

Investigations on Production Traits of Mulards with Experimentally Induced Aflatoxicosis

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Geliş Tarihi / Received: 26.06.2012

ABSTRACT

In this study the toxic effects of aflatoxin B1 (AFB1) on production traits (live body weight, weight gain, feed intake and feed conversion) and relative weights (g/100 g body weight) of visceral organs (liver, kidneys, thymus, spleen, Bursa of Fabricius, heart, gizzard and proventriculus) in mulard ducklings were investigated. The experiment was carried out with four groups of 30 10-day-old ducklings each, over 42 days. 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 2 g/kg Mycotox NG. In experimental groups II and III, the body weight, weight gain, feed intake were lower, feed conversion ratio was higher as well as the relative weights of liver, kidneys, heart, pancreas, gizzard and proventriculus. At the same time, the relative weight of the thymus, bursa of Fabricius and the spleen were considerably reduced. The supplementation of feed of group IV with Mycotox NG protected birds from the negative effects of AFB1 on production traits and prevented changes in the weights of visceral organs.

Key Words: Aflatoxicosis, mulard ducks, production traits

ÖZET

DENEYSSEL OLARAK AFLATOKSİKOZİS İNDÜKLENMİŞ MULARDLARDA ÜRETİM ÖZELLİKLERİ ÜZERİNE İNCELEMELER

Bu çalışmada aflatoksin B1'in (AFB1) mulard ördek palazlarının verim özellikleri (canlı ağırlık, canlı ağırlık kazancı, yem tüketimi ve yemden yararlanma) ve iç organlara (karaciğer, böbrekler, timus, dalak, Bursa Fabricius, kalp, taşlık, ve kursak) ait relatif canlı ağırlıkları (g/100 g vücut ağırlığı) üzerine toksik etkileri incelenmiştir. Deney 10 günlük yaşta ördek palazlarından 30'ar adet 4 grup olacak şekilde ve 42 günde gerçekleştirilmiştir. Gruplar şöyle sıralanabilir; Grup I- kontrol, türüne ve yaşına göre standart yem verilmiştir, grup II- deneysel, 0,5 mg/kg AFB1 yemlerine eklenmiştir, grup III – yemlerine 0,8 mg/kg AFB1 eklenmiştir ve grup 4- yemlerine 0,5 mg/kg AFB1 ve 2 g/kg Mikotoks NG eklenmiştir. Deneysel gruplar II ve III'te vücut ağırlığı, canlı ağırlık kazancı, yem tüketimi daha

düşük iken, yemden yararlanma oranı ile karaciğer, böbrekler, kalp, pancreas, taşlık ve kursağın nispi ağırlıkları artış göstermiştir. Aynı zamanda timus, Bursa Fabricius ve dalağın nispi ağırlıkları önemli ölçüde azalmıştır. Grup4'ün yemine eklenen Mikotoks NG, AFB1'in verim özelliklerine ve iç organların ağırlık değişimleri üzerine negatif etkilerine karşı kuşları korumuştur.

Anahtar Kelimeler: Aflatoksikozis, mulard ördeği, verim özellikleri

Introduction

The contamination of human and animal foods with aflatoxins poses serious health risks (Hussein and Brasel, 2001). Aflatoxins (AF) are secondary toxin metabolites produced by fungi from the genus *Aspergillus* (*A. flavus* and *A. parasiticus*). They are common contaminants of ingredients, used for production of compound poultry feeds (Edds and Bortell, 1983). Among the main aflatoxins are aflatoxin B₁ (AFB₁), aflatoxin B₂, aflatoxin G₁, and aflatoxin G₂. AFB₁ is the most toxic especially in more sensitive animals species, including domestic fowl (Hussein and Brasel, 2001). Aflatoxin B₁ is mutagenic, carcinogenic and teratogenic (Mishra and Das, 2003; Smela et al., 2001). Toxic properties of aflatoxins depend on a number of factors such as their concentration in feeds, the duration of challenge, species, sex, age and health condition (Jewers, 1990). In poultry, aflatoxicosis is manifested with weakness, lethargy, lower feed intake, reduced weight gain, lower meat production, poor feed conversion, lower egg production, higher mortality and changes in the relative weights of visceral organs (Bailey et al., 1998 and 2006; Bintvihok et al., 2002; Edds and Bortell, 1983; Han et al., 2008; Kubena et al., 1998; Mendoza et al., 2006; Shi et al., 2006; Verma et al., 2002). Along with the worse production traits, haematological and blood biochemistry changes are also observed (Bailey et al., 1998; Bintvihok and Kositcharoenkul, 2006; Fernández et al., 1994), as well as liver (Ledoux et al., 1999) and kidney damage (Espada et al., 1992), reduction of humoral and cellular immunity (Qureshi et al., 1998) and consequently, higher susceptibility to infectious diseases (Shashidhara and Devegowda, 2003). Ducklings are the most sensitive to the toxic effect of aflatoxins among bird species (Muller et al., 1970).

