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HISTOPATHOLOGICAL STUDIES ON THE INTERACTION BETWEEN AFLATOX IN AND SOME "mycotic infection in chicks"

BY

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SUMMARY

The present work was conducted to study the interaction between aflatoxin, *Candida albicans* and *Aspergillus Fumigatus*, 300 one day old chicks were used in two experiments, 150 were used in each experiment and the birds in each experiment were divided into 5 groups, 30 chicks in each. The 1st group in both the 1st and 2nd experiments were given diet free from aflatoxin, while the other groups were given diet contained aflatoxin for 6 weeks. After two weeks, all the birds related to the 1st experiment were inoculated intracrop with one ml. of $3x10^3$ *Candida albicans* while those related to the 2nd experiment were intranasally inoculated with one ml. of 4×10^9 spores of Aspergillus fumigatus. The histopathological results indicated that aflatoxin increased the infectivity (as measured by lesion score) in *C. albicans* (66.6% 73.3%, 86% and 93.3%) and also in A. fumigatus (53.3%, 66.6%, 73.3% and 93.3%) respectively.

INTRODUCTION

With the increased demand to animal protein for human consumption, poultry industry has played a great part in the last few years for facing such demand. Poultry are liable to many enviromental and multifactorial stressors (microbial and non microbial) which impair the normal growth and are reflected on their final productivity (meat and eggs).

Aflatoxins are one of these factors which are found to impair the immune mechnisms of birds (Thaxton et al., 1974). Several studies proved that aflatoxin depressed humoral antibody response and cell mediated immune system (Giambrone et al., 1978). Brown and Abrams (1965) isolated salmonella from ducklings and chickens with typical aflatoxicosis. Abrams (1965) also pointed out that not only aflatoxin made birds more susceptible to salmonella but also for other bacterial and viral diseases. Hamilton and Harris (1971) studied the interaction of aflatoxicosis with *Candida albicans* infections and other stresses in chicks.

MATERIAL AND METHODS

Chicks: 300 comercial one day old Arbor acre chicks obtained from Cairo Poultry Company were used in this study. They were housed in electrically-heated cages, where feed and water were available adlibitum untill the end of the 6th , week.

The present study was divided into two experiments as follows:

The first experiment:

150 chicks were used. They were divided into five groups, each contained 30 chicks, then each group subdivided into subgroups A and B. Both the chicks of subgroups 1A and IB were fed feed free from aflatoxin, while the chicks from 2A and 2B to 5A and 5B subgroups received ration contained 125, 250, 500 and 1000 ug/kg aflatoxin respectively untill the end of the 6th. week, the subgroups 1B, 2B, 3B, 4B and 5B were inoculated intracrop after two weeks with one ml of *C.*

albicans suspension contained 3 x 10^3 yeast cell (Huppert et al., 1953). The birds were observed clinically and five chicks from each subgroup were sacrificed after 2 weeks, 3 weeks and 4 weeks post *C. albicans* infection. Crop and proventriculus were collected for mycological and histopathological examinations.

The second experiment

150 chicks were used. They were divided into five groups, each contained 30 chicks, then each group subdivided into subgroup A and B. Both the chicks of subroups 1A and 1B were fed aflatoxin free diet, while the chicks of subgroups 2A and 2B to 5A and 5B subgroups received ration contained 125, 250, 500 and 1000 ug/kg aflatoxin respctively untill the end of the 6th. week. The subgroups 1B, 2B, 3B 4B and 5B were inocualted intranasally after two weeks with one ml of Aspergillus suspension contained 3 x 10^4 spore (Carpenter, 1975) . The birds were observed clinically and five chicks from each subgroup were sacrificed after 2 , 3 and 4 weeks post *Aspergillus fumigatus* infection respectively. Lung tissues were collected for mycological and histopathological examinations.

For histopathological examination the collected specimens were fixed in 10% Formol saline, then routienly processed and embedded in paraffin. The paraffin embeded blocks were cut at 4-6 microns and stained by Haematoxylin & Eosin, and Periodic Acid Schiff technique (PAS) (Carelton, et al., 1967).

RESULTS

The first experiment:

The clinical signs in the birds received 500 or 1000 ug/kg aflatoxin and inoculated with *C. albicans* were retardation of growth and ascities. At necropsy there was marked thickening of the both crop and proventriculus. These findings were clearly observed particularly at the 3rd . and the 4th. weeks as well as *C. albicans* could also be reisolated as shown in (Table 1).

At the 2nd week, the birds received 125 or 250 ug/kg aflatoxin and *C. albicans* showed mild to moderate microscopical alterations, there was vacuolar degeneration of proventricular mucosa with hyperplastic activation in some parts, moreover exfoliated cells were present inside the lumen. Eosinphilic membrane covered most of the mucosa was observed (Fig. 1), which stained positive by PAS technique (Fig. 2). In the same time the similar alterations could be noticed in the birds received 500 or 1000 ug/kg aflatoxin as well as the lamina propria was moderately infiltrated by mononuclear cells (Fig. 3).

