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TOXICITY OF AFLATOXIN B_1 TO CHICK EMBRYO

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Effects of Aflatoxin B_1 on mortality and hatchability of chick embryo were studied. Three different doses 26,81, and 216 ng/egg of aflatoxin B_1 were employed. Aflatoxin B_1 purified from contaminated groundnut meal was used for the first two concentrations and standard aflatoxin B_1 for the third. The lethal dose of 216 ng/egg caused 93 % mortality by the 4th day of incubation period.

Key words: Chick embryo, Aflatoxin B_1, Bioassay.

INTRODUCTION

The aflatoxins are highly toxic carcinogenic, teratogenic and mutagenic compounds produced as secondary metabolites by certain strains of *Aspergillus flavus* and *A. parasiticus*. After their discovery as a result of an empizootic among turkeys [1] in which 100,000 turkey poults died, several tests have been developed to study their toxic effects. First biological assay for aflatoxin was devised using day old ducklings [2] Subsequently other bioassays like chick embryo test [3,4] tissue culture [5] fungi [6] and bacteria [7] were also developed. It has been shown that administration of 100 and 50 ng of AFB/egg showed a 100 and 85 % mortality, respectively, of embryo in fertile eggs [8]. Pure crystalline AFB_1 showed LD_{50} between 0.02 and 0.3 ug/chick embryo [9].

In other bioassays, the tadpole of *Bufo melanostictus Schlneider* and Uperdon species had also been used and found to be quite sensitive to aflatoxin, with the mean LD_{50} of 2.8 ug AFB_1/ml for Bufo larvae and for Uperdon larvae was approximately 0.5 ug/ml [10]. Brine shrimp (Artemia salina L.) has been also used in the biological assays [11,12].

MATERIAL AND METHOD

Aflatoxin contaminated groundnut meal was extracted with chloroform, cleaned-up by column chromatography [13], dried and dilutions were made in ethyl alcohol. Two concentrations containing 26 ng and 81 ng of AFB_1 were prepared. A third concentration of 216 ng was made using pure crystalline AFB_1 standard in ethyl alcohol. Aflatoxin was quantified using densitometer.

Two hundred and forty nest-clean eggs of Singlle Comb White Leghorn were received 48 hours after they had been laid. The flock was fed on feed free from antibiotics, arsenicals and nitrofurazones. The eggs were candled to eliminate misplaced air cells, blood spots, hairline cracks or other shell imperfections and abnormalities in shape, size and calcium deposits within shell thickness. Eggs extremely large or too small were also discarded. A dentist's drill machine was used to make hole of 2mm in diameter at the centre of each air-cell. Care was taken to avoid the entry of shell fragments into the egg and visible fragments were removed with a forcep. All the eggs were kept in a vertical position with the broad end up for one hour to let inoculated material disperse. The selected eggs were randomly divided into 4 groups of 16 eggs each:

Group I. Injected with 20 µl/egg of ethanol.
Group II. Injected with 26 ng of AFB_1/egg.
Group III. Injected with 81 ng of AFB_1/egg.
Group IV. Injected with aflatoxin B_1 standard containing 216 ng AFB_1/egg.

Eggs were candled on the fourth day and on every alternate day thereafter but not after 16th day. From the 18th day of incubation, eggs were no longer turned and placed in hatching trays. All the clear eggs denoting infertility were removed, and the dead embryos during incubating were opened and recorded. The unhatched eggs were opened on the 23rd day and the results were noted.

Table 1 Mortality and hatchability of chick embryo injected with aflatoxin

Group	Treatment	No of eggs/ set	Infertile eggs (%)	Mortality (%)		Total mortality (%)
				Under developed	Developed	
I	Control (Ethyl alcohol)	16	0	0	13	13
II	26 ng AFB_1/egg	16	12 5	14	43	57
III	81 ng AFB_1/egg	16	0	87 5	6 25	94
IV	Standard 216 ng AFB_1/egg	16	12 5	100		100

Table 2. The effect of aflatoxin B_1 on the viability of chicken embryo.

