTS

Nathanael, P. J. R[1]* and Sakunthala, G[2]

[1,2]*Department of Zoology and Aquaculture, Acharya Nagarjuna University Nagarjuna Nagar – 522 510*

E-mail: nathanael_pj@yahoo.co.in, nathanael.zoology@gmail.com

ABSTRACT

Mycotoxic metabolic exudates containing AFB1, liberated by strains of *Aspergillus flavus* and *Aspergillus parasiticus are* the potent carcinogenic, hepatotoxic, mutagenic and nephrotoxic in nature. AFB1 accumulation in poultry feed stuffs causes severe morbid pathological disorders even in molecular level in commercial birds bankrupting the poultry enterprise. The focal theme of our investigation is to analyze certain biochemical changes in liver and serum of one week (Wt 170-180 gm) and two weeks (Wt 220-230 gm) old broilers which were orally intubated with varied doses of AFB1. The results forecast the alterations in the physiology of the broilers leading in alterations in biochemical/molecular mechanisms.

Key words: Mycotoxins, Aflotoxicosis, AFB1, Broilers, liver and serum.

INTRODUCTION

Aflotoxin B1 (AFB1) is potentially carcinogenic, hepatotoxic, mutagenic and terratogenic metabolite produced by two strains of fungi known as Aspergillus *flavus* and *Aspergillus parasiticus.* AFB1 contaminates the poultry feed stuffs such as peanuts, corn, groundnuts, wheat and barley causing severe damage to commercial layers and broilers bankrupting the poultry enterprise (Ruiz et al., 1996; Bajikar, 1999). AFB1 causes severe damage to liver hepatocytes and kidneys. AFB1 causes fatty liver syndrome, hepatocellular carcinoma, negative feed conversions, broodiness in layers and severe immunosupression by causing deterioration of thymus and bursa fabricus (Qureshi, 1998; Toro et al., 2000 , Shivchandra et al., 2004; Lalitha et al., 2014). AFB1 inhibits the activity of adino tri phosphates, glycogensis and glucose transport into the liver and synthesis of proteins and DNA forming toxic intermediary metabolite known as epoxide (Chaturvedi and Sohane, 1998; Nathanael et al., 2010). The present investigations centered on the analysis of the levels of protein, glycogen in liver and total serum proteins and serum transaminases response in commercially procured *Plymouth Rock* broilers (one week and two weeks old) treated with varied doses of AFB1.

MATERIALS AND METHODS

Plymouth Rock strain broilers of one week (Wt 170-180 gm) and two weeks (Wt 220-230 gm) old were obtained locally and kept in open litter system. After acclimatization in laboratory conditions, 0.5 ml of pure AFB1 suspension (obtained from Sigma Ltd. Singapore) at a dose of 0.01ng/bird (Group A) , 0.1ng/bird(Group B)

,0.25ng/bird (Group D) and 0.5 ng/bird (Group E) was intubated orally using a 16 gauge oral feeding needle. These doses were selected after a preliminary study. Two groups (C and F) served as controls for comparison. Five animals from each experimental and control groups were sacrificed at day 1, 4, 8 and 11 of infection. The tissues from liver were collected and processed to study the level of proteins and glycogen using the standard methods of Lowry et al., (1951) and Kemp et al., (1954). Blood was collected through jugular puncture, and serum was separated and processed for total proteins and transaminases by Lowry et al., (1951) and Reitmann and Frankel (1957).

RESULTS AND DISCUSSION

Total proteins in liver: Broilers of group A (which received lowest dose of AFB1 i.e. ., 0.01ng/bird) and B (AFB1@ 0.1ng/bird) shown higher value of proteins when compared to that of controls throughout the experimental tenure. There is an exponential raise of proteins was observed on day 11(group B). in case of group D broilers (AFB1 @ 0.25ng/bird) showed lower levels of protein on day 1 and 3 ; and there is an increase seen on day 8 which is higher than control value ; but there is a sudden decrease occurred on day 11. Birds of group E (which received a high dose of AFB1 @ 0.5ng/bird) showed higher levels of protein on day 1 and 3 (than normal value) and from day 8 to 11 there occurred a steep decline (below normal value) (Table 1).

Glycogen content in liver: In case of broilers of group A, lowered content of glycogen was manifested than the controls, throughout the experimental period. The broilers which received small dose of AFB1 (0.1ng/bird) suspension (group B) showed slightly elevated levels of glycogen than the control broilers throughout the experimental duration. Two weeks old broilers of group D and E showed slightly increased level of glycogen than the

Table 1: Protein content (ng/ml) in the liver of controls (Group C & Group F) and infected (Group A, 0.01ng/bird ; Group B , 0.1ng/bird ; Group D, 0.25ng/bird and Group E, 0.5ng/bird) at different days of experimental period. Values expressed in mean derived from 5observations.

