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Research Article

Immunological, Histopathological and Biochemical Protective Effect of Date Pits (*Phoenix dacrylifera* seeds) Feed Additive Against Aflatoxicated Broiler Chickens

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ABSTRACT

Aflatoxin (AF) is a potent immunotoxin enhancing the incidence and impacts of avian diseases as viral and bacterial pathogens increasing the economic losses attributed to AF exposure. Nowadays, researchers found that different feed additives had the ability to relieve aflatoxicosis, but few researches are carried out on incorporation of date seed powder into the diet for poultry production. So, the present work aimed to study the immunological, histopathological and biochemical protective role of date pits (*phoenix dactylifera* seeds) against aflatoxicosis in broilers. Two hundred and ten-one-day old Arbor Acres broiler chicks were devided into 7 equal groups as the first control (G1) that supplemented by the basal diet, the second (G2) had the basal diet with date pits supplementation 2% (DPS2%) group, the third DPS4% group (G3) fed on the basal diet with date pits 4%, in G4, Aflatoxin (AF) alone was fed a basal diet containing 100 ppb aflatoxin. G5 (AF+HSCAS), fed on a basal diet containing Hydrated Sodium Calcium Aluminum Silicates (HSCAS) 0.3% plus aflatoxin, G6 (AF+DPS2%) fed a basal diet containing date pits 2% plus aflatoxin, and finally G7 (AF+DPS4%) fed a basal diet containing date pits 4% plus aflatoxin. The aflatoxin supplemented to the broiler ration from day one to the end of experiment at 35 days. Aflatoxins supplementation significantly decreased serum proteins and reduced antibody titers against NDV, additionally induced histopathological changes in the lymphoid organs whereas exhibited lymphoid depletion with widening of medulla on the expanse of cortex in bursa of Fabricius (BF) and atrophy of splenic nodule with sever depletion of lymphocytes in white pulp of spleen. However, addition of date pits (2, 4%) and HSCAS (0.3%) to broiler's diet partially ameliorated these hazardous effects of aflatoxins immunologically and histopathologically. Addition of DPS (2 and 4%) gave a better results regarding serum proteins, antibody titers against NDV and histopathological examination of lymphoid organs (BF and spleen) overall compared to HSCAS concluding that date pits could be used as an effective feed additive to control aflatoxicosis in poultry avoiding harmful effect of chemical mycotoxin binders (HSCAS).

Key words: Aflatoxins; Date pits; Antibody titers against NDV; Serum proteins; Histopathological changes; Broilers.

INTRODUCTION

Mycotoxins are naturally occurring toxic contaminants in feeds. They are secondary metabolites which produced by filamentous fungi under appropriate temperature and humidity (Nielsen *et al*., 2009). Cereal grains infected by mycotoxogenic fungi pre- or post-harvest (Glenn, 2007).

Reddy *et al.* (2008) published a review in which they had summarized the major mycotoxins, including aflatoxins (AFs), fumonisins, ochratoxin A (OTA), deoxynivlenol, and zearalenone (ZEN), which had been detected in rice from different countries. Amongst the mycotoxins, aflatoxins are the most intensively sought because of their immunotoxicity which acting on phagocytes and cell-mediated immunity (Bondy and Pestka, 2000).

Aflatoxins have constituted a great threat to the health of animals and humans due to their teratogenic, carcinogenic, mutagenic, and immunosuppressive effects (Yunus *et al*., 2011), and economic losses associated with aflatoxin exposure in broilers include poor growth and

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feed conversion, increased mortality, leg problems, and carcass condemnations. Aflatoxins cause a wide range of metabolic changes in poultry and are associated with liver damage, reduced digestive enzyme activities, and immunosuppression (Edds and Bortell, 1983).

Today there are a variety of commercial adsorbent available in the market in the form of feed additives to control these naturally occurring poisons (Pasha *et al*., 2007). The addition of hydrated sodium calcium aluminosilicates (HSCAS) to AFB1 contaminated diets is demonstrated to greatly reduce the bioavailability of aflatoxin in the gastrointestinal tract (Phillips, 1999). No adsorbent is approved by the Food and Drug Administration for the prevention or treatment of aflatoxicosis (Bintvihok *et al*., 2002).

