

EFFECT OF MYCODOTE ON CLINICAL AND HISTOPATHOLOGICAL CHANGES IN GIMMIZAH AND DOKKI-4 HENS FED ON AFLATOXICATED DIETS

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Abstract

Gimmizah (G) and Dokki-4 (D4) hens of 48 weeks of age were fed diets with two levels of aflatoxin B₁ (AFB₁) 2 and 5 ppm without or with Mycodote by level of 0.1% for three weeks. The clinical and histopathological changes in some organs and glands as affected by treatment were studied.

The results obtained could be summarized as follow:

1- Clinical changes

Liver, kidney, spleen and heart weight as a percentage of live body weight of (G) and (D4) hens were significantly ($P < 0.05$) increased during the treatment period, while weight of bursa of fabricius and thymus % of live body weight decreased significantly in the treated groups compared to the control group.

2- Histopathological changes

Birds fed aflatoxicated diets by 5 ppm without Mycodote showed focal aggregation of lymphoid cells in the hepatic tissues associated with dilatation in the portal vein. The Kupffer cells were proliferated in diffuse manner all over the hepatic tissue. Also, the group fed aflatoxicated (5ppm) plus antitoxin (0.01% Mycodote) showed mononuclear leucocytes inflammatory cells infiltration in the portal area.

The kidney of birds fed 5ppm aflatoxin in the diet showed that there was focal extravasation of red blood cells in between the renal tubules, while, those fed 5ppm aflatoxin plus 0.01% Mycodote, focal aggregation of lymphoid cells was observed in between the degenerated renal tubules.

INTRODUCTION

Aflatoxins are a group of hepatocarcinogenic compounds produced by the mold *Aspergillus flavus* and *Aspergillus parasiticus* when growing on feedstuffs. The severe toxicity and suspected carcinogenicity of aflatoxins and the natural occurrence of these mycotoxins pose a threat, not only to animal industry, but also, to human health through the contamination of feedstuffs (Hamilton and Garlich, 1972). Smith and Hamilton (1970) reported that aflatoxicosis led to enlargement of spleen in chickens. Also, a decrease in body weight gain and enlargement of liver by aflatoxin treatment were reported by El-Shaarawi *et al.* (1993). El-Shaarawi, (1989) indicated that, in chickens, the kidney, pancreas, proventriculus gizzard and thyroid glands were also

target organs which responded to dietary aflatoxins, where enlargement of these organs was observed. Data for all traits were statistically analyzed according to one way analysis of variance design using general linear model (GLM) procedure by computer program of SAS (1985) as the model

$X_{ij} = M + A_j + e_{ij}$. Where :

X_{ij} = represents observation M = overall means

A_j = effect of treatments (diets)

e_{ij} = experimental error.

MATERIALS AND METHODS

One hundred and ninety-two hens 48 weeks of age from (G) and (D4) were fed 0, 2 and 5 ppm aflatoxin B₁ (AFB₁) with Mycodote by level of 0.1% in the diet for three weeks followed by four weeks recovery period (received diet free of both aflatoxin and mycodote). At the end of the treatment period, the birds were held 12 hours prior to slaughter without feed, then, 3 birds from each treatment group of each strain were randomly selected, weighed and slaughtered to obtain organs weight after bleeding, scalding, feather picking by hand and evisceration. Different organs (liver, kidney, spleen, heart and gizzard) were weighed and then, subjected to histopathological examination where the method was as follows:

- 1- Selected organs were taken and fixed in formalin 10% for 10 hours, then, trimmed and washed by water for four hours.
- 2- The fixed tissues were dehydrated by transferring into series of graded concentrated ethyl alcohol (59%, 70% and 100%).
- 3- The tissues were cleared in xylene for 2 hours.
- 4- The cleared tissues were embedded in paraffin wax at 60-70°C for four hours.
- 5- They were cut by using Reichert-Jung 2040 microtome, the sections were dewaxed by xylene for 10 minutes, then in methylalcohol for 3 minutes and washed by distilled water for 5 minutes.
- 6- The sections were routinely stained with hematoxylin and eosin (H and E).
- 7- The stained sections were washed by tap water and mounted in Canada balsam.

RESULTS AND DISCUSSION

1-Clinical symptoms

During the treatment period, birds fed on AFLB₁ (2 or 5 ppm) without Mycodote showed loss in their appetite and decrease in activity at the first week. At the end of treatment period, the symptoms were sharply decreased body weight, feed consumption, fertility percentage, and then, death occurred. The group of birds fed on AFLB₁ plus Mycodote appeared normal healthy and no mortality was observed during this period.