Days of necropsy	One week old broilers			Two weeks old broilers		
	Group A	Group B	Group C	Group D	Group E	Group F
1	84.1	42.6	26.3	22.8	73.0	32.8
3	57.3	42.6	26.5	25.3	42.1	33.0
8	42.0	33.4	26.4	51.0	27.8	32.9
11	32.9	159.6	26.2	14.2	16.8	33.0

Table 2: Glycogen content (mg/gm) in the liver of controls (Group C & Group F) and infected (Group A, 0.01ng/bird ; Group B , 0.1ng/bird ; Group D, 0.25ng/bird and Group E, 0.5ng/bird) at different days of experimental period. Values expressed in mean derived from 5observations.

Days of necropsy	One week old broilers			Two weeks old broilers		
	Group A	Group B	Group C	Group D	Group E	Group F
1	6.44	7.71	6.42	7.2	6.68	6.52
3	6.20	7.62	6.49	6.6	6.92	6.49
8	5.91	6.63	6.51	6.9	6.62	6.51
11	5.20	6.59	6.46	6.4	6.81	6.46

normal levels. The decreased content of glycogen clearly indicates the malfunctioning of liver and onset of chronic hepatic ailments due to AFB1 exposure as suggested by Zimmerman (1970) and Viveka Vardhani and Nathanael (2011).

The disturbance in protein /glycogen metabolism in liver (the target organ) might have resulted from DNA strand breakage and release of ROS species due to severe stress manifested by broilers of group A and B caused by AFB1(Table2).

Total serum proteins: In the present study, a significant decrease in the serum total proteins has been noticed in the experimental broilers as compared to controls. Proteins form the major portion of the dissolved solution in the in the plasma. The serum protein content is significantly decreased in Groups A, B, C and D throughout the infection period when compared

Table 3: Serum Protein content (mg/ml) in the serum of controls (Group C & Group F) and infected (Group A, 0.01ng/bird; Group B, 0.1ng/bird; Group D, 0.25ng/bird and Group E, 0.5ng/bird) at different days of experimental period. Values expressed in mean derived from 5observations.

Days of necropsy	One week old broilers			Two weeks old broilers		
	Group A	Group B	Group C	Group D	Group E	Group F
1	0.55	0.21	0.63	0.37	0.37	0.79
3	0.34	0.20	0.63	0.33	0.33	0.77
8	0.33	0.18	0.63	0.32	0.31	0.74
11	0.30	0.13	0.63	0.20	0.29	0.78

Table 4: Serum Glutamate Oxaloacetic Transaminase (SGOT) (AST) activity (Units/ml) in serum of controls (Group C & Group F) and infected (Group A, 0.01ng/bird; Group B, 0.1ng/bird; Group D, 0.25ng/bird and Group E, 0.5ng/bird) at different days of experimental period. Values expressed in mean derived from 5observations.

Days of necropsy	One week old broilers			Two weeks old broilers		
	Group A	Group B	Group C	Group D	Group E	Group F
1	13.5	24.5	19.2	17.5	18.8	12.5
3	22.75	28.25	19.7	22.3	24.3	12.2
8	29.37	36.62	19.5	23.3	29.5	12.3
11	34.12	39.12	19.3	37.5	37.2	12.4

Table 5: Serum Glutamate Pyruvate Transaminase (SGPT) (ALT) activity (Units/ml) in serum of controls (Group C & Group F) and infected (Group A, 0.01ng/bird; Group B, 0.1ng/bird; Group D, 0.25ng/bird and Group E, 0.5ng/bird) at different days of experimental period. Values expressed in mean derived from 5observations.

Days of necropsy	One week old broilers			Two weeks old broilers		
	Group A	Group B	Group C	Group D	Group E	Group F
1	17.62	18.75	18.2	24.5	18.5	12.7
3	22.75	23.5	18.7	29.3	23.5	12.7
8	29.37	29.0	18.5	36.5	29.2	12.7
11	37.0	37.25	18.3	37.2	34.2	12.7

with the controls. Clinical observations would suggest that hepatic diseases might disturb the protein synthesis in liver which in turn reflects on the level of serum proteins (Zimmerman and Seeff, 1970). Lafarge and Frayssinet (1970) reported that aflotoxin inhibits RNA polymerase *in vivo* and deranges the protein synthesis mechanism (Table 3).

Serum aspertate transaminase content: In Group A (0.5 ng/bird), there was an increase of AST from day 1 to 11in comparison with the normal value. In Group B (0.5ng/bird), there was a highly significant increase from day 1, to 11 when compared to the normal value; there was a twofold rise from day 3 to 11 of experimental period and was at peak on day 11. Broilers which received two varied doses showed an almost equal response on day 11 in the elevation of AST. A close correlation between chronic hepatic toxicity and increased oxidative stress has been reported in humans and experimental animals. During the present investigation the stress of aflotoxicosis and/or pathological reactions could have direct effect on the liver by increasing the production and/or releasing transaminases into the blood. Sharma (1980) reported that the level of AST and ALT in liver or their release into the blood stream forms a specific indicator of liver status. The level of serum enzymes are important to know whether liver metabolism been activated during hepatotoxicity (Meghna et.al; 2007). These results also state that hepatocytes are damaged as the hepatocytic damage is related to increased ALT levels (Table-4).

