Histopathological changes of some internal organs in broilers fed aflatoxin

Ekhlas K. H
Coll.of Edu. /Unive.of Mosul

Abstract

Forty, one-day-old male broiler chicks (Ross 308), were randomly distributed at one day of age to 2 experimental groups consisting of 10 birds with two replicates for 35 days. Group 1 fed control diet with no Aflatoxin (AF) (negative control),while group 2 fed AF contaminated diet at a rate of 2.5ppm. Scarifying birds done at the end of the experiment, bursa of Fabricius, liver and kidney were sectioned for microscopical examination .It was shown that AF affected Liver, kidney and the immune system organ (bursa of Fabricius), which are considered to be the target organs for AF and these are primarily affected in aflatoxicosis.

Key words: Aflatoxin, broilers, histopathology

Introduction

Aflatoxins, secondary metabolites of various Aspergillus spp., commonly contaminate a wide variety of tropical and subtropical food/feed stuffs. These mycotoxins are known to have strong hepatotoxic and carcinogenic effects and are regulated by feed/food law in at least 100 countries (1). Chemically, aflatoxins are difuranocoumarin compounds and include B1, B2, G1, G2, M1, and M2 (2). These mycotoxins contaminate a wide variety of agricultural commodities including oilseed meals, dried fruits, spices, and cereals (3). Aflatoxins M1 and M2 however, mainly occur in milk (AFM1 in small quantities also reported in eggs) as metabolites of the B1 and B2. Among the various types of aflatoxins, aflatoxin B1 (AFB1) is most commonly encountered and it is also considered to have higher toxicity than other aflatoxins (4).Aflatoxins are highly toxic and carcinogenic mycotoxins produced by *Aspergillus flavus* and *A. parasiticus* (5). Poultry feeds and ingredients are vulnerable to fungal growth and aflatoxin formation. Aflatoxins are relatively stable in feed products. Aflatoxin BI is the most toxic, and bepatotoxicity is the primary effect in nearly all animals. Aflatoxin producing fungi and aflatoxin contaminatedanimal feedstuffs are recognized worldwide , usually with adverse implications for poultry production (6). Aflatoxicosis occurs in poultry worldwide (7). The direct and indirect effects of aflatoxicosis include increased mortality from heat stress (broiler breeders) (8); loss of egg production (leghorns) (9); anemia, hemorrhages, liver condemnations (10), paralysis, lameness (11), and impaired performance of broilers (12); nervous signs (13), and mortality (ducks) (9); impaired ambulation and paralysis (quail) (14); impaired immunization (turkeys) (15); and increased susceptibility to infectious disease in many species (16). Cases of concurrent aspergillosis and aflatoxicosis confirmed that Aspergillus spp. Threaten poultry production in the feed, litter, and environment (17). The pathology of experimental aflatoxicosis is similar to the naturally occurring disease.. Aflatoxicosis in chickens caused yellow, ocher discoloration of the liver, with multifocal hemorrhage and a reticulated pattern on the capsular surface. In time, the livers developed white foci as hepatic lipid content increased. Histologic lesions occurred as fatty vacuolation of hepatocyte cytoplasm; karyomegaly and prominent nucleoli in hepatocytes; proliferation of bile ducts; and fibrosis. Basophilic, vacuolated, regenerative hepatocytes, and inflammation by heterophils and mononuclear cells occurred in the portal zones (18). For these studies in chickens, no aflatoxin-related lesions were reported in either the kidney or major lymphoid tissues (19).The aim of the study was to describe the effect of feeding 2.5 ppm Aflatoxin to broilers for 5 weeks on the histopathological changes in some of the internal organs .