The measures implemented for reduction of the toxic effects of aflatoxins on animals consist of a variety of methods for treatment of aflatoxin-contaminated feeds. Such methods are the use of mould inhibitors, microbial fermentation, physical separation of contaminated seeds, heat inactivation, treatment with ammonia (CAST, 1989), degradation of aflatoxins with ozone (Mckenzie et al., 1997), extraction of aflatoxins with organic solvents (hexane and acetone) (Rayner and Doller, 1968), grinding of contaminated grain (Brekke et al., 1975), and use of adsorbents (Phillips et al., 1990). The major disadvantages of these methods are that they are expensive, laborious and only partly effective. At present, one of the most promising and practical approaches for detoxication of contaminated feeds is the use of adsorbents. Added to aflatoxin-contaminated feeds, mycosorbents could bind AF during feed digestion and thus, mycotoxins pass safely through the organism (Davidson et al., 1987; Phillips et al., 1990). The main advantages of adsorbents are their safety for animals, and the ease of application via supplementation to feeds. Nevertheless, not all adsorbents are equally useful for protection of domestic fowl against the toxic effects of aflatoxins. Some binders (hydrated sodium calcium aluminosilicate, ethacal, novasil, perlite, and zeobrite) could be detrimental for utilisation of nutrients (Abdel-Wahhab et al., 1995; Kubena et al., 1998; Scheideler, 1993). Dale (1998) established that many of mycosorbents available at the market, were not tested for in vivo efficacy but were approved for use on the basis of in vitro tests. Despite that, in vitro tests are not always a reliable indicator for the ability of the adsorbent to bind aflatoxins (Scheideler, 1993). The mycotoxin binding efficacy and the effect on nutrient utilization of supplements should be assessed in vivo. The use of mycosorbents as aluminosilicates (Kubena et

al., 1998; Ledoux et al., 1999), zeolites (Kececi et al., 1998; Miazzo et al., 2005; Scheideler, 1993), bentonites (Rosa et al., 2001; Santurio et al., 1999) and clinoptilolites (Oguz and Kurtoglu, 2000; Oguz et al., 2000a and 2000b) are preferred due to the high extent of AF binding in the gastrointestinal tract.

The purpose of this study was to investigate the toxic effects of AFB₁ on productive traits and the relative weights of visceral organs in mulards after independent intake of AFB₁ or in combination with Mycotox NG and to evaluate the potential for prevention of aflatoxicosis.

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 B₁.

Group III – experimental. Mulards received the standard feed supplemented with 0.8 mg/kg aflatoxin B₁.

Group IV – experimental. Mulards received the standard feed supplemented with 0.5 mg/kg aflatoxin B₁ (purity 99%) and 2 g Mycotox NG /kg feed (0.2%) (Ceva Sante Animale, France). Mycotox NG, per 100g contains: Thymol – 5.0 g and Micronised yeast and inorganic adsorbent *qs* – 100.0 g.

The starter, grower and finisher diets were formulated to meet the nutrient requirements according to NRC (1994) (Table 1).

The average live body weight of mulards before the experiment's start was 201.5±1.83 g (group I), 201.1±1.87 g for group II, 202.3±2.03 g for group III and 207.0±1.64 g for group IV.

Aflatoxin B₁ 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 15th day; it was 20°C by the 28th day and thereafter – +18°C, with relative air humidity 60–75% (Ordinance, 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 m²-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. For determination of visceral organs' weight, the birds were euthanised by cervical dislocation.

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

The statistical processing was performed using the computer program GraphPad. The statistical analysis was performed with one-way, ANOVA. In case of significant P-values (P<0.05), the non-parametric Tukey-Kramer Multiple Comparison test was then applied.

