EFFECT OF DIMETHYL DIPHENYL BICARBOXYLATE (DDB) ON BROILER CHICKENS DURING EXPERIMENTAL AFLATOXICOSIS

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ABSTRACT

In vivo trial was conducted to test the efficacy of dimethyl diphenyl bicarboxylate (DDB), to reduce the deleterious effect of aflatoxins (AF) on broiler chickens from 14 to 42 days of age. A total of one hundred 14-d-old Hubbard broiler chickens were randomly assigned to 4 treatment groups: control, AF, DDB, and AF plus DDB, of 25 chickens each. AF and DDB either alone or in combination were incorporated in the diet with concentrations of 2.75 and 30 mg/kg food, respectively. Chickens fed AF alone were adversely affected. The AF significantly decreased feed intake and body weight gain by 15.33% and 26.62%, respectively and increased feed conversion ratio by 15.76%, compared to control. Addition of DDB to an AF-containing diet significantly improved the previous performance traits. When compared to control, addition of DDB to AF-free diet improved feed conversion ratio by 7.88%. A significantly lower proportions of chickens were recorded as feeding, foraging and in comfort activities in AF-fed group. AF treatment significantly increased the percentages of chickens that were inactive (sleeping, standing, sitting) and those engaged in abnormal behaviour patterns (feather pecking). Comfort behaviour was found more often and chickens performed inactive and abnormal behaviour less frequently when DDB was added to AF-containing diet. Compared to control, addition of DDB to AF-free diet did not significantly alter the expression of behaviour patterns, except for decreased percentages of inactive behaviour. Moreover, frequency of feeding behaviour as well as time spent feeding were significantly lower in the focal animal samples in AF-fed chickens compared to control. AF treatment significantly decreased serum total protein and albumin and increased uric acid, creatinine, alanine amino transferase (ALT) and aspartate amino transferase (AST) concentrations. AF significantly decreased values of red blood cell counts, haemoglobin and increased values of white blood cell counts. The adverse effect of AF was significantly reduced by DDB. Compared to control, addition of DDB to AF-free diet did not significantly alter the biochemical and haematological characters. Feeding AF significantly increased relative weights of liver, kidney, heart and proventriculus while decreased relative weight of spleen. Moreover, histopathologic degenerative changes were observed in livers and kidneys of AF consuming chickens. Addition of DDB significantly decreased the severity of lesions in AF-fed group, while addition of DDB to AF-free diet did not produce any significant changes compared with control. The presence of AF alone in the diet significantly depressed the secondary immune response of chickens to Newcastle disease vaccine as measured by haemagglutination inhibition (HI) test. DDB was significantly effective in ameliorating the suppressive effect in AF treated group. Chickens receiving DDB showed no significant changes in antibody titers, compared to control. These results suggest that DDB addition was effective to ameliorate the detrimental effect of AF on broiler chickens.
INTRODUCTION

Aflatoxins (AF) are the most frequently found mycotoxins in poultry feed, produced by *Aspergillus flavus* and *Aspergillus parasiticus*, that cause liver damage in poultry and livestock (28). The fungal attack and production of aflatoxins may occur during preharvest or post harvest, during storage and transportation of feed, and at farm level itself like in feed troughs. High temperature, humidity and increased moisture content in the feed grains are among the contributing factors in the tropical regions that encourage fungal growth and aflatoxin production (23).

Aflatoxins are differentiated into B (B1 & B2), and G (G1 & G2) subtypes based on structure, chromatographic and fluorescent characteristics (39). M1 & M2 are derivatives of B1 and are comparatively less toxic. In terms of occurrence, B1 is more predominately found than others and is the most toxic type to poultry and livestock including human beings. Aflatoxicosis lowers the profitability of poultry production by decreased growth, feed conversion efficiency, egg production and immunity leading to heavy economic losses (1,11,35). Also associated with aflatoxicosis is anorexia, anaemia, haemorrhage and increased relative weights of several organs (15,18,41). Hepatotoxicosis is one of the major signs of aflatoxicosis in poultry (22). Livers characteristically are pale and enlarged as a result of aflatoxicosis, with microscopic changes including fatty change, hepatic necrosis, and biliary hyperplasia (14). Chronic aflatoxicosis in poultry may be diagnosed by determining the serum biochemical and haematological alterations before clinical symptoms become apparent (19,27). Decreased serum concentrations of total protein and albumin are consistent indicators of hepatotoxicity of AF in chickens (25,26). Moreover, broiler chickens fed 2.5 mg AF/kg diet have shown not only decreased values of red blood cell counts, haemoglobin but also an increased values of white blood cell counts (19,36).