Because of the concerns about the side effects of conventional medicine, the use of natural medicinal plants as an alternative to conventional treatment of various diseases can be on the rise in the last few decades. A larger number of these plants and their isolated constituents showed beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects (Dattner, 2003). Among the promising medicinal plants, Date pits (*Phoenix dactylifera L*.) is playing an important role in the economic and social life of the people in the date producing countries (Basuni and AL-Marzooq, 2010).

Therefore, the potential uses of date pit seeds (DPS) in different industries are promising especially with their availability at a very low cost (Golshan *et al*., 2017). A large number of date seeds can be obtained from the date industries or the waste products annually. Date seeds are containing high levels of valuable bioactive compounds as gluco-which improves the nutritive value of this product along with its dietary natural antioxidant content (Hoerr, 1997, Al–Farsi and Lee, 2011 and Sharifi *et al*., 2017).

Date pit seed contains 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, 0.9–1.8% ash and 22.5–80.2% dietary fiber. Also, it contains high levels of phenolics (3102– 4430 mg gallic acid equivalents/ 100 g), antioxidants and dietary fiber (78–80 g/100 g) (Al-Farsi *et al*., 2007). Date pit seed powder is also used for addition to animal, poultry and fish feed as it was reported to enhance growth, improve feed efficiency and also meat palatability in animals (Al-Farsi and Lee, 2011).

Many studies have been carried out on DPS in Egypt focusing mainly on their chemical composition but, lacking the effects of date seeds on aflatoxicosis in poultry farms. Therefore, the effective use of date pits and their biochemical immunological and histopathological protective role in reducing the effects of aflatoxicosis in broiler have been investigated in the current experiment through evaluation of antibody titers against NDV, serum proteins and histopathological changes of lymphoid organs in comparison to HSCAS (0.3%) supplementation in feed.

MATERIALS AND METHODS

Production of aflatoxin

Aspergillus parasiticus NRRL - 2999 pure culture was used for aflatoxin production (National Research Centre, Cairo, Egypt) via fermentation of rice using the method of Shotwell *et al*. (1966).

Feed additives (Mycotoxin binders)

a. Phoenix dactylifera seeds collection and preparation

Purchased from a local dates pitting factory, washed, air-dried and finally coarse powdered and supplemented at percentage of 2% and 4% to chicken diets according to each treatment.

b. Hydrated sodium and calcium aluminum Silicates (HSCAS)

The experimental used adsorbent was HSCAS (a commercial mycotoxin adsorbent Toxi-Mold Plus®) obtained from Egyco-Vet Company. It was well mixed with basal diet through a dose of 3 kg per ton for their bird groups.

Experimental design

The present study is affirmed by the Ethics of Animal Experiments Committee, Damanhour University, Egypt. Briefly, two hundred and ten 1-day-old unsexed Arbor Acres broiler chicks were purchased from a local commercial hatchery (NASCO Egypt, Alexandria) and randomly allocated into 7 equal groups at the first day of age. Each one group was subdivided into three replicates (10 birds per replicate) and floor reared. Feed (starter type from 1st to 21st days and grower type from 22nd to 35th days). and water were supplied *ad-libitum* for 35 days of age and optimum managemental factors were applied regarding ventilation, temperature, lighting and litter management all over the experimental period.

Furthermore, the birds were vaccinated for ND & IBV at 5th day using (Polimun® ND Clon 124 + IB H120-BioTestlab Vasilkov, Ukraine). Gumbro intermediate (Bursine 2® vaccine, Zoetis, Parsippany, CA, USA) and ND (Nobilis® ND LaSota, Intervet, Netherlands) both at 12th and 20th days via eye drop.

The diet composition was formulated according to the recommendation of National Research Council Nutrient Requirements for Arbor Acres broiler chickens NRC (1994) prepared without any feed additives rather than the compounds under study.

Seven chicken groups were used as follows: the first group (G1) fed on a commercial broiler diets without supplement (control); G2 (DPS2%) fed on the basal diet with date pits 2% supplementation, G3 (DPS4%) fed on the basal diet with date pits 4%, G4 (AF) had aflatoxin as 100µg /kg feed (100 ppb), G5 (AF+HSCAS) fed a basal diet containing HSCAS 0.3% plus aflatoxin, G6 (AF+DPS2%) had a basal diet containing date pits 2% plus aflatoxin, and finally G7 (AF+DPS4%) had a basal diet containing date pits 4% plus aflatoxin. Also, the basal diets were tested for possible residual AF before feeding and there were no detectable levels present. A recorded daily observation for health problems and mortality were carried out all over 35 days of age.

