

## Investigations on Liver Function in Mulards with Experimentally Induced Aflatoxicosis

*Nely GROSEVA, Ivan VALCHEV, Rumen BINEV\*, Dian KANAKOV, Tsanko HRISTOV, Lazarin LAZAROV, Krasimira UZUNOVA, Yordan NIKOLOV*

*Faculty of Veterinary Medicine; Trakia University, Student's Campus, 6000 Stara Zagora, Bulgaria*

*\*Corresponding Author: Rumen BINEV Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University, Student's Campus 6000 Stara Zagora, Bulgaria,*

*e-mail: binew@abv.bg*

**Geliş Tarihi / Received: 08.10.2012**

### ABSTRACT

Ducklings are among the most sensitive avian species to the toxic effects of aflatoxin B1 (AFB1). In this experiment, the toxic effects of AFB1 on liver morphology, blood plasma aspartate amino-transferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH),  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), albumin, blood glucose, and plasma total protein (TP) were established in mulard ducks. The experiment was carried out with four groups of 20 10-day-old ducklings each. Each group included three subgroups with 10 birds. The groups were as followed: group I – control (which received standard feed according to the species and age), group II – experimental, which received compound feed with 0.5 mg/kg AFB1, group III– receiving compound feed supplemented with 0.8 mg/kg AFB1 and group IV – compound feed supplemented with 0.5 mg/kg AFB1 and 2g/kg Mycotox NG. The experiment lasted for 42 days. Macroscopically, livers were enlarged, rounded, with yellowish colour and a frail consistency in group II and III. Histopathologically, a various extent of dystrophy was detected depending on the dose of ingested toxin. The supplementation of compound feed with the mycosorbent Mycotox NG improved deviations in blood biochemical parameters and substantially reduced the severity and prevalence of histological lesions.

**Key Words:** Aflatoxicosis, toxicity, duckling, liver damage, mycosorbent (Mycotox NG)

### ÖZET

#### AFLATOKSİKOZİS İLE DENEYSEL OLARAK UYARILMIŞ MULARDLARDA KARACİĞER FONKSİYONLARI ÜZERİNE İNCELEMELER

Kanatlılarda aflatoksin B1 (AFB1)'in toksik etkilerine en hassas olan ördek yavrularıdır. Bu çalışmada mulard ördeklerde AFB1'in karaciğer morfolojisi, plazma aspartat amino-transferaz (AST), alanin amino-transferaz (ALT), alkalik fosfataz (ALP), laktat dehidrogenaz (LDH),  $\gamma$ -glutamiltransferaz ( $\gamma$ -GT), albümin, kan glukoz, ve plazma total protein (TP) üzerine toksik etkileri incelenmiştir. Deney 20 adet 10 günlük yaştaki ördek yavrularından 4 grup ile gerçekleştirilmiştir. Her grupta 10 ördekten oluşan 3 alt grup bulunmaktadır. Grupların oluşturulmasında; grup I – kontrol (türe ve yaşa göre standart olan başlangıç yemi verilmiştir), grup II – deneysel, 0,5 mg/kg AFB1 içeren yem

verilmiştir, grup III–0,8 mg/kg AFB<sub>1</sub> içeren yem verilmiştir ve grup IV – yeme 0,5 mg/kg AFB<sub>1</sub> ve 2 g/kg Mycotox NG katılmıştır. Deney 42 gün sürmüştür. Grup II ve III’te makroskopik olarak karaciğerin büyümüş, yuvarlaklaşmış, sarı renkte ve gevrek yapıda olduğu gözlenmiştir. Histopatolojik olarak toksin alınma oranına göre değişen derecelerde distrofi tespit edilmiştir. Yeme mikosorbent Mycotox NG katılması kan biyokimyasal parametrelerde sapmaları düzeltilmiş ve histolojik lezyonların şiddetini ve yaygınlığını azaltmıştır

**Anahtar Kelimeler:** Aflatoksikozis, toksisite, ördek yavrusu, karaciğer hasarı, mikosorbent (Mycotox NG)

### Introduction

Mycotoxins are secondary toxic metabolites produced by moulds growing on numerous nutrients as maize, sorghum, peanuts and wheat (Hussein and Brasel, 2001). The cause of mycotoxicoses in animals is the consumption of cereal and rough fodders contaminated with mycotoxins (Chu, 1991). Aflatoxins are exceptionally toxic chemical compounds produced by some members of the order *Aspergillus* (*A. flavus* and *A. parasiticus*) (Fernández et al., 1995). They are encountered in field conditions as contaminants of cereal crops after harvesting, during the storage in storehouses and after processing (Council for Agricultural Science and Technology, 1989). The moisture content and temperature are the primary regulators of fungal growth and of aflatoxin formation in nutritive substrates. Optimal conditions for growth of fungi and for toxin production in cereal crops and oil plant seeds are a temperature between 12–42 °C and humidity 18% and 9–10%, respectively (WHO, 1979). Aflatoxins cause substantial economic losses in poultry and livestock industry. In many instances, feeding aflatoxin-contaminated feeds could result in poor economic results in poultry husbandry (Hamilton, 1984). The biochemical activity of aflatoxins is manifested by impaired metabolism of the energy, carbohydrates, proteins, lipids and nucleic acids (Ellis et al., 1991). Their biological effects include also carcinogenicity, mutagenicity, teratogenicity and hepatotoxicity (Coulombe, 1991). Aflatoxins are a frequent cause for reduced production of poultry meat with poor technological traits (Leeson et al., 1995). Several factors are responsible for these adverse effects of aflatoxins: lower activity of pancreatic enzymes, reduced bile secretion (Coulombe, 1991), impaired locomotion due to sciatic nerve injury (Leeson and Summers,