Materials and methods

This study was carried out on 40 one-day-old male broiler chicks (Ross 308), procured from commercial hatchery, during 2011 year. The study carried out in a private poultry farm in Arbil.. Birds were randomly distributed at one day old to 2 experimental groups each containing 10 birds with two replicates. They reared on deep litter system. The temperature degree and humidity percentages daily measured and recorded approximately 35 ± 2.0 C^o at the first day, then the temperature degree gradually decreased weekly by 2.5 C^o with age until the end of the experiment at 35 days. All diets formulated to provide the nutrient requirements according to (20). The ration based on yellow-corn soya bean contained 23.99% crude protein and 3188 Kcal metabolizable energy. Offering Feeds and water were *ad libitum* . Continuous lighting program (24hr) used during the experimental period. Production of Aflatoxin was by culturing rice by *Aspergillus flavus (*CECT 2687) according to the method reported by (21). The toxin was calculated using Neogen ELISA kit (Neogen Corporation) with XL_{800} reader. The toxin containing rice was added to experimental diets, and mixed to homogeneity by means of a twin –shell blender. Checking the experimental ration to contain no detectable levels of aflatoxins, Ochratoxins, Zearalenone, and T-2 toxin was by the method reported by (22). The experimental treatments were as follows: Group 1 has no toxin (negative control). Group 2 contains 2.5ppm AF. Birds vaccinated against Newcastle disease and infectious bronchitis by spray method at one day of age, Newcastle disease at 8 days and infectious bursal disease at 14 days of age. Scarifying birds done at the end of the experiment, pieces of bursa of Fabricius, liver and kidney were put in 10% buffered formalin, fixed in paraffin, and then sectioned at thickness of 5µ for microscopical examination. Staining sectioned organs was by Hematoxylin and eosin (23).

Results

Histologicaly of livers, kidneys and bursa of Fabricius in control group killed at the end of the experiment showed a fairly well preserved texture.The histopathological changes in studied organs of broilers fed AF were as follows:

Kidneys:

Kidneys of broilers fed Aflatoxin revealed dilatation of proximal tubules with necrosis undergoing in their epithelium; with some of the nuclei have enlarged bizarre forms. Kidney sections also show focal aggregation of lymphoid cells in between the degenerated renal tubules. In addition, thickening of glomerular basement membrane and collapse of glomerular tuft found in the bird fed the toxin (figure 1&2).

Liver:

Grossly, Aflatoxin feeding to chickens caused yellow, ocher discoloration of the liver. In microscopic picture, dilatation in the portal veins and sinusoids seen with inflammatory heterophils and mononuclear cells in the portal zones. The main lesions were chiefly cytoplasmic vacuolation (fatty change), and the bile duct hyperplasia (figure 3,4&5).

The bursa of the Fabricius:

AF fed group revealed a loss normal bursal architecture. , thinning of the cortical layer, medulary lymphoid depletion with Lymphocytolysis. Edema and interfollicular fibrosis were noticed. There was hyperplasic, discontinued and corrugated bursal epithelial layer (figure 6&7).

