PATHOLOGICAL FINDINGS IN EXPERIMENTAL AFLATOKICOSIS IN CHIKS

H.Nesrin BİLGİÇ (*) Tahsin Yeşildere (*)

SUMMARY: In this study 100 day old Broiler chiks were fed for 0-30 days with 0,1,2 and 4 $_{u}$g AFB_1 per gram of feed. Liver autopsies were collected at 10.20 and 30 days of experiment. Macroscopically in the initial stages petechial haemorrages were observed. While in the advanced stages of Aflatoxicosis liver were putty like coloured and friable. Microscopically parenchymal and fatty degeneration, microfocal necrosis, nodular and ductular hyperplasia, proliferation in the bile ducts, hyperplasia of the mucosae of bile duct and gall bladder were observed.

INTRODUCTION

Aflatoxins are produced by various types of fungi belonging to Aspergillus, Penicillium, Rhizopus, Clodosporium and Alternaria. Basically they are produced by A.flavus and A.parasiticus fungi (6,9).

The Aflatoxins are mainly composed of AFB_1, AFB_2, AFG_1, AFG_2 components depending upon their flourescence characteristic observed under the ultra violet light (6,10). Aflatoxins along with toxic nature also have carcinogenic and mutagenic effect (7,11). Bird are sensitive to Aflatoxins in the following order; duck, turkey, phesant, hen and quail (16).

Key words-AFB_1, Broiler chicken, liver

Experiments performed in each type of birds have shown that in acute and chronic cases of Aflatoxicosis the most clearcut reaction is the proliferatio of the bile ducts (12,15).In addition, in parenchimal cells vacuolisation, fatty degeneration and picknosis resulting in necrosis of the hepatic cells (12), microfocal necrosis is observed (17). These changes have been reported to occur in the periportal region of liver in hen (12).

MATERIAL AND METHOD

One hundred day old Arbor-Acres broiler chiks were used as experimental birds. Birds were fed with 0,1,2 and 4 $_{u}$g AFB_1 per gram of feed. Birs were fed ad libitum for 30 days. Liver autopsies were performed on day 10,20,30 of experiment. H and E staining was performed for microscopic slides. Suspected section were stained with Scharlach oil stain, Gomori reticulum stain, Gomori iron reaction, Van Gieson and PAS stain.

Preparation of the ration containing AFB1

To rule out the possibility of AFB1 in the basal ration analysis was performed with HPLC (5). After assuring the absence of AFB1 in the ration crystallin AFB1 (Sigma Chemical Co) was dissolved in 5 ml of (98+2) benzen asetonitril mixture. From this mixture 50 $_{u}$l was injected into HPLC and the observed peak was compared with the standart peak. In thes way the quantity of ABF1 present in the packet was measured. After making dilutions from the AFB1 containing benzen asetonitril mixture the feed was soaked. Fed was let to be dried at 50 C , in an incubator having air circulation, for over night. Stock feed containing AFB1 was mixed to the basal ration with the help of Waring blender. The quantity of AFB1 in feed samples was determined with HPLC. During the entire experimental period feeds for control and experimental birds were stored at 4 C .

RESULTS

Macroscopic findings

No lesion was observed in the control group at autopsy.

The 10th day:

In the first group (1 ppm) 4 livers with petechial haemorrages and 4 with putty like colour were observed. In one

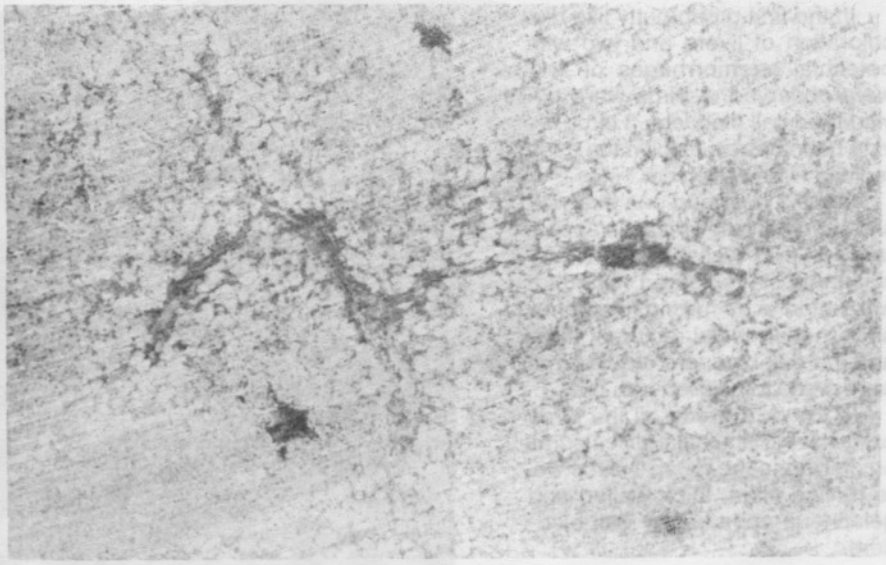

Fig 1: Fatty degeneration starting from the portal area (Peripheral fatty degeneration of thi lever) H.E X 115