Table 1: Studies of the interaciton between aflatoxin in diet and *Candida albicans* infection.

Group	Total No.	No. of Sacrifled	Lesions of crop & proventriculus			%	Reisolation of C.A. crop/ proventriculus		
			2W	3W	4W		2W	3W	4W
1B	15	5	3/5	1/5	1/5	26.6	5	1	1
1A	15	5	-	-	-	0	-	-	-
2B	15	5	4/5	3/5	3/5	66.6	5	3	2
2A	15	5	-	-	-	0	-	-	-
3B	15	5	5/5	4/5	2/5	73.3	5	4	3
3A	15	5	-	-	-	0	-	-	-
4B	15	5	5/5	5/5	3/5	86.6	5	5	4
4A	15	5	-	-	-	0	-	-	-
5B	15	5	5/5	5/5	4/5	93.3	5	5	4
5A	15	5	-	-	-	0	-	-	-

W: Week.

At the 3rd weeks, the birds received 125 or 250 ug/kg aflatoxin and *C. albicans* revealed that the proventricular mucosa was completely necrosed and covered by eosinphilic membrane.

The lamina propria was heavily infiltrated by mononuclear cells and heterophils, funthermore the submucosa was also infiltrated by mononuclear cells and exhibited vascular dilation. In the birds received 500 and 1000 ug/kg aflatoxin and *C. albicans* , The eosinophilic membrane could be clearly observed and the submucosal glands were completely necrosed and replaced by mononuclear cells, moreover the crop showed hyperkeratosis with the appearance of pseudohyphae deeply penetrating its mucosa (Fig. 4). The birds received aflatoxin only revealed degenerative changes of proventricular mucosa with cells desquamation in some parts, while the control birds appeared to be normal.

The second experiment:

The post mortem examination of the birds in this experiment revealed that the birds received 500 or

Fig. 1: Proventriculus showing clear thick eosinophilic membrane covering the epithelial lining. H&E x400.

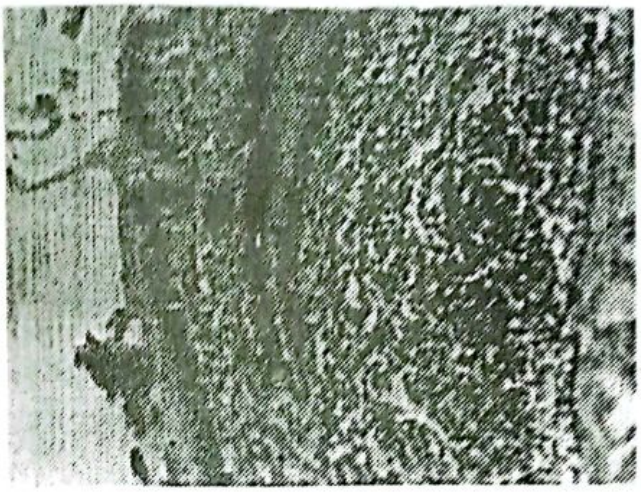

Fig. 3: Proventriculus showing infiltration of the lamina propria by mononuclear cells. H&E x400.

Fig. 2: Proventriculus showing PAS POSITIVE membrane covering the epithelial cells. PAS x400.

Fig. 4: Crop showing pseudohyphae of *C. albicans* organism deeply penetrating the crop mucosa. PAS x 400.

1000 ug/kg aflatoxin and *A. fumigatus* showed severely congested and oedematous lung as well air sacs were thick and opaque. These findings were noticed obviously at the 3rd and the 4th weeks, more over *A. fumigatus* could also be reisolated from lung tissue (Table 2).

Table 2: Studies of the interaciton between aflatoxin in diet and *Aspergillus Fumigatus* infection.

Group	Total No.	No. of Sacri-fled	Lesions of crop & proventriculus			%	Reisolation of A.F. crop/ proventriculus		
			2W	3W	4W		2W	3W	4W
1B	15	5	3/5	2/5	2/5	46.6	4	2	1
1A	15	5	-	-	-	0	-	-	-
2B	15	5	3/5	4/5	1/5	53.3	4	4	2
2A	15	5	-	-	-	0	-	-	-
3B	15	5	4/5	3/5	3/5	66.6	5	3	3
3A	15	5	-	-	-	0	-	-	-
4B	15	5	4/5	4/5	3/5	73.3	5	5	2
4A	15	5	-	-	-	0	-	-	-
5B	15	5	5/5	5/5	4/5	93.3	5	5	4
5A	15	5	-	-	-	0	-	-	-

W.: Week.

At the 2nd week the birds received 125 or 250 ug/kg aflatoxin and *A. fumigatus* revealed that the primary bronchi contained desquamated cells with mucous exudate and the lamina proporia showed hyperplastic lymphoid follicle with vascular dilation (Fig. 5). The birds received 500 or 1000 ug/kg aflatoxin and *A.fumigatus* revealed more or less severe pulmonary lesions, the pulmonry bronchi still contained desquamated cells, mucous and inflammatory cells. Furthermore, the lamina propria showed small to large histocytic granulomes.