Serum sampling

a. Hemagglutination inhibition test

The blood samples were collected from wing vein by venous puncture at 2nd, 3rd, 4th and 5th weeks by using a sterile syringe in clean dry non-coated tubes. Each blood sample was left to coagulate at room temperature and centrifuged at 3000 rpm for 5 minutes and the clear serum was collected individually in sterile Eppendorf tubes.

Serum samples were used for detection of antibody titers against Newcastle viruses were measured using Hemagglutination Inhibition (HI) method described by Sever (1962) using LaSota antigen with 4 HA units.

b. Serum proteins

The collected sera were subjected to determination of total protein, albumin following the instructions enclosed in the manufactured kits produced by Biodiagnostic Company, Egypt. Also, serum globulin levels were calculated by subtraction of albumin value from the total protein value of the same sample **(Coles, 1986)** and albumin / globulin ratios of the same samples were calculated by subdividing the value of the serum albumin on the value of serum globulin of the same sample.

Histopathological examination of lymphoid organs

At the end of experiment on day 35, three chickens from each group were euthanized, bursa of Fabricius and spleen samples were obtained and submitted for histopathology to evaluate lesions and abnormalities. Fixation of samples was applied in 10 % buffered formalin solution for one week. Blocks and staining were carried out according to (Drury and Wallington, 1980).

Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means ($P \leq 0.05$) by SPSS.20® (IBM Cooperation, Armonk, NY, USA).

RESULTS

Determination of geometric means of antibody titers against NDV in broilers

The data obtained in Table (1) revealed that the geometric means of antibody titer against Newcastle disease virus (NDV) in broilers were non significantly ($P \geq 0.05$) affected in 2, 3, 4 and 5 weeks among different experimental groups. But at 5th week, antibody titer against NDV was increased numerically following DPS supplementation in G2 and G3 (as 6 and 5.4 $\log_2$) when compared to control G1 (5.2 $\log_2$). The highest titer was observed in G2 as 6.0 $\log_2$ and the lowest titer which was observed in the aflatoxicated non treated G4 as 4.2 $\log_2$. Also, antibody titer against NDV was increased numerically in aflatoxicated and treated groups G5, G6 and G7 as 5.6, 5 and 4.4 $\log_2$, respectively when compared to aflatoxicated non treated G4.

Serum proteins

The total protein, albumin, globulin and A/G ratio of broilers were non-significantly ($P \geq 0.05$) affected at 2nd, 3rd and 4th week. At the 5th week, the total protein and globulin levels were decreased significantly ($P \leq 0.05$) and serum albumin levels decreased non-significantly ($P \geq 0.05$) in G4 (AF) and in all aflatoxicated and treated groups (G5, G6 & G7) when compared to control, while serum albumin levels were increased non-significantly ($P \geq 0.05$) in G2 & G3 when compared to control (Table 2).

The total protein, albumin and globulin levels were increased numerically in G5, G6 and G7 when compared to group treated with AF alone (G4), despite in these 3 groups, there are no significant differences between each other. A/G ratio levels had no significant ($P \geq 0.05$) changes at 5th week.

Histopathological examination of bursa of fabricius and spleen

The noticeable lesion of BF of birds in G1, G2 and G3 was normal histological structure (Fig. 1, 2 & 3, resp.), while the lesion of BF of birds in G4 was lymphoid depletion and widening of medulla on the expanse of cortex (Fig. 4). The examined BF of birds in G5 showed mild lymphoid depletion (Fig. 5), while, that of birds in G6 showed relatively normal histological structure (Fig. 6). Additionally, the BF of birds in G7 showed mild lymphoid depletion (Fig. 7).

The examined spleen of birds in G1, G2 and G3 showed normal histological structure (Fig. 8, 9 & 10, resp.), while the spleen of birds in G4 showed atrophy of splenic nodule and sever depletion of lymphocytes in white pulp (Fig. 11.A & 11.B, resp.). The spleen of birds in G5 and G6 showed normal histological structure (Fig. 12 &13, resp.), while the spleen of birds in G7 showed atrophy of splenic nodule (Fig. 14).

Finally, no significant toxic microscopic lesions and normal histology were evident in spleen and bursa of fabricius sections of birds in G1 (control) or G2, G3 fed DPS 2% and 4% respectively and all these 3 groups had no mortalities.

