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EFFICACY OF SOME NATURAL AND BIOLOGICAL FEED ADDITIVES IN CONTROLLING DUCKLING AFLATOXICOSIS

BY

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ABSTRACT

In a completely randomized design, 360 one day-old white pekin ducklings were assigned to twelve treatments with two replicates of 15 birds for each replicate to study and to evaluate the efficacy of Betoms[®] (20%), papaya dry fruit (1g/kg), Ginger root (10g/kg) and Liquorice (1g/kg) individually and in combined with each other to counteract the toxic effects of aflatoxin (AF) at a level of 0.5mg/kg ration on growth performance and serum biochemistry in white pekin ducklings. On a weekly basis duckling weight, growth performance, enzymatic activity (ALT, AST, ALP), creatinine and total protein were measured and data were subjected to statistical analysis. Mortalities were recorded on daily basis. Treatment of aflatoxicated birds with any one of the used feed additives have some diminished inhibitory effects to AF adverse effects on signs, growth performance and mortalities. All dietary treatments of aflatoxicated birds induced some improvements on organs weights ratio and biochemical parameters when compared with untreated positive aflatoxin control group. Combination of all dietary treatments at the same ratio did not result in any improvements in parameters affected by aflatoxin.

In conclusion, our results showed that dietary supplementation of Betmos, Papaya, Ginger or Liquorice may reduce the adverse effects of AF on white Pekin ducklings.

Key words: Aflatoxin, Betmos[®], Performance, Serum biochemistry, ducklings.

INTRODUCTION

Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *penicillium puberulum* (Wilson and Payne, 1994).

Aflatoxins are hepatotoxic and hepatocarcinogenic agents for several animal species, especially young poultry (Shank, 1981; Angsubhakorn, 1983 a,b and Bintivihok et al. , 1987 a,b). Furthermore, aflatoxins were found to cause high mortality, reduced growth rate, increase feed conversion, suppress immunity and increase susceptibility of birds to various infectious agents (Mahmoud and Korshom, 1996 and Abu El-ela, 2013).

Among the different potential decontaminating microorganisms is *saccharomyces cerevisiae* (Hassan et al., 2012). Estrified glucomannan is a toxin binder extracted from cell wall of *saccharomyces cerevisiae* that has a large surface area containing pores of different sizes to trap a wide range of mycotoxins (Cole, 1999). There are several feed additives have been reported to be of some value in reducing the toxicity of aflatoxin such as ginger (Vimala et al., 1999 and Abdelhamid et al., 2002), liquorice (Al-daraji , 2012) and papaya fruit extracts (Hassan et al. , 2013).

Liquorice (*Glycyrrhiza glabra*) is a tropical Asian and Mediterranean plant (Sharma and Agrawal, 2013) and it was reported to have antiviral, anticancer, antioxidant, Immune-stimulant and hypotensive hepato-protective activities (Zore, 2005 and Sahu and Vaghela, 2011).

Ginger (*Zingiber officinale*) is a well-known plant that widely used as a specie and medical treatment for certain ailments in traditional medicine (Mohd-Yusof et al., 2002 and Tapsell et al., 2006). It has anticarcinogenic, antioxidant and anti-inflammatory activities (RamaKrishnan, 2013).

Papaya fruit (*Carica papaya* L) belongs to family *caricaceae* has been used as a remedy against a variety of diseases (Mello et al., 2008 and Munoz et al., 2000). Papaya have a biological activities as immunostimulating and antioxidant activity (Aruoma et al., 2006), treating activities for healing wounds and burns (Mahmood et al., 2005) and bacteriostatic activity (Osato et al., 1993).

The present study was designed and conducted to investigate and evaluate the efficacy of some biological feed additive as Betmos (Estrified Glucomannan) and Natural feed

additives including papaya fruit powder, liquorice and ginger root powder either individually or in combination to counteract the adverse toxic effects of aflatoxin in white Pekin ducklings.

MATERIALS AND METHODS

Ducklings and dietary treatments:

Three hundred and sixty (one-day-old) white Pekin ducklings obtained from a commercial hatchery, individually weighed and randomly distributed into 12 treatments with two replicates of 15 birds for each replicate. Ducklings were fed basal diet obtained from EL-Barka Company with continuous Lighting program. Feed and water were available *ad libitum* until day 21 of age.

Aflatoxin production

Aflatoxin production was performed by inoculation of *Aspergillus parasiticus* (NRRL 2999) on rice as described by Shotwell et al. (1966). Moldy rice was autoclaved, dried and ground to fine powder. Aflatoxin levels in rice powder were measured by HPLC method in the mycotoxins central lab and food safety of the National Research center. Milled rice was added to the basal diet to provide the level of 0.5mg Aflatoxin /Kg diet.

The dietary treatments were:

First treatment used as negative control fed on basal ration containing neither aflatoxin nor treatment , second treatment used as positive control (diet containing AF at 0.5mg /Kg) , third treatment used as basal diet containing 1gm /Kg Betmos, without AF , fourth treatment used as diet containing AF at 0.5mg /Kg plus 1gm /Kg Betmos , fifth treatment used as basal diet containing 1gm /Kg papaya fruit powder , six treatment used as diet containing AF at 0.5mg/Kg plus 1gm /Kg papaya fruit powder , seventh treatment used as basal diet containing 10gm /Kg Ginger powder , eighth treatment used as diet containing AF at 0.5mg /Kg plus 10gm /Kg Ginger powder , ninth treatment used as basal diet containing 1gm /Kg Liquorice , tenth treatment used as diet containing AF at 0.5mg/Kg plus 1gm /Kg Liquorice , eleventh treatment used as basal diet containing concomitant Betmos , Papaya , Ginger and Liquorice at the recommended doses. Twelfth treatment used as Diet containing AF at 0.5mg /Kg plus concomitant Betmos, Papaya, Ginger and Liquorice at the recommended doses.

