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EFFECTS OF DIETARY AFLATOXIN B_1 ON PERFORMANCE, ON HEMATOLOGIC, PATHOLOGIC AND IMMUNOLOGIC CHANGES IN BROILER CHICKENS

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ABSTRACT: *The influences of dietary aflatoxin B_1 on performance, on hematologic, pathologic and immunologic changes in broiler chickens were studied. One hundred and fifty hatched broiler chickens were fed with diet containing aflatoxin B_1 (1.0 ppm and 2.5 ppm) for three weeks. Blood samples, serum, and immune organs were obtained to investigate hematological, clinico-chemical, and histopathologial changes. Body weight gain and feed intake were significantly decreased. The liver and kidney were increased, whereas the bursa of Fabricius, spleen and thymus were decreased. Serum protein, albumin, globulin and cholesterol were significantly decreased. Involution of the cortex was present in the thymus, and loss of follicles and involution of the cortex were also present in the bursa of Fabricius from broiler chicken fed aflatoxin B_1. Formation of hepatic cells into cylinderic ductlike structures with a centrally placed lumen was seen in the liver, and proximal-tubules were dilated, epithelium was undergoing necrosis in the kidney from broiler chicken fed aflatoxin B_1, whereas AST and ALT were significantly increased in the aflatoxin B_1* treated group.

Key Words: *Aflatoxin B_1, Broiler chicken, Clinicochemical value, Hematological analysis, Histopathology, Immune organs.*

INTRODUCTION

A lot of literatures have been accumulated on various aspects of aflatoxicosis since its discovery in 1960. The biological and toxicological effects have been reported in a number of laboratory and domestic animals, and birds (Brown, 1965;

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Garlich *et al.*, 1973; Smith *et al.*, 1976). The impairment of reticuloendothelial activity, phagocytic activity of leukocytes and alveolar macrophages, primary immune response, and complement system have been reported (Michael *et al.*, 1973; Thaxton *et al.*, 1974; Richard and Thurston, 1975; Chang and Hamilton, 1979).

Susceptibility to the toxic and carcinogenic effects of aflatoxin varies markedly between species (Newberne and Butler, 1969; Wogan and Shank, 1970). The duck, trout and rat are highly susceptible to the carcinogenic effects of aflatoxin, whereas the monkey, mouse and hamster are relatively resistant (Wogan, 1973; Hsieh and Wong, 1981).

Presence of aflatoxins in poultry diets is associated with growth retardation, inefficient feed conversion, increased contaminations (Smith and Hamilton, 1970), leukocytic changes (Thaxton *et al.*, 1974), depressed antibody formation (Tung *et al.*, 1975) and susceptibility to disease with acute effects of aflatoxin related to rates of metabolism and elimination (Wong and Hsieh, 1980). Although genetic differences in response to aflatoxin have been demonstrated in chickens (Brown and Abramas, 1956; Gumbmann *et al.*, 1970), most studies involved broilers and layers rather than young egg-type birds.

It is known that animals exposed to aflatoxin B_1-contaminated grains are more susceptible to disease caused by bacteria, viruses and protozoa (Miller *et al.*, 1978). Serious economic losses to the livestock industry have been attributed to aflatoxin B_1.

Therefore, the purpose of the present study was to investigate the effect of dietary aflatoxin B_1 on performance, hematologic, pathologic and immunologic changes in broiler chicken.

MATERIALS AND METHODS

Animal Husbandry

One hundred and fifty hatched broiler chickens were obtained from a commerical hatchery (Cheonho Hatchery Co.). They were housed in electrically heated batteries under continuous lighting, and feed and water available *ad libitum*. Aflatoxicosis was induced by incorporating known amounts of aflatoxin B_1 (Sigma Chemical Co., St. Louis, MO) into a commercial broiler starter ration (Jeil Co.) devoid of all medication and detectable aflatoxin. The toxin containing diets were fed from hatching until the birds were 3 weeks of age at which time the experiments were terminated.

Animal Treatment

There were three replicates of 10 birds at each treatment and the experimental design was completely randomized. The treatment levels were control, aflatoxin B_1 1.0 μg/g of diet, and aflatoxin B_1 2.5 μg/g of diet.