Results

The effect of compound feed supplementation with either AFB₁ only or with the combination Mycotox NG and AFB₁ on live body weight, average daily feed intake, feed conversion and average daily weight gain, in mulards over 6 weeks are presented in Tables 2, 3, 4 and 5. Biometric studies showed that by the end of the experiment, the average live body weight of ducklings fed a ration with 0.5 mg AFB₁/kg (experimental group II) was by 485 g lower than that of controls (P<0.001), (17.3%),

whereas those receiving 0.8 mg AFB₁/kg (group III) – by 756 g lower ($P<0.001$) (26.97%). Compared to control group, mulards treated with 0.5 or 0.8 mg/kg AFB₁, exhibited statistically significantly lower average daily feed intake and average daily weight gain ($P<0.05$ – $P<0.001$). Feed consumption for one unit weight gain increased by 12.74% and 17.97% ($P<0.01$ – $P<0.001$) in experimental groups II and III, respectively. In group IV, the supplementation of feed with 0.5 mg AFB₁/kg and 2 g Mycotox NG /kg feed (0.2%) had a beneficial effect on productive traits, with insignificant differences vs the control birds ($P>0.05$).

Table 1. Ingredient composition and nutrient levels of the basal diets (as fed) g/kg.

Tablo 1. Bazal diyetlerin (yemleme olarak) g/kg içerik bileşimleri ve besin değerleri.

Ingredients	Starter	Grower and Finisher
Corn	570	734
Soybean meal 48%	360	205
Vegetable oil	30	25
Limestone	16	14
Dicalcium phosphate	14	12
Salt	4	4
Vitamin:mineral premix ¹	3	3
Methionine	3	2
Lysine HCL	0	1
	1,000.0	1,000.0
Calculated analyses		
Crude protein, g/kg	230	160
Metabolisable energy (kcal/kg)	3,000	3,150
Calcium	9.5	8.2
Nonphytate phosphorus, g/kg	4	3.3
Arginine	14.5	9.9
Lysine	12.9	9.1
Methionine	6.4	4.6
Methionine+cysteine	10.4	7.4
Threonine	8.2	6
Tryptophan	3	2
Valine	10	7.5

¹Content of vitamins and minerals in diet per g of premix are as follow: Vit. A 1828 IU; Vit. D₃, 881 IU; Vit. E, 3.67 IU; Menadion sodium bisulfate, 1.46 mg; Riboflavin, 1.83 mg; d-pantothenic acid, 3.67 mg; Niacin, 14.69 mg; Choline chloride, 257 mg; Vit. B12, 4.4 µg; Biotin 18.4 µg; Thiamine mononitrate 735 µg; Folic acid, 330 µg; Pyridoxime hydrochloride, 1.1 mg; I, 370 µg; Mn, 22.02 mg; Cu, 1.48 mg; Fe, 14.69 mg; Zn, 14.69 mg; Se, 100 µg.

The relative weights of visceral organs (g/100 g body weight) (liver, kidneys, heart, spleen, bursa of Fabricius, thymus, pancreas, gizzard and proventriculus) are presented in Table 6. It shows that feeding a diet contaminated with increasing doses of AFB₁ resulted in statistically significant increase in relative weights of the liver, kidneys, heart, pancreas, gizzard, and proventriculus compared to ducklings fed standard compound feed ($P<0.01$ – $P<0.001$). Also, the relative weights of the thymus, bursa of Fabricius and the spleen were substantially lower ($P<0.01$ – $P<0.001$). There were no statistically significant differences in relative weights between control mulards and mulards receiving 0.5 mg AFB₁/kg and 0.2 mg Mycotox NG /kg feed (experimental group IV) ($P>0.05$). Nevertheless, the relative weight of the liver remained slightly higher compared to that in controls.

Discussion

The most important economical effect of aflatoxicosis in domestic fowl results from stunted growth, lower feed intake and increased feed conversion (Goswami et al., 1998; Scheideler, 1993). The results of this study indicated that feeding a ration containing 0.5 or 0.8 mg AFB₁/kg had an effect on productive traits in mulards. Our results about production traits corresponded to those of other authors in broiler chickens (Fernandez et al., 1994). The adverse influence of AFB₁ on the growth of chickens is related to the less efficient feed energy and protein utilisation (Verma et al., 2002), and probably results from the worse digestive and metabolic processes in birds. The higher feed conversion and the lower weight gain in experimental groups of mulards, receiving feed supplemented only with AFB₁ could be attributed to the dystrophy of parenchymal organs (Goswami et al., 1998; Scheideler, 1993). According to other authors, the adverse effects of AFB₁ on production traits result from anorexia, apathy, protein synthesis and lipogenesis inhibition (Oguz and Kurtoglu, 2000; Oguz et al., 2000a and 2000b). The impaired liver function and the lower conversion