1988), DNA damage (Huff et al., 1986a; 1986b), as well as disturbed metabolism (carbohydrates, vitamins, proteins, amino acids, lipids). One of the most important toxic effects of the toxin in the inhibition of protein synthesis which results in considerable reduction of plasma protein concentrations, mainly of  $\alpha$  and  $\beta$  proteins and albumin (Espada et al., 1997). Also, the changes in serum and plasma activity of numerous enzymes as AST, AP,  $\gamma$  GT, LDH and creatine kinase (CK), are used as evaluation criteria to determine the severity of aflatoxicosis in chickens, ducklings and turkey poults (Rao and Joshi, 1993; Quist et al., 2000). The presence of small amounts of AFB<sub>1</sub> in poultry feeds causes stunted growth, reduces the feed conversion, egg hatchability and increases the susceptibility to infectious and parasitic diseases (Coulombe, 1993). In 1973, the EEC approved the maximum allowances of AFB<sub>1</sub> in feeds for different livestock species (European Economic Community, 1974). At present, the EEC allowances in compound feeds for cattle, goats and sheep are 50 ng/g and 20 ng/g for poultry and swine and another 20 ng/g and 5 ng/g for feed additives (European Economic Community, 1991). Aflatoxicosis-related morphological changes in important visceral organs such as the liver, is very important for diagnosing this mycotoxicosis. Gross changes in the liver are enlarged size, rounded borders and discoloration (yellowish tint). These changes are histologically seen as dystrophy and necrosis of hepatocytes, hyperplastic epithelium of biliary ductules and peribular infiltration of mononuclear cells (Bata et al., 1996; Bintvihok et al., 1991b).

Data provided by the Food and Agriculture Organization of the United Nations (FAO) at least 25% of cereal crops produced at a worldwide scale are contaminated with

aflatoxins (Dowling, 1997). Therefore, a variety of methods for feed detoxication have been developed. These methods not only decrease the concentrations of toxins to safe levels (under the specified allowances), but prevent the formation of new toxic compounds from aflatoxin degradation without decreasing the nutritional value of treated foods. A variety of methods (physical, chemical, biological) were investigated with regard to their efficacy for inactivation of aflatoxins in animal feeds aimed to bind or destroy the toxin. The supplementation of feeds with various non-nutrient sorbents (aluminosilicates) decreases the gastrointestinal absorption of fungal metabolites (Piva et al., 1995).

The aim of the current research was to evaluate the toxic effects of AFB<sub>1</sub> on liver morphology and the related alterations in blood biochemistry in mulard ducklings. Our other aim in this experiment was to determine the possibility of reducing the toxic effects of AFB<sub>1</sub> in one of the experimental groups (group IV), by adding to the feed mikosorbent Mycotox NG (Ceva Sante Animale, France).

### Materials and Methods

The experiment was conducted with 80 10-day-old female mulards. They were divided into 4 groups, 20 birds in each, and further subdivided in 2 equal subgroups.

The experimental design was as followed:

Group I – control. Mulards of the control group were fed balanced compound feed according to their age, manufactured at the Zoohraninvest, Stara Zagora. They were fed pelleted starter grower, and finisher feeds.

Group II – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B<sub>1</sub>.

Group III – experimental. Mulards received the standard feed supplemented with 0.8 mg/kg aflatoxin B<sub>1</sub>.

Group IV – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B<sub>1</sub> (purity 99%) and 2 g Mycotox NG

(containing per 100 g Thymol– 5.0 g and Micronised yeast and inorganic adsorbent *qs* – 100.0 g) /kg feed (0.2%) (Ceva Sante Animale, France).

The average live body weight of mulards before the experiment's start were 201.5±1.83 g (group I), 201.1±1.87 g for group II, 202.3±2.03 g for group III and 200.7±1.64 g for group IV.

Aflatoxin B<sub>1</sub> was produced by *Aspergillus flavus* (99% purity) and purchased from Sigma-Aldrich, Germany. It was ground before being mixed with feed for better homogenisation. During the experiment, the liver body weight, the weight gain, feed conversion and the daily feed intake were determined for each subgroup on post treatment days 14, 28 and 42. The access to feed and water was free (*ad libitum*). The mulards were reared in conditions compliant with the hygienic norms for this category birds. The microclimatic parameters were optimal and equal for all groups. In the beginning of the experiment, ambient air temperature was 35°C and decreased by 1°C daily until the 15<sup>th</sup> day; it was 20°C by the 28<sup>th</sup> day and thereafter +18°C, with relative air humidity 60–75% (Ordinance 44/2006). The duration of the light day was 24 h throughout the trial. The control and experimental groups of ducklings were housed in separate 4 m<sup>2</sup>-sections in the same premise. The sections were bedded with a 5 cm-layer of clean dry wood shaving. During the first week, the feeding width was 1 cm and thereafter – 10 cm.