Protective additives:

- Betmos (Mannan oligo saccharides and Beta glucan) supplied by Biomedical Pharmaceutical Industries.
- Papaya fruit powder from local market.
- Ginger root powder from local market.
- Liquorice powder from local market.

Symptoms, mortalities and gross lesions:

Birds in all groups were observed daily. Mortalities were recorded and dead ducklings were exposed to postmortem examination for AF lesion.

Performance and organ weights:

Body weight was recorded at days 1, 7, 14 and 21 of age and feed consumption were recorded daily, body weight gain and feed conversion ratio were calculated. At day 21 of age, 4 birds of each group were randomly chosen and sacrificed and relative organ weights were calculated.

Blood collection and biochemical assay:

Individual blood samples were collected from jugular vein at day 7, 14 and 21 of age. Sera were separated and frozen (-20C°) for biochemical assays. Serum total Protein, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) were measured using commercial kits.

- AST and ALT were determined according to the method of Reitman and Frankel (1957).
- Alkaline phosphatase was determined according to the method of Berth and Delangh (2004).
- Total protein was determined according to Weichselbaum (1946) and Gornal et al., (1949).
- Creatinine was determined according to Bartels et al., (1971) and Popper et al., (1937).

Statistical analysis

Data were statistically analyzed by SPSS, 1994. All statements of significance were based on the 0.05 level of probability.

RESULTS

Mycotoxicosis is condition associated with fungal contamination of feed ingredients and the intake of harmful fungal metabolites. Aflatoxins are the most predominant and major fungal toxins among mycotoxins which attributed to induction of potential problems in livestock (Nilipour, 2002).

No clinical signs were seen in negative controls all over the experimental periods. Aflatoxicated ducks (Group 2) suffered from depression, anorexia, lameness, abnormal vocalization, feather picking and purple discoloration of legs that was less pronounced in aflatoxin-treated groups (Group 4, 6, 8, 10 and 12).

Aflatoxin gross lesions were more obvious in sacrificed duck at day 21 of age and the most sever ones appear in positive control (aflatoxin treated non-additive group). Lesions were enlarged pale or yellowish friable liver with lobular pattern and distended tan color gall bladder, enlarged and pale kidneys, and enlarged and mottled spleen.

Aflatoxin administration (0.5mg/kg ration) to one day old ducklings for 21 days produced 40% mortalities that started at day 13 of age and peaked on the 3rd weeks of age (Table 1). On mortality basis ginger addition to aflatoxin contaminated ration appeared to be the most protective anti-aflatoxin among the tested materials. 6.7% higher mortalities occurred in ducklings fed aflatoxicated ration treated with either papaya or Betmos than those treated with ginger. Liquorice seems to be the least active modulators to the toxic effects of aflatoxin among the tested ones. Surprisingly the combination of ginger, papaya, Betmos and Liquorice at the same levels of its individual usage do not have any advantage in counteracting the adverse effect of aflatoxin and is much worst in protection than the individual usage of such materials.

Body weights (table2) were significantly reduced by aflatoxin (500ppb) addition in group (2) when compared to control non medicated group (G1). This significant reduction started by the end of week 1 post feeding aflatoxicated diet &in awards. All feed additives used with aflatoxicated diets failed to decrease the toxic effect of aflatoxin on body weights except papaya and Betmos where they significantly diminished the decrease in body weights caused by AF by week2&3 respectively.

All single feed additives (papaya, ginger, Liquorice) addition to normal ration significantly improves body weights of birds in comparison with negative control group (G1). Group fed ration with no aflatoxin additive but included with all natural feed additives

(papaya, ginger and Liquorice) do not have any improvement in body weight all over the experimental period than negative control group.

The lowest weight gain, feed intake and the highest FCR (table 3) were found in the positive control group by week 2, 1, 3, and inwards respectively. Addition of any of all feed additives to aflatoxin contaminated diet did not significantly improve weight gain and feed intake all over the experimental periods.

Although feed conversion ratio was insignificantly increased by AF in the first and second weeks, yet it was increased significantly in the third week. There was no significant impact of different feed additives on FCR during 1st and 2nd weeks of the experiment except Betmos in group (4). Betmos addition to aflatoxicated ration significantly improves FCR when compared to AF-positive control group all over the experimental periods. In week 3 FCR improved significantly ($p < 0.05$) by all feed additives either individually or mixed.

Relative liver weights (Table 4) significantly increased by feeding 500ppb aflatoxin for 21days but not the relative organ weights of spleen, bursa of fabricious and thymus. Relative organ weights of liver, Spleen, Bursa and Thymus did not significantly improved by addition of feed additives individually or mixed.

In non aflatoxin supplemented groups there was no significant difference in relative organ weights between different treated groups.

Effects of different treatments on blood biochemical parameters are shown in table (5). Aflatoxin had significant effects on serum components, increasing AST and ALT levels in 1st and 2nd weeks but increased ALT level in the 3rd week. It significantly increased ALP level in 2nd and 3rd weeks.

Total protein (TP) was significantly ($p < 0.05$) decreased by aflatoxin administration in 2nd and 3rd weeks of the experiment while creatinine level was insignificantly ($p > 0.05$) increased in weeks 2, 3.