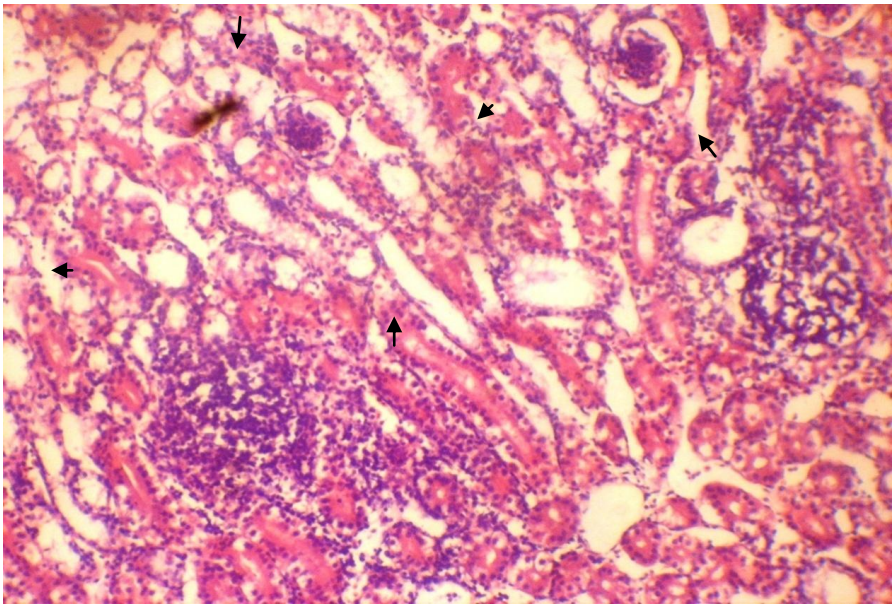

Figure 1 :dilatation of proximal tubules with necrosis undergoing in their epithelium, focal aggregation of lymphoid cells in between the degenerated renal tubules, thickening of glomerular basement membrane and collapse of glomerular tuft. some of the nuclei have enlarged bizarre form (arrows).(165X)

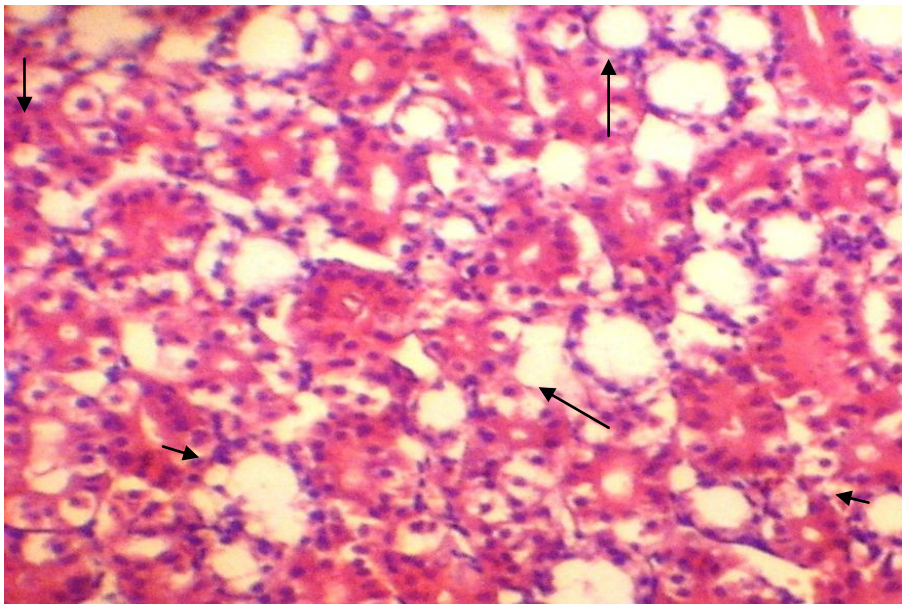

Figure 2 : dilatation of proximal tubules with necrosis undergoing in their epithelium, some of the nuclei have enlarged bizarre form (arrows).(165X)