Blood samples were collected from v. metatarsalis medialis on days 21 and 42 by using sterile heparinized vacutainers (FL medical, Italy) for assay of enzyme activities of ALT, AST, AP,  $\gamma$ -GT, LDH, and concentrations of total protein, albumin, blood glucose. Blood samples were centrifuged within 30 min after collection at 1500 rpm, 4°C. Immediately after that they were frozen at -20°C until analyzed. on a biochemical analyzer BS-120, Mindray, China.

After the end of the experiment, the birds were euthanised by cervical dislocation. Liver specimens for histological examination were

fixed in 10% formalin. The samples were embedded in paraffin using an ethanol series. Blocks of 5  $\mu$ m were cut on a microtome Leica model RM 2235 and sections were stained with haematoxylin-eosin.

Data were statistically processed by one-way ANOVA with Turkey-Kramer as post hoc test.

## Results

**1. Blood biochemical studies:** Blood plasma glucose, total protein and albumin changes in the control groups of mulards and in experimental groups are presented in Table 1. Blood glucose decreased statistically significantly on day 21 in experimental groups II and III ( $P<0.05$ ,  $P<0.001$ ) compared to controls. By the 42<sup>nd</sup> day, the changes vs controls were distinctly marked ( $P<0.001$ ). As shown on Table 1, plasma total protein and albumin were statistically significantly lower ( $P<0.01$ ,  $P<0.001$ ) in mulards which received only AFB<sub>1</sub>. Blood activity of aminotransferases (AST and ALT) and lactate dehydrogenase, gamma glutamyl transferase and

alkaline phosphatase were considerably elevated ( $P<0.01$ ,  $P<0.001$ ) in ducks treated only with AFB<sub>1</sub> in the feed (Table 2). The observed changes in chemical parameters depended to AFB<sub>1</sub> concentrations in feed and the duration of exposure. Blood biochemical parameters in experimental group IV, where AFB<sub>1</sub> administration was combined with the mycosorbent Mycotox NG showed in general lower values compared to experimental groups II and III, which received only AFB<sub>1</sub> at increasing levels, but remained statistically significantly lower than controls ( $P<0.05$ ,  $P<0.001$ ).

**2. Gross anatomy studies:** Ducklings fed a ration containing AFB<sub>1</sub> at 0.5 mg/kg feed exhibited an enlarged liver, brown-yellowish in colour, with striated and petechial haemorrhages (Figure 1).

The liver of ducklings treated with AFB<sub>1</sub> at 0.8 mg/kg feed was markedly enlarged, with rounded borders and frail consistency. Its colour was clay-brownish, with much enlarged gall bladder overfilled with bile (Figure 2).

**Table 1.** Effect of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) alone or combined with Mycotox NG on blood plasma total protein, albumin and glucose concentrations in mulard ducklings.

**Table 1.** Mulard ördeklerinde aflatoksin B<sub>1</sub> (AFB<sub>1</sub>)'in tek başına ve Mycotox NG ile birlikte kan plazma total protein, albümin ve glukoz değerlerine etkisi.

Groups	Total protein (g/l)		Albumin (g/l)		Glucose (mmol/l)	
	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
I	42.0± 1.09	40.2± 1.22	26.0± 1.39	24.1± 1.10	19.00± 0.73	17.27± 0.74
II	32.6± 1.36 <sup>1c</sup>	25.0± 1.62 <sup>1c</sup>	19.6± 1.04 <sup>1c</sup>	13.4± 0.94 <sup>1c</sup>	16.04± 0.35 <sup>1c</sup>	11.71± 0.37 <sup>1c</sup>
III	24.2± 1.42 <sup>1c,2c</sup>	19.00± 1.57 <sup>1c,2a</sup>	15.5± 0.92 <sup>1c,2a</sup>	10.0± 0.69 <sup>1c,2a</sup>	13.55± 0.67 <sup>1c,2a</sup>	9.86± 0.40 <sup>1c</sup>
IV	37.2± 0.71 <sup>1a,2a,3c</sup>	35.0± 0.84 <sup>1a,2c,3c</sup>	20.1± 0.65 <sup>1b,3a</sup>	19.5± 0.65 <sup>1b,2c,3c</sup>	15.58± 0.46 <sup>1b,3a</sup>	14.71± 0.52 <sup>1a,2b,3c</sup>

Data are presented as mean  $\pm$  SEM; n =20 ducklings in each group; <sup>a</sup> $P<0.05$ ; <sup>b</sup> $P<0.01$ ; <sup>c</sup> $P<0.001$ ; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

**Table 2.** Effect of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) alone or combined with Mycotox NG on blood plasma AST, ALT,  $\gamma$ GT, LDH and AP in mulard ducklings.**Tablo 2.** Mulard ördeklerinde aflatoksin B<sub>1</sub> (AFB<sub>1</sub>)'in tek başına ve Mycotox NG ile birlikte kan plazma AST, ALT,  $\gamma$ GT, LDH ve AP değerlerine etkisi.