Addition of all feed additives individually or combined was insignificantly improve liver enzymes (AST and ALT) all over the experimental periods except papaya addition to aflatoxicated birds significantly improve levels of AST and ALT in the 2nd week and ALT in the 1st week when compared with AF-positive control group (G2). But all additives significantly reduced ALP level in the 2nd and 3rd weeks of the experiment in comparison with aflatoxin-treated group.

Addition of any of the feed additives in non aflatoxin supplemented groups were not significantly ($p > 0.05$) affect AST and ALT levels all over the experiment except papaya

addition in group (5) significantly reduce AST level in the 2nd week. There was no significant difference in creatinine level between all groups of the experiment.

TP insignificantly improved ($p>0.05$) by addition of Betmos, Papaya, Ginger, Liquorice individually or mixed with each other to aflatoxin contaminated diet when compared to aflatoxin positive control group (G2) all over the experimental periods.

Table (1): Effects of various additives and aflatoxin on mortality of ducklings:

Groups	Total number of birds	No of dead birds	Mortality percent%
Control negative	30	0	0
Control positive(AF)	30	12	40%
Betmos control	30	0	0
AF+Betmos	30	6	20%
Papaya control	30	0	0
AF+Papaya	30	6	20%
Ginger control	30	0	0
AF+Ginger	30	4	13.33%
Liquorice control	30	0	0
AF+Liquorice	30	8	26.66%
All additives control	30	0	0
AF+All additives	30	9	30%

Table (2): Effects of various additives and aflatoxin on body weights of birds: (Mean± SE)

Groups	Body weights			
	Day 1	Day 7	Day 14	Day 21
Control negative	56.3±1.47 ^a	183.8±5.23 ^{cd}	367.3±9.52 ^d	691.0±19.05 ^e
Control positive(AF)	63.0±1.89 ^{abc}	169.3±4.23 ^{ef}	281.6±9.22 ^f	401.9±20.008 ^f
Betmos control	62.5±1.77 ^{abcd}	214.0±5.002 ^{ab}	398.7±10.74 ^{bc}	713.5±17.62 ^{bc}
AF+Betmos	61.2±1.70 ^{abcd}	180.3±4.55 ^{de}	319.3±8.18 ^e	469.2±10.28 ^d
Papaya control	58.0±1.43 ^{de}	224.7±4.36 ^a	429.7±10.96 ^a	763.8±12.66 ^a
AF+Papaya	61.5±1.15 ^{abcd}	178.8±3.38 ^{de}	309.8±10.54 ^{ef}	455.6±13.80 ^{de}
Ginger control	61.0±1.27 ^{abcde}	212.0±3.41 ^{ab}	425.3±9.60 ^{ab}	739.8±19.03 ^{ab}
AF+Ginger	58.7±1.33 ^{cde}	160.3±3.87 ^f	284.3±8.23 ^f	428.6±13.58 ^{def}
Liquorice control	64.7±1.55 ^a	211.2±5.12 ^b	442.7±10.36 ^a	759.5±15.61 ^a
AF+Liquorice	59.2±1.24 ^{bcdde}	168.2±3.70 ^{ef}	285.5±8.82 ^f	425.0±14.02 ^{def}
All additives control	58.2±1.27 ^{cde}	194.5±4.05 ^c	373.5±11.52 ^{cd}	687.8±15.50 ^c
AF+All additives	63.7±1.77 ^{ab}	168.5±4.89 ^{ef}	281.0±6.72 ^f	417.1±17.78 ^{ef}

Means in the same column with different subscript differ significantly ($P \leq 0.05$).

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Table (3): Effects of various additives and aflatoxin on feed consumption, body weight gain and feed conversion ratio. (Mean± SE)