Aflatoxicosis affected flocks are refractory to vaccination. The inhibitory effect of AF was demonstrated against Newcastle disease vaccination in broiler chickens (17), and layer-breeders (2).

Aflatoxin B1 has a significant toxic action on developmental patterns of behavior in newborn rats, particularly on development of motor coordination (20). Research in poultry has shown that T-2 toxin, a member of the tricothecene family of mycotoxins, causes abnormal behaviour in chickens (52), however to our knowledge no reports on the effect of aflatoxicosis on the expression of different behavioural activities in poultry are available. Aflatoxin has been reported to cause classic
physiological stress in chickens (51). Powell (40) has pointed out that modification of
the ethogram which accompany environmental changes may be interpreted either as
evidence of limitations it places on behaviour or as a behavioural adaptation to the
new environment. Moreover, presence or absence of behavioural abnormalities has
been proposed for assessing the welfare status of husbandry system (6,13). Some
effects of these behaviours are aggression, injurious behaviours such as feather
pecking and stereotypies. Therefore, further studies are then required to characterize
behavioral effects of aflatoxicosis in chickens where the occurrence of
disadvantageous behaviour patterns would be expected in such inappropriate
environment.

Dimethyl diphenyl bicarboxylate (DDB; Fig. 1) is an intermediate process of
synthesizing Schizandrin C, a natural compound isolated from Fructus schizandrae
chinensis. Currently, DDB is widely used in Asian countries as a hepato-protective
drug with high effectiveness in normalizing liver
functions and very low side effects. Pharmacological studies revealed that they
increased liver protein and glycogen synthesis and antagonized liver injuries (29).

![Fig. 1. Chemical structure of DDB.](image)

DDB significantly suppressed the AFB₁-induced hepatic damage as evidenced by the
decrease of AFB₁-elevated serum marker enzymes; aspartate aminotransferase (AST)
and alanine aminotransferase (ALT) in rats (32,33). DDB protected rats against AFB₁
hepatotoxicity by increasing the detoxifying metabolism of AFB₁ in the liver. The
protective effect of DDB against chemical induced fatty liver in rats was also reported
(21). However, the use of DDB to suppress the harmful effect of aflatoxin in chickens
has not been investigated.

Therefore, the objective of the present study was to evaluate the efficacy of DDB to
counteract the deleterious toxic effect of AF in broiler chickens by evaluating its
effect on performance traits, behavioural expression, various biochemical and
haematological profiles, relative organ weights, pathological changes and immune
response.
MATERIALS AND METHODS

Animals and housing conditions:
A total of one hundred, 1-d-old Hubbard broiler chicks of both sexes were obtained from a commercial hatchery, and maintained at experimental heated pens under continuous lighting with feed and water available ad libitum from 1 to 14 d. At day 14, individually weighed chicks were randomly allotted into 4 treatment groups, of 25 chicks each, and housed in pens measuring (2.33 x 3.17 m, height 3.0 m; 3.4 bird/m²). The 4 dietary treatments were; 1) CONT: basal diet; 2) AF: basal diet plus 2.75 mg AF/kg diet; 3) DDB: basal diet plus 30 mg DDB/kg diet; 4) AF plus DDB: basal diet plus 2.75 mg AF/kg diet plus 30 mg DDB/kg diet.

The Chicks were fed a commercial corn-soyabean meal based broiler starter diet up to 28 d. containing 21% CP, 3,100 kcal/kg ME and finisher ration until slaughter at 42 d. containing 18% CP, 3,100 kcal/kg ME without added antibiotics, coccidiostats or growth promoters.

Aflatoxin production:
The AF was produced by fermentation of rice by Aspergillus parasiticus NRRL 2999 (obtained from The National Research Center, Cairo, Egypt) as described by Shotwell et al. (45) and modified by Demet et al. (4). Fermented rice was autoclaved, dried and ground to a powder and the AF content was measured by HPLC (44,49). Of the total AF content in the rice powder, 78.3% was AFB₁, 11.2% was AFG₁, 8.72% was AFB₂, and 1.78% was AFG₂. The rice powder was incorporated into the basal diet and confirmed by HPLC to provide the desired level of 2.75 mg/kg of diet. The basal diet did not contain detectable levels of AFB₁, AFB₂, AFG₁, or AFG₂ (detection limits <10 µg/kg).

