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Evaluation of antimycotoxin effects of Curcuma longa and Zingiberofficinale on broilers toxicated with aflatoxin

Doaa A.H.* and Ghada A.A. **

Animal health research institute, agriculture research center,Dokki,Giza,Egypt, Al-mansoura provincial Lab.*Department of mycology, ** Department of poultry disease

Abstract

This study was conducted to evaluate the efficacy of turmeric powder (Curcuma longa) at a dose of 80 mg/kg ration ,ginger (Zingiber officinale) at a dose of 0.75gm/kg ration and hydrated sodium calcium aluminosilicate (HSCAS) at a dose of 0.5% to counteract the toxic effects of aflatoxin B1 at dose of 2.5ppm/kg ration in broiler chickens. Hundred sixty chicks of one day age were distributed into 8 groups at 7 day of age. First group kept as control , 2nd group fed ration contain AFB1, 3rd group received ration contain AFB1+ HSCAS,4th group received ration contain AFB1 + Curcuma longa powder,5th group fed ration contain AFB1+ Zingiber officinale, 6th group fed ration contain AFB1 + HSCAS + Curcuma longa + Zingiber officinale 7th group fed ration contain AFB1 + Curcuma longa + HSCAS, 8th group fed ration contain AFB1 + Zingiber officinale + HSCAS. The feeding program continued till 45 day of age. Body weight was recorded weekly, serum samples we collected for biochemical studies at the end of experiment while organs (liver, kidney and intestine) were collected for pathological studies at 21 and 45 day of age. The results cleared that chicks of the 2nd group showed significant decrease in body weight while 3rd ,4th ,5th ,6th ,7th ,8th groups showed a significant increase in body weight which appeared clearly at 45 day of age ,5th and 6th groups recorded the highest body weight .Regarding to biochemical parameters, the results revealed that the chicks of group 2 posses the lowest values of serum total protein, albumin, globulin and showed significant increase in liver enzymes AST, ALT and ALP concentration. Also, a significant increase in serum uric acid, urea, creatnine and total cholesterol concentration level .The use of Curcuma longa and/or Zingiber with or without (HSCAS) showed a significant improvement of the values constituent and returned to normal level. In group 2 the liver, kidney and intestine showed necrotic changes. While groups treated with Curcuma longa and/or Ginger showed improvement in histopathological effects of aflatoxin B1 especialy in group (6) at 45day of age.

Key words: Aflatoxins, curcuma longa, ginger, broiler, performance, biochemical parameters, histopathological change .