The body weights and the feed consumed were measured weekly, and mortality was recorded daily. At the termination of the experiments, the birds were weighed, killed by cervical dislocation, and the liver, kidney, spleen, thymus, and bursa of Fabricius were removed and weighed on a group basis.

The blood samples were obtained from carotid artery from 10 cockerels randomly selected for determination of hematological analysis and serological analysis. Hematological parameters were RBC, hemoglobin and hematocrit, and serological parameters were AST, ALT, total protein, albumin, globulin, and cholesterol.

Hematological Analysis

The hematological parameters were Red Blood Cell (RBC), the capacity of red blood cell (Ht), and the quantity of hemoglobin (Hb). The blood was drawn to 0.5 graduation in RBC pipette and diluted with the Gower solution. The number of red blood cell was measured by using the hemacytometer under the microscope. We measured the ratio of the blood corpuscle/blood plasma with decipherment board after hematocrit centrifugation. The quantity of hemoglobin was measured by using hemoglobin diagnostic reagent, Drabkin solution.

Clinico-chemical Values

Blood samples were allowed to clot for 30 min at room temperature. After centrifuging the specimen, the obtained serum was stored at $-20°C$ until analyzed

Table 1. Formula and chemical composition of experimental diet

Ingredients	%
Corn, yellow 50.01	
Wheat	15.05
Soybean oil meal	14.88
Fish meal (cp 52%)	5.33
Fish meal (cp 63%)	3.00
Sunflower seed meal	4.00
Wheat bran	1.96
Limestone	0.55
Tricalcium phosphate	0.06
Yellow greese	0.52
DL-methionine (50%)	0.29
L-lysine (80%)	0.01
Salt	0.21
Vitamin mixture	0.68
Pigments	0.01
Total	100.00
Chemical composition	
Metabolizable energy (kcal/kg)	3,000*
Crude protein (%)	20.36
Crude fat (%)	3.95
Crude fiber (%)	3.87
Crude ash (%)	4.98
Calcium (%)	0.91
Phosphate (%)	0.63

*Calculated value

for clinico-chemical values. Clinico-chemical values (total protein, albumin, globulin, AST, ALT and cholesterol) were determined by using Photometer 40 20.

Histopathology

Liver, kidney, spleen, thymus and bursa of Fabricius were removed and fixed in 10% buffered formalin. Samples were dehydrated with automatic tissue processor and embedded in paraffins. Sections were cut by microtome and stained with hematoxylin and eosin, and examined under microscope.

Statistical Analysis

All data were examined for their statistical significances with the Student's t-test.

RESULTS AND DISCUSSION

The formula and chemical composition of the experimental diet used in this experiment are presented in Table 1. The changes of the body weight gain for three weeks were shown in Figure 1. Dietary aflatoxin B_1 significantly reduced body weight gain in a dose-dependent fashion. Intakes of feed were reduced in aflatoxin B_1 treated group compared to the control group (Figure 2). The results