Groups	1st week			2nd week			3rd week		
	Feed intake(gm)	Weight gain (gm)	FCR	Feed intake(gm)	Weight gain (gm)	FCR	Feed intake(gm)	Weight gain (gm)	FCR
Control negative	199.66 ± 12.12 ^{cd}	127.5 ± 19.83 ^{abc}	1.57 ± 0.04 ^{cd}	397.66 ± 13.85 ^{bc}	183.5 ± 15.17 ^b	2.19 ± 0.17 ^{cd}	673.66 ± 1.15 ^a	323.6 ± 7.66 ^a	2.07 ± 0.03 ^d
Control positive(AF)	177.66 ± 0.96 ^d	106.3 ± 6.67 ^c	1.67 ± 0.06 ^a	272.73 ± 7.73 ^d	112.5 ± 12.19 ^a	2.43 ± 0.08 ^a	399.29 ± 13.40 ^b	132.4 ± 14.83 ^b	3.02 ± 0.09 ^a
Biomos control	222.99 ± 2.11 ^{ab}	6.15 ^{cd} ± 151.5	1.46 ± 0.02 ^{bc}	393.33 ± 3.85 ^a	184.6 ± 4 ^b	2.13 ± 0.005 ^{bc}	668.16 ± 0.50 ^a	314.8 ± 8.17 ^a	2.12 ± 0.02 ^d
AF+Biomos	175.50 ± 0.86 ^d	119.1 ± 4.16 ^{abc}	1.47 ± 0.03 ^{bc}	298.68 ± 1.16 ^d	139.02 ± 6.68 ^a	2.15 ± 0.05 ^b	363.75 ± 3.46 ^{bc}	149.5 ± 4.36 ^b	2.43 ± 0.06 ^a
Papaya control	231.83 ± 3.56 ^a	166.6 ± 14 ^a	1.39 ± 0.04 ^a	483.50 ± 1.44 ^{ab}	205 ± 0.0 ^{cd}	2.11 ± 0.005 ^{bc}	703.33 ± 2.11 ^a	334.1 ± 9.16 ^a	2.10 ± 0.04 ^d
AF+papaya	194.33 ± 0.76 ^{ab}	117.3 ± 1 ^{ab}	1.65 ± 0.0 ^a	285.91 ± 3.32 ^d	130.6 ± 7.33 ^a	2.19 ± 0.04 ^{cd}	365.74 ± 9.38 ^{bc}	146.1 ± 4.7 ^b	2.49 ± 0.02 ^a
Ginger control	210.16 ± 0.28 ^{bc}	151.0 ± 1 ^{ab}	1.38 ± 0.003 ^a	455.50 ± 7.79 ^a	213.3 ± 17 ^{ab}	2.14 ± 0.06 ^{bc}	688.16 ± 6.06 ^a	314.5 ± 8.17 ^a	2.18 ± 0.01 ^d
AF+ginger	167.33 ± 0.57 ^f	101.6 ± 8.33 ^a	1.65 ± 0.07 ^a	275.86 ± 3.15 ^d	123.8 ± 13.84 ^a	2.24 ± 0.11 ^{cd}	348.83 ± 6.37 ^a	144.5 ± 3.76 ^b	2.41 ± 0.08 ^a
Liquorice control	211.33 ± 4.42 ^{ab}	146.5 ± 1.83 ^{abc}	1.49 ± 0.02 ^{bc}	441.16 ± 11.45 ^a	231.5 ± 8.5 ^a	1.90 ± 0.01 ^a	677.49 ± 4.13 ^a	316.8 ± 9.83 ^a	2.13 ± 0.02 ^d
AF+liquorice	179.49 ± 1.82 ^{cd}	109.0 ± 5.66 ^{ab}	1.64 ± 0.03 ^a	270.70 ± 0.17 ^d	117.3 ± 6.66 ^a	2.31 ± 0.07 ^{cd}	377.12 ± 20.89 ^{bc}	139.5 ± 0.96 ^b	2.70 ± 0.13 ^b
All additives control	202.33 ± 3.65 ^{bc}	136.3 ± 0.66 ^{abcd}	1.48 ± 0.02 ^{bc}	401.33 ± 15.39 ^{bc}	179 ± 22.33 ^b	2.25 ± 0.07 ^{cd}	684.33 ± 0.96 ^a	314.3 ± 12.33 ^a	2.18 ± 0.04 ^d
AF+all additives	172.16 ± 0.28 ^f	104.8 ± 8.50 ^a	1.65 ± 0.07 ^a	264.46 ± 0.69 ^d	112.2 ± 0.06 ^a	2.35 ± 0.005 ^{cd}	389.22 ± 9.74 ^{bc}	136.9 ± 5.19 ^b	2.83 ± 0.008 ^b

Means in the same column with different subscript differ significantly (P<0.05).

Table (4): Effect of various additives and aflatoxin on relative organs weights of sacrificed ducklings at 21 days of age: (Mean± SE)

Group	Relative organs weight(gm)			
	Liver wt	Spleen wt	Bursa wt	Thymus wt
Control negative	6.06 ± 0.71 ^c	0.24 ± 0.03 ^a	0.19 ± 0.01 ^a	0.34 ± 0.03 ^a
Control positive(AF)	8.25 ± 0.36 ^a	0.41 ± 0.10 ^a	0.11 ± 0.01 ^a	0.26 ± 0.02 ^a
Betmos control	6.75 ± 0.37 ^{bc}	0.30 ± 0.008 ^a	0.21 ± 0.005 ^a	0.32 ± 0.008 ^a
AF+Betmos	7.50 ± 0.31 ^{abc}	0.34 ± 0.018 ^a	0.20 ± 0.02 ^a	0.30 ± 0.01 ^a
Papaya control	6.82 ± 0.23 ^{abc}	0.28 ± 0.01 ^a	0.22 ± 0.01 ^a	0.31 ± 0.005 ^a
AF+papaya	7.33 ± 0.36 ^{abc}	0.33 ± 0.02 ^a	0.22 ± 0.03 ^a	0.32 ± 0.011 ^a
Ginger control	6.20 ± 0.67 ^c	0.26 ± 0.02 ^a	0.20 ± 0.008 ^a	0.33 ± 0.02 ^a
AF+ginger	7.62 ± 0.37 ^{abc}	0.36 ± 0.03 ^a	0.19 ± 0.02 ^a	0.30 ± 0.01 ^a
Liquorice control	6.67 ± 0.62 ^{bc}	0.33 ± 0.02 ^a	0.22 ± 0.03 ^a	0.31 ± 0.02 ^a
AF+liquorice	7.89 ± 0.34 ^{ab}	0.37 ± 0.02 ^a	0.17 ± 0.01 ^a	0.32 ± 0.01 ^a
All additives control	6.81 ± 0.22 ^{abc}	0.29 ± 0.01 ^a	0.19 ± 0.02 ^a	0.35 ± 0.02 ^a
AF+all additives	8.01 ± 0.26 ^{ab}	0.38 ± 0.01 ^a	0.14 ± 0.02 ^a	0.28 ± 0.02 ^a

Means in the same column with different subscript differ significantly (P<0.05).

