

## AFLATOXICOSIS IN MOULARD DUCKLING

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### ABSTRACT

The current study was conducted to evaluate the potential toxic effects of aflatoxins (AFs) in Moulard ducklings. A total of 20 one-day-old Moulard ducklings were classified into two groups, with 10 ducklings in each group. Ducklings in the control group (G1) were fed on an AFs-free diet. In group 2 (G2), ducklings received a naturally AFs-contaminated feed with 50 ppb total AFs for 25 days. Feed intake, weight gain and feed conversion ratio (FCR), symptoms, postmortem changes, hematological and biochemical changes were investigated. Results showed an increase in feed intake with a bad feed conversion ratio (FCR), liver, kidney and thigh muscle hemorrhage in PM lesions, and a significant decrease in body, liver, and gizzard absolute weight of AFs exposed ducks. Also, there was a significant increase in aspartate aminotransferase (AST) and alanine transaminase (ALT) levels, a significant decrease in urea level, and a non-significant decrease in hematological parameters such as Hb, RBCs, WBCs, and platelets in comparison with the control group.

**Key words:** Aflatoxins (AFs); ducklings; feed conversion ratio; AST; ALT

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### INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi such as *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, and *Claviceps* genera (Arroyo-Manzanares *et al.*, 2021). They are capable of contaminating food and feed worldwide (Mupunga *et al.*, 2014) and causing severe health problems to humans and animals upon their entry into the body system through the food chain

(Bennett *and* Klich, 2003). These metabolites are non-essential for fungal growth and reproduction but act as fungal virulence factors (Puschner, 2002).

The most popular mycotoxins that act as a risk for health and cause economic disturbances include aflatoxins (AF), fumonisins (F), zearalenone (ZEN), ochratoxins (OT), trichothecenes, and ergot alkaloids (Hussein *and* Brasel, 2001). The disease condition caused by mycotoxins is called mycotoxicosis and is characterized by the following points: It is not transmissible; drug and antibiotic treatments have little or no effect; outbreaks are often seasonal and usually associated with a specific food or

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feed. Finally, the examination of the suspected food or foodstuff often reveals signs of fungal activity (Marin *et al.*, 2013).

Aflatoxins were discovered and isolated as a result of the mysterious Turkey X disease in 1960 in the United Kingdom due to the contaminated peanut meal that was imported from Brazil (Blount, 1961).

There are eighteen different types of AFs produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (Bennett *et al.*, 2007). Chemically, AFs are difuranocoumarins (Bennett and Klich, 2003; Nakai *et al.*, 2008) and divided into difurocoumarocyclopentenone, which includes AFB1, AFB2, AFM1, and AFM2, and difurocoumarolactone, which involves AFG1 and AFG2 (Stroka and Anklam, 2002). The potencies of AFs were arranged from the most potent to the least potent as follows: AFB1, AFG1, AFB2, and AFG2, respectively, according to their chemical nature (Wogan, 1966). AFB1 is the most potent and widespread in the world (Cullen and Newberne, 2013), where it represents 75% of total AFs in food and feed (Ayub and Sachan, 1997) and has carcinogenic, immunotoxic, teratogenic, and mutagenic effects on humans and animals (Ostry *et al.*, 2017). IARC of the World Health Organization in 1993 classified AFB1, AFB2, AFG1, and AFG2 as Group 1 carcinogens (human carcinogens) (Li *et al.*, 2009), and AFM1 as Group 2B (possible human carcinogens) (Kara and Ince, 2014).

Aflatoxicosis is the sickness condition caused by AFs exposure. It has a major negative health effect on humans, animals, and poultry. Human exposure to AFs may be direct through contaminated food or indirect via consuming polluted animal or poultry products and by products that were previously fed on AFs-contaminated rations (Leong *et al.*, 2012). Adverse effects of AFs vary according to animal and/or poultry (species, sex, and age) and AFs (type, dose, and period of exposure) (Marin *et al.*,

2013). Compared to mammals, poultry are more susceptible to AFs. The most susceptible species in poultry are ducks, followed by turkey > quail > chicken (Diaz and Murcia, 2019), while the most susceptible domestic animals are dogs > pigs > calves > cows > sheep (Kidanemariam and Fesseha, 2020).