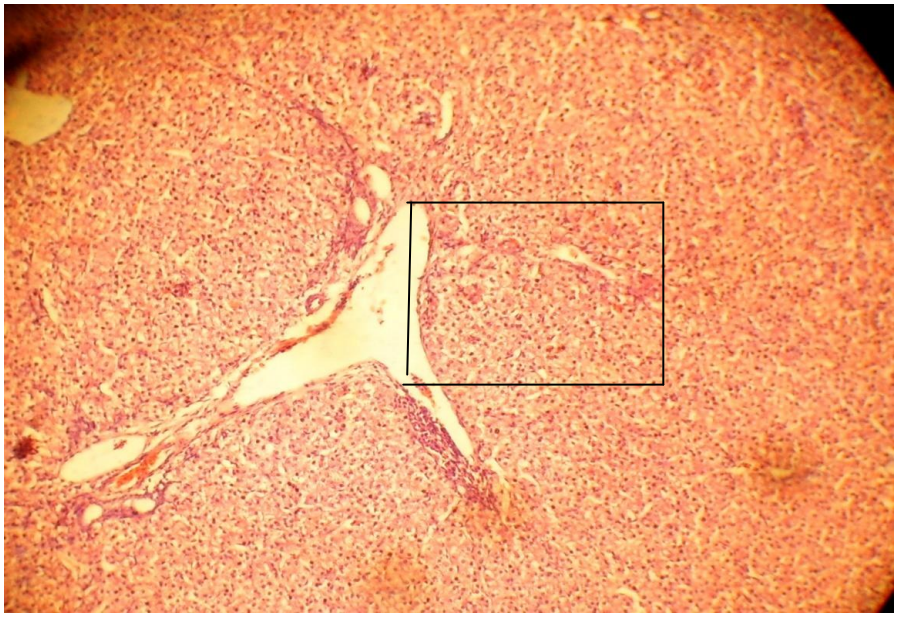

Figure 3: dilatation in the portal veins and sinusoids seen with inflammatory cells in the portal zones.(35X)

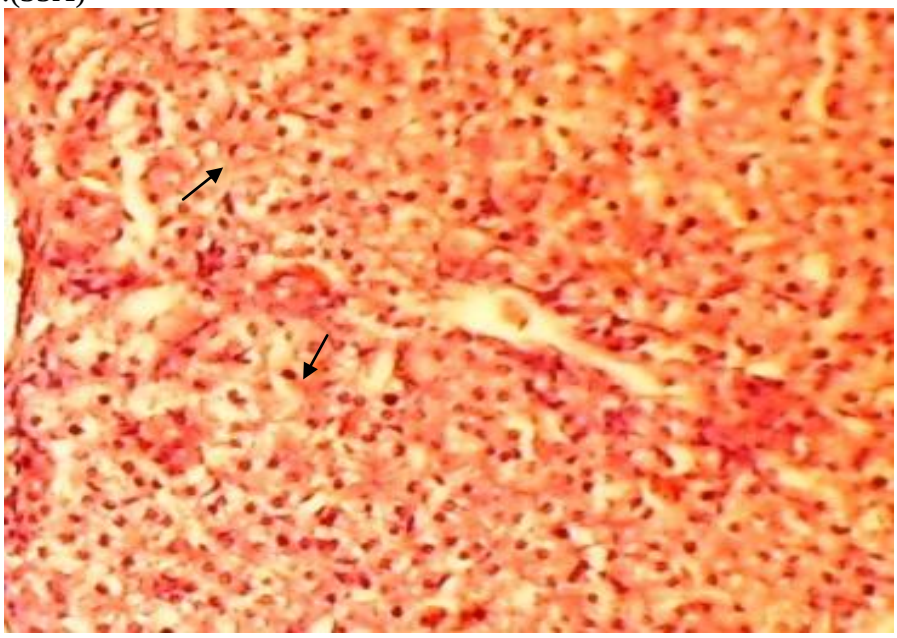

Figure 4: Magnification of figure 3 . cytoplasmic vacuolation (fatty change)(arrows) of hepatocytes .(200X)