Table (5): Effects of various additives and aflatoxin on serum biochemical parameters: (Mean± SE)

Group	1 st week						2 nd week						3 rd week								
	ALT(U/L)	AST(U/L)	ALP(U/L)	CR(mg/dl)	TP(g/dl)	ALU(U/L)	AST(U/L)	ALP(U/L)	CR(mg/dl)	TP(g/dl)	ALU(U/L)	AST(U/L)	ALP(U/L)	CR(mg/dl)	TP(g/dl)	ALU(U/L)	AST(U/L)	ALP(U/L)	CR(mg/dl)	TP(g/dl)	
Control negative	8.73 ± 1.3 ^a	12.1 ± 83.6 ^a	1705.0 ± 77.3 ^a	0.35 ± 0.05 ^a	3.40 ± 0.39 ^{abcd}	22.84 ± 0.5 ^{abcd}	27.94 ± 3.1 ^{abcd}	836.0 ± 175.6 ^f	0.37 ± 0.06 ^a	4.02 ± 0.66 ^a	15.13 ± 1.4 ^a	39.44 ± 2.7 ^a	1150.4 ± 39.2 ^{abcd}	0.40 ± 0.04 ^a	5.21 ± 0.27 ^a						
Control positive(AF)	21.45 ± 1.1 ^a	48.07 ± 3.9 ^a	2339.5 ± 191.9 ^a	0.55 ± 0.06 ^a	2.20 ± 0.30 ^a	32.77 ± 4.1 ^a	38.13 ± 2.8 ^a	1881.0 ± 96.9 ^a	0.44 ± 0.08 ^a	1.62 ± 0.20 ^a	33.18 ± 5.1 ^a	40.99 ± 2.7 ^a	1530.8 ± 72.1 ^a	0.50 ± 0.06 ^a	1.12 ± 0.19 ^a						
Beesmas control	11.36 ± 0.9 ^a	13.11 ± 3.2 ^a	1429.2 ± 171.2 ^a	0.30 ± 0.15 ^a	3.75 ± 0.34 ^{abcd}	19.21 ± 1.9 ^{ab}	29.00 ± 2.2 ^{abcd}	1012.8 ± 61.2 ^{cd}	0.32 ± 0.05 ^a	5.07 ± 0.88 ^a	15.50 ± 2.4 ^b	37.84 ± 2.9 ^a	904.25 ± 14.2 ^f	0.43 ± 0.10 ^a	5.52 ± 0.45 ^{ab}						
AF+Beesmas	19.61 ± 1.3 ^{ab}	45.96 ± 2.8 ^a	1031 ± 265.8 ^a	0.26 ± 0.05 ^a	2.75 ± 0.65 ^{abcd}	28.83 ± 1.7 ^{ab}	35.64 ± 3.2 ^a	1492.2 ± 115.9 ^{bc}	0.36 ± 0.05 ^a	2.14 ± 0.46 ^a	30.29 ± 1.7 ^a	40.94 ± 3.9 ^a	1150 ± 64.2 ^{bc}	0.48 ± 0.04 ^a	1.33 ± 0.17 ^a						
Papaya control	7.99 ± 0.5 ^a	11.29 ± 1.6 ^a	1688.5 ± 265.8 ^a	0.28 ± 0.08 ^a	4.66 ± 0.35 ^a	18.78 ± 1.5 ^a	18.29 ± 1.6 ^a	967.05 ± 89.8 ^d	0.33 ± 0.05 ^a	5.04 ± 0.22 ^a	14.34 ± 1.1 ^b	35.83 ± 1.6 ^a	717.95 ± 27.8 ^a	0.40 ± 0.04 ^a	6.11 ± 0.11 ^a						
AF+Papaya	16.5 ± 21.4 ^b	30.23 ± 3.7 ^a	1990.6 ± 284.4 ^a	0.24 ± 0.02 ^a	2.34 ± 0.30 ^{abcd}	22.84 ± 2.3 ^{abcd}	29.40 ± 1.3 ^{abcd}	1386.0 ± 80.0 ^{bc}	0.34 ± 0.02 ^a	2.38 ± 0.24 ^a	26.38 ± 4.5 ^a	35.19 ± 3.9 ^a	1022.6 ± 36.0 ^{bc}	0.47 ± 0.04 ^a	1.87 ± 0.29 ^a						
Ginger control	9.96 ± 1.7 ^a	12.53 ± 2.5 ^a	1658 ± 57.9 ^a	0.34 ± 0.09 ^a	4.0 ± 7.45 ^{ab}	20.80 ± 0.8 ^{ab}	25.15 ± 3.0 ^{abcd}	1257.8 ± 63.8 ^{cd}	0.37 ± 0.04 ^a	5.37 ± 0.74 ^a	12.42 ± 2.3 ^b	35.23 ± 1.8 ^a	928.40 ± 21.8 ^{cd}	0.43 ± 0.03 ^a	5.17 ± 0.49 ^b						
AF+Ginger	20.66 ± 1.3 ^{ab}	45.75 ± 3.9 ^a	2086.4 ± 277.2 ^a	0.29 ± 0.05 ^a	2.48 ± 0.40 ^{abcd}	26.18 ± 3.9 ^{abcd}	31.15 ± 2.1 ^{abcd}	1552.0 ± 104.6 ^{cd}	0.36 ± 0.02 ^a	2.19 ± 0.29 ^a	28.25 ± 1.4 ^a	38.13 ± 2.7 ^a	1128.8 ± 44.2 ^{abcd}	0.50 ± 0.06 ^a	1.15 ± 0.12 ^a						
Liquorice control	8.84 ± 0.9 ^a	9.40 ± 0.8 ^a	1722.3 ± 278.3 ^a	0.33 ± 0.07 ^a	3.75 ± 0.21 ^{abcd}	20.58 ± 1.9 ^{ab}	23.26 ± 3.2 ^{abcd}	1312.4 ± 51.4 ^{cd}	0.34 ± 0.03 ^a	4.34 ± 0.50 ^a	14.07 ± 2.3 ^b	34.39 ± 1.9 ^a	1076.5 ± 25.1 ^{cd}	0.46 ± 0.06 ^a	5.54 ± 0.15 ^{ab}						
AF+Liquorice	18.59 ± 1.5 ^{ab}	38.40 ± 4.5 ^a	1937.4 ± 246.9 ^a	0.25 ± 0.02 ^a	2.76 ± 0.90 ^{abcd}	26.57 ± 1.9 ^{abcd}	31.86 ± 2.1 ^{abcd}	1516.0 ± 99.9 ^{bc}	0.37 ± 0.02 ^a	2.08 ± 0.19 ^a	28.15 ± 2.2 ^a	37.13 ± 2.5 ^a	1146.9 ± 25.0 ^{abcd}	0.50 ± 0.04 ^a	1.35 ± 0.08 ^a						
All additives control	11.34 ± 1.9 ^a	13.98 ± 1.8 ^a	1789.2 ± 305.5 ^a	0.34 ± 0.06 ^a	3.71 ± 0.35 ^{abcd}	20.43 ± 1.4 ^{ab}	30.19 ± 3.3 ^{abcd}	1200.4 ± 34.1 ^{cd}	0.35 ± 0.03 ^a	4.06 ± 0.60 ^a	15.50 ± 1.9 ^a	39.23 ± 3.4 ^a	1038.9 ± 28.8 ^{cd}	0.49 ± 0.04 ^a	5.83 ± 0.16 ^{ab}						
AF+all additives	21.26 ± 1.2 ^a	46.61 ± 2.5 ^a	2048.2 ± 159.3 ^a	0.30 ± 0.02 ^a	2.24 ± 0.22 ^{abcd}	28.10 ± 1.3 ^{abcd}	34.81 ± 1.9 ^{abcd}	1384.4 ± 162.4 ^{cd}	0.40 ± 0.03 ^a	2.01 ± 0.32 ^a	31.89 ± 1.7 ^a	41.72 ± 1.8 ^a	1282.5 ± 25.5 ^{cd}	0.50 ± 0.28 ^a	1.22 ± 0.12 ^a						