Data collection:
Performance traits:
Individual body weight gain and group feed consumption were recorded at weekly intervals and mortality was recorded as it occurred.

Behavioural observations:
Behavioural observations started in week 4 and ended in week 6 of birds’ age. Birds in each pen were observed four times a week for a period of 20 min each, twice
in the morning (between 09:30 and 12:00 h) and twice in the afternoon (between 14:00 and 16:00 h). Data from morning and afternoon sessions were combined. Behavioural data were collected by one observer. All pens were observed in a different random order on each occasion. During the 20-min session, 15 min were directed for scan sampling observation where the behaviour of each bird was recorded once every 5 min from a single "on the beep" observation (34), according to one of 10 mutually exclusive categories: inactive behaviour patterns (sleeping, standing, sitting), feeding, drinking, walking, wing flapping, comfort activities (preening, body shaking, head scratching, wing-leg stretching, billwiping), dustbathing, foraging, abnormal behaviour (non-aggressive feather pecking; gentle, often repeated, without withdrawal by the recipient) and agonistic behaviour patterns (fleeing and threatening). Each scan of the 25 birds took 2 to 3 min to complete. The percentages of chickens engaged in each behaviour was calculated from the total number of birds observed during all scan samples in a pen. Because of the emphasis on feeding in this study, focal animal observation were carried out to obtain more detailed information. Focal animal observation commenced after end of the 15-min scan sampling of a pen and lasted for 5 min, where the frequencies of feeding bouts for 3 selected focal animals as well as bout durations were recorded. The frequencies of feeding bouts were calculated per bird per hour, and bout durations as the mean duration of feeding bout per bird.

**Immune function :**
The chicks were vaccinated against Newcastle Disease Virus (NDV) at 7 and 21 days of age by intraocular dropping. Individual blood samples were collected from the wing veins of 5 chickens per treatment, randomly selected at 24, 28, 31 days of age and serum concentrations of antibodies to VDV were determined by haemagglutination inhibition (HI) test.

**Relative organ weights and histopathology :**
At 42 d of birds' age, the experiment was terminated and 5 birds per treatment were slaughtered and selected internal organs (liver, kidney, heart, proventriculus and spleen) were excised and weighed. Organs weights were expressed as relative organ weights (grams of organ per 100 g of body weight). Samples from the liver and kidney of the 5 selected birds were taken for histopathological examination. Tissue samples were fixed in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin wax, sectioned at 4 µm, and stained with hematoxylin and eosin.
Serum biochemical and haematological analysis:

Blood samples were collected from the same birds and serum concentrations of total protein, albumin, uric acid, creatinine, as well as activities of alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined on a clinical chemistry analyzer according to the recommendation of the manufacturer's procedure. Red blood cell (RBC) and white blood cell (WBC) counts were determined by haemocytometer method using Natt-Herrik solution. Haemoglobin (Hb) amounts were determined by the cyanmethaemoglobin method.

Statistical analysis:

Data for all variables were subjected to two-way analyses of variance (ANOVA) to assess the effect of AF, DDB and their interaction using the general linear models procedure in the SPSS® statistical software (47). The means for treatments showing significant differences in the ANOVA were compared using Tukey HSD test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Data presented in table 1 show the effects of dietary treatments on performance traits including feed consumption (FC), body weight gain (BWG) and feed conversion ratio (FCR) of broiler chickens from 14 to 42 d of age. Compared to control, feeding AF alone significantly decreased FC and BWG and increased FCR. Addition of DDB to AF-containing diet, significantly improved the adverse effect of AF on FC, BWG and FCR. The overall FC was reduced by 15.33% in AF-fed chickens and by only 10.94% when DDB was added, compared to control. Similarly, overall BWG was significantly decreased in AF group by 26.62%, but by only 17.22% in AF plus DDB group. FCR was increased by 15.76% in AF-treated chickens, but by only 7.88% when DDB is added, compared to control. Addition of DDB to AF-free diet had no significant effect on BWG. However, DDB decreased overall FC by 4.37% and improved FCR by 7.88% when added to AF-free diet, compared to control.