The effect of AFLB₁ on relative organ and glands weight is shown in Tables 1 and 2, where it shows that, at the end of treatment period, treated groups fed on aflatoxificated diets without Mycodote was significantly ($P < 0.05$) increased in liver, kidney, spleen and heart relative weight of both Gimmizah (G) and Dokki-4 (D4) birds compared to control groups. Results in the same tables revealed that the increment of relative weight increased by the increment of AFLB₁ from 2 to 5 ppm. On the other hand, the lowest increase in the relative weight of these organs was obtained by hens fed on aflatoxificated diet containing 2 ppm AFLB₁ supplemented by 0.1% Mycodote. At the end of recovery period, after the withdrawal of AFLB₁, hens fed low aflatoxin level (2ppm) with 0.1% Mycodote recorded lesser organs weight than those fed a high level of aflatoxin (5ppm) with Mycodote. These results are in agreement with those reported by Kobena *et al.* (1990 a, b and 1993). The same tables showed that there were significant ($P < 0.05$) decrease in bursa of fabricius and thymus gland of birds fed on aflatoxificated diet without Mycodote. The effect was highly observed in treated groups fed high level of AFLB₁ (5ppm) than those fed on low level (2ppm). These results agreed with those obtained by Hamilton and Garlich (1972), Huff *et al.* (1988), Kubena *et al.* (1990b), Abd El-Hamid *et al.* (1992), and Edrington *et al.* (1997), who found that the relative weight of liver, kidney, and spleen increased, while those of bursa and thymus glands decreased by aflatoxicosis. However, the birds fed on aflatoxificated diets with 0.1% Mycodote the detoxifier alleviated the effect of aflatoxin on organs weight.

2- Histopathological changes

There was no histopathological alteration in liver in all birds fed the control diet as well as the diet containing antitoxin. The birds fed on 2ppm aflatoxin diet showed focal aggregation of mononuclear leucocytic inflammatory cells located in the portal area as well as in the other hepatic tissue in association with severe dilation of portal veins and sinusoids in which the latter showed massive number of leucocytic cells (Fig 1). In the portal area, there were infiltration of granular acidophilic cells as well as mononuclear leucocytic inflammatory cells associated with severe dilation of portal veins and sinusoids, Kupffer cells were proliferated in diffuse manner all over the hepatic tissue.

The birds fed 5ppm aflatoxin diet showed besides the previous changes, many of Kupffer cells proliferated in diffuse manner all over the hepatic tissue (Fig 2).

As in the liver, birds fed on diet without aflatoxin with or without Mycodote showed no histopathological alteration in kidney tissues, while, those fed diet containing 2ppm aflatoxin showed focal aggregation of lymphoid cells in between the renal tubules and glomeruli in the cortical portion in association with hyperemic blood vessels. The epithelial cells lining the renal tubules showed degenerative changes with pyknosis in some of their nuclei in focal manner (Fig 3).

In birds fed 5ppm aflatoxin in the diet, there was focal extravasation of red blood cells in between the renal tubules (Fig 4).

The clinical symptoms obtained in both studied strains were found to be almost similar as shown in Tables 1 and 2.

Table 1. Effect of antitoxin (Mycodote) as detoxifier for aflatoxin B₁ on relative organs and glands weight (%) of body weight of Gimmizah hens during treatment (0-3 weeks) and recovery (4-7 weeks) periods.

Items	Treatment period (Start to 3 weeks)						Recovery period (4-7 weeks)					
	AFB ₁ level (ppm)			Mycodote %			AFB ₁ level (ppm)			Mycodote %		
	0	0.1	0.0	0.1	0.0	0.1	0	0.1	0.0	0.1	0.0	0.1
Body weight	1542.6 ^b	1729.7 ^b	1464.0 ^a	1570.3 ^c	1290.4 ^a	1349.0 ^d	1616.0 ^b	1747.0 ^a	1511.6 ^{cd}	1557.6 ^b	1360.1 ^d	1435.3 ^{cd}
Liver	2.91 ^f	2.83 ^e	4.18 ^d	3.72 ^e	5.91 ^b	4.78 ^b	2.71 ^d	2.63 ^e	3.38 ^e	3.12 ^b	3.57 ^e	3.19 ^c
Kidney	1.03 ^f	1.01 ^e	1.39 ^e	1.20 ^d	1.87 ^a	1.65 ^b	1.08 ^f	1.04 ^f	1.02 ^b	1.13 ^b	1.29 ^a	1.24 ^b
Spleen	0.221 ^c	0.217 ^b	0.288 ^{ab}	0.261 ^d	0.396 ^a	0.34 ^{bc}	0.223 ^b	0.219 ^a	0.231 ^c	0.225 ^d	0.245 ^f	0.238 ^e
Heart	0.264 ^a	0.312 ^a	0.365 ^d	0.234 ^d	0.481 ^a	0.425 ^{cd}	0.258 ^b	0.277 ^a	0.295 ^c	0.291 ^d	0.346 ^f	0.320 ^e
Bursa of Fabricius	0.329 ^a	0.317 ^a	0.233 ^b	0.251 ^b	0.185 ^c	0.241 ^c	0.236 ^b	0.321 ^a	0.302 ^c	0.312 ^d	0.272 ^f	0.293 ^e
Thymus	0.366 ^a	0.352 ^a	0.253 ^{bc}	0.277 ^{cd}	0.165 ^{cd}	0.219 ^e	0.394 ^b	0.425 ^a	0.329 ^e	0.336 ^d	0.297 ^f	0.318 ^e

a, b, ... = Mean on the same row are differently superscripted are significantly different (P < 0.05).
AF = aflatoxin B₁.