DISCUSSION

The crucial component of the immune system and one of the first lines of defense against foreign invaders is the natural antibody and complement production which can also provide information on the changes in the innate immune system during aflatoxicosis (Cotter, 2012). So,

Table 1: Experimental results regarding antibody titers against Newcastle disease virus (NDV) by Heamagglutination inhibition test (HI) for all broiler groups at 2nd, 3rd, 4th and 5th week.

Group	2nd week	3rd week	4th week	5th week
G1 (Control)	3.2±0.49[a]	4.0±0.31[a]	4.4±0.51[a]	5.2±0.66[a]
G2 (DPS2%)	3.4±0.51[a]	4.4±0.51[a]	5.2±0.66[a]	6.0±0.31[a]
G3 (DPS4%)	3.8±0.66[a]	4.2±0.37[a]	4.6±0.60[a]	5.4±0.74[a]
G4 (AF)	3.2±0.37[a]	3.6±0.67[a]	4.0±0.54[a]	4.2±0.66[a]
G5 (AF+HSCAS)	3.6±0.24[a]	4.8±0.20[a]	5.0±0.44[a]	5.6±0.51[a]
G6 (AF+DPS2%)	3.4±0.40[a]	3.8±0.20[a]	4.2±0.80[a]	5.0±0.54[a]
G7 (AF+DPS4%)	3.0±0.63[a]	3.8±0.49[a]	4.1±0.44[a]	4.4±0.92[a]

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). Means within the same column under the same category carry different superscripts are significantly different ($P \leq 0.05$). Values are expressed as means ± SE.

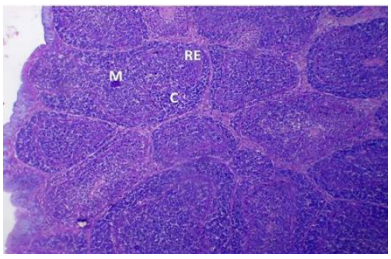

Fig. 1: Bursa of Fabricius of a chicken of G1 showing normal histological structure. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

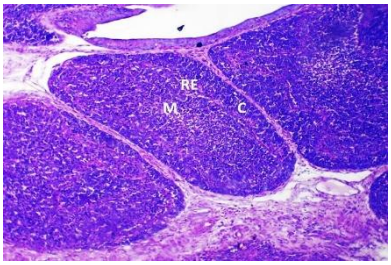

Fig. 2: Bursa of Fabricius of a chicken of G2 s Showing normal histological structure. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

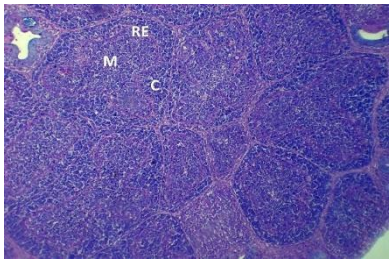

Fig. 3: Bursa of Fabricius of a chicken of G3 showing normal histological structure. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

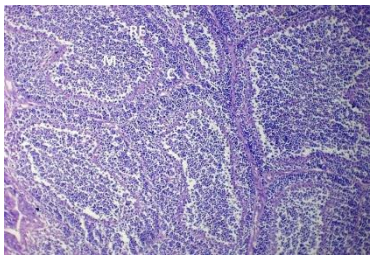

Fig. 4: Bursa of Fabricius of a chicken of G4 showing lymphoid depletion and widening of medulla on the expanse of cortex. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

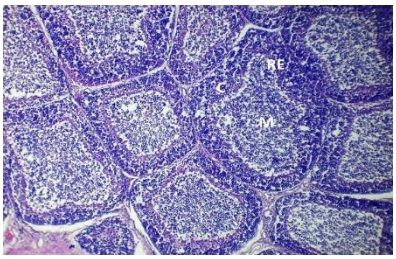

Fig. 5: Bursa of Fabricius of a chicken of G5 showing mild lymphoid depletion. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

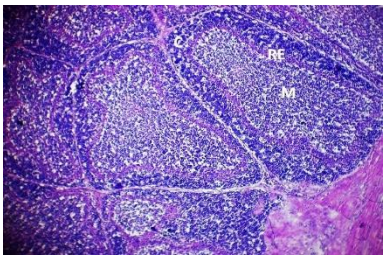

Fig. 6: Bursa of Fabricius of a chicken of G6 showing relatively normal structure. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

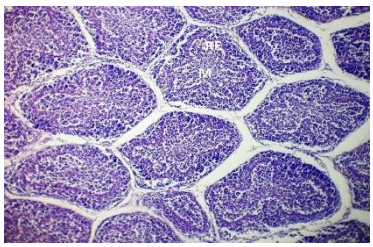

Fig. 7: Bursa of Fabricius of a chicken of G7 showing mild lymphoid depletion. (M: medulla C: cortex RE: reticulo-endothelium). H&E. (x160).