of feed proteins and fat accompanying aflatoxicosis, influence the growth and health of birds (Espada et al., 1992; Fernandez et al., 1994; Kececi et al., 1998). A possible cause for the stunted growth could be the lower absorption rate of nutrients and the reduced secretion of pancreatic enzymes consequently to the lower utilisation of proteins and energy (Osborne and Hamilton, 1981). The adverse effects on live body weight, weight gain, feed intake and conversion in ducklings were observed from the second week of the trial onward and progressed until its end. The presence of aflatoxins in compound feeds reduce the activity of numerous enzymes involved in the metabolism of carbohydrates, proteins, fats and nuclei acids in broiler chickens (Campbell et al., 1983). Aflatoxins cause a nutritional deficiency and

subsequently, lower weight gain, higher liver and proventriculus weight (Huff et al., 1986). The nutritional deficiency, on its part, could lead to impaired activity of digestive enzymes and to reduced absorption of nutrients (Boden and Jensen, 1985). An experiment with 1-day-old broiler chickens whose ration was supplemented with 4 mg AFB₁/kg, Ledoux et al. (1999), investigated the protecting effect of 1% hydrate calcium sodium aluminosilicate on production traits and relative weights of visceral organs. The authors established no difference with the control groups with respect to feed intake, weight gain, feed conversion and relative weights of visceral organs. In general, mycosorbents were effective for prevention or alleviation of toxic effects of aflatoxins in chickens.

Table 2. Effect of aflatoxin B₁ (AFB₁) alone or combined with Mycotox NG on live body weight in mulard ducklings.

Tablo 2. Tek başına veya Mycotoks NG ile birlikte aflatoksin B₁ (AFB₁)'in Mulard ördek palazlarında canlı ağırlık üzerine etkisi.

Groups	Live body weight (g)				
	Initial weight (g)	Day 14	Day 28	Day 42	Difference %
I	201.5±1.83	707±19.26	1624±13.51	2804±21.09	100
II	201.1±1.87	585±10.13 ^{1c}	1333±14.14 ^{1c}	2319±22.97 ^{1c}	17.30
III	202.3±2.03	515±11.47 ^{1c,2a}	1152±29.62 ^{1c,2c}	2048±20.20 ^{1c,2c}	26.97
IV	207.0±1.64	714±17.96 ^{2c,3c}	1630±13.66 ^{2c,3c}	2807±28.52 ^{2c,3c}	0

Data are presented as mean ± SEM; n =20 ducklings in each group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

Table 3. Effect of aflatoxin B₁ (AFB₁) alone or combined with Mycotox NG on average daily feed intake in mulard ducklings.

Tablo 3. Tek başına veya Mycotoks NG ile birlikte aflatoksin B₁ (AFB₁)'in Mulard ördek palazlarında yem tüketimi üzerine etkisi.

Groups	Average daily feed intake (g)			
	Day 14	Day 28	Day 42	Difference %
I	90.92±1.76	185.75±0.26	257.73±0.86	100
II	82.85±1.09 ^{1c}	177.65±0.69 ^{1c}	242.41±0.076 ^{1c}	5.96
III	72.33±1.08 ^{1c,2c}	171.20±1.51 ^{1c,2c}	228.34±0.096 ^{1c,2c}	11.41
IV	91.01±0.19 ^{1c,2c}	186.87±0.19 ^{2c,3c}	257.56±0.68 ^{2c,3c}	0

Data are presented as mean ± SEM; n =20 ducklings in each group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

Table 4. Effect of aflatoxin B₁ (AFB₁) alone or combined with Mycotox NG on average daily weight gain in mulard ducklings.**Tablo 4.** Tek başına veya Mycotoks NG ile birlikte aflatoksin B1 (AFB₁)'in Mulard ördek palazlarında günlük ortalama canlı ağırlık artışı üzerine etkisi.

Groups	Average daily weight gain (g)			
	Day 14	Day 28	Day 42	Difference %
I	36.10±1.45	65.49±2.02	84.28±1.77	100
II	27.41±0.70 ^{1c}	53.42±1.34 ^{1c}	70.42±1.45 ^{1c}	16.44
III	11.31±0.81 ^{1c,2a}	45.49±2.21 ^{1c,2a}	63.99±2.42 ^{1c,2c}	24.07
IV	36.64±1.25 ^{2c,3c}	65.42±1.88 ^{2c,3c}	84.06±2.36 ^{2c,3c}	0

Data are presented as mean ± SEM; n =20 ducklings in each group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

Table 5. Effect of aflatoxin B₁ (AFB₁) alone or combined with Mycotox NG on feed conversion in mulard ducklings.**Tablo 5.** Tek başına veya Mycotoks NG ile birlikte aflatoksin B1 (AFB₁)'in Mulard ördek palazlarında yemden yararlanma üzerine etkisi.