Table 2. Effect of antitoxin (Mycodote) as detoxifier for aflatoxin B₁ on relative organs and glands weight (%) of body weight of Dokki 4 hens during treatment (0-3 weeks) and recovery (4-7 weeks) periods.

Items	Treatment period (Start to 3 weeks)						Recovery period (4-7 weeks)					
	AFB1 level (ppm)			Mycodote %			AFB1 level (ppm)			Mycodote %		
	0		0.1	0.0		0.1	0		0.1	0.0		0.1
	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1
Body weight	1513.5 ^b	1684.7 ^{ab}	1421.2 ^f	1521.3 ^e	1212.3 ^e	1311.4 ^d	1597.3 ^b	1679.0 ^a	1485.6 ^c	1540.7 ^b	1398.4 ^d	1398.2 ^c
Liver	2.53 ^{af}	2.91 ^c	4.27 ^e	3.58 ^d	5.82 ^b	4.41 ^b	2.64 ^d	2.56 ^d	3.30 ^{ab}	2.95 ^c	3.52 ^a	3.19 ^{ac}
Kidney	1.06 ^c	1.04 ^e	1.34 ^{ab}	1.16 ^d	1.83 ^a	1.70 ^b	1.05 ^e	0.96 ^f	1.18 ^{ab}	1.08 ^c	1.23 ^a	1.21 ^{bc}
Spleen	0.215 ^e	0.211 ^e	0.257 ^{bc}	0.232 ^c	0.358 ^d	0.297 ^a	0.221 ^b	0.222 ^a	0.295 ^c	0.225 ^d	0.241 ^f	0.239 ^e
Heart	0.246 ^c	0.297 ^b	0.342 ^b	0.314 ^c	0.459 ^a	0.396 ^b	0.262 ^b	0.285 ^c	0.303 ^c	0.229 ^d	0.337 ^f	0.316 ^e
Bursa of Fabricius	0.308 ^a	0.284 ^a	0.226 ^b	0.236 ^b	0.175 ^d	0.221 ^{cd}	0.18 ^b	0.310 ^a	0.299 ^c	0.287 ^d	0.265 ^e	0.272 ^e
Thymus	0.378 ^a	0.361 ^a	0.272 ^c	0.288 ^a	0.187 ^e	0.229 ^{bc}	0.421 ^a	0.424 ^a	0.332 ^b	0.345 ^b	0.314 ^c	0.325 ^c

a, b, ... = Mean on the same row are differently superscripted are significantly different (P < 0.05).
AF = aflatoxin B₁.

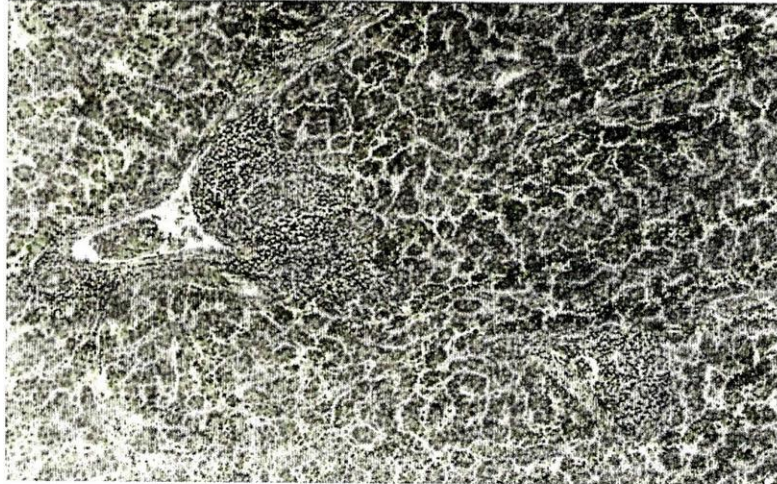


Fig. 1. Liver of birds of experimental group fed on aflatoxicated diet (2 ppm) showing dilation in portal veins and sinusoids with monoclear aggregations in the portal area.