Groups	AST (U/L)		ALT (U/L)		$\gamma$ GT (U/L)		LDH (U/L)		AP (U/L)	
	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
I	44.7± 1.14	48.1± 2.85	15.5± 0.74	16.4± 0.60	15.8± 0.90	17.1± 1.00	270.0± 11.42	281.0± 11.19	76.0± 3.41	79.0± 4.01
II	62.9± 1.96 <sup>1c</sup>	79.0± 2.29 <sup>1c</sup>	27.0± 1.21 <sup>1c</sup>	31.7± 0.89 <sup>1c</sup>	26.1± 1.13 <sup>1c</sup>	37.8± 2.46 <sup>1c</sup>	356.5± 11.55 <sup>1b</sup>	470.0± 15.96 <sup>1c</sup>	100.0± 2.58 <sup>1c</sup>	117.3± 3.30 <sup>1c</sup>
III	81.8± 2.12 <sup>1c,2c</sup>	108.9± 3.03 <sup>1c,2c</sup>	40.4± 1.65 <sup>1c,2c</sup>	49.8± 2.30 <sup>1c,2c</sup>	32.7± 1.83 <sup>1c,2a</sup>	48.0± 1.71 <sup>1c,2b</sup>	440.0± 21.89 <sup>1c,2b</sup>	527.9± 26.98 <sup>1c,2b</sup>	122.3± 4.00 <sup>1c,2c</sup>	147.2± 5.15 <sup>1c,2c</sup>
IV	51.9± 1.03 <sup>1a,2c,3c</sup>	62.3± 1.46 <sup>1c,2c,3c</sup>	20.9± 0.91 <sup>1a,2b,3c</sup>	23.9± 1.42 <sup>1c,2c,3c</sup>	20.6± 0.90 <sup>1a,2c,3c</sup>	27.1± 1.81 <sup>1b,2b,3c</sup>	328.0± 13.06 <sup>1a,3c</sup>	381.2± 12.96 <sup>1b,2b,3c</sup>	87.9± 1.34 <sup>1a,2a,3c</sup>	98.4± 3.27 <sup>1a,2a,3c</sup>

Data are presented as mean  $\pm$  SEM; n =20 ducklings in each group; <sup>a</sup>P<0.05; <sup>b</sup>P<0.01; <sup>c</sup>P<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

**Figure 1.** Haemorrhages in liver parenchyma in ducklings treated with AFB<sub>1</sub> at 0.5 mg/kg feed.

**Şekil 1.** Yemlerine 0,5 mg/kg AFB<sub>1</sub> katılan ördeklerde karaciğer parankimasındaki hemorajiler.

**Figure 2.** Fatty liver dystrophy in ducklings treated with AFB<sub>1</sub> at 0.8 mg/kg feed.

**Şekil 2.** Yemlerine 0,8 mg/kg AFB<sub>1</sub> katılan ördeklerde karaciğer yağlanması.

The birds treated with 0.5 mg/kg AFB<sub>1</sub> and Mycotox NG had a slightly enlarged liver, with red-brownish to dark red colour depending on the degree of congestion, and enlarged gall bladder (Figure 3).

**3. Histopathological studies:** Histopathological changes in the liver of mulards treated with 0.5 mg AFB<sub>1</sub>/kg feed resulted in strong dilation of capillaries, pericapillary oedema and Kupffer cells' activation. In some of ducklings, haemorrhages were observed. Hepatocytes exhibited granular degradation and

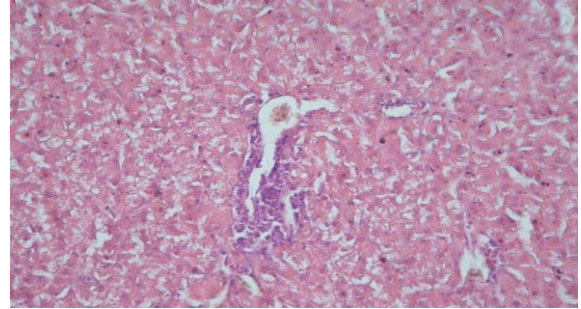
cytoplasm vacuolation, karyorrhexis, karyopyknosis and initial fatty dystrophy (Figure 4 and 5).

Ducklings treated with 0.8 mg AFB<sub>1</sub>/kg feed exhibited more intense liver changes with predomination of fatty dystrophy. Perivascular mononuclear infiltrations were also observed (Figure 6). At some areas, there were small areactive necrotic foci and biliary thrombi (Figure 7). The epithelium of biliary ductules was hyperplastic.



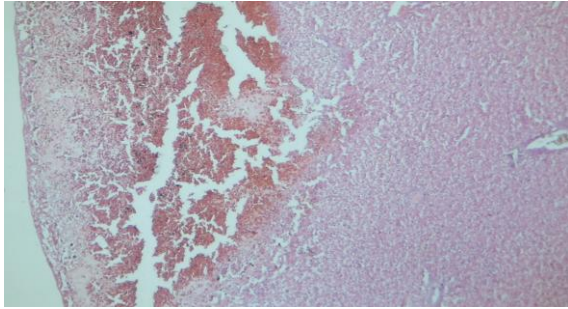
**Figure 3.** Liver hyperaemia in ducklings treated with AFB<sub>1</sub> at 0.5 mg/kg feed and with Mycotox NG