Groups	Feed conversion (g feed/g weight gain)			
	Day 14	Day 28	Day 42	Difference %
I	2.55±0.096	2.85±0.090	3.06±0.066	100
II	3.01±0.082 ^{1c}	3.35±0.088 ^{1a}	3.45±0.074 ^{1a}	12.74
III	3.27±0.098 ^{1c}	3.84±0.19 ^{1c}	3.61±0.13 ^{1c}	17.97
IV	2.50±0.080 ^{2c,3c}	2.87±0.083 ^{2c,3c}	3.08±0.088 ^{2a,3b}	0

Data are presented as mean ± SEM; n =20 ducklings in each group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

Table 6. Effect of aflatoxin B₁ (AFB₁) alone or combined with Mycotox NG on relative visceral organ weights in mulard ducklings.**Tablo 6.** Tek başına veya Mycotoks NG ile birlikte aflatoksin B1 (AFB₁)'in Mulard ördek palazlarında iç organ ağırlıkları üzerine etkisi.

Groups	Liver	Kidneys	Heart	Bursa of Fabricisus	Thymus	Spleen	Pancreas	Proventriculus	Gizzard
I	2.98±0.044	0.54±0.012	0.70±0.018	0.078±0.001	0.39±0.010	0.127±0.002	0.45±0.038	1.17±0.011	3.64±0.027 ^{1c}
II	3.30±0.035 ^{1c}	0.67±0.011 ^{1c}	0.87±0.016 ^{1c}	0.091±0.001 ^{1c}	0.29±0.005 ^{1c}	0.093±0.001 ^{1c}	0.60±0.011 ^{1c}	1.30±0.011 ^{1c}	4.10±0.034 ^{1c}
III	4.08±0.048 ^{1c,2b}	0.78±0.020 ^{1c,2b}	0.96±0.015 ^{1c,2b}	0.104±0.001 ^{1c,2c}	0.25±0.007 ^{1c,2a}	0.075±0.001 ^{1c,2b}	0.71±0.060 ^{1c,2b}	1.41±0.015 ^{1c,2c}	4.43±0.055 ^{1c,2c}
IV	3.10±0.032 ^{2c,3c}	0.59±0.012 ^{2c,3c}	0.76±0.012 ^{2c,3c}	0.074±0.001 ^{2c,3c}	0.37±0.042 ^{2c,3c}	0.115±0.006 ^{2c,3c}	0.48±0.012 ^{3c,2b}	1.21±0.012 ^{2c,3c}	3.80±0.047 ^{2c,3c}

Data are presented as mean ± SEM; n =20 ducklings in each group; ^aP<0.05; ^bP<0.01; ^cP<0.001; 1 – vs the control group; 2 – vs experimental group I; 3 – vs experimental group II.

The liver, kidneys and immune system organs are target organs to the toxic effects of aflatoxins and the first to be affected following intake of contaminated feeds (Espada et al., 1992; Fernandez et al., 1994; Ledoux et al., 1999). The higher relative weight of the liver results from the accumulation of lipids, which on its part causes the typical for aflatoxicosis in broiler chickens fatty enlargement and frailty (Ibrahim et al., 1998; Kubena et al., 1993). Hepatotoxic effects of aflatoxins entail the disorders in the lipid, carbohydrate and protein metabolism (Kubena et al., 1993 and 1998; Ledoux et al., 1999), as well as haemopoietic disorders (Oguz et al., 2000a). Increased relative weights of the liver, kidneys, heart, pancreas, gizzard and proventriculus confirm the results with broiler chickens treated with AFB₁ in feed. The addition of the adsorbent Mycotox NG to the feed of ducklings in this investigation had a protective effect on increasing of organs' weights. The addition of adsorbents to poultry feeds was efficient for prevention and improvement of changes in visceral organs' weights (Kubena et al., 1993). The lower live weight gain and the feed intake in experimental groups II and III, shown in this experiment with mulards, agrees with results from chicken researches on lowering AFB₁ effects with respect to production traits (Edds and Bortel, 1983). The addition of the adsorbent Mycotox NG to feed had a completely opposite effect on the AFB₁-induced inhibition on ducks' growth. Similar results were reported in chickens receiving mycosorbents with contaminated feed, such as hydrated calcium sodium aluminosilicate (Ledoux et al., 1999). It is proved that mycosorbents bind aflatoxins in the intestinal tract, allowing for their safe passage through the organism (Davidson et al., 1987; Phillips et al., 1990). The use of adsorbents is thought to be the most promising and economically justified approach for prevention of adverse effects of mycotoxins in animals (Dakovic et al., 2005). Some adsorbents as bentonites, zeolites, synthetic aluminosilicates, are able to bind aflatoxins and thus, to prevent or decrease their harmful effects on animals (Abdel-Wahhab et al., 1999). The main mechanism of action of adsorbents consists in