Introduction

Aflatoxins (AF) are mycotoxins produced by the toxigenic fungi mainly by Aspergilus flavus and Aspergilus parasiticus. AF was ingested by the bird accumulate in different tissues, organs and eggs and entered into human food chain which posses a major risk to human health. AFB1 is the most biological active form of aflatoxins that found on food **(Batt et al.,2001).** AFB1cause lesions in liver, kidney, spleen and other vital organs which lead to decrease growth and performance in poultry **(Sandhu et al., 2005).** The clinical signs affected birds are decreased food intake ,decreased body weight, poor skin, decreased egg production and increase suceptability of the bird to infectious diseases which indicate impaired immune system **(Bakshi et al.,2000).** Aflatoxins in broiler chickens has been widely investigated as carcinogenic, mutagenic, teratogenic **(Wildet al.,2000,Sur, 2003)**. The adverse effect of aflatoxin depends on age, species, nutrational status of birds as well as dose and period which it is consumed **(Smith et al.,1995).** It is inhibit the enzymatic activity involved in the metabolism of carbohydrates, proteins, lipids and nucleic acid as well as blood total proteins, cholesterol and urea concentration **(Dewegoda and murthy,2005). Maurice et al.(1983)** demonstrated that oral intake of low concentration (50 or100 ug/kg) aflatoxins with feed could disturb the normal metabolism in broiler chickens, according to the American food and drug Agency (FDA) permissible dietary aflatoxin concentrations for poultry are up to 20 ppb**(Aravind et al.,2003).** Certain plant compounds like flavonids and curcuminoids posses antioxidant property, inhibiting the biotransformation of AFB1 to their active epoxide derivatives **(Lee et al.2001).** The most recent dietary formulation approach to prevent mycotoxicosis in poultry is an incorporation of antioxidant with adsorbent **(Surai et al.,2001).** Tumeric is a medical plant has been reported to have antimicrobial, anti-inflammatory,antiviral antioxidant and anticancer effect on various labolatory animals **(Anto et al.,1998)**. Curcuminoids (TCMN) yellowish pigments present in turmeric powder have shown antioxidant and protective effects against AFB1**(Nisarani et al.,2009).Gowda et al., (2008)** reported that addition of turmeric to AFB1 diet significantly ($P<0.05$) improved weight gain of chicks and normalized the altered activities of LDH and ALT induced by AFB1 **(Nayak and Sashidhar 2010)**, addition (HSCAS) to the diet AFB1 significantly ($P<0.05$) improved feed intake and weight gain and reduced relative liver weigh. The addition of turmeric and HSCAS ameliorated the adverse effects of AFB1 on some of the serum chemistry parameters and reduction in the severity of hepatic microscopic lesions **(Sapcota et al., 2009)** reported that using Curcuma longa in commercial broilers during aflatoxicosis (300ppm of AFB1) improved carcass parameters, livability, performance index. They concluded that inclusion dry powder of Curcuma longa at dose 1.5g/Kg of feed can be used to reduce the negative effect of aflatoxin as ameliorating agent. In addition inhibited the spore count and the AF

production **(Gowda et al.,2004)**. Ginger is a perennial herbaceous plant that is apart of zingiberacease family .The use of Z.officinal rhizome has gaind popularity among modern physicians in recent years **(Abo-Ghanema et al.,2012).** Z.officinale were used as food additive to improve the health state, performance and productivity of many farm animals **(El makki et al., 2013).** Ginger as carminative, diuretic tonic and disinfectant compound contain glucosinolate, sterols and trite pens **(AL yahya, 1998).**Using aqueous extract of Zingiber officinale effectively ameliorated the deviation induced in both kidney and heart of animals administrated AFB1 **(Azza and Nadia, 2009). Najafi and Taherpour(2014)** reported that using 0.8% ginger could be suggested as effective alternative for virginimycin with respect to feed efficiency and health parameters, adding of turmeric rhizome powder at 3% concentration to diet significantly decreased serum total cholesterol, Aspartate amino transferase (AST) and Alanin amino transferase (ALT) (**Zhang et al.2009)**. The supplementation of ginger 3% have some positive effects on production performance and some blood metabolites of lying hens **(Malekizadeh et al.,2012).** The supplementation of laying hen diet with ginger root at rate of 0.5-0.75% diet positively influence egg production and plasma antioxidant status of laying hens, decrease feed concentration of egg yolk cholesterol with no adverse effect on egg weight and feed conversion of hens **(Abdollah et al.,2014).** The most recent economical and promising approach to prevent the aflatoxins in poultry is combined using of adsorbent and turmeric compound **(Gowda et al.,2008and Diaz et al., 2009)** .

Hence, this research study was conducted to investigate and evaluate the efficacy feed additives containing HSCAS, turmeric powder and ginger either singly or in combination to counter the adverse toxic effect of aflatoxin B1 in broiler chickens.