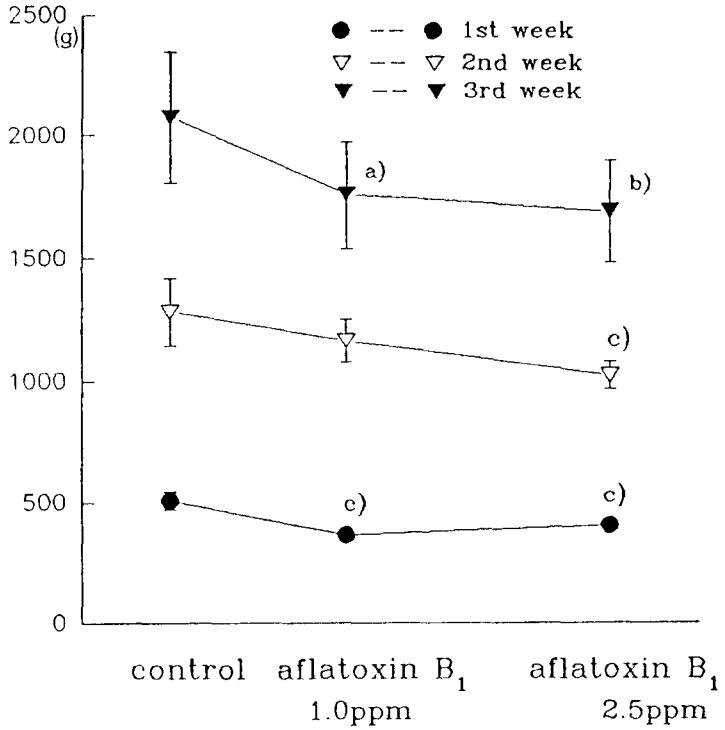

Figure 1. Effect of dietary aflatoxin B_1 on the body weight gain of broiler chickens.
a). Significantly different from control group at $P<0.1$, b). Significantly different from control group at $P<0.05$, c). Significantly different from control group at $P<0.01$.

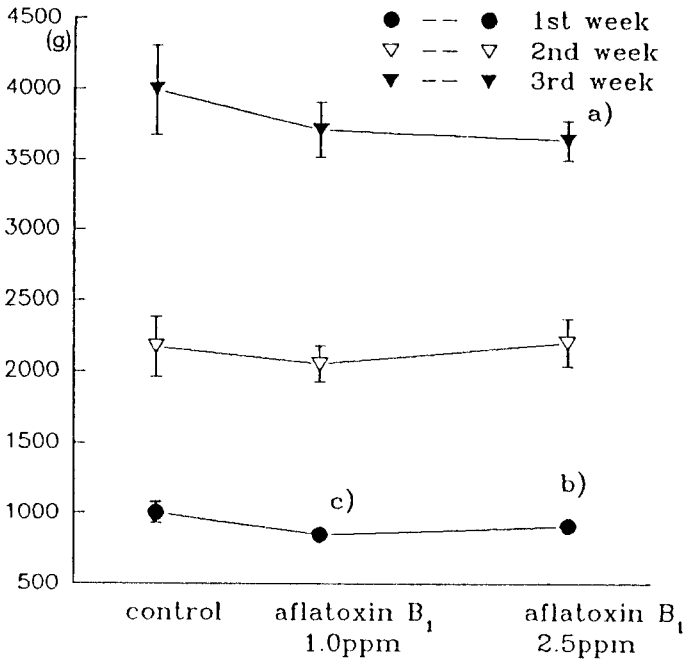

Figure 2. Effect of dietary aflatoxin B_1 on the feed intake of broiler chickens.
a). Significantly different from control group at $P<0.1$, b). Significantly different from control group at $P<0.05$, c). Significantly different from control group at $P<0.01$.

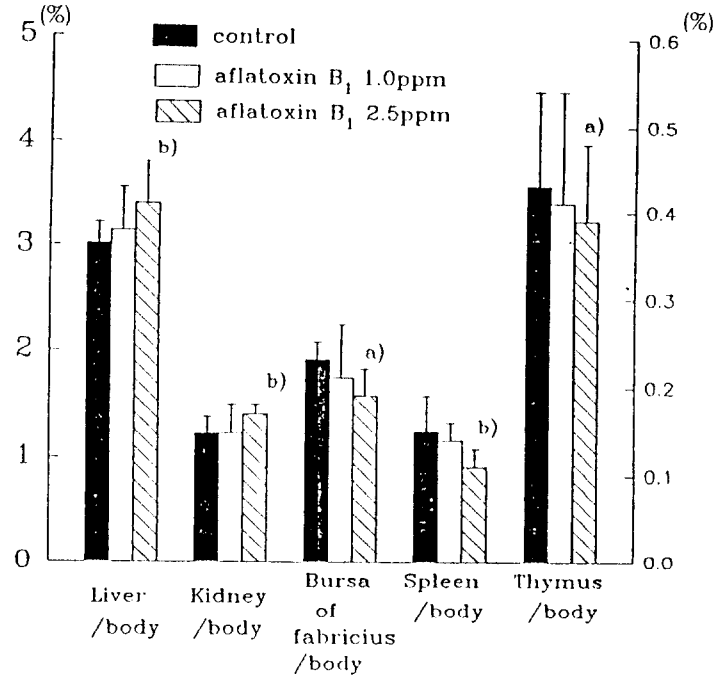

Figure 3. Effect of dietary aflatoxin B_1 on the relative organ weights of broiler chickens.
a). Significantly different from control group at $P<0.1$, b). Significantly different from control group at $P<0.05$.

of relative organ weight changes were shown in Figure 3. Relative weights of liver and kidney were increased in a dose-dependent fashion in aflatoxin B_1 treated group. The immune organs, such as bursa of Fabricius, spleen and thymus were significantly decreased in a dose-dependent fashion.