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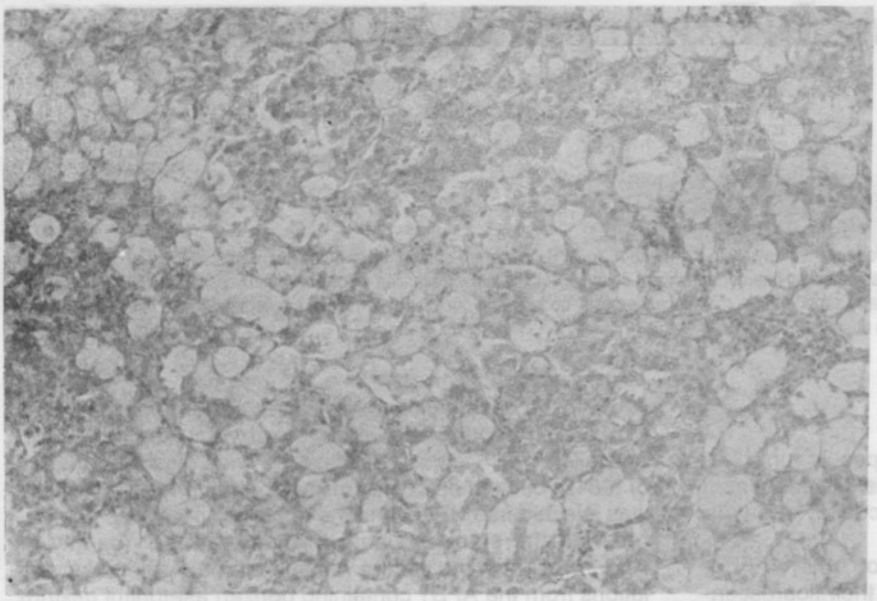

Fig 2: Extensive fatty degeneration of the paranchmal cells. H.E X 315

bird petechial haemorrhage in the breast muscles was also observed.

In the 2 nd group (2 ppm) one bird died on the 7 th day of experiment. 4 birds with petechial haemorrhage in liver, 2 birds with putty like coloured livers; one bird however, with read yellow mosaic appearance and 4 birds with colour change in the pars caudodorsalis were observed. In one bird subcutaneous haemorrhage in the breast region was noted whereas 5 birds were found to be having haemorrages in the medial side thighs.

In the 3 rd group (4 ppm) one bird was died on 7 th day of reach. While findings 7 birds with putty like discoloration of liver, 5 birds were however noted to have colour change in the pars caudodorsalis region.

The 20 th day:

In the first group putty like discoloration of livers and two with petechial haemorrhages of liver were observed. 6 birds were having filled gall bladders. 3 birds having haemorragic focci in the thigh regions were observed.

In the 2 nd group one bird was showing red yellow mosaic appearance of liver while in others liver was putty like coloured. One bird was found to be having haemorrahage in the medial region of thigh and the gall bladders of two birds were noted to be filled with bile.

In the 3 rd group all the birds were having putty like discolourati on of liver. In the group 7 birds were having filled, thick walled and gelatinous appearance gall bledders.

The 30 th day:

In the first group, 9 birds having tense capsule blunt edges and friable consistency of livers were noted. 6 birds were having red yellow mosaic appearance of livers. Gall bladders in 3 birds were observed to be filled and gelatinous.

In the 2 nd group livers from 3 birds were having mosaic appearance; 6 were having putty like colour appearance with tense capsules. While noticing haemorrahages the thigh region (in 5 birds) in breast regions (in 3 birds), pea size haemorrages were noted under the wings in 4 birds. Fully filled, thick walled and gummy appearance gall bladders were observed in 8 birds.

In the 3 rd group one bird died 23 rd day of experiment. Livers with putty like discolaration blunt ends and friable consistency were observed in 8 birds. 2-2,5 cm in wing and pea size bleeding focci in thighs were noted in 4 birds. Call bladders were filled, gelatinous and gummy in appearance in all the birds.