There have been no specific treatments or antidotes for AFs till now (Gupta *et al.*, 2022). The current study aimed to evaluate the toxic effects of AFs in duckling hepatorenal and hemopoietic systems, which are the most affected systems in aflatoxicosis. The chosen duckling model for the current study is intended as there are limited studies on duck aflatoxicosis, despite its high susceptibility to AFs.

## MATERIALS AND METHODS:

### 1. Materials:

#### Ethical approval

All the applicable ethical guidelines for ducklings were followed during handling and sample collection. Adequate measures were taken to minimize pain or discomfort according to the animal welfare ethics approved by the Faculty of Veterinary Medicine at El-Minia University with approval number IRB-FVM-MU-2023-104 with a date 5/ 9/ 2023.

#### Chemicals

- Poultry feed contaminated with 50 ppb total AFs was prepared in the Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Assiut University, through the addition of aflatoxigenic *Aspergillus flavus* to previously analyzed feed free from AFs and were analyzed by ultra-performance liquid chromatography (UPLC) in the Central Lab. of Faculty of Veterinary Medicine, Assiut University according to Benvenuti and Burgess (2010).

- Biochemical kits such as AST, ALT, total protein, albumen, urea, and creatinine were

obtained from Biomed and Diamond Company, Egypt and analyzed by spectrophotometer.

### **Birds (ducklings) and experimental design**

Twenty one-day-old Moulard Ducklings were obtained from a private farm in Day rout, Assiut governorate. Ducklings were housed in two groups (10each).G1 ducklings were fed on AFs-free rations and acted as a control group. G2 ducklings received a naturally AFs-contaminated ration with 50ppb total AFs, daily consumption for 25 days.

### **Time schedule for samples collection and preparation**

After 25 days of experimentation, only five ducks per group were slaughtered and blood samples were collected in two tubes: one for hematology (EDTA tube) and the other to collect serum for further biochemical analysis. Liver, kidney, spleen, gall bladder, gizzard, and proventriculus were collected, examined for PM lesions, weighted and preserved in formalin 10% for further pathological studies.

## **2. Adopted methods**

**2.1. Ducks performance:** Clinical signs, mortalities and P/M findings were recorded during the experiment.

### **2.2. Body and absolute organ weight**

The body weight of each duckling was recorded at the initial, during, and at the end of the experiment. Liver, kidney, spleen, gall bladder, gizzard, and proventriculus were

removed, stripped of fatty tissues, blotted, examined macroscopically, and weighed.

**2.3. Feed conversion ratio** was calculated according to Elkafrawy(2020).

### **2.4. Hematological parameters**

A blood sample collected in an EDTA tube was used for CBC analysis by the CBC Analyzer (MS4Se Vet) from mslab, Austria.

### **2.5. Liver and kidney function tests:**

Blood samples were centrifuged at 3000 rpm for 15 min. for serum collection and used to evaluate AST (aspartate aminotransferase), ALT (alanine transaminase) according to Bergmeyer *et al.* (1977), total protein according to Kingsley (1939) and Yatzidis(1987), albumin, creatinine, and urea according to Tietz (1995) with the Spectrophotometer Mindray BA-88A.

### **2.6. Statistical analysis**

Results were expressed as mean  $\pm$  SE using the computer SPSS program for Windows, version 20.0, with an independent T test according to Jinn (2011) to compare G1 and G2.