Total protein, albumin, globulin and A/G ratio were shown in Figure 4. Total protein and globulin were significantly decreased in a dose-dependent fashion in aflatoxin B_1 treated group. Cholesterol was significantly decreased, whereas AST and ALT were significantly increased in a dose-dependent fashion in aflatoxin B_1 treated group (Figure 5). The hematological changes were shown in Figure 6. RBC, hemoglobin and hematocrit were not affected by aflatoxin B_1 treatment in this study.

Involution of the cortex was present in the thymus, and loss of follicles and involution of the cortex were also present in the bursa of Fabricius from broiler chicken fed aflatoxin B_1. Formation of hepatic cells into cylinderic ductlike structures with a centrally placed lumen was seen in the liver, and proximal-tubules were dilated, epithelium was undergoing necrosis in the kidney from broiler chicken fed aflatoxin B_1. But considerable changes were not observed in the spleen in this study.

Dietary aflatoxin B_1 reduced body weight gain with the depression being dose-related as expected (Agric *er al.*, 1980) and suggested mechanisms for this effect include inhibition of ribonucleic acid (RNA) (Clifford and Rees, 1966) and deoxyribonucleic acid (DNA) synthesis (Rogers and Newberne, 1967), as well as

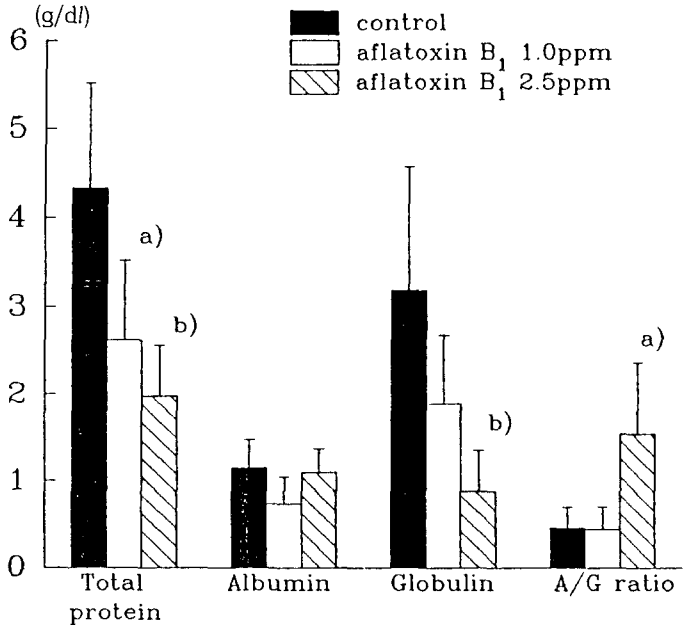

Figure 4. Effect of dietary aflatoxin B_1 on the serum proteins of broiler chickens
a). Significantly different from control group at $P<0.1$, b). Significantly different from control group at $P<0.05$.