Behavioural activities of the birds were significantly affected by feeding AF (table 2). Percentages of chicks recorded as feeding, foraging and in comfort behaviour activities significantly decreased, whereas inactive (sleeping, standing, sitting) and abnormal behaviour patterns (feather pecking) increased in AF-consuming chicks compared to control. Addition of DDB to AF-fed group significantly increased comfort behaviours and decreased inactive and abnormal
behaviours. When compared to control, DDB-alone group showed significantly lower percentages of inactive behaviour. However, there were no significant differences between control and DDB-alone group regarding the expression of other various behavioural activities. In the focal animal samples (table 3), frequency of feeding behaviour in AF-fed chickens was less than half that in control group (7.00±0.50 versus 16.33±1.53, P < 0.001) and significantly little time was spent in feeding bouts.

The effects of dietary treatment on serum biochemical values of broiler chickens at 42 d of age are presented in table 4. Feeding aflatoxin alone significantly decreased total protein, albumin, while increased uric acid, creatinine, ALT and AST concentrations. Adding DDB to AF-containing diet significantly improved the adverse effect of AF. However, the addition of DDB to AF-free diet did not significantly alter the biochemical values compared to control.

Table 5 shows the effect of treatment on haematological values of broiler chickens. Values of RBC counts and Hb decreased, whereas values of WBC counts increased in AF-consuming chickens compared to control. A significant improvement was observed in the AF-related changes in the haematological parameters when DDB was added to AF-fed chickens. No significant differences in the haematological parameters were found in DDB-alone group compared to the control chickens.

Intake of AF caused a significant increase in the relative weights of liver, kidney, heart, proventriculus, while decreased the relative weight of spleen compared to control (Table 6). These changes were significantly altered by DDB. However, relative organ weights were not significantly different among control, DDB-alone and AF plus DDB groups.

No histopathological changes were found in the livers and kidneys of the control chickens. Marked hyperaemic changes were found in the livers and kidneys of AF-fed chickens with focal mononuclear leucocytic inflammatory cells aggregation in the hepatic and intertubular tissues (fig. 2a, 3a). The portal area showed severe dilatation in the portal vein, coagulative necrosis in the epithelial cells lining the bile duct and inflammatory cell infiltration. The addition of DDB greatly reduced the severity of lesions in livers and kidneys of AF-consuming chickens (Fig. 2b, 3b). Addition of DDB to AF-free diet did not cause significant histologic changes compared to control.

The results of antibody titers against NDV are presented in table 7. Feeding AF significantly reduced the secondary immune response to NDV as measured by HI test at day 3, 7 and 10 post-immunization compared to control. DDB significantly increased HI titers in AF-fed chickens at day 7 and 10 post-immunization, while no
significant increase was detected at day 3. DDB-alone group showed no significant differences in antibody titers compared to control.

Table 1. Effect of DDB on feed consumption (FC), body weight gain (BWG) and feed conversion ratio (FCR) for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>DDB</th>
<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FC (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3-6</td>
<td>3481.6±0</td>
<td>3329.62±0</td>
<td>2947.7±0</td>
<td>3100.76±0</td>
</tr>
<tr>
<td>Week 3-6 (change from control%)</td>
<td>0±0</td>
<td>-437±0</td>
<td>-1533±0</td>
<td>-1094±0</td>
</tr>
<tr>
<td><strong>BWG (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3-6</td>
<td>1713±57.78a</td>
<td>1782±44.55a</td>
<td>1257±19.84b</td>
<td>1418±19.60c</td>
</tr>
<tr>
<td>Week 3-6 (change from control%)</td>
<td>0±0</td>
<td>+4.03±0</td>
<td>-26.62±0</td>
<td>-17.22±0</td>
</tr>
<tr>
<td><strong>FCR (g feed/g gain)</strong></td>
<td>2.03±0</td>
<td>1.87±0</td>
<td>2.35±0</td>
<td>2.19±0</td>
</tr>
<tr>
<td>Week 3-6</td>
<td></td>
<td>-7.88±0</td>
<td>+15.76±0</td>
<td>+7.88±0</td>
</tr>
<tr>
<td>Week 3-6 (change from control%)</td>
<td>0±0</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Values within rows with no common superscripts are significantly different (p<0.05), according to ANOVA.
Values represent the mean±SEM of 10 broiler chickens per treatment.