At the 3rd and the 4th weeks, the lesions were more or less in the all groups of birds but it differes in severity. In the birds received 125 0r 250 aflatoxin and *A. fumigatus,* lung exhibited degenerative changes in the bronchial epithelium with cells desquamation in some parts which were mixed with inflammatory cells (Fig.6). The peribronchial and perialveolar blood vessels were congested as well as serofibrinous exudate and mononuclear cells infiltration could be noticed in the interlobar spaces (Fig. 7).

In the birds received 500 or 1000 ug/kg aflatoxin and *A. fumigatus*, the formentioned lesions were still present but more severe, moreover corpora amylacea was noticed inside the secondry bronchi (Fig. 8). The birds received aflatoxin only revealed mild degenerative changes in the pri bronchi, while the control birds did not reveal pathological alterations.

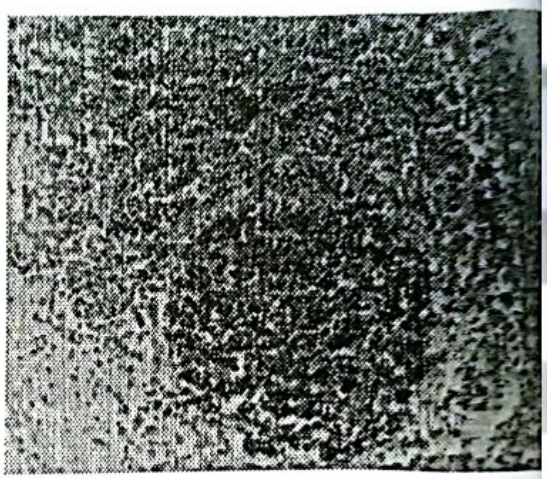

Fig. 5: Lung showing hyperplasia of peribronchial phoid follicle. H & E x 400.

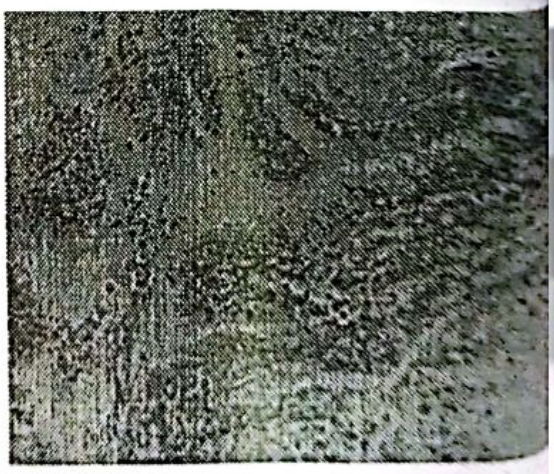

Fig. 6: Pulmonary bronchi showing degen changes in the epithlium and the lumen desquamated cells, inflammatory cells and mucous. H&E x400.

Fig. 7: Lung showing diffuse mononuclear cells infiltration in the interlobar space - H&E x400.

Fig. 8: Lung showing corpora amylacia within secondry bronchi and interlobar oedema.. H&E x400.

DISCUSSION

The effect of aflatoxin on the immune system was studied by many investigators to increase the susceptibility of chicks to salmonellosis, asperigellosis, coccidosis, marek's disase and *C. albicans* infection, Edds et al., (1973), Hamilton, et al. (1971) and Richard, et al. (1973) and also its effects on the hinderance of the humoral and cell mediated immune responses (Campbell, et al., 1983 and Giambrone et al., 1978).

In the present study trials were done to produce experimental infection with *C. albicans* in four groups of chicks, these groups fed ration contained different doses of aflatoxin . The post mortem examination revealed that the crop and proventriculus were more or less affected in the all infected groups, moreover the infectivity percentage were 66.4, 73.3, 86.6 and 93.3 respectively (Table 1). These results were coincided with that mentioned by (Hamilton and Harris 1971). The results were also confirmed by histopathological lesions which characterized by the pesence of eosinophilic membrane on the proventricular mucosa, the submucosal glands were necrosed and completely replaced by mononuclear cells, furthermore the crop mucosa was penetrated by pseudohyphae of albicans. These results come in harmoney with that of (Hache, 1966).

Concerning to the interaction between *Aspergillus fumigatous* and aflatoxin, the post mortem examination in birds received *A. fumigatous* and aflatoxin with different doses revealed that their lungs were affected with different degrees according to aflatoxin doses and the infectivity percentage was similar to *C. alicans.* The results were also confirmed by the histopathological lesions which were characterized by the presence of mucous exudate and inflammatory cells inside the primary bronchi as well as few numbers of cellular granulomas could be noticed adjacent to the secondry and tertiary bronchi. These results were coincided with (Richard, et al., 1973), who added that these granulomas might be suffered from caseous necrosis.

In conclusion the effect of aflatoxin on the resistance of birds to microbial infections seems to be varied according to its dose level.

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