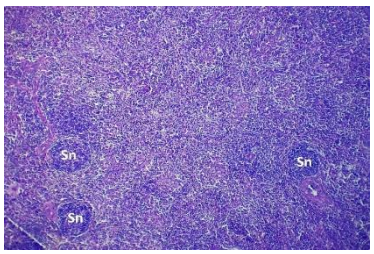

Fig. 8: Spleen of a chicken of G1 showing normal histological structure. (Sn: splenic nodules). H&E. (x160).

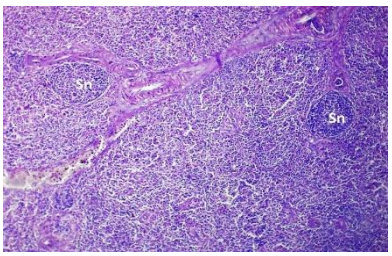

Fig. 9: Spleen of a chicken of G2 showing normal histological structure. (Sn: splenic nodules). H&E. (x160).

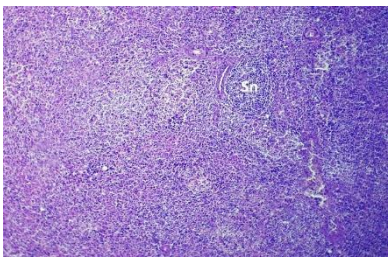

Fig. 10: Spleen of a chicken of G3 showing normal histological structure. (Sn: splenic nodules). H&E. (x160).

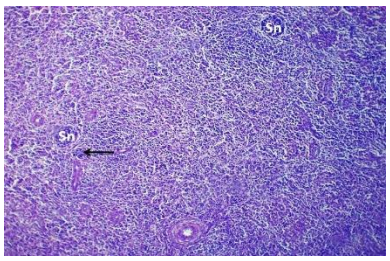

Fig. 11.A: Spleen of a chicken of G4 showing atrophy of splenic nodule (arrow). (Sn: splenic nodules). H&E. (x160).

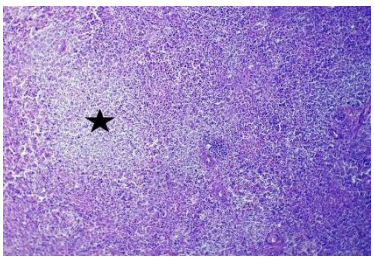

Fig. 11.B: Spleen of a chicken of G4 showing severe depletion of lymphocytes in white pulp (star). H&E. (x160).

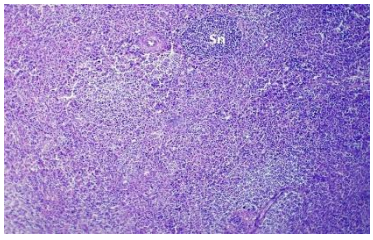

Fig. 12: Spleen of a chicken of G5 showing normal histological structure. (Sn: splenic nodules). H&E. (x160).

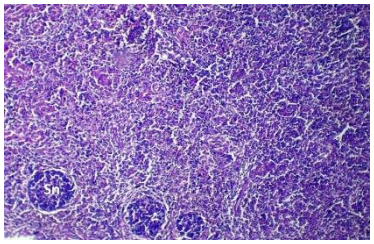

Fig. 13: Spleen of a chicken of G6 showing normal histological structure. (Sn: splenic nodules). H&E. (x160).

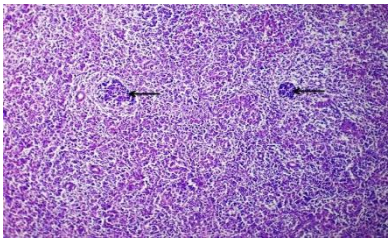

Fig. 14: Spleen of a chicken of G7 showing atrophy of splenic nodule (arrows). (Sn: splenic nodules). H&E. (x160).

Table 2: Experimental results regarding serum total protein (g/dl), albumin (g/dl), globulin (g/dl) and albumin / globulin ratio for all broiler groups at 2nd, 3rd, 4th and 5th week.