Materials and Methods

- **AFB1 production**: was done according to **Davis et al.(1966)** using Aspergilus flavus standard toxigenic strain which obtained from microbiology department faculty of veterinary medicine Mansoura university. The fungus was culture on potato dextrose agar for four days at 25˚C. Rice was autoclaved three times in succeeding days. A 6mm block of fungus from the edge of a growing colony was inoculated onto 100gm sterilized rice and incubated at 25˚C for 7days, it was then autoclaved twice to kill the fungus then rice dried in oven at 56˚C for 48hrs .The concentration of AFB1 was measured using HPLC compared with standard aflatoxin B1 which was obtained from Sigma chemical company .The amount of AFB1 to be mixed in feed of each calculated, the quantities of fermented rice containing required amount of AFB1were weighed and extracted by soaking into three fold quantity of chloroform (100-300) for overnight and filter through cotton cloth .All chloroform was evaporated, the concentration residues were resuspended into 100ml

polyethylene glycol, the suspention was evenly mixed in 0.5kilograms and finally in the required quantity of feed .

- **Hydrated sodium calcium aluminosilicate (HSCAS):** is a feed additive, adsorbent and toxin binder was obtained from veterinary pharmacy at Mansoura.

- **Turmeric powder** (Curcuma longa) and **Ginger** (Zingibero fficinale) were obtained from Sigma Aldrich.

- **Experimental chickens** :One hundred and sixty(Cobb500) ,day old broiler chicks were purchased from a commercial hatchery. The birds were kept in cleaned and disinfected pens. Birds were maintained on 14 hours continuous light schedule throughout the experimental period (45days).chickens were vaccinated against Newcastle and Gumboro diseases .

-**Experimental design**: At seven day of age chicks were randomly distributed into 8 groups each contain 20 chicks and used to evaluate the efficacy of turmeric powder at dose of 80mg/kg ration **(Wafaa et al.2013)**, ginger at dose of 0.75gm/kg ration **(Abdollah et al.2011)** and HSCAS at dose of 0.5% **(Dawoud et al.2002)** to counteract the toxic effects of AFTB1 at dose of 2.5ppm /kg ration **(Wafaa et al.2013)** 1st group fed standard ration and kept as control . 2nd group fed standard ration contaminated with AFB1, 3rd group received ration contain HSCAS+AFB1,4th group fed ration contain AFB1 + Curcuma longa powder, 5th group fed ration contain AFB1 + Zingiber officinale, 6th group fed ration contain AFB1 + HSCAS + Curcuma longa + Zingiber offinciale, 7th group fed ration contain AFB1 + Curcuma longa + HSCAS. 8th group fed ration contain AFB1 + Zinger officinalis + HSCAS. This feeding program continued till 45 day of age.

- **Growth performance parameters:** The body weight of chickens were recorded weekly .The body weight gain and FCR were recorded at the end of the experiment.

- **Biochemical studies :** blood samples were collected from 10 chicks of each group at the end of experiment period and centrifuged to obtain serum samples for measurement of biochemical parameters which include serum total protein, albumin, globulin, ALT, AST, uric acid, urea, creatinine, alkaline phosphatse and cholesterol. All tests were measured calorimetrically using commercial kits (bio-merieux-co, Marcy l E Toil France)

- **Pathological studies:** Clinical signs and post mortem findings were recorded**.** At 21 day and 45 day of age 5 chickens from each group were scarified. Specimen from liver, kidney and intestine were collected and fixed in 10% buffered neutral formalin solution , dehydrated in gradual ethanol (70-100%) cleared in xylene and embedded in paraffi, five micron thick paraffin sections were prepared and then routinely stained with

hematoxlyine and eosin (H&E) dye **(Bancroft and Gamble, 2007)** and then examined microscopically.

- **Quantification of aflatoxin residues:** Other specimens from liver were collected for quantification of aflatoxin residues using HPLC.

-**Statistical analysis** :The obtained data were analyzed using the liner model programs of SAS(1990).The difference among means were tested using Duncan Multiple range test (**Duncan,1955).**

Results and Discussions

-Growth performance parameters:

Body weight: The results in table (1) showed, that there were a significant difference ($p\leq$ o.05) in body weight between positive control group (G2) and all other groups at all the period of the experiment. At 14 day of the age there were not a significant difference in body weight between negative control (G1) and (G3,G6,G7 &G8). At 21 day there were a significant increase in body weight between (G5,G7&G8) when compared to (G1). At 28 days of age there were a significant increase in body weight between (G5,G6&G8) and negative control (G1). At 35day of age there were a significant increase in body weight between (G3,G5,G6,G7&G8) and (G1). At 45 day of age there were a significant increase in body weight between all intoxicated and treated groups and negative control (G1). The highest body weight were (1968,1868&1824 gm) for (G6,G8&G5) respectively.