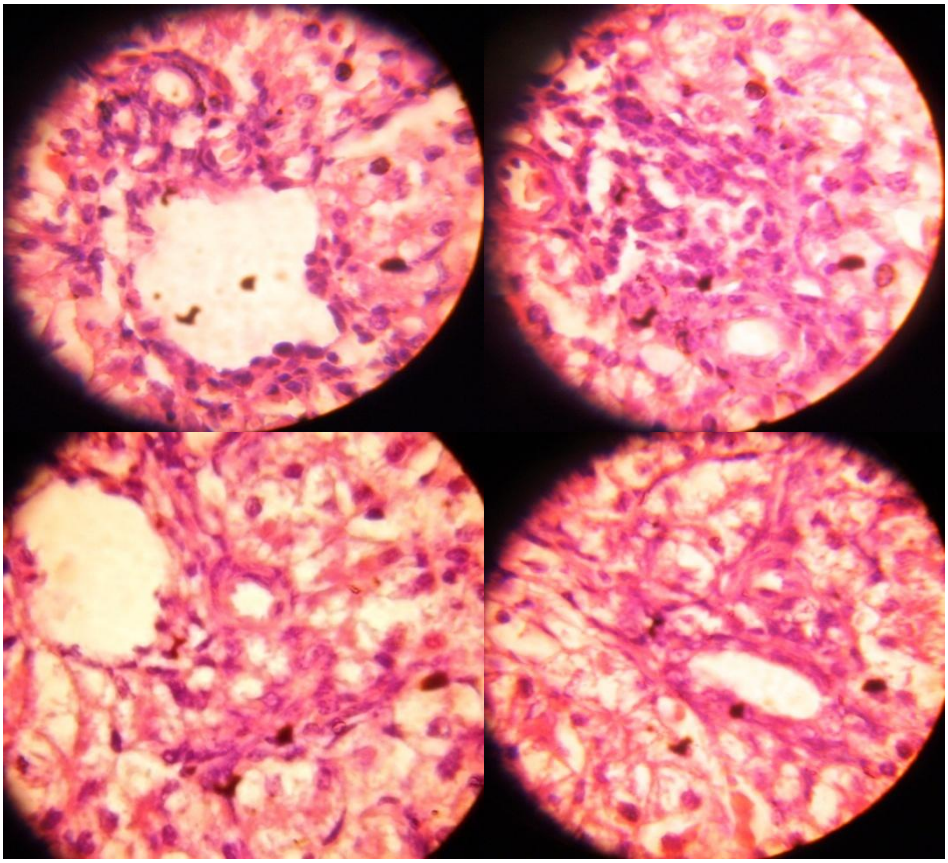

Figure 5: cytoplasmic vacuolation (fatty change), and the bile duct hyperplasia(200X)

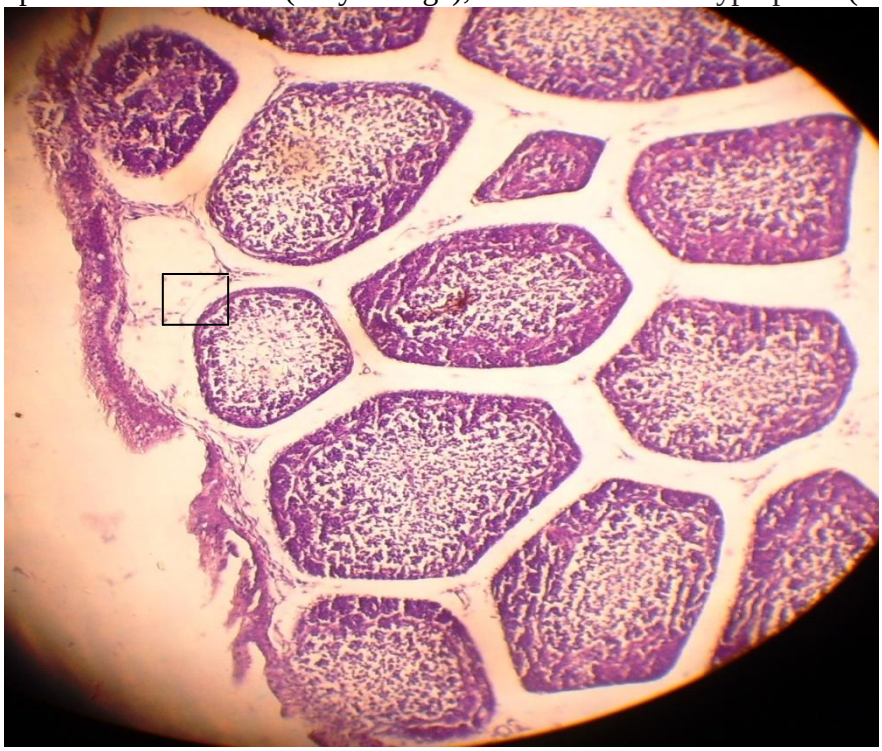

Figure 6 :Loss of normal bursal architecture with generalized lymphoid depletion, Lymphocytolysis, edema and interfollicular fibrosis and Discontinuity and corrugation of hyperplastic epithelial layer..(115X)