chemical adsorption of aflatoxins in the gastrointestinal tract and subsequent decrease in their utilisation (Abdel-Wahhab et al., 1999; Phillips et al., 1990).

The thymus, spleen and bursa of Fabricius are organs, responsible for the humoral and cellular immunity, so they should remain active over lifetime (Huff et al., 1992; Qureshi et al., 1998; Sakhare et al., 2007). The lower relative weights of these organs in groups treated with AFB₁ only results from the atrophy, sclerosis, necrosis and lower lymphoid cell counts (Huff et al., 1992; Sakhare et al., 2007). The increased relative weights of the gizzard and the proventriculus, kidneys and the pancreas result from the increased doses of AFB₁ in feed and confirm the data reported from previous studies in broiler chickens (Huff et al., 1986; Miazzo et al., 2005; Safamehr, 2008; Xin-Yan et al., 2008). The higher relative weights of the gizzard and the proventriculus could be attributed also to inflammation of the gastric mucosa and its swelling (Kubena et al., 1997 and 1998). The demonstrated increased relative weights of the heart in mulards from experimental groups II and III confirms the findings from previous studies with broiler chickens receiving AFB₁ with feed (Kubena et al., 1993; Ledoux et al., 1999).

Conclusion

The present results indicated that the presence of AFB₁ in compound feeds for mulards was related to reduced energy value resulting in lower live body weight, lower feed intake, lower daily weight gain and higher feed conversion ratio. At the same time, the tested aflatoxin caused changes in relative weight of the thymus, bursa of Fabricius and the spleen. The supplementation of feed contaminated with 0.5 mg/kg AFB₁ with Mycotox NG provided a full protection of birds from the negative effects of AFB₁ on production traits and changes in the relative weights of visceral organs.

REFERENCES

- Abdel-Wahhab, M.A., Abo-Norag, M., Edrington, T.S., Kubena, L.F., Harvey, R.B., Philips, T.D., 1995. Influence of hydrated sodium calcium aluminosilicate and virginiamycin on