**Şekil 3.** Yemlerine 0,5 mg/kg AFB<sub>1</sub> ve Mycotox NG katılan ördeklerde karaciğer hiperemisi.



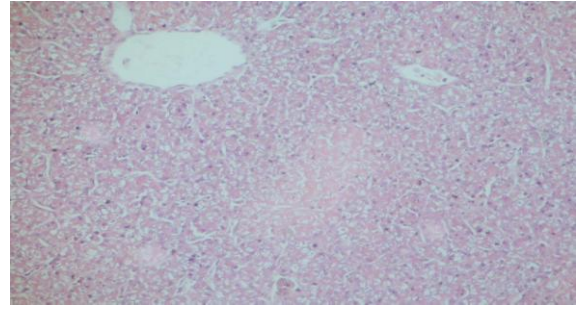
**Figure 6.** Perivascular mononuclear infiltrations in liver parenchyma ducklings treated with AFB<sub>1</sub> at 0.8 mg/kg feed. H/E. Bar=20 µm.

**Şekil 6.** Yemlerine 0,8 mg/kg AFB<sub>1</sub> katılan ördeklerde karaciğer parankimasında perivasküler mononükleer infiltrasyonlar. H/E. Bar=20 µm.



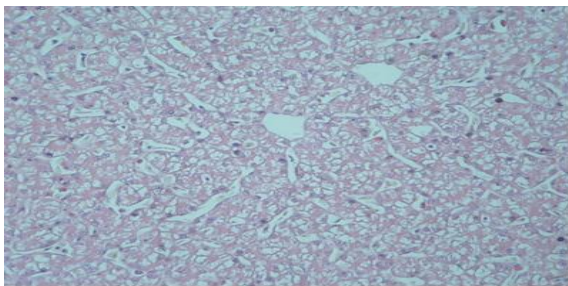
**Figure 4.** Haemorrhages in the liver parenchyma in ducklings treated with AFB<sub>1</sub> at 0.5 mg/kg feed. H/E. Bar=20 µm.

**Şekil 4.** Yemlerine 0,5 mg/kg AFB<sub>1</sub> katılan ördeklerde karaciğer parankimasındaki hemorajiler. H/E. Bar=20 µm.



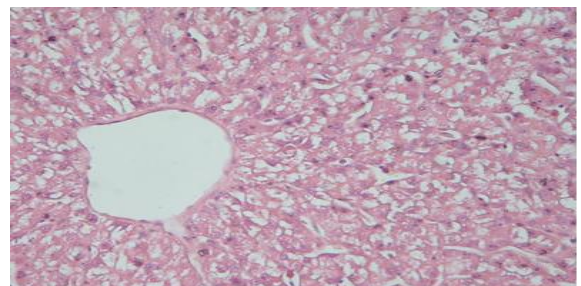
**Figure 7.** Areactive necrosis and biliary thrombi in the parenchyma of hepatocytes in ducklings treated with AFB<sub>1</sub> at 0.8 mg/kg feed. H/E. Bar=20 µm.

**Şekil 7.** Yemlerine 0,8 mg/kg AFB<sub>1</sub> katılan ördeklerde hepatosit parankimasında nekroz ve safra kanallarında tromboz. H/E. Bar=20 µm.



**Figure 5.** Strong vacuolisation and granular cytoplasmic degradation in liver hepatocytes of ducklings treated with AFB<sub>1</sub> at 0.5 mg/kg feed. H/E. Bar=20 µm.

**Şekil 5.** Yemlerine 0,5 mg/kg AFB<sub>1</sub> katılan ördeklerde karaciğer hepatositlerinde kuvvetli vakulizasyon ve granüler sitoplazmik dejenerasyon. H/E. Bar=20 µm.



**Figure 8.** Granular and mild fatty dystrophy with activation of capillary endothelium in hepatocytes of ducklings treated with AFB<sub>1</sub> at 0.5 mg/kg feed and 2g/kg feed Mycotox NG. H/E. Bar=15 µm.

**Şekil 8.** Yemlerine 0,5 mg/kg AFB<sub>1</sub> ve 2g/kg Mycotox NG katılan ördeklerin hepatositlerinde kapillar endoteliumunun aktivasyonu ile granüler ve hafif yağlı distrofi.

The dystrophic changes in hepatocytes of mulards treated with 0.5 mg AFB<sub>1</sub> /kg feed and Mycotox NG, were weak to moderate – cloudy swelling with granular dystrophy and less frequently, fatty dystrophy (Figure 8). Gross anatomy and histopathological changes in the liver of control mulards were not observed.

### Discussion

Aflatoxins cause considerable losses to poultry health (Allameh et al., 2005). Metabolic disorders in aflatoxicosis are characterized with protein synthesis inhibition and consequent reduction of plasma protein and albumin, and increased AST, AP,  $\gamma$  GT and LDH activities (Huff et al., 1986a; Zhao et al., 2010). These biochemical indices are used for evaluation of severity of liver changes accompanying aflatoxicosis (Denli et al., 2005; Bintvihok and Kositcharoenkul, 2006). Serum AST and ALT changes in liver damage are specific and used for monitoring cell viability and enhanced cell membrane permeability (Novelli et al., 1995). Clinically, serum AST, AP,  $\gamma$  GT, LDH and ALT are biomarkers of liver function. They are localised in the mitochondria of hepatocytes and play an important role in protein metabolism. In this experiment, the increased activity of these enzymes provided evidence for their release from damaged hepatocytes. Similar changes have been observed in other investigations in chickens, having received different doses of aflatoxins (Aravind et al., 2003). The increased activity of AP and  $\gamma$  GT is in agreement with the reports of other researchers (Bintvihok et al., 1991a; 1991c), about increased liver enzyme activity in chickens with aflatoxicosis and reduction occurring after feed supplementation with detoxication agents. Furthermore, the observed histological alterations in the liver confirmed data from previous studies of Bryden (1985) on one-day-old Peking ducklings treated with 0.1, 0.2, 0.4 and 0.8 mg aflatoxin B<sub>1</sub>/kg feed for 7, 14 and 21 days. Reduced blood total protein and the related hypoalbuminaemia results from binding of aflatoxins with DNA and RNA and intracellular proteins in hepatocytes, a mechanism that impairs the protein synthesis