Microscopic findings

The 10 th day:

In the first group (1 ppm) hydropic and fatty degeneration of was observed as cells hepatocytes were swollen filled albumin granules, having foggy appearance under the capsula there was more dense microfocal necrosis and along with this an increase in fibroblasts and fibrocytes was observed. Around the central veins coagulative necrosis was seen. Activation of the epithelial and reticular cells was found around the Kiernan space. Proliferation and expansion of bile ducts, fibrinoid degenariton of the bile duct

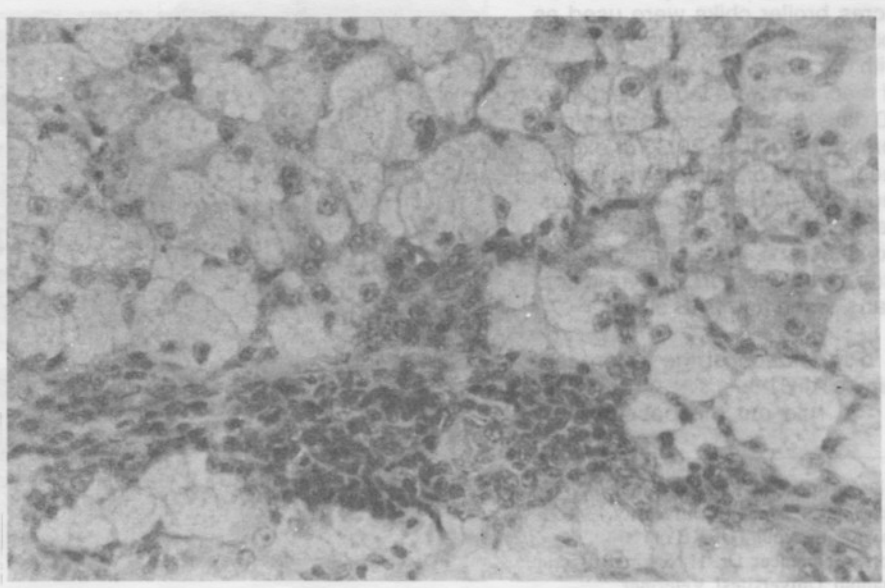

Fig 3: Clear appearance of extensive fatty degeneration of the parnchymal cells. H.E X 485

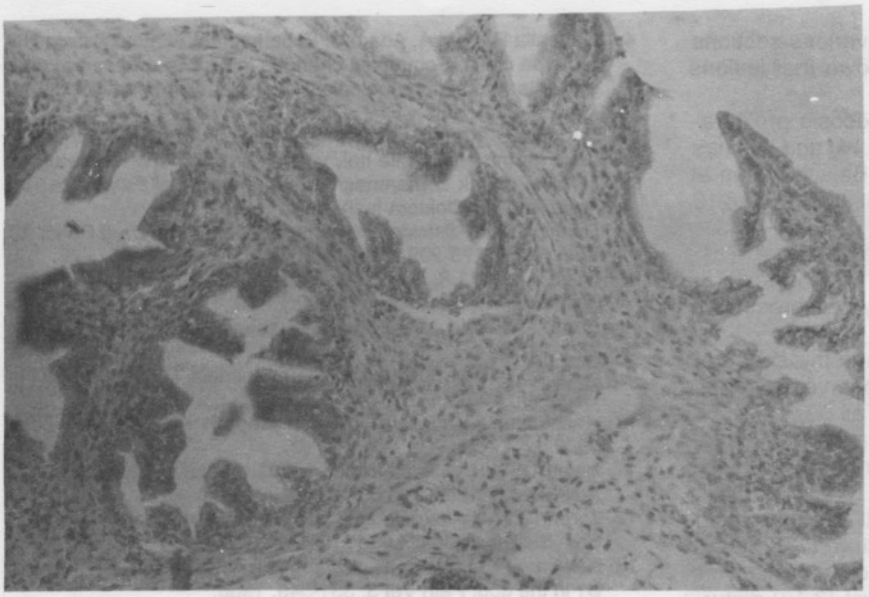

Fig 4: Adenomateous formation in the mucosal epithelium of the bile ducts. H.E X 485

wall and a belt of extensive coagulative necrosis was observed around the bile ducts. Mucosal epithelial cells of the bile duct and gall bladder have undergone extensive hyperplasia and were projecting like fingers. This finger like process were observed to be adenomatous in formation (Fig 4).

Moreover in the 2 nd group, here and there thickening of capsules marginal hyperchromacy of hepatic epithelial cells and division of hepatic paranchyma into small islets due to reticular cells and fibroblasts was observed. However in the 3 rd group along with ductular hyperplasia pseudobile ducts were observed.