## **RESULTS**

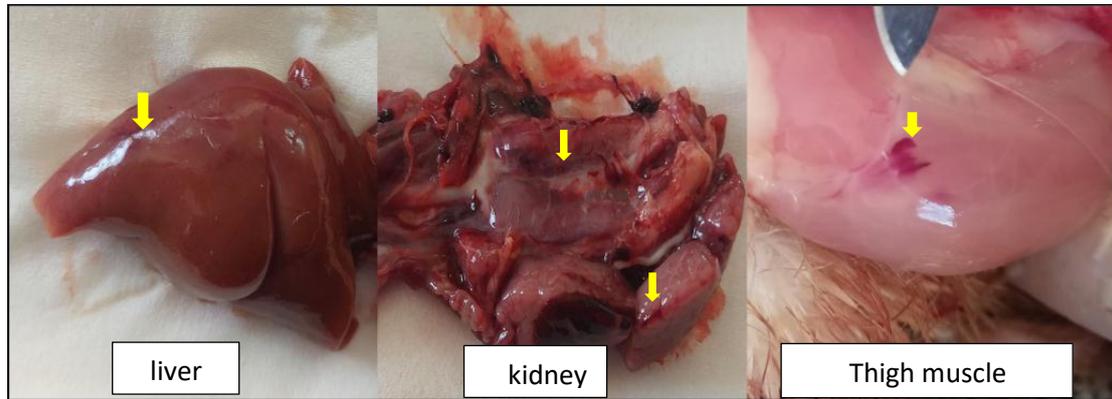
Ducks performance (clinical signs, mortalities, and PM findings): the overall behavior of ducks that feed on contaminated diets with AFs (50 ppb) was similar to that of the control group (G1) during the experiment but showed an increase in feed intake, a decrease in growth rate (Fig. 1), ruffled feathers, and brownish diarrhea. There were no mortalities during the study.



**Fig. 1:** Decreased growth rate in ducks fed on an AFs-contaminated diet (b) in comparison with the control group (a)

PM findings of ducks fed on a contaminated diet with 50 ppb AFs for 25 days showed hemorrhages in liver, kidney, and thigh

muscles (Fig. 2) in comparison with the control group.



**Fig. 2:** PM findings of ducks fed on an AFs-contaminated diet showed hemorrhages in liver, kidney, and thigh muscles (arrow).

The body weight was significantly decreased in G2 in comparison with the

control group during the experiment (Table 1).

**Table 1:** Effects of AFs on ducks body weight (g/duck).

Group/days	0	7	14	21	25
<b>Control (G1)</b>	48.55 ± 1.62	211.09 ± 5.12	550.36 ± 13.92	1092 ± 27.08	1232.55 ± 30.48
<b>AFs (G2)</b>	47.73 ± 0.91	176.00 <sup>a</sup> ± 5.48	438.73 <sup>a</sup> ± 13.39	798.91 <sup>a</sup> ± 23.53	878.81 <sup>a</sup> ± 28.80

Data represented the body weight as mean ± S.E. in treated and control ducks. (N= 10), where (a) indicates a significant difference in comparison with the control group.

The liver, kidneys, spleen, gizzard, proventriculus, and gall bladder of ducks were weighted after slaughtering, and the results showed that liver and gizzard absolute weights significantly decreased

in G2 in comparison with the control group. Kidneys, spleen, proventriculus, and gall bladder showed non-significant variation in G2 compared with G1, according to Table 2.

**Table 2:** Effects of AFs on ducks absolute organ weight.

Group/organs	Liver	Kidney	Spleen	Gizzard	Proventriculus	Gall bladder
<b>Control (G1)</b>	36.89 ±3.18	11.24 ± 0.67	0.89 ± 0.10	51.65 ± 2.50	4.61 ± 0.28	1.41 ± 0.03
<b>AFs (G2)</b>	21.84 <sup>a</sup> ± 1.51	9.48 ± 0.45	0.95 ± 0.06	41.79 <sup>a</sup> ± 1.08	4.85 ± 0.31	1.79 ± 0.24

Data represented the absolute organ weight as mean ± S.E. in treated and control ducks. (N= 5), where (a) indicates a significant difference in comparison with the control group.

Feed intake, weight gain, and feed conversion ratio results showed that the feed intake was higher than in ducks fed on AFs-contaminated feed with low weight gain in

comparison with the control group. As a result, the feed conversion ratio was bad in this group compared with ducks fed on free AFs rations, as shown in Table 3.