- aflatoxicosis in broiler chicks. *Poultry Science* 74, 626-632.
- Abdel-Wahhab, M.A., Nada, S.A., Amra, H.A., 1999.** Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. *Journal of Applied Toxicology* 19 (3), 199-204.
- 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.
- Bailey, R.H., Kubena, L.F., Harvey, R.B., Buckiey, S.A., Rottinghaus, G.E., 1998.** Efficacy of various inorganic sorbents to reduce the toxicity of aflatoxin and T-2 toxin in broiler chickens. *Poultry Science* 77, 1630-1632.
- 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 B₁. *Toxicol* 47, 41-46.
- Bintvihok, A., Thiengnin, S., Doi, K., Kumagai, S., 2002.** Residues of aflatoxins in the liver, muscle and eggs of domestic fowls. *Journal Veterinary Medicine Science* 64, 1037-1039.
- Boden, S., Jensen, L., 1985.** The effect of marginal levels of calcium, fish meal, torulas yeast and alfalfa meal on feed intake, hepatic lipid accumulation, plasma estradiol and egg shell quality among laying hens. *Poultry Science* 64, 937-946.
- Brekke, O.L., Peplinski, A.J., Nelson, G.E.N., Griffen, E.L., 1975.** Pilot-plant dry milling of corn containing aflatoxin. *Cereal Chemistry* 52, 205-211.
- Campbell, M.L., May, J.D., Huff, W.E., Doevr, J.A., 1983.** Evaluation of immunity of young broiler chickens during aflatoxicosis and ochratoxicosis. *Poultry Science* 62, 2138-2144.
- CAST, 1989.** Council for Agricultural Science and Technology Task Force Report 16. Mycotoxins: Economic and Health Risks. CAST, Ames, IA.
- Dakovic, A., Tomasevic-Canovic, M., Dondur, V., Rottinghaus, G.E., Medakovic, V., Zanic, S., 2005.** Adsorption of mycotoxins by organozeolites. *Colloids and Surfaces B: Biointerfaces* 46 (1), 20-25.
- Dale, N., 1998.** Mycotoxin binders: It's time for real science. *Poultry Digest* 57, 38-39.
- Davidson, J.N., Babish, J.G., Delaney, K.A., Taylor, D.R., Phillips, T.D., 1987.** Hydrated sodium calcium aluminosilicate decreases the bioavailability of aflatoxin in the chicken. *Poultry Science* 66 (Suppl. 1), 89.
- Edds, G.T., Bortell, R.R., 1983.** Biological effects of aflatoxin in poultry. In: Diener, U. L., Asquith, R. L., Dickens, J. W. (Eds) *Aflatoxin and Aspergillus flavus in Corn*. Southern Cooperative Services Bulletin, 279, Alabama Agricultural Experiment Station, Auburn University, AL, pp. 55-61.
- Espada, Y., Domingo, M., Gomez, J., Calvo, M.A., 1992.** Pathological lesions following an experimental intoxication with aflatoxin B₁ in broiler chickens. *Research in Veterinary Science* 53, 275-279.
- 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.
- Goswami, S., Mukit, A., Bhattacharya, M., 1998.** Histochemical study on chronic aflatoxicosis in ducks. *Indian Journal of Animal Science* 68 (11), 1181-1183.
- Han, X.Y., Huang, Q.C., Li, W.F., Jiang, J.F., Xu, Z.R., Han, X.Y., Huang, Q.C., Li, W.F., Jiang, J.F., Xu, Z.R., 2008.** Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B₁ levels. *Livestock Science* 119 (1), 216-220.
- Huff, W.E., Kubena, L.F., Harvey, R.B., Hagler, V.M., Swanson, S.P., Phillips, T.D., Creger, C. R., 1986.** Individual and combined effects of aflatoxin and deoxynivalenol (DON, vomitoxin) in broiler chickens. *Poultry Science* 65, 1291-1298.
- Huff, W.E., Kubena, L.F., Harvey, R.B., Phillips, T.D., 1992.** Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. *Poultry Science* 71, 64-69.
- Hussein, H.S., Brasel, J.M., 2001.** Review: Toxicity, metabolism and impact of mycotoxins on humans and animals. *Toxicology* 167, 101-134.
- Ibrahim, I.K., Al-Joubory, K.M.T., Shareef, A.M., 1998.** Reducing aflatoxicosis in growing chicks by dietary sodium bentonite. *IPA Journal of Agricultural Research* 8, 130-138.
- Jewers, K., 1990.** Mycotoxins and their effect on poultry production. *Options Mediterraneennes Serie A* 7, 195-202.