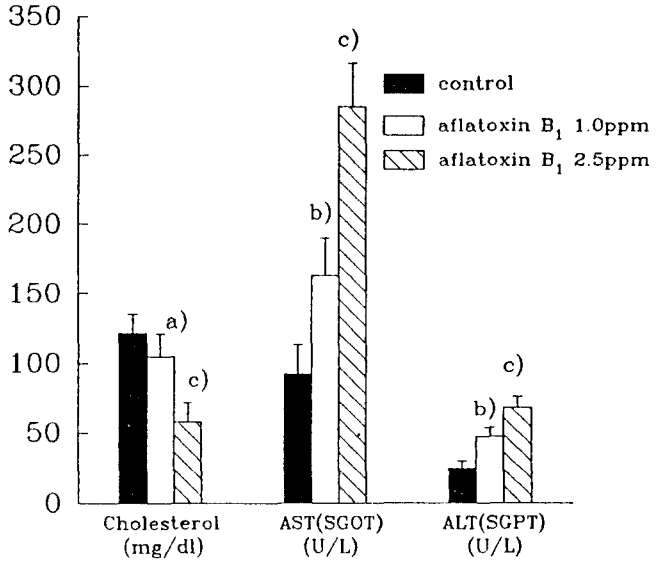

Figure 5. Effect of dietary aflatoxin B_1 on the clinico-chemical values of broiler chickens. a). Significantly different from control group at $P<0.1$, b). Significantly different from control group at $P<0.05$, c). Significantly different from control group at $P<0.01$.

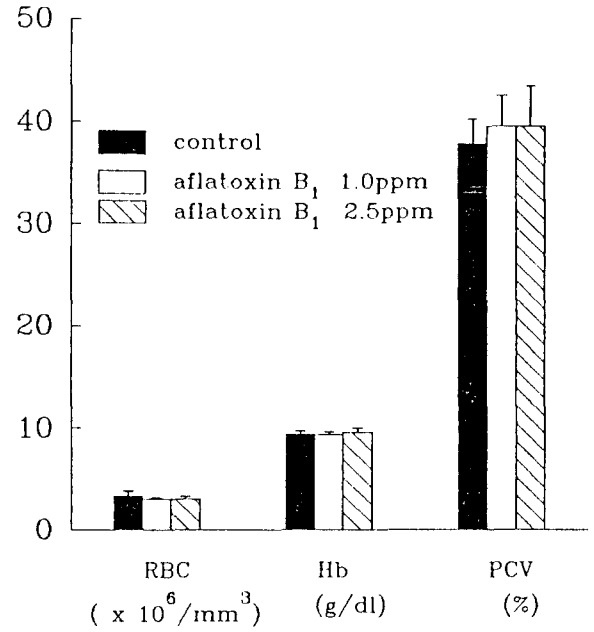

Figure 6. Effect of dietary aflatoxin B_1 on the hematological values of broiler chickens.

decreased RNA polymerase activity (Gelboin *et al.*, 1966). Consequences of partial inhibition of RNA and DNA synthesis involve reduced protein synthesis, which would depress growth. Sharlin *et al.* (1980) postulated that depressed appetite or palatability reduced consumption of feed containing aflatoxin. The pattern for

feed consumption was consistent with that for body weight. Reductions in feed consumption were very similar to those for body weight. The reductions at the higher levels were similar to those reported by Dalvi and MacGown (1984), who fed 2500 and 5000 ppb of dietary aflatoxin to broilers. These results are consistent with those observed with chicks and roosters (Wyatt *et al.*, 1975).

Aflatoxin reduced the bursa weight with the changes on the relative weight basis. These results were consistent with previous report that aflatoxins reduce bursa size (Thaxton and Hamilton, 1974) and suggest that the effect of aflatoxin on bursa weight is greater than on body weight. Aflatoxin B_1 reduced the thymus weight. Negative effect of aflatoxins on thymus size have been previousely reported. Although the population by aflatoxin level interaction for relative spleen weight was significant, there was no consistent pattern to describe the interaction. Aflatoxin B_1 reduced the spleen weight in this study.

The liver is the primary site of metabolism of ingested aflatoxin B_1, as well as the primary location of residues. The metabolism of aflatoxin results in the alteration of various metabolic processes within hepatocytes which leads to the gross and microscopic lesion observed (Heathcote and Hibbert, 1978). Lesions of chronic aflatoxicosis include pale yellow-to tan liver that is microscopically characterized by hepatocyte vacuolation, karyomegaly and bile ductule proliferation (Miller *et al.*, 1978). Acute aflatoxicosis in swine is characterized by centrilobular hepatic necrosis (Edds, 1973). Electron microscopic alteractions of hepatocytes associated with aflatoxin ingestion have been described in the rat, monkey, guinea pig, and duckling (Theron, 1965). The changes were generally restricted to the nucleus, endoplasmic reticulum and ribosomes.