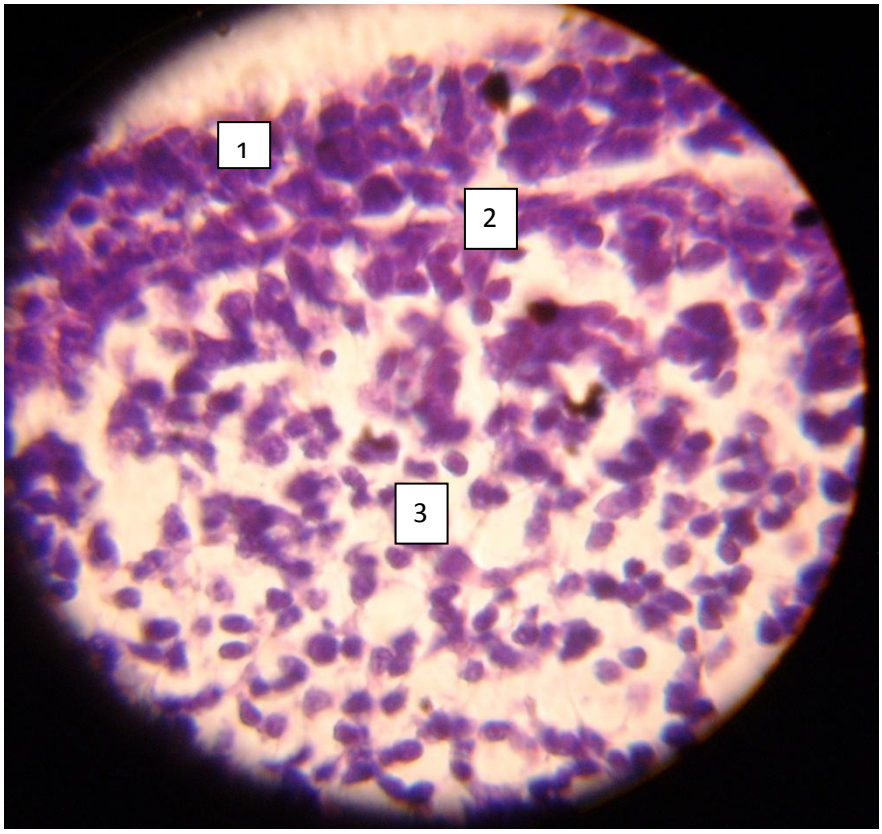

Figure 7: magnification of figure 6, showing the Presence of interepithelial cell layer(2), thinning of the cortical layer (1), medulary lymphoid depletion with Lymphocytolysis(3).(900X)