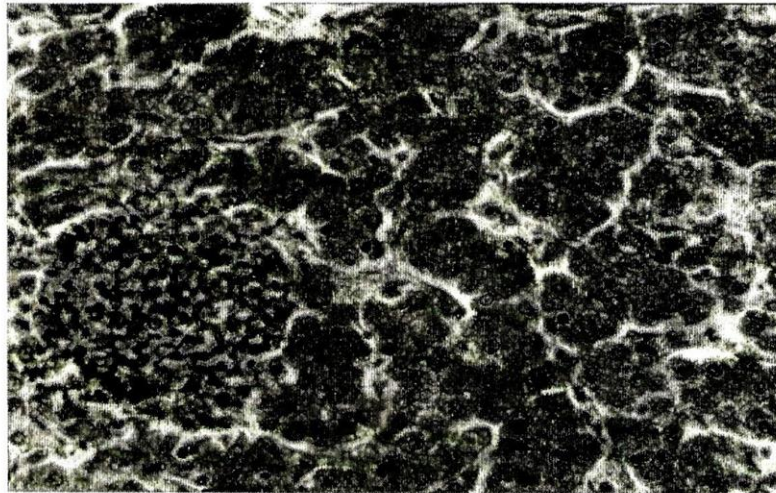


Fig. 2. Liver of birds of experimental group fed on (5ppm) aflatoxicated diet showing granular acidophilic cells in portal area with Kupffer cells proliferation.



Fig. 3. Kidney of birds fed (2ppm) aflatoxicated diet showing lymphoid cells aggregation with hyperemic blood vessels.

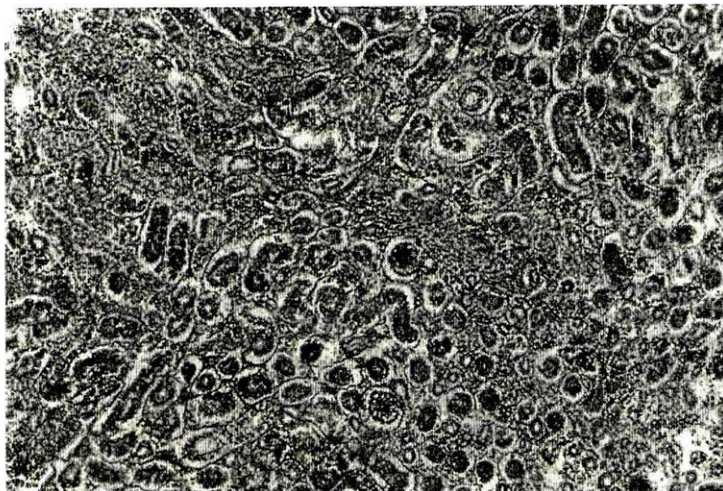


Fig. 4. Kidney of birds fed (5ppm) aflatoxicated diet showing focal extravasation of red blood cells.

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تأثير اضافة الميكودوت على التغيرات المرضية والهستوباثولوجية فى دجاج الجميزة ودقى-٤ المغذى على علائق تحتوى على الأفلاتوكسين ب١

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٣. المركز القومى لبحوث وتكنولوجيا الأشعاع

تمت تغذية دجاج عمر ٤٨ أسبوعا على مستويين من أفلاتوكسين ب١ (٢ و ٥ جزء فى المليون) مع اضافة ميكودوت (مضاد لسموم الفطريات) بمستوى ٠,١% أو بدون اضافة لمدة ٣ أسابيع لدراسة التغيرات المرضية والهستوباثولوجية فى بعض الأعضاء الداخلية والغدد لدجاج الجميزة ودقى-٤ بتأثير كل من الأفلاتوكسين والميكودوت. وكانت النتائج المتحصل عليها كالتالى:

التغيرات المرضية :

١. إزداد الوزن بالنسبة للوزن الحى لكل من الكبد والكلى والقلب زيادة مؤكدة خلال فترة المعاملة بينما انخفض الوزن النسبى لغدة البرسا والطحال مقارنة بمجموعة المقارنة.
٢. التغيرات الهستوباثولوجية:
فى الطيور التى تمت تغذيتها على عليقة بها ٥ جزء فى المليون أفلاتوكسين بدون ميكودوت ظهرت تجمع متواصل من الخلايا الليمفاوية فى أنسجة الكبد مصحوبة بتدهور فى الوريد البابى، كما انتشرت خلايا كوبفر فى جميع أنسجة الكبد وكذلك فان الأفراد المغذاه على الأفلاتوكسين والميكودوت ظهرت بها خلايا أحادية النواة (مونونوكيولار ليكوسيتك) المسببة للألتهاب وذلك فى منطقة الوريد البابى.
وفى الكلوة لدم الحمراء منتشرة بين القنوات البولية فى الطيور المغذاه على الأفلاتوكسين بدون ميكودوت أما الطيور التى حصلت على ميكودوت فى العليقة فقد ظهر تتجمع متواصل من الخلايا الليمفاوية خلال القنوات البولية المتدهورة.