ITEM	Group	2nd Week	3rd Week	4th Week	5th Week
Total Protein (g/dl)	G1 (Control)	2.92±0.26^{a}	2.92±0.03^{a}	2.93±0.03^{a}	2.95±0.11^{a}
	G2 (DPS2%)	2.93±0.26^{a}	2.94±0.01^{a}	2.96±0.16^{a}	2.99±0.13^{a}
	G3 (DPS4%)	2.92±0.23^{a}	2.94±0.02^{a}	2.95±0.13^{a}	2.98±0.16^{a}
	G4 (AF)	2.91±0.23^{a}	2.88±0.05^{a}	2.72±0.12^{a}	2.26±0.10^{b}
	G5 (AF+HSCAS)	2.92±0.15^{a}	2.92±0.04^{a}	2.80±0.08^{a}	2.42±0.12^{b}
	G6 (AF+DPS2%)	2.92±0.15^{a}	2.91±0.15^{a}	2.82±0.06^{a}	2.48±0.11^{b}
	G7 (AF+DPS4%)	2.92±0.22^{a}	2.90±0.12^{a}	2.78±0.06^{a}	2.36±0.17^{b}
Albumin (g/dl)	G1 (Control)	0.33±0.02^{a}	0.32±0.03^{a}	0.33±0.04^{a}	0.33±0.04^{abc}
	G2 (DPS2%)	0.32±0.03^{a}	0.34±0.05^{a}	0.37±0.04^{a}	0.39±0.06^{a}
	G3 (DPS4%)	0.31±0.03^{a}	0.33±0.04^{a}	0.35±0.03^{a}	0.37±0.04^{b}
	G4 (AF)	0.32±0.05^{a}	0.29±0.06^{a}	0.25±0.04^{a}	0.21±0.04^{c}
	G5 (AF+HSCAS)	0.32±0.03^{a}	0.32±0.04^{a}	0.28±0.02^{a}	0.24±0.04^{bc}
	G6 (AF+DPS2%)	0.32±0.05^{a}	0.32±0.04^{a}	0.29±0.05^{a}	0.26±0.04^{bc}
	G7 (AF+DPS4%)	0.32±0.03^{a}	0.31±0.02^{a}	0.27±0.03^{a}	0.22±0.03^{c}
Globulin (g/dl)	G1 (Control)	2.59±0.27^{a}	2.60±0.03^{a}	2.60±0.09^{a}	2.62±0.15^{a}
	G2 (DPS2%)	2.61±0.24^{a}	2.60±0.04^{a}	2.59±0.20^{a}	2.60±0.14^{a}
	G3 (DPS4%)	2.61±0.21^{a}	2.61±0.05^{a}	2.59±0.14^{a}	2.61±0.19^{a}
	G4 (AF)	2.59±0.18^{a}	2.59±0.10^{a}	2.47±0.16^{a}	2.05±0.08^{b}
	G5 (AF+HSCAS)	2.60±0.14^{a}	2.60±0.08^{a}	2.52±0.07^{a}	2.18±0.10^{b}
	G6 (AF+DPS2%)	2.60±0.16^{a}	2.59±0.16^{a}	2.53±0.07^{a}	2.22±0.13^{b}
	G7 (AF+DPS4%)	2.60±0.24^{a}	2.59±0.11^{a}	2.51±0.05^{a}	2.14±0.14^{b}
A / G ratio	G1 (Control)	0.12±0.01^{a}	0.12±0.01^{a}	0.13±0.01^{a}	0.12±0.02^{a}
	G2 (DPS2%)	0.12±0.01^{a}	0.13±0.02^{a}	0.14±0.02^{a}	0.15±0.02^{a}
	G3 (DPS4%)	0.11±0.01^{a}	0.12±0.01^{a}	0.13±0.01^{a}	0.14±0.02^{a}
	G4 (AF)	0.12±0.01^{a}	0.11±0.02^{a}	0.10±0.02^{a}	0.10±0.02^{a}
	G5 (AF+HSCAS)	0.12±0.01^{a}	0.12±0.02^{a}	0.11±0.02^{a}	0.11±0.01^{a}
	G6 (AF+DPS2%)	0.12±0.02^{a}	0.12±0.02^{a}	0.11±0.02^{a}	0.11±0.02^{a}
	G7 (AF+DPS4%)	0.12±0.02^{a}	0.11±0.01^{a}	0.10±0.01^{a}	0.10±0.01^{a}