Discusion

Aflatoxin used in the present study was produced by culturing of *Aspergillus favus* (CECT 2687). This strain has been known to produce both aflatoxin B1 and aflatoxin B2 (24).Although poultry is considerably resistant to aflatoxin, due to which the acute intoxication is relatively rare, so the chronic intoxication with aflatoxin demands ingestion of aflatoxin for several weeks (one week minimum), and hence our study lasted 35 days.The aim of this study was to elucidate the microscopic changes during chronic aflatoxicosis in broilers through one broiler production period (35 days) with AF levels of 2.5 ppm. which occur within the range of many studies which have been performed to observe the toxic effects of AF on the target organs with higher levels of AF (1–5 ppm) (25). It was shown that AF affected the organs belonging to the haematopoietic, immune and reticulo-endothelial systemsm, i.e., Liver, kidney and the immune system organ (bursa of Fabricius), which are considered to be the target organs for AF and these are primarily affected in aflatoxicosis cases (26). These changes in the vital organs induce negative effects on the performance, humoral immunity of the broilers (27). Since the liver is the main target for AF, so the reduced appetite during aflatoxicosis could be due to impaired liver metabolism caused by the liver damage by the aflatoxins as reported by (28).The main histopathological changes included were fatty change, cellular dissociation, necrosis, cellular infiltration, and bile duct hyperplasia, which were in the line of many histopathological changes in the livers of chickens exposed to AFB1 reported in the literature on avian aflatoxicosis (29) directly related to the level of toxin in the feed and duration of exposure (30).The mentioned studies have stated that the

bile-duct hyperplasia findings, in particular, may constitute chronic aflatoxicosis cases and indicate the regenerative changes in the liver. The marked cytoplasmic vaculoation in the AF fed birds in the present study is perhaps the result of inhibition of protein synthesis in the hepatocytes by the toxin, involving different pathways, (31), or to the increase in the lipid deposits in the liver due to impaired fat metabolism mediated through inhibition of phospholipids synthesis and cholesterol. which in-turn affects the transportation of lipid from the liver.Our results of the histopathological changes may be typical to those reported in the literatures (32) who stated that AFB1 treatment of broiler chicks induced a severe cytotoxicity and inhibition of hepatocytes cell proliferation (33).The changes in the kidneys of birds fed AF at a rate of 2.5 ppm are renal tubules necrosis, mononuclear cell infiltration which were consistent with the observations of earlier studies (34). Glomerular basement membrane thickening in the AF fed birds was reported in the earlier studies,that the glomerular basement membrane thickening, karyomegaly and collapse of the glomerular tuft in the AF fed birds also suggest the cumulative interaction of these toxins in inducing severe changes in the kidneys(35).Cellular depletion in the follicle medulla of the bursa Fabricius indicating that chronic aflatoxicosis can be produced by feeding level of AF (2.5 ppm) over one broiler production period(35 day). In this regard, dietary AFB1 has been found by (36) to result in degeneration in bursal follicles associated epithelium (FAE). These lesions were similar to earlier reports (37). Histopathological changes in broiler chickens fed aflatoxin and cyclopiazonic acid (35).A significant reduction in the relative size of bursa of fabracius was recorded even lower rate of AF; i.e., at 200, 400 and 600 ppb AF level. AF is known to cause immune suppression in chicken and concomitantly reduce the relative size of the bursa of fabracius is responsible for immunological competence reported by (38,39,40). From above, is also important to be stressed on that a reliable evaluation of AF toxicity should be performed not only by macroscopic investigations of organs alone but also by histopathological examination.

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دراسة نسجية مرضية لبعض الأعضاء الداخلية لأفراخ فروج اللحم المستهلكة لسموم الافلا

إخلاص خليفة حامد
كلية التربية / جامعة الموصل

الخلاصة

تمت تربية وتوزيع أربعون من أفراخ فروج اللحم نوع Ross إلى مجموعتين بواقع 10 أفراخ لكل منهما وبمكررين، الأولى استهلكت عليقة خالية من سموم الافلا والثانية احتوت عليقتها على 2.5 جزء بالمليون من سموم الافلا . تم ذبح الأفراخ عند نهاية التجربة وأخذت عينات من كل من كل من الكبد والكلية وغدة فابريشيا لغرض التقطيع النسجي . أظهرت المقاطع النسجية تغيرات مرضية نسجية في كل هذه الأعضاء بفعل سموم الافلا وإنها الأعضاء الهدف لهذه السموم.

الكلمات المفتاحية: سموم الافلا ، أفراخ فروج اللحم، التقطيع النسجي