The 20 th day:

In the first group wide haemorrhagic focci encircled with fibrous connective tissue were observed. Swollen, foggy hepatic cells due to extensive parenchymal and fatty degenaration of cells was observed throughout the lingth of Remark cords (Fig 1,2,3). Microfocal necrosis was noticed under the capsule. Areas of basophillic nodular hyperplasis were observed in the liver. The arrengement of Remark cords have been disturbed due to formation radial chains by reticular cells, fibroblasts and Kuppfer cells. Proliferation of bile duct, fibrinoid degeneration of the wall of bile duct and areas of extensive hyperplasia of mucosal epithelium of bile duct and gall bladder were noticed. Moreover in the 3 rd group ductular hyperplasia was observed.

The 30 th day:

In the first group around the Kiernan space 25-30 cells by undergoing fatty degeneration have formed small fatty lakers. Under the capsular area more profound necrosis and increase in the activity of fibroblasts and fibrocytes was noted. Basopyhlic cyncitial chains formed by reticular cells, fibroblasts and histiocytes have disturbed the arrengement of Remark cords. Hyperplasia of the mucosal epithelium of bile duct and gall bladder was noticed (Fig 5). Moreover nodular and ductular hyperplasia was observed in 2 nd group.

DISCUSSION

In our research although there was a severe colour change in the area between pars caudodorsalis and pars caudoventralis but no fissure was not observed and this colour change dissappeared in the advanced stages of toxication (2). Moreover, on the evaluation of histopathologic lesions it was noted that pars caudodorsalis has been less affected by toxic effects (2).

In thigh petechial and echymotic haemorrhages are in the shape of haemorrhage reported by Dafalla et all (4). However it is different from the reported study as regards the period of appearance of haemorrhage, used dose and extensive haemorrhagic focci of advance stage.

Haemorragic, red yellow mosaic appearance and the advanced stage putty like discoloration and friable consistency of liver observed by us agrees with the findings of reported in the literature (1,2,3,4,15,15).

Oedema and thickening of the wall of gall bladder noted by us agrees with that reported by Dafalla et al (4).

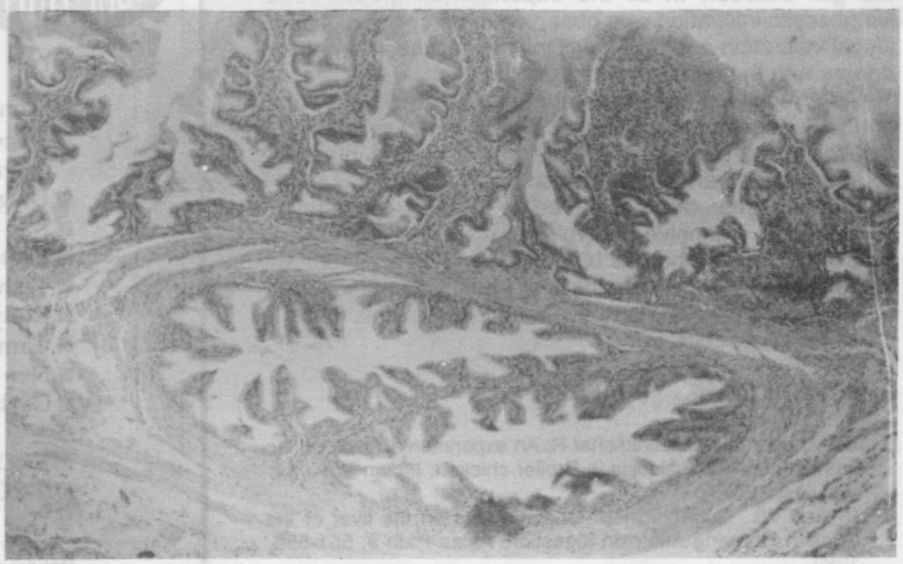

Fig 5: Severe proliferation and adenomateous formation in the epithelial cells of the mucosa of gall bladder. H.E X 240

Histopathologic studies performed on various sections of every lobe and caudodorsalis have shown that lesions were light in degree (14).

In acute and chronic cases of Aflatoxicosis proliferation of bile duct have been reported by (12,14) and this has been reported to happen at 28 th day by Balachandran et all (1) but we have first time observed that it appeared at 10 th day.

Findings related to Aflatoxicosis are localized in the periportal zone in hen (12). Light degree of degeneration of hepatocytes of the midlobule observed by us agrees with the literature (4).

In the 2 nd group coagulative necrosis in the shape of single circle around the Vena sentralis observed by us is similar to that reported by Balachandran et all (1).

In all the experimental group, degeneration of microfocal necrotic focci in a cleare and very severe form were observed under the capsule.