Alanine transaminase content: In the present investigation, there is a significant increase in the levels of serum AST and ALT in infected broilers when compared to controls. There was a marked increase in the both the experimental groups. In case of group A approximately there was a 2 fold increase on day 1 and 3 fold rise on day 8 and day 11. In case of group B, there was an increase of ALT throughout the period of experimentation and was peak on day 11. It is clear from these results that both the doses of 0.25ng and 0.5ng disturbed the physiology of birds resulting in the elevated levels of ALT. These results manifest clear evidence that AFB1 might have disturbed the metabolism of transaminases in liver which is the target organ, and caused the destruction of hepatocytes. This may result in the leakage of transaminases into the serum (Table-5).

ACKNOWLEDGEMENTS:

The Author expresses his gratitude to his beloved Mom, ***P. V. Sumitra*** for her financial support during his research career. This paper is dedicated in the fond memory of ***Ms. Daisy***.

REFERENCES

1. **Bajikar, M. R. (1999).** Why and how of hazard analyses critical control point (HACCP) in poultry industry. Poultry Punch. **15**: 79 - 84.
2. **Chaturvedi, V. B. and Sohane, R. K. (1998).** Importance of aflotoxin in poultry industry. Indian Poultry Review, **26**:19-25.
3. **Chennaiah K, Sivasankar R and Sugunakar Y.** (2013). impact of agrimin and fishmin on the carbohydrate metabolic enzymes in the muscle and liver tissue of the different fish (*c.catla. l. roh),* Bolife.**1(3),** 130-135.
4. **Kemp, A., Vankits. and Haljnimgem, A.J.M. (1954).** A colorimetric method for the determination of glycogen in tissue. Biochem. J. 646-648.
5. **Lafarge, C. and Frayssinet, C. (1970).** International Journal of Cancer**. 6**: 74-83.
6. **Lalitha Kumari, S., Nathanael, P.J.R. and Viveka Vardhani, V. (2014).** Anomaly in the levels of protein and DNA of the lymphoid organs of two weeks old broilers intubated with AFB1 suspension. Res.J.of Pharm.Biol.and Che.Sci . **5(3):** 1407-1412.
7. **Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951).** Protein measurement with folin phenol reagent. Jou Biol Chem, **177**: 751 – 766.
8. **Meghnad, G. joshi., Rekha, Tangadi., Aruna, Kanase. and Bamane, S.R. (2007).** Effect of calcium-zinc bioactive ceramics on liver and kidney functions. Ind Jour Comp Ani Phy, **25(2);** p.36-45.

9. **Nathanael, P.J.R., Lalitha kumari, S. and Viveka Vardhani, V. (2010).** Influence of aflotoxin B1 on protein and DNA content of lymphoid organs of one week old broilers during induced aflotoxicosis Journal of Bioscan. **5; (3):** 469-471.
10. **Qureshi , A.A. (1998).** Hydro pericardium and kidney lesion. Poultry International**. 27:** 48-49
11. **Reitman, S. and Frankel, S. (1957).** A colorimetric method for the the determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. Ame. I. Clin. Patho, **28**:56-58.
12. **Ruiz, J.A ., Bentabola, A., Gallego, C., Anguala, R.A. cousta, I. and Jodral, M. (1996)**. Aflotoxin producing strains of A.flavus in the mould flora of the different green house substrates for the cultivation of cucumber (*Cucumis satisvus* L.) . Int.J.Food Microbilogy. **29:**193-199.
13. **Shivachandra, S.B., Sah, R.L., Singh, S.D., Kataria, J.M. and Manimaran, K. (2004).** Comparative pathological changes in aflotoxin fed broilers infected with hydropericardium Syndrome. Indian Journal of Animal Sciences. **74(6)** : 600-604.
14. **Toro, H., Gonzabez, C., Cerdal, Hess, M., Reyes, E. and Geisse, C. (2000).** Chick anemia virus and fowl adenovirus: Association to induced inclusion body hepatitis/hydropericardum syndrome. Avian diseases**. 44**: 51-58.
15. **Viveka Vardhani, V. and Nathanael, P. J. R. (2011).** The panoramic view of changes in physiology and biochemical aspects in broilers suffering due to experimental aflotoxicosis: a review. Ind. J. Comp. Ani. Physiol**. 29 :**1-7
16. **Zimmerman, H.J., and Seeff, L.B. (1970).** Enzymes in hepatic disease. In coodley, EL(ed): Diagnostic enzymology, Philadelphia, Lea and Febiger.**1**-38.

DOI:https://dx.doi.org/10.5281/zenodo.7220595
Received: 10 July 2014;
Accepted; 20 August 2014;
Available online : 9 September 2014