G: group. DPS: date pits (2 or 4%). AF: aflatoxin (100 µg/kg feed). HSCAS: hydrated sodium calcium aluminosilicate (0.3%). Means within the same column under the same category carry different superscripts are significantly different (P≤0.05). Values are expressed as means ± SE.

the geometric mean of antibody titers against NDV was an important parameter to be measured in this study and it didn't significantly (P≥0.05) affected among different experimental broiler groups at 2nd, 3rd and 4th week. But at 5th week, antibody titer against NDV was decreased numerically showing the worst titer as 4.2 $\log_2$ in G4 (AF) when compared to negative control (G1) as 5.2 $\log_2$, indicating that aflatoxin severely affected the immune response of the birds and reduce the titer of NDV. Some studies found that AFB1 can similarly diminish innate and adaptive humoral immune capabilities and antibody titers are often reduced, whether measured as total serum levels of IgA, IgG and IgM (Chen *et al*., 2014a), or as production of specific antibodies in response to sheep red blood cells (Verma *et al*., 2004) or as exposure response to infectious bronchitis virus, infectious bursal disease virus and Newcastle disease virus vaccines as AFB1 can impair the effectiveness of vaccination and reduce antibodies production against these diseases (Azzam and Gabal, 1998; Oguz *et al*., 2003).

Also, from the obtained results at 5th week, we noticed that supplementation of broilers with ration containing DPS at levels 2% and 4% improved broiler immunity producing non-significant numerically higher antibody titers against NDV in G2 as 6.0 $\log_2$ and G3 as 5.4 $\log_2$ than negative control G1 as 5.2 $\log_2$, with the highest titer was observed in G2. At the same week, NDV antibody titers were increased numerically in the aflatoxicated and treated groups (G6 and G7) as 5 and 4.4 $\log_2$ respectively, when compared the aflatoxicated non treated G4, indicating that date pits supplementation non-significant increased HI-titer of chicks against NDV. El-Far *et al*. (2016) explained a substantial enhancement of immunity, and antioxidant status by DPS supplementation (without aflatoxin) in broiler that might be related to the antioxidant and immune-stimulant constituents of *Phoenix dactylifera* seeds and the authors found that at 21st day, the birds fed diets containing DP at levels 2 and 4% had a significant antibody titer against NDV, respectively, when compared with control one; also on the 42nd day the DPS2, DPS4, and DPS6 showed a significant antibody titer against NDV this higher titers may be related to the prolonged experimental period for 42 days rather than 35 days in our experiment.

Additionally, at 5th week, antibody titer against NDV was increased numerically with showing the second highest titer in G5 (HSCAS+AF) as 5.6 $\log_2$ when compared to the aflatoxicated non treated G4 as 4.2 $\log_2$, indicating that addition of HSCAS to broiler's diet was effective in ameliorating the bad effect of AF on the HI-titer in chicks vaccinated against ND. Also, Pasha *et al*. (2007) reported the titer against ND was severely decreased after addition of AF and improved by inclusion of 0.5% simple sodium bentonite. While, Chen *et al*. (2014b) who reported the supplementation of HSCAS did not affect the serum natural antibody or the complement system.

Histopathologically, the effect of AF on lymphoid tissues could be also explained, whereas bursa of Fabricius (BF) of birds in G4 (AF) showed lymphoid depletion and widening of medulla on the expanse of cortex, while the spleen showed atrophy of splenic nodule

and sever depletion of lymphocytes in white pulp. These results were agreed with previous studies in which aflatoxin lead to severe depletion of lymphoid tissue such as BF and spleen (Ali, 2014).

Inclusion of DPS to diet contain 100 ppb AF as in G6 and G7 reduce the severity of pathological changes in BF and spleen and it seems that the effect of mannanoligosacharides (MOS) in DPS on AF toxicity not only related to the binding capacity with AF but also these MOS have the ability to prevent colonization of opportunistic bacterial pathogens in the gastrointestinal tract (Olsen, 1995). Also, Vayalil (2002) showed that the induced protection against aflatoxicosis occurred via decreased the level of liver enzyme activity as well as decreased the free radical propagation.