Means in the same column with different subscript differ significantly (P<0.05).

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, CR: creatinine and TP: total protein.

DISCUSSION

Administration of aflatoxin (500 ppb) to ducklings resulted in 40% mortality in day 21 of age and it started to appear at day 13 from feeding aflatoxin. This result disagreed with **Abo El-ela, 2013** who stated that daily mortalities in duckling fed AF-contaminated diet (700ppb) started to appear 4 days after feeding aflatoxin contaminated diet and total mortality percent was 100% at day 21 of ducks age. This may attributed to the high dose used. Our result disagreed with **Zhao et al ., 2010** who reported 28% mortalities in broiler chickens fed aflatoxin contaminated diet for 21 days.

Aflatoxin at 500ppb of the diet had a negative effect on growth and FCR during most of the experimental periods which coincided with the decrease in feed intake. This agreed with the results reported by **Shen et al., (1988)** who found a negative effect of AF on productive performance of mule ducklings. The reduced feed intake due to aflatoxin ingestion may be contribute to protein catabolism, increase of blood ammonia, the kidney injury and impairment of glomerular filtration (**Tessari et al., 2006**). Lack of negative effect of AF during 1st and 2nd weeks on FCR could be attributed to the diminished effect on feed intake or the low number of experimental replicates.

Weight gain of aflatoxicated ducks reduced significantly. This result agreed with **Afzal and Saleem (2004)**. This reduction in weight gain might be due to aflatoxin-interference with normal metabolic pathway through inhibition of protein synthesis and enzyme system that involved in carbohydrate metabolism and energy release (**Hassan et al., 2000**).

Body weights of aflatoxicated birds in group (2) were reduced significantly when compared to negative control group. This result agreed with Huff et al., 1986 who noted that aflatoxin treatment significantly decreased body weights of birds.

Significant increase of relative weight of liver in aflatoxin positive control group (G2) in comparison to negative control group (G1) agreed with **Kubena et al., 1990; 1993; Ledoux et al., 1999 and Abousadi et al., 2007**. The higher relative weight of liver is due to excessive fat accumulation consequently to the impaired fat metabolism (**Abousadi et al., 2007**). Aflatoxin administration (500ppb) to ducklings resulted in non-significant differences in relative weights of spleen, thymus and bursa. This result disagreed with **Valchev et al., 2013** who found that aflatoxicosis resulted in significant increase in relative weight of spleen and significant decrease in relative weights of bursa and thymus.

The effect of dietary supplementation of aflatoxin on serum parameters cleared that levels of AST and ALT were significantly increased and this result agreed with **Mahmoud and Koroshom, 1996; Aravind et al., 2003 and Denli et al., 2009** who reported an increase in AST and ALT activities upon feeding diet contaminated with aflatoxin. Alkaline phosphatase increased significantly during aflatoxicosis in this study and this agreed with **Dafala et al., 1987 and Celyk et al., 2003**. This increase in liver's enzyme profile in aflatoxicated birds was most likely reflects liver tissue damage and alternated hepatocyte membrane integrity with leakage of enzymes into blood (**Duncan and Prasse, 1986**).