Table 2. Effect of DDB on the percentages of chickens engaged in different activities in scan samples for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>DDB</th>
<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inactive behaviour</strong></td>
<td>23.22±0.92a</td>
<td>15.34±0.75</td>
<td>42.09±0.80b</td>
<td>25.51±1.91c</td>
</tr>
<tr>
<td><strong>Feeding</strong></td>
<td>16.56±0.92a</td>
<td>15.98±0.88b</td>
<td>11.53±0.55b</td>
<td>14.39±0.66ab</td>
</tr>
<tr>
<td><strong>Foraging</strong></td>
<td>19.67±0.67ac</td>
<td>21.90±0.23a</td>
<td>11.75±1.12b</td>
<td>17.17±0.58c</td>
</tr>
<tr>
<td><strong>Comfort behaviour</strong></td>
<td>17.99±0.17a</td>
<td>20.84±0.15a</td>
<td>10.68±0.37b</td>
<td>18.19±1.00c</td>
</tr>
<tr>
<td><strong>Feather pecking</strong></td>
<td>0.11±0.10c</td>
<td>0.00±0.00c</td>
<td>1.28±0.32b</td>
<td>0.00±0.00c</td>
</tr>
</tbody>
</table>

*Values within rows with no common superscripts are significantly different (p<0.05), according to ANOVA.
Values represent mean±SEM of 3 pooled scan sample observations per treatment.
Table 3. Effect of DDB on the frequency of feeding behaviour per individual per hour and duration of feeding bouts (s) for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
<tr>
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<th>DDB</th>
<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding frequency (per individual per hour)</td>
<td>16.33±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.33±0.58&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>7.00±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00±1.32&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration of feeding bouts (s)</td>
<td>48.19±3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.51±2.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.22±2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.73±2.09&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Values within rows with no common superscripts are significantly different (<i>p</i>&lt;0.05), according to ANOVA.

Values represent mean ± SEM of 3 pooled focal sample observations per treatment.

Table 4. Effect of DDB on serum albumin (ALB), total protein (PROT), uric acid (UA), creatinine (CR), aspartate amino transferase (AST) and alanine amino transferase (ALT) for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
<tr>
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<th>DDB</th>
<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB (g/dl)</td>
<td>1.52±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>PROT (g/dl)</td>
<td>3.65±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.71±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51±0.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.64±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>5.33±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.10±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.06±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.63±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CR (mg/dl)</td>
<td>0.14±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>131.40±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.40±1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>185.00±2.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147.80±4.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>0.63±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.88±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Values within rows with no common superscripts are significantly different (<i>p</i>&lt;0.05), according to ANOVA.

Values represent mean±SEM of 5 broiler chickens per treatment.
Table 5. Effect of DDB on values of red blood cell (RBC) counts, haemoglobin (Hb) and white blood cell (WBC) counts for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
<tr>
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<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6 mm^3)</td>
<td>2.19±0.05a</td>
<td>2.16±0.05b</td>
<td>1.57±0.04b</td>
<td>1.98±0.02b</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.96±0.05a</td>
<td>8±0.05a</td>
<td>6.09±0.05b</td>
<td>6.74±0.09c</td>
</tr>
<tr>
<td>WBC (10^3 mm^3)</td>
<td>34.75±0.20a</td>
<td>34.47±0.26a</td>
<td>39.16±0.29b</td>
<td>37.25±0.26c</td>
</tr>
</tbody>
</table>

Values within rows with no common superscripts are significantly different (p<0.05), according to ANOVA. Values represent mean ± SEM of 5 broiler chickens per treatment.

Table 6. Effect of DDB on relative organ weights for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
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<th>AF</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.33±0.07a</td>
<td>2.36±0.04a</td>
<td>3.22±0.21b</td>
<td>2.57±0.03a</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.77±0.04a</td>
<td>0.79±0.02a</td>
<td>1.05±0.04b</td>
<td>0.84±0.04a</td>
</tr>
<tr>
<td>Heart</td>
<td>0.54±0.03a</td>
<td>0.51±0.02a</td>
<td>0.7±0.02</td>
<td>0.59±0.02</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>0.67±0.01a</td>
<td>0.65±0.02a</td>
<td>0.8±0.01b</td>
<td>0.71±0.02a</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.13±0.01a</td>
<td>0.12±0.01a</td>
<td>0.07±0.00</td>
<td>0.11±0.01</td>
</tr>
</tbody>
</table>

Values within rows with no common superscripts are significantly different (p<0.05), according to ANOVA. Values represent mean ± SEM of 5 broiler chickens per treatment.