However, the addition of HSCAS to the aflatoxicated diet in G5 couldn't fully improve the organ, s functions as there were mild lymphoid depletion in BF which indicated that HSCAS didn't completely protect broilers against aflatoxicosis but partially ameliorated its effect which may be attributed to the dose of HSCAS as mentioned previously by Neeff *et al.* (2013), who reported that HSCAS didn't completely protect broilers against aflatoxicosis, but was effective in reducing aflatoxin residues in liver and kidney of chicks fed 2.5 mg of AFB1/kg of diet from 0-21 days. Also, Phillips (1999), said that the protective effect of HSCAS resulted from the rapid binding capacity of HSCAS to aflatoxins in the gastrointestinal tract of chickens, thus preventing its absorption and normal distribution to the liver. This effect could be increased through higher HSCAS dose supplemented in feed. This agreed with Pasha *et al.* (2007), who reported that the mortality increased significantly in broilers to 40% with the addition of 100 mcg/kg AF and was restored to 16.6%, with the dietary inclusion of 0.5% simple sodium bentonite.

Serum biochemical measures can often help diagnose aflatoxicosis even before the evidence of decreased performance; for instance, decreased serum protein concentration is a dependable biomarker of hepatotoxicity induced by AF (Kececi *et al*., 1998). In the current study, serum proteins are more affected, whereas at the 5th week, the total protein (TP) and globulin levels were decreased significantly ($P \leq 0.05$) and serum albumin (ALB) levels decreased non significantly ($P \geq 0.05$) in G4 (AF) when compared to control G1 indicating the chronic cumulative effect of aflatoxins. The reduction in protein levels during aflatoxicosis could be attributed to the inactivation of biosynthetic enzymes of protein synthesis and impairment of amino acid transport in liver due to liver/kidney disorder in which protein is not digested or absorbed properly and as a result of altering protein metabolism and DNA production, prevention of the replication and transcription of RNA occurs.

These results agreed with Kubena *et al*. (1990), Zhao *et al*. (2010), Liu *et al*. (2016) and Raja *et al*. (2017) explaining that the reduction in serum TP, ALB, and globulin, as indicators of protein synthesis, were observed in chicks consuming diets containing AFB1. AFB1 adducts with biomolecules causing damage to hepatocytes that impairs metabolic functions of the liver during AFB1 exposure which reduced TP levels as the liver is responsible for production of most circulating proteins. Also, Priyadarshni and Narasareddy (2010) attributed the reduced globulins to the depressant effect of AF on protein synthesis. Moreover, disease states affect the relative changes in albumin and globulins in different ways and a low A/G ratio may reflect overproduction of globulins or under production of albumin. The A/G ratio in the present study was not significantly ($P \geq 0.05$) different among all the groups.

At 5th week of age, date pits supplementation to aflatoxicated diet increased numerically the levels of total protein, albumin and A/G ratio in G6 (DPS2%+AF) when compared to G4 which treated with AF alone. The mechanism for the protective effect of date seeds powder against AF toxicity seems to involve the sequestration of the toxin in the gastrointestinal tract with subsequent elimination of the toxin. Also, Raju and Devegowda (2000) observed a 25.4% reduction in TP of broilers fed AF and grown until d 35, whereas addition of esterified glucomannan was able to reduce this variable to 14.7%. While Orabi and Shawky (2014) reported that date seed caused a significant decrease of TP without significant difference in albumin between date seed supplemented and control groups.

Moreover, the addition of HSCAS to aflatoxicated diet in G5 could increase the levels of total protein, albumin and A/G ratio numerically compared to G4 at 5th week, indicating supplementation of HSCAS can reduce the toxic effect of AF. The improvement effect of alluminosilicates against aflatoxin in terms of serum biochemical profile has been previously reported by Kubena *et al*. (1990), Zhao *et al*. (2010) and Chen *et al*., 2014b who concluded that these adsorbents partially restored the alterations in serum chemistry associated with AFB1 contamination whereas a higher addition levels were more effective than a lower level at restoring ALB, TP, and globulin.

Conclusions

Addition of date pits (2, 4%) to broiler's diet induced a partial protective effect against aflatoxicosis and this protection is dose-related as 2% supplementation gave better protection than the higher dose 4%. Also, incorporation of DPS in broiler chicken's diet increased the antibody titers against NDV, significantly decreased serum total protein and globulin and ameliorated the histopathological changes in bursa of Fabricius and spleen caused by AF. Date pits can be an attractive alternative for aflatoxin binders, since they are efficient, applicable and cheap avoiding the harmful chemical mycotoxin binders causing appreciable losses in nutritive value and palatability. Concomitantly, further studies specially with lower doses of date pits targeting the spectrum of activity of date pits regarding other types of mycotoxins should be applied.

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