Aflatoxin administration significantly decreased level of serum total protein. Our findings agreed with **Tung et al., 1970; Huff et al., 1986; Kubena et al., 1990** who found hypoproteinemia in birds suffering from aflatoxicosis. This reduction in total serum protein level was due to suppression of liver protein synthesis as a consequence of mitochondrial injury and the lowered rate of ATP synthesis (**Brown and Abrams, 1965**).

Creatinine level did not significantly affected by aflatoxin administration (500ppb) to ducklings. Our findings disagreed with **Denli et al., 2005 and Bintvihok and Kositcharoenkul, 2006** who reported that there were alterations in kidney parameters in birds fed on aflatoxin. But there was insignificant increase in creatinine level in comparison with negative control group (G1). This insignificant increase indicated some renal tissue damage (**Abd El-Ghany et al., 2013**).

All feed additives addition to aflatoxin contaminated diet reduced mortalities in ducks in comparison with aflatoxin positive control group.

Betmos supplementation to aflatoxicated diets resulted in 20% mortality. This indicate that half of the mortalities caused by 500 ppb AF could be overcome by addition of 1% Betmos, plus improvements in FCR. This result agreed with **Kamalzadeh et al., 2009** who reported that Estrified glucomannan supplementation 1g/kg diet reduced mortality to 50% and improve FCR of aflatoxicated broiler chickens. Furthermore Betmos supplementation in group (4) significantly improves body weights of aflatoxicated ducks in 2nd and 3rd weeks of experiment when compared to positive control group. This result agreed with **Aravind et al., 2003**. Betmos addition to normal diet in (G3) significantly improve body weights in 2nd and 3rd weeks of experiment and improve feed intake in 1st week when compared to negative control group (G1). **Kocher et al. (2004)** acknowledged that mannanoligosaccharides (MOS) can influence the utilization of nutrients in the intestine and was capable of stimulating

specific microbial populations resulting in improved fiber fermentation with a reduction in starch and sugar utilizing bacterial populations. Betmos addition to AF-contaminated diet caused insignificant improvement in relative weight of liver in comparison with AF-positive control group. This result disagreed with **Raju and Devegowda, 2000; Aravind et al., 2003 and Girish and Devegowda, 2006**. Furthermore it resulted in insignificant improvement in relative weights of spleen, bursa and thymus. Liver enzymes (AST and ALT) improved insignificantly by Betmos addition. This result agreed with **Aravind et al., 2003** who found that AST and ALT remained unaltered when compared to AF-positive control group. Moreover Betmos caused significant improvement in ALP in 2nd and 3rd weeks of the experiment. This result agreed with **Raju and Devegowda, 2000**.

Papaya supplementation to aflatoxin-treated ration drop mortality percent to 20% instead of 40% in AF-treated group. It resulted in significant improvement in body weights of aflatoxicated ducks in 3rd weeks of the experiment and improvement in FCR in the 3rd week. This indicate that papaya fruit had some protective effects against some adverse effect of aflatoxin which might be due to free radical scavenging activity as well as normalization of organism's superoxide level by papaya (**Krishna et al., 2008**). Papaya supplementation to AF-contaminated ration caused insignificant improvement in relative organ weights (liver, spleen, bursa of fabricious and thymus) when compared to aflatoxin treated group. It resulted in significant improvement in liver enzymes. This result agree with **Hassan et al., 2013** who reported that liver enzymes significantly improved in rats fed AF-contaminated diet and treated with extracts of papaya fruit. **Raj Kapoor et al (2002)** reported that papaya extract improve liver function through the production of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells.

Ginger supplementation to aflatoxin-treated ration resulted in lowest mortality percent (13.33%) in all aflatoxin treated groups and significant improvement in FCR in week 3 of the experiment. **Ramakrishnan (2013)** stated that ginger was natural antioxidant and anticarcinogenic dietary component also protect against lipid peroxidation (**Aeschbach et al., 1994 and Kuo et al., 1999**). Ginger addition to basal diet resulted in significant improvement in body weights of birds all over the experimental period. It significantly improved FCR in 1st week. It significantly improved feed intake in 2nd week when compared to negative control group. This result agreed with **Farinu et al., 2004** who reported that ginger addition to broiler chicken improved growth performance. According to **Herawati. (2010)** the improved performance attributed to two types of digestive enzymes in ginger (protease and lipase)

which are present as a part of the plant natural protective mechanisms (**Zhang et al., 2009**). Ginger addition to aflatoxin-contaminated ration insignificantly improved relative organ weights (liver, spleen, bursa and thymus) when compared to AF-positive control group, and caused insignificant improvement in liver enzymes. This result disagreed with **Yousef (2009)** who demonstrated that ginger along with mixed herbal extracts supplied to aflatoxicated albino rats resulted in improvement in the liver cancer tissues compared to control group, where it decreased the elevated liver enzymes of aflatoxicated rats.

Liquorice addition to AF-contaminated diet sequelled in mortality 26.66% and significant improvement in FCR in 3rd week of the experiment. This result agreed with **Al Daraji et al., 2005a** who found that addition of liquorice to diet containing AF improved FCR of broiler chicken. Liquorice caused insignificant improvement in body weights and weight gains of aflatoxicated birds when compared to AF-positive control group. This result disagreed with **Al Daraji et al., 2005a**. Liquorice addition to AF-contaminated diet caused insignificant improvement in relative weight of liver compared to aflatoxicated group, and insignificant improvement in AST, ALT and TP. Our results are in disagreement with **Al-Daraji et al., 2005b** who showed that the addition of liquorice extract to AF-containing diet significantly ameliorated the negative effect of AF on liver enzymes and TP.