**Table 3:** Effects of AFs on ducks feed intake (FI), weight gain (WG), and feed conversion ratio (FCR).

Group/days		0-7	8-14	15-21	22-25
Control (G1)	FI	1944	5490	8744	5406
	WG	1788	3732	5265	2239
	FCR	1.087	1.471	1.661	2.414
AFs (G2)	FI	2279	6276	8968	6056 <sup>a</sup>
	WG	1411 <sup>a</sup>	2890 <sup>a</sup>	3962 <sup>a</sup>	879 <sup>a</sup>
	FCR	1.615 <sup>a</sup>	2.172 <sup>a</sup>	2.264 <sup>a</sup>	6.889 <sup>a</sup>

The data represented the periodically absolute feed intake (g), weight gain (g), and feed conversion ratio in treated and control ducks (N = 10), where (a) indicates a significant difference in comparison with the control group.

Hematological parameters such as Hb, RBCs, WBCs, HCT, Lymph, and platelets showed non-significant variation between ducks fed on AFs-contaminated diet and the control group as represented in Table 4.

**Table 4:** Effects of AFs on ducks hematological parameters.

Groups /hematological parameters	Hb g/dl	RBCs x100 <sup>3</sup> cells/ $\mu$ l	WBCs x10 <sup>3</sup> /mm <sup>3</sup>	HCT %	Lymph	Platelets x10 <sup>3</sup> /mm <sup>3</sup>
Control (G1)	12.98 $\pm$ 0.47	5.12 $\pm$ 0.43	6.13 $\pm$ 0.82	44.52 $\pm$ 1.84	88.87 $\pm$ 1.22	480.33 $\pm$ 101.84
AFs (G2)	10.55 $\pm$ 0.52	4.12 $\pm$ 0.075	4.12 $\pm$ 0.29	38.17 $\pm$ 1.28	83.44 $\pm$ 2.52	170.00 $\pm$ 5.51

Data represented the hematological parameters as mean  $\pm$  S.E. in ducks fed on AFs-contaminated diet and the control group (N= 5).

Liver and kidney function test results showed a significant increase in AST and ALT levels in ducks fed on AFs-contaminated diets in comparison with the control group, as well as a significant decrease in urea levels in ducks

fed on AFs in comparison with the control group. Albumin, total protein, and creatinine results showed non-significant variation in AFs and the control group as shown in Table 5.

**Table 5:** Effects of AFs in ducks liver and kidney function tests

Groups	Liver function tests				Kidney function tests	
	AST g/dl	ALT g/dl	Albumin g/dl	Total protein g/dl	Urea mg/dl	Creatinine mg/dl
Control (G1)	34.60 $\pm$ 5.86	19.93 $\pm$ 2.23	1.90 $\pm$ 0.06	3.93 $\pm$ 0.03	6.03 $\pm$ 0.12	0.40 $\pm$ 0.00
AFs (G2)	64.27 <sup>a</sup> $\pm$ 10.98	37.3 <sup>a</sup> $\pm$ 5.75	1.40 $\pm$ 0.20	3.20 $\pm$ 0.30	3.83 <sup>a</sup> $\pm$ 0.17	0.33 $\pm$ 0.03

Data represented liver and kidney function tests as mean  $\pm$  S.E. in ducks fed on AFs- contaminated diets and the control group (N=5). where (a) indicates a significant difference in comparison with the control group.

## DISCUSSION

AFs alter the animal and poultry production, where it is considered the main enemy for poultry industry. It raises mortality rates,

lowers nutrient absorption and growth rates, weakens immune systems, and reduces productivity.

Results showed a significant decrease in body weight, liver, and gizzard absolute weight in AFs-contaminated diet group in comparison with the control group. Also hemorrhages in the liver, kidney, and thigh muscles as postmortem lesions in AFs-contaminated diet group in comparison with the control group. All these disturbances in duck growth and performance are considered a consequence of AFs hepatic intoxication and protein metabolism disturbances, which agrees with Andretta *et al.* (2011) results and interpretations.

Ducks fed on an AFs-contaminated diet showed increased feed intake, decreased growth rate, increased feed conversion ratio, ruffled feathers, and brownish diarrhea. The increase in feed conversion ratio in the current study due to AFs exposure agreed with Abu El-Ela *et al.* (2013 and 2019), showed an increase in feed intake and a decrease in weight gain, so a bad feed conversion ratio occurs related to low body weight in ducks fed on AFs-contaminated feed. The absolute organ weight results of the current study agreed with Wan *et al.* (2013), who observed the depletion of liver weight in ducklings due to exposure to AFs, but disagreed with the study conducted by Tansakul *et al.* (2017), which revealed that the liver and spleen weights were elevated by AFs-contaminated feed, but their results were in harmony with the current study in the decrease of duck body weight.