(Abdel-Rahman et al., 2002; Quesada et al., 2000). The observed dystrophic and necrotic changes in the liver in aflatoxicoses could be due to damage of primary cell macromolecules (lipids, proteins and DNA). These injuries are secondary to oxidative stress caused by aflatoxins – a mechanism triggering oxidative changes in DNA and lipid peroxidation (Hashem et al., 2009). Furthermore, the intracellular accumulation of calcium in aflatoxicoses results in reduced formation of adenosine triphosphate (Quezada et al., 2000). The higher blood activity of  $\gamma$ -GT indicates cholestasis or hyperplasia of biliary ductules in birds (Brugere et al., 1987). Higher  $\gamma$ -GT activity is observed in broiler chickens fed rations naturally contaminated with aflatoxins, which was probably due to hepatocytic degeneration and consequent absorption of this enzyme in blood circulation (Afzali and Devegowda, 1999). Serum LDH concentrations consist of isoenzymes of different origin. From a clinical point of view, the differentiation of the various LDH types would be beneficial (Cardinet, 1989). In birds, high enzyme levels were established in the liver, kidney and the heart (Campbell and Coles, 1989). LDH activity is elevated parallelly to AFB<sub>1</sub> dose in feed and liver size (Fernández et al., 1994). The higher activity of studied enzymes could be also caused by the cholestasis, observed in this experiment as well (Fernández et al., 1994).

The liver is a target organ of the toxic effect of AFB<sub>1</sub>, as this is the organ where most aflatoxins are bioactivated to reactive 8,9-epoxide, which binds to DNA and proteins. As a result, the liver structure is damaged (Bailey et al., 2006; Pasha et al., 2007). Aflatoxins are hepatotoxic for all vertebrates, causing fatty infiltration, hepatocytic degeneration and necrosis (Riley and Pestka, 2005). The low blood glucose levels are due to the lower feed intake and/or lower activity of enzymes, involved in carbohydrate metabolism (Zhao et al., 2010). It is proved that aflatoxins inhibit protein synthesis (Tung et al., 1975a; 1975b). As a result, hypoproteinaemia is frequently encountered in aflatoxicosis (Huff et al., 1986b). Kubena et al. (1990a; 1990b) reports that despite the addition

of hydrated sodium calcium aluminosilicate to poultry feed contaminated with aflatoxin, blood total protein and albumin concentrations remained low. Kececi et al. (1998) indicated that the changes in biochemical parameters in chickens having received 2.5 mg AF/kg feed, could be alleviated by addition of 5 mg/kg bentonite to feed.

The result of present experiments confirmed previous reports and showed that the utilisation of specific sorbents could greatly reduce the toxic effects of aflatoxins in growing birds (Kubena et al., 1990a; 1990b). At present, none of studied mycosorbents could provide a full protection against any of mycotoxins. The main mechanism of prevention against the toxic effects of aflatoxins consists in isolation and binding of mycotoxin to the mycosorbent in the gastrointestinal tract and thus, reduction of aflatoxin bioavailability (Phillips et al., 1990).

### Conclusion

The results of the present study proved that the tested concentrations of AFB<sub>1</sub>, added to compound feed, had an adverse effect on liver function in mulard ducklings manifested by increased enzyme activity of blood AST, ALT, LDH,  $\gamma$  GT and AP. Simultaneously, the tested aflatoxin doses provoked reduction of blood total protein, albumin and blood glucose concentrations. Increasing AFB<sub>1</sub> doses (0.5 or 0.8 mg/kg feed) provoked specific for aflatoxicosis histological changes of liver (vacuolar and granular dystrophy, Kupffer cell activation, karyorrhesis, karyopyknosis, perivascular swelling and proliferations, biliary thrombi and hyperplasia of biliary ductules). In this experiment, the addition of 0.2% (2 g/kg feed) Mycotox NG to a ration containing 0.5 mg/kg AFB<sub>1</sub>, could effectively improve the severe changes in monitored blood biochemical indices and the histological lesions secondary to aflatoxicosis.