Table 7. Effect of DDB on antibody titers; haemagglutination inhibition to Newcastle disease virus at 3, 7 and 10 d post-immunization for broiler chickens fed on diet containing 2.75 mg aflatoxins (AF)/kg feed at 14 to 42 d of age.

<table>
<thead>
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<th>AF</th>
<th>AF+DDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 Post-immunization</td>
<td>2.80±0.58a</td>
<td>3.20±0.37a</td>
<td>0.80±0.37a</td>
<td>1.00±0.32a</td>
</tr>
<tr>
<td>Day 7 Post-immunization</td>
<td>3.80±0.37a</td>
<td>4.20±0.58a</td>
<td>1.60±0.24b</td>
<td>3.20±0.20a</td>
</tr>
<tr>
<td>Day 10 Post-immunization</td>
<td>4.40±0.40bc</td>
<td>5.20±0.20a</td>
<td>2.20±0.20a</td>
<td>3.60±0.24a</td>
</tr>
</tbody>
</table>

Values within rows with no common superscripts are significantly different (p<0.05), according to ANOVA. Values represent mean ± SEM of 5 broiler chickens per treatment.
Fig. 2: Liver. (A) AF-treated group: Severe dilatation in the portal vein, coagulative necrosis of the epithelial cells lining the bile duct and mononuclear leucocytic inflammatory cells infiltration in the portal area, (B) AF plus DDB group: Hyperaemic central vein and sinusoids.

Fig. 3: Kidney. (A) AF-treated group: Hyperaemia in the blood vessels and capillaries with focal mononuclear leucocytic inflammatory cells aggregation in the intertubular tissue, (B) AF plus DDB group: Hyperaemic blood vessels and capillaries in between the renal tubules.
DIscussion

AF losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (19). Aflatoxicosis is primarily a hepatic disease causing liver damage (27). AF are considered unavoidable contaminants of feed, even where good manufacturing practices have been followed. Therefore, the use of agents which antagonise the potent toxic effects of AF have therapeutic and economic importance. A new approach to the detoxification of AF is the addition of antihepatitis drug, DDB. Clinical studies in rats have revealed the protective effect of DDB against AF–induced damage (33). Moreover, enhanced effectiveness of DDB against chemicals –induced hepatic injury were also reported (21,53).

Several measurements have been used in our study in order to determine whether DDB could protect against the toxic effects of AF in broiler chickens. The profile comprises measurements of performance, behavioural changes, biochemical and haematological alterations, pathological changes and immune function.

The minimum concentration of AF needed to cause adverse effects on the performance of broiler chickens is 2.7 mg/kg feed (5), very close to the concentration used in our study. The most prevalent and important effect of aflatoxicosis is poor body weight gain which greatly affects profit in poultry industry. In the present study, feeding 2.75 mg/kg feed significantly decreased BWG and FC in broiler chickens from 14 to 42 d. of age compared with the control. AF had also a significant adverse effect on FCR. These findings are in accordance with Kubena et al. (23), and Oguz and Kurtoglu (35) who reported similar results in broiler chicks given 2.5 mg AF/ kg diet for 3 weeks. Our results also agree with other reports on experimental aflatoxicosis in quails (38,42). Such adverse effects of AF on FC, BWG and FCR may be attributed to anorexia, listlessness, impaired general health and the inhibitory effects of AF on protein synthesis and lipogenesis (19,22).

DDB is a compound isolated from Shizandra fruits, one of the most important herbs in traditional Chinese medicine. Addition of 30 mg DDB/kg diet to the AF-containing diet significantly ameliorated the reported adverse effect of AF on FC, BWG and FCR in broiler chickens. These improvements may be referred to the promoting action of DDB to enhance the over all well-being and vitality. There have been numerous reports on Shizandra extracts’ ability to increase efficiency, help in digestion and regulate gastric acid release (31).
Feeding DDB alone to the chickens decreased FC by 4.37% and improved BWG and FCR by 4.03% and 7.88%, respectively compared to control chickens. However, these improvements were not statistically significant.