Focal fat deposition, resulting from fatty degeneration in the Kiernan space agrees with the literature (1,3). In our research radial chains formed by reticular cells, histiocytes and mesenchymal originating from bile duct (3,13) disturbed the arrangment of Remark cords (1,8,12,13,14).

Margial hyperchromacy of hepatic cells and appearance of nuclei like inclusion bodies agrees with the literature (3,13). Basophylic nodular hyperplastic areas observed are similar to the findings reported by Newberne et all (13).

It was interesting to observe the arrangement of degenerating hepatic cells in a serias around a central lumen which resemble with a duct resulting in ductular hyperplasia. It agrees with the findings of the literatures (1,3,12).

Hyperplasia of the mucosal epithelium of bile ducts observed by us agrees to reported literature (3,4). However hyperplastic areas observed by us, were in the form of finger like adenomatous extensions. Newberne et all (13) have reported the formation of pseudobile duct formation in Aflatoxicosis in dogs and it was interesting to note them in the hen.

At all stages of study we have found the severe mucosal epithelial hyperplasia and adenomatous formation of the gall bladder. In all the experimental groups focal lymphocytic infiltration was observed to be changed in multifocal infiltration and heterophil infiltration was also noted it agrees with the reported literature (1,4,15).

As a results in experimental Aflatoxicosis, paranchymal and fatty degenariton of hepatic parenchymal cells, microfocal necrosis, peripheral fatty degeneration and in advance stage appearance of toxic hepatitis due to diffuse fatty degenaration has been observed. In addition, proliferation of bile ducts, extensive hyperplasia of the mucosal epithelium of bile ducts and gall bladder, being as an important characteristic for the diagnosis of Aflatoxicosis has been noted. Persistency of the above mentioned events leads to the chronic hepatitis.

REFERENCES

1. Balachandran C, Ramakrishar R: An experimental study on the pathology of Aflatoxicosis in Broiler chicken. Indian Vet J 11: 911-914, 1987.
2. Bryden WL, Cumming RB: Observations on the liver of the chicken following Aflatoxin ingestion. Avian Path 9: 551-556, 1980.
3. Carnaghan RBA, Lewis G, Patterson DSP, Allcroft R: Biochemical and pathological aspects of groundnut poisoning in chickens. Path Vet 3: 601-615, 1966.
4. Daffealla R, Yagi A, Adam S: Experimental Aflatoxicosis in Hybro type chiks: Sequential changes in growth and serum constituents and histopathological changes. Vet Hum Toxicol 29 (3): 222-226, 1987.
5. Dağoğlu C: Kanatlı yemlerinde Aflatoksin türevlerinin ince tabaka kromatografisi ile itel, yüksek basınçlı sıvı kromatografi ile nicel olarak saptanması. İstanbul Üniv Vet Fak Farm Tok Anabilim Dalı (Doktora tezi), 1991.
6. Denning DW: Aflatoxin and human disease. Adv Drug React Ac Pois Rev 4: 175-209, 1987.
7. Dunn JJ, Lee LS, Ciegler A: Mutagenicity and toxicity of Aflatoxin precursors. Environ Mut 4: 19-26, 1982.
8. Edds CT: Acute Aflatoxicosis: A reviev. JAVMA 162 (4): 304-308, 1973.
9. Hamilton PB: Mycotoxins and farm animals. Refush Vet 39 (1-2): 17-44, 1982.
10. Hesseltine CW: Mycoto0ins. Mycopath App 39: 371-383, 1969.
11. Hsieh DPH: Biological reactive intermediates of mycotoxins. Adv Exp Med Biol 197: 597-610, 1986.
12. Jordan FTM: Aflatoxicosis In: Poultry Diseases. Cordon RF Eds. Bailliers Tindall London 270-271, 1982.
13. Newberne PM, Russo R, Wogan GN: Acute toxicity of Aflatoxin B1 in the dog. Path Vet 3: 331-340, 1966.
14. Newberne PM, Butler WH: Acute and chronic effect of Aflatoxin on the liver of domestic and laboratory animals: A rewiev. Cancer Res 29: 236-250, 1969.
15. Newberne PM: Chronic Aflatoxicosis. JAVMA 163 (1): 1262-1267, 1973.
16. Osweiler CD: Mycotoxins and poultry health. Zootecnica 5: 48-50, 1987.
17. Popescu O, Cotofan O, Coman 1: Diagnostic stratagy of mycotaxicoses revealed by extrapolation of results an experimental mycotaxicosis in broilers. Lucrari Ştiıntifice 29: 8587, 1985.
18. Wannop CC: The histopathology of Turkey "X" disease in Great Britain. Avian Diseases 5: 371-381, 1961.

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