Group	Treatments		Days												
			2	3	4	5	8	9	10	12	16	18	20	23	87
I	Ethyl alcohol	Alive	16	16	16	16	16	16	16	16	16	16	16	14	87
		Dead	–	–	–	–	–	–	–	–	–	–	–	02	13
II	26 ng AFB_1/egg	Alive	14	14	14	14	14	14	14	13	12	12	8	6	43
		Dead	–	–	–	–	–	–	–	1	1	–	4	2	57
III	81 ng AFB_1/egg	Alive	16	16	16	16	15	14	09	03	–	–	2	01	06
		Dead	–	–	–	–	1	1	5	6	–	–	1	1	94
IV	Standard 216 ng	Alive	14	4	1	1	1	–	–	–	–	–	–	–	0
	AFB_1/egg	Dead	1	9	3	–	–	–	1	–	–	–	–	–	100

RESULTS AND DISCUSSION

Toxicity of aflatoxin B_1 to chicken embryo of Single Comb White Leghorn via the air cell route was determined. Mortality during the development period as a result of injection of different levels of AFB_1 is given in Table 1. Fifty seven percent mortality was observed at 26 ng AFB_1/egg. Group III receiving 81 ng AFB_1/egg resulted in 94 % mortality. Hatchability is shown in Table 2. Group I showed maximum hatchability 87 % whereas in groups II, III and IV hatchability was 43, 6 and 0 % respectively. Most of the embryos died before 12th day of development in aflatoxin treated groups.

In group II 14 % of underdeveloped and 43 % of developed embryos died, thus bringing total mortality to 57 %. The higher toxic dose, 81 ng/egg resulted in death of embryos by the 12th day of development. The highest toxic dose of 216 ng/egg, killed 93 % of the embryos by the 4th incubation day and there was 100 % mortality by the 10th day (Table 2). At highest dose (216 ng/egg) mortality was apparent much earlier during the incubation period, since most of the embryos did not survive longer than the 4th day. However, with a dose of 26 ng and 81 ng AFB_1 many embryos did survive longer than the 9th day. It has been shown that LD_{50} of pure crystalline AFB_1 for chick embryo ranges from 20 ng to 300 ng [9]. The present study shows that lethal toxic dose ranges from 26 ng to 216 ng/egg and toxic effects with highest dose is exhibited on the embryo by the 4th day of incubation period.

The study indicates that the chick embryo bioassay is a useful technique which can demonstrate the possible toxic effect of aflatoxins. The assay is also useful for testing the toxicity of other chemical compounds [14].

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REFERENCES

1. W.P. Blount, J. Brit. Turkey Fed., **9**, 52 (1961).
2. F.D. Asplin and R.B.A. Carnagham, Vet. Rec., **73**, 1215 (1961).
3. B.S. Platt, R.J. Stewart and S.R. Gupta, Proc. Nutr. Soc., **21**, 30 (1962).
4. M.J. Verrett, J.P. Marliac and J.C. Modughlin Jr., J. Assoc. Agr. Chem., **47**, 1003 (1964).
5. J. Gablicks, W. Schaeffer, L. Friedman and G.N. Wogan, J. Bacterial., **90**, 720 (1965).
6. H.R. Burmeister and C.W. Hesseltine, Appl. Microbiol., **14**, 403 (1966).
7. N.L. Clements, J. Assoc. Off. Anal. Chem., **51**, 611 (1968).
8. D.P.H. Hsieh, A.S. Salhab, J.J. Wong and S.L. Yang, Toxicol. Appl. Pharmacol., **30**, 237 (1974).
9. M. Archer, Mycopathol. Mycol. Appl., **54**, 453 (1974).
10. S.N. Arseculeratne, L.M. De Silva, C.H.S.R. Bandunatha, G.E. Tennekoon, S. Wigesundera and K. Balasubriamaniam, Br. J. Exp. Pathol., **50**, 285 (1969).
11. R.F. Brown and J.D. Wildman, J. Assoc. Off. Anal. Chem., **51**, 905 (1968).
12. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobson, D.E. Nichols and J.L. McLaughli, Planta Medica, **45**, 31 (1982).
13. *Official Methods of Analysis,* Chapter 26, (Association of Official Analytical Chemists, 1984), 14th ed.
14. J. Richard, P. Miroslav and R. Zdenek, Indian J. Exp. Biol., **23**, 588 (1985).