- 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., Edrington, T.S., Harvey, R.B., Buckley, S.A., Phillips, T.D., Rottinghaus G.E., Casper, H.H., 1997.** Individual and Combined Effects of Fumonisin B1 Present in *Fusarium moniliforme* Culture Material and T-2 Toxin or Deoxynivalenol in Broiler Chicks. *Poultry Science* 76, 1239-1247
- Kubena, L.F., Harvey, R.B., Bailey, R.H., Buckley, S.A., Rottinghaus, G.E., 1998.** Effects of hydrated sodium calcium aluminosilicate [T-Bind] on mycotoxicosis in young broiler chickens. *Poultry Science* 77, 1502-1509.
- Kubena, L.F., Harvey, R.B., Huff, W., Elissalde, M.H., Yersin, A.G., Phillips, T.D., Rottinghaus, G.E., 1993.** Efficacy of a hydrated sodium calcium aluminosilicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol. *Poultry Science* 72, 51-59
- Ledoux, D.R., Rottinghaus, G.E., Bermudez, A.J., Alonso-Debolt, M., 1999.** Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. *Poultry Science* 78, 204-210.
- McKenzie, K.S., Sarr, A.B., Mayura, K., Bailey, R.H., Miller, D.R., Rogers, T.D., Norred, W.P., Voss, K.A., Platner, R.D., Kubena, L.F., Phillips, T.D., 1997.** Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. *Food and Chemical Toxicology* 35, 807-820.
- Mendoza, D.A., Perez-Arevalo, M., Gomez, C., Molero, G., Novoa, E., Rinco, H., Ascanio, E., 2006.** Effect of foodstuff contaminated with aflatoxin B-1 (0.07 mg/kg) on liver morphology and serum enzymes (AST and ALT) activity in broiler chickens. *Revista Científica-Facultad De Ciencias Veterinarias* 16 (1), 39-47.
- Miazzo, R., Peralta, M.F., Magnoli, C., Salvano, M., Ferrero, S., Chiacchiera, S.M., Carvalho, E.C.Q., Rosa, C.A.R., Dalcero, A., 2005.** Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. *Poultry Science* 84, 1-8.
- Mishra, H.N., Das, C., 2003.** A review on biological control and metabolism of aflatoxin. *Critical Reviews in Food Science and Nutrition* 43 (3), 245-264.
- Muller, R.D., Carlson, C.W., Semeniuk, G., Harshfield, G.S., 1970.** The response of chicks, duckling, goslings, pheasants and poults to graded levels of aflatoxins. *Poultry Science* 49, 1346-1350.
- NRC (1994).** National Research Council, Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
- Oguz, H., Kececi, T., Birdane, Y.O., Onder, F., Kurtoglu, V., 2000a.** Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis. *Research in Veterinary Science* 69, 89-93.
- Oguz, H., Kurtoglu, V., 2000.** Effect of clinoptilolite on fattening performance of broiler chickens during experimental aflatoxicosis. *British Poultry Science* 41, 512-517.
- Oguz, H., Kurtoglu, V., Coskun, B., 2000b.** Preventive efficacy of clinoptilolite in broilers during chronic aflatoxin (50 and 100 ppb) exposure. *Research in Veterinary Science* 69, 197-201.
- Ordinance, 2006.** Ordinance 44/20.04.2006 for veterinary medical requirements to animal rearing facilities, Official Gazette 41 Appendix 9/II, 61-62.
- Osborne, D.J., Hamilton, P.B., 1981.** Decreased pancreatic digestive enzymes during aflatoxicosis. *Poultry Science* 60, 1818-1821.
- 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.
- Qureshi, M.A., Brake, J., Hamilton, P.B., Hagler, W.M., Nesheim, S., 1998.** Dietary exposure of broiler breeders to aflatoxin results in immune dysfunction in progeny chicks. *Poultry Science* 77, 812-819.
- Rayner, E.T., Dollear, F.G., 1968.** Removal of aflatoxins from oilseed meals by extraction with aqueous isopropanol. *Journal of the American Oil Chemistry Society* 45, 662-624.
- Rosa, C.A.R., Miazzo, R., Magnoli, C., Salvano, M., Chiacchiera, S.M., Ferrero, S., Saenz, M., Carvalho, E.C.Q., Dalcero, A., 2001.** Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Poultry Science* 80, 139-144.

- Safamehr, A., 2008.** The performance and hematological characters in broiler chicks fed ammonia treated aflatoxin contaminated feed. *Journal of Animal and Veterinary Advances* 7 (3), 331-336.
- Sakhare, P.S., Harne, S.D., Kalorey, D.R., Warke, S.R., Bhandarkar, A.G., Kurkure, N.V., 2007.** Effect of Toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *Veterinarski Arhiv* 77, 129-146.
- Santurio, J.M., Mallmann, C.A., Rosa, A.P., Appel, G., Heer, A., Diageforde, S., Botticher, M., 1999.** Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxin. *British Poultry Science* 40, 115-119.
- Scheideler, S.E., 1993.** Effects of various types of aluminosilicates and aflatoxin B₁ on aflatoxin toxicity, chick performance, and mineral status. *Poultry Science* 72, 282-288.
- Shashidhara, R.G., Devegowda, G., 2003.** Effect of mannan oligosaccharide on broiler breeder production traits and immunity. *Poultry Science* 82, 1319-1325.
- Shi, Y.H., Xu, Z.R., Feng, J.L., Wang, C.Z., 2006.** Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks. *Animal Feed Science and Technology* 129, 138-148.
- Smela, M.E., Currier, S.S., Bailey, E.A., Essigmann, J.M., 2001.** The chemistry and biology of aflatoxin (B₁): from mutational spectrometry to carcinogenesis. *Carcinogenesis* 22 (4), 535-545.
- Verma, J., Swain, B.K., Johri, T.S., 2002.** Effect of various levels of aflatoxin and ochratoxin A and combinations on protein and energy utilisation in broilers. *Journal of the Science of Food and Agriculture* 82 (11), 1412-1417.
- Xin-Yan, H., Qi-Chun, H., Wei-Fen, L., Jun-Fang, J., Zi-Rong, X., Xin-Yan, H., Qi-Chun, H., Wei-Fen, L., Jun-Fang, J., Zi-Rong, Xu., 2008.** Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B₁ levels. *Livestock Science* 119, 216-220.