### REFERENCES

- Abdel-Rachman, S.S., Sheble, M.A., Abdel-Azeem, A., Rawi, S.M., 2002.** Biochemical and pathological studies on hepatic and renal alteration of male albino rats given aluminium chloride and/or aflatoxin B<sub>1</sub>. *Egypt Journal Comparative Pathology and Clinical Pathology* 15 (1), 359-367.
- Afzali, N., Devegowda, G., 1999.** Ability of modified mannanoligosaccharide to counteract aflatoxicosis in broiler breeder hens. *Poultry Science* 78 (Suppl. 1), 228-228.
- Allameh, A., Safamehr, A., Mirhadi, S.A., Shivazad, M., Razzaghi-Abyaneh, M., Afshar-Naderi, A., 2005.** Evaluation of biochemical and production parameters of broiler chicks fed ammonia treated aflatoxin contaminated maize grains. *Animal Feed Science and Technology* 122, 289-301.
- Aravind, K.L., Patil, V.S., Devegowda, G., Umakantha, B., Ganpule, S.P., 2003.** Efficacy of esterified glucomannan to counteract mycotoxicosis with naturally contaminated feed on performance and serum biochemical and hematological parameters in broilers. *Poultry Science* 82, 571-576.
- Bailey, C.A., Latimer, G.W., Barr, A.C., Wigle, W.L., Haq, A.U., Balthrop, J.E., Kubena, L.F., 2006.** Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *Journal Applied and Poultry Research* 15, 198-206.
- Bata, A., Glavits, R., Vanyi, A., Salyi, G. 1996.** More important mycotoxicoses of poultry clinico-pathological. Review article. *Magyar Allatorvosok Lapja* 51, 395-408.
- Bintvihok A., Klainil, P., Intraraksa, R., Sutherat, S., 1991c.** Aflatoxin detoxification by mycoinhibitor. *Proceedings of the 10<sup>th</sup> Annual Livestock Conference*, 522-532.
- Bintvihok, A., Kositcharoenkul, S., 2006.** Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B<sub>1</sub>. *Toxicol* 47, 41-46.
- Bintvihok, A., Shoya, S., Sutherat, S., Nagasawa, S., Sato, M., 1991b.** Effects of aflatoxin B<sub>1</sub> in ducklings: effect on liver lesions. In: Champ, B.R., Highley, E., Hocking, A.D., Pitt, J.I. (Eds.), *Fungi and Mycotoxins in Stored Products ACIAR Proceedings* 36, 233-235.
- Bintvihok, A., Uaphua, D., Klainil, P., Intraraksa, R., Sutherat, S., Wisutharom, K., Boonmeerod, M., Hayashi, M., 1991a.** Aflatoxin and toxic residue: its influence with regard to jeopardize the chicken and tissues. *Journal Thai Veterinary Medicine Association* 42 (4), 209-217.



- Brugere, J., Brugere, H., Basset, I., Sayad, N., Vaast, J., Michaux, J.M., 1987.** Biochimie clinique en pathologie aviare. Interet et limites des dosages enzymatiques chez la poule. *Recueil Medicine Veterinaire* 24, 1091-1099.
- Bryden, W.L., 1985.** Mycotoxins and duck production. In: DJ. Farrell and P. Stapleton (eds.), *Duck production science and world practice*. Armidale, NSW, Australia, University of New England, pp. 149-167.
- Cambell, T.W., Coles, E.H., 1989.** Patología Clínica de Aves. In: *Diagnóstico y Patología Veterinaria*, 4<sup>th</sup> edn, México, Editorial Interamericana 285-308.
- Cardinet, G.H., 1989.** Skeletal muscle function. In: Kaneko, J.J. (Ed.) *Clinical Biochemistry of Domestic Animals*, San Diego, California, Academic Press, Inc., pp. 462-495.
- Chu, F.S., 1991.** Mycotoxins: food contamination, mechanism, carcinogenic potencial and preventive measures. *Mutation Research* 259, 291-296.
- Coulombe, R.A., 1993.** Biological action of mycotoxins. *Journal Dairy Science* 76, 880-891.
- Coulombe, R.A., 1991.** Aflatoxins. In: Sharma, R. P., D. K. Salunkhe (Eds.), *Mycotoxins and Phytoalexins*. CRC Press, Boca Raton, FL, USA.
- Council for Agricultural Science and Technology, 1989.** In: *Mycotoxins: Economic and Health Risks*. K. A. Nisi (ed.), Council for Agricultural Science and Technology, Ames, IA., pp. 1-91
- Denli, M., Okan, F., Doran, F., Inal, T.C., 2005.** Effect of dietary conjugated linoleic acid (CLA) on carcass quality, serum lipid variables and histopathological changes of broiler chickens infected with aflatoxin B<sub>1</sub>. *South African Journal of Animal Science* 35, 109-116.
- Dowling, T.S., 1997.** Fumonisin and its toxic effects. *Cereal Foods World* 42, 13-15.
- Ellis, W.O., Smith, J.P., Simpson, B.K., 1991.** Aflatoxin in food: occurrence, biosynthesis, effects on organisms, detection and methods of control. *Critical Reviews in Food Science and Nutrition* 30 (4), 403-439.
- Espada, Y., Ruiz, G.R., Cuadras, C., Cabanes, F.J., 1997.** Fumonisin mycotoxicosis in broilers: Plasma proteins and coagulation modifications. *Avian Diseases* 41, 73-79.
- European Economic Community, 1974.** Council Directive 74/63/EEC on the fixing of maximum permitted levels for undesirable substances and products in feedingstuffs. *Official Journal of the European, Community* No. L38.
- European Economic Community, 1991.** EEC Council Directive 91/126/EEC. Amending the annexes to Council Directive 74/63/EEC on undesirable substances and products in animal nutrition. *Official Journal of the European, Community* No. L 60.
- Fernández, A.M., Verde, T., Gomez, J., Gascon, M., Ramos, J.J., 1995.** Changes in the prothrombin time, haematology and serum proteins during experimental aflatoxicosis in hens and broiler chickens. *Research in Veterinary Science* 58 (2), 119-122.
- Fernández, A.M., Verde, T., Gomez, J., Gascon, M., Ramos, J.J., Gomez, J., Luco, D.F., Chavez, G., 1994.** Variations of clinical biochemical parameters of laying hens and broiler chickens fed aflatoxin-containing feed. *Avian Pathology* 23, 37-47.
- Hamilton, P.B., 1984.** Determining safe levels of mycotoxins. *Journal of Food Protection* 47 (7), 570-575.
- Hashem, M.A., Mohamed, M.H., 2009.** Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. *Veterinaria Italiana* 45 (2), 323-337.
- Huff, W.E., Kubena, L.F., Harvey, R.B., Corrier, D.E., Mollenhauer, H.H., 1986b.** Progression of aflatoxicosis in broiler chickens. *Poultry Science* 65 (10), 1891-1899.
- Huff, W.E., Kubena, L.F., Harvey, R.B., Hagler, V.M., Swanson, S.P., Phillips, T.D., Creger, C.R., 1986a.** Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poultry Science* 65 (7), 1291-1298.
- Hussain, H.S., Brasel, J.M., 2001.** Review: Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* 167, 101-134.
- Kececi, T., Oguz, H., Kurtoglu, V., Demet, V.O., 1998.** Effects of polyvinylpyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *British Poultry Science* 39, 452-458.
- Kubena, L.F., Harvey, R.B., Huff, W., Corrier, D.E., Phillips, T.D., Rottinghaus, G.E., 1990b.** Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and T-2 toxin. *Poultry Science* 69 (7), 1078-1086.