Research on the use of behaviour as a mean of assessing welfare should receive much emphasis. Behavioural studies were interested in the effect of AF on neural disturbance (52) and motor coordination (20) in rats but not in poultry. To our knowledge, this study is the first to investigate the effect of AF on the development of different behavioural activities in broiler chickens. As we expected, feeding AF to chickens significantly altered the expression of various behaviours. AF-fed chickens spent relatively little time on feeding which is consistent with the adverse effect exerted by AF on performance traits in our study. AF significantly increased percentages of inactive chickens (sleeping, standing and sitting) and decreased expression of comfort activities and foraging behaviour. These findings are in agreement with the AF-induced listlessness and general depression reported in previous studies (19). Higher percentages of feather pecking, an abnormal behaviour pattern in poultry, were observed in birds fed on AF. Feather pecking has been previously reported to be associated with stress (9). Decreased comfort activities with increased expression of abnormal behaviour in AF-fed chickens imply that these dietary treatment was the least appropriate for birds. Feeding DDB significantly improved the adverse effect of AF on behaviour of broiler chickens. The most interesting finding was that feeding DDB alone significantly reduced percentages of inactive birds. This may be explained by the tonic effect of DDB enhancing the birds activities.

Biochemical analyses revealed significant decreases in serum total protein and albumin of AF-fed chickens. Our results are in agreement with previous studies (7,19,27,36,41). Decreases in total protein and albumin are frequent findings in chickens with aflatoxicosis that could be attributable to inhibition of protein synthesis in the liver as reflected in impaired growth in our study. It has been reported that aflatoxins block RNA synthesis and inhibit protein synthesis in liver (12, 24,50). In the present study, concentrations of ALT and AST significantly increased in AF-fed group as previously reported by Shukia and Pachauri (46) indicating cellular damage of the liver. This hepatic alterations lead to increased cellular permeability resulting in release of enzymes into the serum. Serum uric acid and creatinine were also significantly increased by AF. These results are consistent with the findings of Santurio et al. (43), reflecting impaired kidney function. Addition of DDB to AF-
containing diet provided a significant improvement in all biochemical characters. These findings support the previous results where DDB significantly suppressed AFB₁-induced hepatic damage as evidenced by the decrease of AFB₁-elevated key liver enzymes levels; ALT and AST in rats (32). It has been reported that DDB protected rats against AFB₁ hepatotoxicity by increasing the detoxifying metabolism of AFB₁ in the liver. Treatment with DDB was shown to slightly increase the level of AFM₁, the less toxic metabolite (33). Moreover, it was found that DDB would affect the wall of hepatocytes through dual hepatocellular protective action, the first one is through antioxidant like effect acting as a scavenger for the free radicals, the second action is through the potentiation of the cell wall fluidity to stand by the intra and extra cellular injurious agents.

Various studies have reported that Hb and RBC counts were decreased by aflatoxicosis in poultry (3,18,19,36) reflecting the depressing effect of AF on haemopoietic tissue. This decrease in the haematological values has been reported to be related to the AF-induced inhibition of protein synthesis (24). Our results are in consistence with the previous studies. In the present study, there was a significant increase in WBC count in AF-fed chickens suggesting that AF is inducing inflammatory response in the chickens (19). A significant improvement of all haematological parameters was observed when DDB is added to AF-consuming chickens. These improvements may be due to the reported function of DDB to support protein synthesis in liver by regulating hepatic cell metabolism (21).

Increases in the relative weights of liver, kidney, heart, proventriculus of AF-fed chickens in the present study have been previously reported (24,27,37). In contrary to Edrington et al. (7, 8) and Ortatatli and Oguz (37) a significant decrease in relative spleen weight was observed in our study as shown by Stoev et al. (48). The AF-induced increase in relative liver weights may be attributed to an accumulation of lipids in liver, producing the characteristic enlarged, friable fatty livers associated with aflatoxicosis in chickens (16,24). The decrease in relative spleen weights in AF-fed group may be a consequence of degenerative changes and decreased lymphoid tissue (48). Addition of DDB to AF-containing diet resulted in organs weights similar to those of controls. In our study, the histopathological changes observed in livers and kidneys of AF-fed chickens confirm the results of previous AF studies (14,27,37). Addition of DDB to AF diet significantly improved the AF-associated pathology in livers and kidneys. The improvements achieved by DDB indicates the ability of DDB to ameliorate the AF-impairment effect in protein, carbohydrate and lipid metabolism. Feeding AF to chickens, caused a depressed immune response to NDV vaccine. These
results are consistent with those reported in previous studies (10, 17,30). DDB was significantly effective in reducing the immunosuppressive effect of AF as measured by HI titers, confirming the reported ability of DDB to promote the immune function (31).