Addition of all mixed feed additives to AF-contaminated diet caused significant improvement in FCR in the third week of experiment and insignificant improvement in other growth performance parameters. Furthermore it not alleviates the adverse effect of AF on relative organ weights (liver, spleen, bursa and thymus) and on liver enzymes activities and TP when compared to AF-positive control group. This result indicate that mixing of all feed additives might have antagonistic effect on ducks fed aflatoxin contaminated ration.

In summary AF dramatically depressed growth performance parameters and resulted in high mortality of white pekin ducklings. Birds fed AF-contaminated ration had enlarged livers and impaired liver functions with decreased serum proteins under the conditions of this experiment. All used feed additives have some variable protective effect against aflatoxicosis (0.5 mg/kg diet) in duckling. Papaya supplementation to aflatoxicated ducks had the best but not absolute protective effect in controlling aflatoxicosis in this study.

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المخلص العربي

تأثير بعض اضافات الأعلاف الطبيعية والبيولوجية على التحكم في التسمم بالأفلاتوكسين في البط

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في دراسته الحاليه استخدم الزنجبيل وعرق السوس وفاكهة البابايا المجففه ومركب البيتموس (مستخلص جدار خميرة السكرومييسس سيرفاسي) للحد من التأثيرات السلبية الناجمه عن الأفلاتوكسين المضاف لعلائق البط البكىنى. واستخدم فيها ٣٦٠ بطه بكينى عمر يوم واحد ، تم توزيعها عشوائيا على اثنى عشر معاملة يتكون كلا منها من ٣٠ طائر ومكررين وكانت معاملات التجربه كما يلى : (١) مجموعه المقارنته بدون اضافة افلاتوكسين او اى اضافات اعلاف . (٢) اضافة افلاتوكسين الى العليقه بتركيز ٠,٥ جزء بالمليون . (٣) اضافة مركب البيتموس بتركيز ١ جم /كجم علف . (٤) اضافة ٠,٥ جزء بالمليون افلاتوكسين + ١ جم بيتيموس / كجم علف . (٥) اضافة فاكهه البابايا المجففه بتركيز ١ جم / كجم علف . (٦) اضافة ٠,٥ جزء بالمليون افلاتوكسين + ١ جم بابايا مجففه / كجم علف . (٧) اضافة الزنجبيل بتركيز ١٠ جم / كجم علف . (٨) اضافة ٠,٥ جزء بالمليون افلاتوكسين + ١٠ جم زنجبيل /كجم علف . (٩) اضافة عرق السوس بتركيز ١ جم /كجم علف . (١٠) اضافة ٠,٥ جزء بالمليون افلاتوكسين + ١ جم عرق السوس / كجم علف . (١١) اضافة خليط من اضافات الأعلاف بالتركيزات المستخدمه فى الدراسه / كجم علف . (١٢) اضافة ٠,٥ جزء بالمليون افلاتوكسين + خليط من اضافات الأعلاف بالتركيزات المستخدمه فى الدراسه. وتم ابقاء الطيور على هذه العلائق حتى عمر ٣ اسابيع .

اظهرت النتائج ان ادخال الأفلاتوكسين فى علائق البط البكىنى ادى الى وفيات بدأت من اليوم الثالث عشر ووصلت الى ٤٠ ٪ فى اليوم الحادى والعشرين من عمر البط. الا ان اضافة مركب البيتموس وفاكهة البابايا المجففه والزنجبيل وعرق السوس وخليط من كل هذه الاضافات ادى الى معدل وفيات ٢٠ ٪ ، ٢٠ ٪ ، ٣٣،١٣ ٪ ، ٦٦،٢٦ ٪ ، ٣٠ ٪ بالترتيب .

ايضا ادت سموم الأفلاتوكسين الى نقص فى معدل نمو الطائر واختلافات فى اوزان الأعضاء (الكبد والطحال و غدة فابريشوس والغدة الثيموسيه) وايضا الى زيادة ملحوظه فى انزيم اسبرتات الامينو ترانسيفيراز وانزيم الالنين امينو ترانسيفيراز خلال فترة التجربه . وزيادة فى انزيم الفوسفاتيز القلوى فى الاسبوع الثانى والثالث من التجربه . وسببت سموم الافلاتوكسين زياده غير معنويه فى مستوى الكرياتينين فى الدم ونقص معنوى فى مستوى البروتين الكلى فى الدم. لكن استخدام اضافات الأعلاف المختلفه مثل مركب البيتموس والبابايا والجوزبيل وعرق السوس ادى الى التخفيف من بعض التأثيرات السلبية للأفلاتوكسين .

يستنتج من الدراسه الحاليه ان معدلات النمو واوزان الاعضاء والقياسات البيوكيميائيه للبط قد تاثرت بالمعامله بالأفلاتوكسين وان استخدام مركب البيتموس والبابايا والزنجبيل وعرق السوس بالتركيزات المحدده قد ساهم فى الحد من التأثيرات السلبية للأفلاتوكسين على هذه الصفات. لكن فاكهه البابايا المجففه قد اظهرت افضل تأثير واقى ضد الاثار الضاره للأفلاتوكسين فى البط البكىنى .