Modification of cellular integrity of these tissues by chemical or physical agents result in immunosuppression (Glick, 1967). It should be noted that regression of bursa and the suppression of the HA responses occurred with concentrations of aflatoxin that do not inhibit growth (Smith and Hamilton, 1970). Since the potential of lymphoid tissue to produce antibodies dependent on bursa and thymus, the regression of these organs by aflatoxin would be expected to result in impaired immunological performance. A similar mechanism is postulated for the effect of actinomycin D, a carcinogenic antibiotic, on the immune system (Kaufman, 1971).

Certain serum biochemical changes have been associated with aflatoxin ingestion. Because the liver is the major organ affected by aflatoxins and their metabolites, alterations in liver-specific serum enzymes have been reported with both acute and chronic aflatoxicosis (Gumbmann and Williams, 1969). The present study showed that the activities of serum ALT and AST increased greatly in aflatoxin B_1 treated group. Aflatoxin B_1 is a relatively hepatotoxic agent. The histological findings that periportal necrosis and formation of hepatic cells into cylinderic ductlike structures appeared after Aflatoxin B_1 treatment correlated well with the biochemical finding of a sharp argument of the activities of serum ALT and AST. It appeared that the histological and biochemical data from chickens treated with Aflatoxin B_1 in the present study were chronologically correlated.

The serum total protein and globulin had been reduced in the aflatoxin treated group. It was like the same result with the report that aflatoxin makes the activity

of RNA polymerase suppressed and brings abnormality in the protein metabolism. The result that aflatoxin controls the immune system seems like the reduction of the immune elements such as complement, transferin, the increase of an Ab-Ag complex formation, the increase of immune constraint elements like creative protein and the reduction of globulin.

We could not observe remarkable changes in RBC, Ht, and PCV in this study. But if we exposed them longer, certain effects in the hematopoietic organs and the blood elements can be expected.

Aflatoxin B_1-induced immunosuppression has been observed in many animal species; in general, cellular immune processes were compromised. Michael et al. (1973) reported inhibition of phagocytic activity in chickens. Impairment of phagocytosis in isolated rabbit alveolar macrophages has been reported (Richard and Thurston, 1975). Thuston *et al.* (1972) observed a significant reduction in complement activity in the serum of guinea pigs dosed with aflatoxin B_1. Aflatoxin B_1 exposure in swine decreased responses to mitogens and inhibited macrophage migration and delayed-type hypersensitivity (Miller *et al.*, 1978).

In conclusion, results described in this paper demonstrate that dietary aflatoxin B_1 causes the histopathologic lesion and suppression of the immune system in the broiler chickens. The suppression of the immune system by aflatoxin B_1 decreases the resistance to disease, increases the susceptibility to various secondary toxicants and then brings depression of growth and productivity. Therefore, it is important that further studies should be conducted to define the cell types and functions of the immune system altered by aflatoxin B_1.

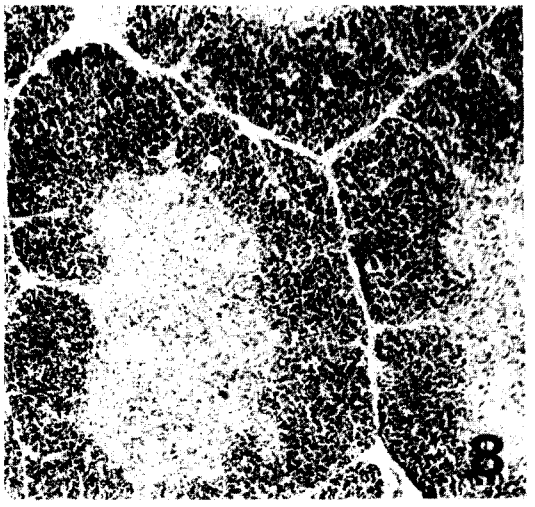

Figure 7. Thymus from broiler chicken fed aflatoxin B_1. Involution of the cortex are present. Hematoxylin and Eosin stain. ×160.

Figure 8. Thymus from broiler chicken fed normal diet. Lobes have a distinct cortex and medulla. Hematoxylin and Eosin stain. ×160.