The current study revealed hepatic damage in AFs-contaminated diet group which caused protein synthesis impairment and poor duck performance. Liver and kidney function test results showed a significant increase in AST and ALT levels in ducks fed on AFs-contaminated diets in comparison with the control group, as well as a significant decrease in urea levels in ducks fed on AFs in comparison with the control group. Albumin, total protein, and creatinine results showed a non-significant decrease in the AFs group compared with control ducks. Disturbances in

liver and kidney function tests act as markers for liver and kidney dysfunction.

These current results were in harmony with the studies that were conducted by Abdalla *et al.* (2012), which showed the liver biochemical disturbances of aflatoxin in chickens, and He *et al.* (2013), which revealed the increase of hepatic enzyme activity in ducks. Tansakul *et al.* (2017) studied the toxicological effects of different doses of AFs in laying duck liver and showed an increase in AST enzyme and a non-significant variation in protein level in serum, and Abu El-Ela *et al.* (2019) revealed the elevation of liver enzymes, especially ALT, AST, and ALP, in white Pekin ducklings. Finally, the results partially agreed with El-Sheshtawy *et al.* (2021), where the ALT, AST enzyme activities, and creatinine were significantly elevated and the serum total protein and albumin were significantly reduced in AFs intoxicated Pekin ducklings.

Hematological parameters showed a non-significant variation in ducks fed on AFs-contaminated diet and agreed with the study, which was designated by Tansakul *et al.* (2017) and showed non-significant variation between different duck groups fed on different doses of AFs, which may be due to exposure to low dose of AFs. Disagree with the studies that were conducted by He *et al.* (2013) and Rattanasinthuphong *et al.* (2017), which observed a decrease in Hb, PCV, and RBCs in ducks fed diets containing AFs.

## CONCLUSION

The current study results revealed the main adverse effects of 50 ppb AFs in ducks feed for 25 days as follows: poor performance of ducks, decreased body, liver, and gizzard weight, and increased the liver enzymes. In future studies, we will try different methods to ameliorate the toxic effects of AFs in ducks.

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## التسمم بالأفلاتوكسين في صغار البط المولار

طارق محب صبحي ، زكريا مختار زكي ، صفوت علي ، هبه فوزي كمال

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تعد الأفلاتوكسينات من أخطر السموم الفطرية التي تفرز من الأسبراجلس فلافس و الأسبراجلس باراسيتكس وتهدد صحة الإنسان والحيوان علي مستوي العالم. ولقد أجريت الدراسة الحالية لدراسة التسمم بالأفلاتوكسينات في صغار البط المولار حيث تم إجراء التجربة علي عدد ٢٠ من صغار البط المولار عمر يوم والتي تم تقسيمهم إلي مجموعتين: ١٠ بالمجموعة الأولى والتي تمثل المجموعة الضابطة للتجربة و ١٠ بالمجموعة الثانية التي تم تغذيتها علي أعلاف بها ٥٠ جزء من البليون من الأفلاتوكسينات يومياً لمدة ٢٥ يوم وأوضحت النتائج سوء معدل التحويل الغذائي، وجود نزف في الكبد، الكلي والعضلات. كما أوضحت النتائج نقص في وزن الجسم، والكبد والقونصة في المجموعة التي تعرضت للأفلاتوكسين. أيضاً أوضحت الدراسة وجود زيادة معنوية في انزيمات الكبد، ونقص معنوي في مستوي البوريا ونقص غير معنوي في اختبارات الدم مثل الهيموجلوبين، خلايا الدم الحمراء والبيضاء والصفائح الدموية مقارنة بالمجموعة الضابطة بالتجربة. خلصت هذه الدراسة إلي سمية الأفلاتوكسينات علي الكبد في صغار البط ومن ثم تأثيرها علي التحويل الغذائي وجميع أنشطة الجسم في الطائر.