The significant interaction between AF and DDB found in the present study to be associated with most of the measured parameters could be best described as antagonistic. This interaction indicates the protective effect of DDB against the toxic effects of AF in broiler chickens.

REFERENCES


تأثیر الحبة الصفراء (دى -دى -دى -دى) فی بداری التسمین علی فطر الافلاتوكسین

هبة الليثى 1 و هشام الزوربة 2

قسم الصحة والرعاية البيطریة، كلیة الطب البيطری، جامعة القاهرة


إضافة فطر الافلاتوكسین وكذلك الحبة الصفراء (دى–دى–دى–دى) على حدي أو مجتمعان إلى عطیة الطیور وذلك بتتكز 2.75 و 30 مجم / كجم عطیة على التوالي.

و كانت نتائج الدراسة كالتالي: 1- كانت تغذیة الطیور على عطیة الحبة الصفراء على فطر الافلاتوكسین مفردة لها تأثیراً ضاراً ملحوظاً. أدى الافلاتوكسین إلى خفض كميات العلف المستهلكة وكذلك معدلات الزيادة في الوزن بنسبة 15.33%.

2- أد ت تأثیرة الحبة الصفراء (دى–دى–دى–دى) على عطیة المجموعة الضابطة إلى فطر الافلاتوكسین واستفادة جميع القيادات الإنتاجية السابقة في بداری التسمین. 3- أد ت تأثیرة الحبة الصفراء بموازنة إلى عطیة الطیور إلى تعیین معدل التحول الغذائي بنسبة 7.88%.

4- كانت نسبة سلوكیات الأکل، الاستکشاف والراحة أقل حدوناً في المجموعة المغذاة على فطر الافلاتوكسین.

5- أد ت تأثیرة الافلاتوكسین بمفرداء إلى زيادة نسبة الطیور المشاركة في سلوكіات الخمول (نوم -وقوف -جلوس).

6- واد ت نتائج الدراسة في الأمام السلوکیة الفی بی طبیعة (نقر الريش).

7- واد ت تأثیرة الحبة الصفراء إلى عطیة المجموعة الضابطة على فطر الافلاتوكسین.

8- أد ت تأثیرة الافلاتوكسین على خفض حبیة الدم الحمراء، العيموجلوبي بی ماده قیمة الدم البارال.

9- أد ت أيضا الافلاتوكسین إلى خفض قیم خلايا الدم الحمراء، الهیموجلوبي بينما زادت قیم خلايا الدم البيضاء.
10- أدت إضافة الحبة الصفراء إلى خفض التأثير الضار لفطر الافلاتوكسین بصورة ملموسة بينما لم يحدث أي تغير ملموسة في الخصائص البيوكيميائية وكذلك صورة الدم لبداري التسمين عند إضافة دي – بي بمفردها على طيور.), 11- أدت تغذية الطيور على فطر الافلاتوكسین إلى زيادة الأوزان النسبية للأعضاء الداخلية المختلفة مثل الكبد، الكلي، القلب بينما انخفضت الأوزان النسبية للطحال كما وُجدت تغيرات هستوبيولوجية في الكبد وكلي الطيور المغذاة على فطر الافلاتوكسین. 12- أدت إضافة الحبة الصفراء إلى خفض شدة الإساءة في الطيور المغذاة على الفطر بينما لم تحدث إضافة الحبة الصفراء بمفردها أي تأثير مقارنة بالمجموعة الضابطة. 13- أدت تغذية الطيور على فطر الافلاتوكسین بمفردهة إلى خفض مستويات الأجسام المناعية الثانوية المضادة للفاح مرض النيوكامل. 14- كان لإضافة الحبة الصفراء تأثيراً ملموسة في تحسين حالة المناعية المنخفضة لدى الطيور المغذاة على فطر الافلاتوكسین. 15- لم تحدث إضافة الحبة الصفراء بمفردها تغيرات هامة في مستويات الأجسام المناعية مقارنة بالمجموعة الضابطة. أوضحت هذه النتائج التأثير النفاذ للحبة الصفراء (دي – بي) لتحسين التأثير الضار الناتج من تغذية بداري التسمين على فطر الافلاتوكسین.