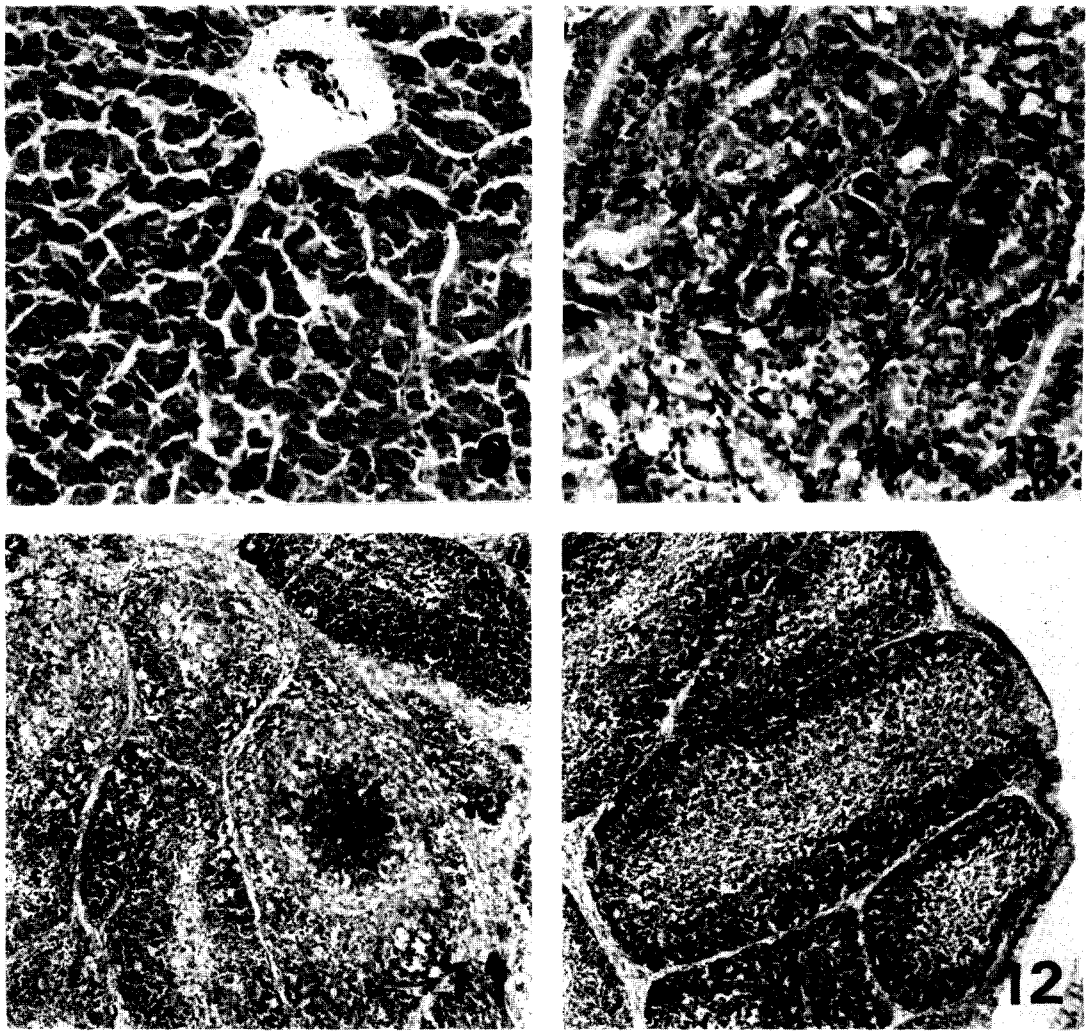

Figure 9. Liver from broiler chicken fed aflatoxin B_1. Formation of hepatic cells into cylinderic ductlike structures with a centrally placed lumen are seen. Hematoxylin and Eosin stain. ×160.
Figure 10. Kidney from broiler chicken fed aflatoxin B_1. Proximal-tubules are dilated, epithelium is undergoing necrosis. Hematoxylin and Eosin stain. ×160.
Figure 11. Bursa of Fabricius from broiler chicken fed aflatoxin B_1. Loss of follicles and involution of the cortex are present. Hematoxylin and Eosin stain. ×63.
Figure 12. Bursa of Fabricius from broiler chicken fed normal diet. Internal fold packed with lymphoid follicles that have a distinct cortex and medulla. Hematoxylin and Eosin stain. ×63.

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