- Kubena, L.F., Harvey, R.B., Phillips, T.D., Corrier, D.E., Huff, W., 1990a.** Diminution of aflatoxicosis in growing chickens by the dietary addition of a hydrated, sodium calcium aluminosilicate. *Poultry Science* 69 (5), 727-735.
- Leeson, S., Diaz, G.J., Summers, J.D., 1995.** Poultry metabolic disorders and mycotoxins. University Books, Guelph, Ontario, Canada 249-298.
- Leeson, S., Summers, J.D., 1988.** Some nutritional implications of leg problems in poultry. *British Veterinary Journal* 144, 81-92.
- Novelli, E.L., Rodrigues, N.L., Ribas, B.O., 1995.** Superoxide radical and toxicity of environmental nickel exposure. *Human Experimental Toxicology* 14, 248-251.
- Ordinance 44/20.04.2006.** For veterinary medical requirements to animal rearing facilities, *Official Gazette* 41 Appendix 9/II, 61-62.
- Pasha, T.N., Farooq, M.U., Khattak, F.M., Jabbar, M.A., Khan, A.D., 2007.** Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. *Animal Feed Science and Technology* 132, 103-110.
- Phillips, T.D., Clement, B.A., Kubena, L.F., Harvey, R.B., 1990.** Detection and detoxification of aflatoxins: Prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate. *Veterinary and Human Toxicology* 3, 15-19.
- Piva, G., Galvano, F., Pietri, A., Piva, A., 1995.** Detoxification methods of aflatoxins. A Review. *Nutrition Research* 15 (5), 767-776.
- Quesada, T., Cuellar, H., Valdivia, A.G., Reyes, J.J. 2000.** Effects of aflatoxin B<sub>1</sub> on the liver and kidney of broilers chickens during development. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 125 (3), 265-272.
- Quist, C.F., Bounous, D.I., Kilburn, J.V., Nettles, V.F., Wyatt, R.D., 2000.** The effect of dietary aflatoxin on wild turkey poults. *Journal of Wildlife Diseases* 36, 436-444.
- Rao, V.N., Joshi, H.C., 1993.** Effect of certain drugs on acute induced aflatoxicosis in chicken (4 mg AFB<sub>1</sub>/ kg b.wt.). *Indian Veterinary Journal* 70, 344-347.
- Riley, T., Pestka, J., 2005.** Mycotoxins: Metabolism, mechanism, and biochemical markers. In: Diaz D.E. (ed.), *The Mycotoxin Blue Book*, Nottingham University Press, Nottingham, pp. 279-294.
- Tung, H.T., Cook, F.W., Wyatt, R.D., Hamilton, P.B., 1975a.** The anemia caused by aflatoxin. *Poultry Science* 54, 1962-1969.
- Tung, H.T., Wyatt, R.D., Thaxton, P., Hamilton, P.B., 1975b.** Concentrations of serum proteins during aflatoxicosis. *Toxicology and Applied Pharmacology*, 34, 320-326.
- WHO (World Health Organization), 1979.** Environmental health criteria, safety evaluation of certain food additives 1-127.
- Zhao, J., Shirley, R.B., Dibner, J.D., Uraizee, F., Officer, M., Kitchell, M., Vazquez-Anon, M., Knight, C.D., 2010.** Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. *Poultry Science* 89, 2147-2156.