Body weight gain and Food conversion ratio: Table (2) showed that there were significance difference ($p\leq 0.05$) in body weight gain between control group (G1) and aflatoxicated group (G2) and aflatoxicated treated groups. Among the treated groups the highest body weight gain were (1685, 1577, 1557) for (G6, G8&G5) respectively. Also there were significance difference ($p\leq 0.05$) in FCR between aflatoxicated group (G2) and aflatoxicated treated groups. The lowest FCR were (1.47, 1.52& 1.56) for (G6, G7&G8) respectively.The reduction in body weight, body weight gain and increase in FCR in aflatoxicated group(2) in agreement with the results obtained by **(Tedesco et al.,2004,Shi et al., 2006,Denli et al.,2009 and Wafaa et al.,2013).**The depression in growth upon feeding aflatoxin could be attributed to reduced protein synthesis as reported by **(Verma et al.,2002)** reduce appetite by **(Sharlin et al.,1980),**increase lipid excreation in dropping **(Osbrneand Hamilto,1981).Hassan et al,. (2000)** stated that the toxicity of AFB1 as characterized by reduction in body weight gain as interfere with normal metabolic pathway throught the inhibition of protein synthesis and enzymes system that is involved in carbohydrate metabolism and energy release .Another point of view was discussed by **(Nelson et al.,1982).**who postulated that aflatoxin reduce the ability of the bird to digest dry

matter .There is a significant improvement in growth performance parameters in treated groups when compared to negative control(G1).The highest improvement were recorded in(G6,G7&G8) respectively. HSCAS making AFB1unavailable for gut absorbtion and allowing the mycotoxin to pass harmless through the animal **(Mabbett,2005 and Zhao et al.,2010).**On the other hand the growth promoting effect exerted by curcuma longa on AFB1 was studied by**(Yarru et al.,2009,Rangsaz and Ahangaran,2011)** they demonstrated that supplementation of turmeric extract in diet containing AFB1 improve performance because the major antioxidant ingredient of turmeric is known to inhibit biotransformation of aflatoxin to aflatoxicol in liver **(Lee et al.,2001).**As well as dietary supplementation with ginger led to increase body weight it may be contributed to that, ginger contains volatile oils as zingibaine,zingiberol,gingrol and resin which speed digesion and enhance protein digesting enzymes ,these results agree with that obtained by **(Demir et al.,2013 and Iqbal et al.,2011)**

-Biochemical parameters: Table (3) showed that there was high significant decrease in total protein in second group(G2) when compared to control group(G1) and compared to all other test groups.The reduction in serum protein might be contributed to the binding of aflatoxin to DNA,therefor, aflatoxin hinder transcreption and translation in return decrease protein synthesis **w**hile groups, (3,4,5,7,8) showed non significant changes compared to control group1 and to all other experimental groups. Whereas group (G6) showed significant increase in total protein compared to control group. These findings in accordance with that obtained by **(Kalorey et al.,2005,Cavin and Schilter 2008 and Tejada-Castaneda et al.,2008).** Albumin in second group showed high significant decrease compared to control group **(Ramadevi et al.,2000)** while serum albumin return to normal level in groups (5,6). Globulin showed high significant decrease in second group while in groups (5,6,7,8) the level of serum globulin return to normal level while groups (3,4) showed significant increase compared to control group (G1). Urea, uric acid and creatnine showed significant increase in second group which indicated renal tissue damage due to aflatoxin these results in agreements with data reported by **(Denli et al.,2005,Bintvihoka and Kositcharoenkul 2006 and Yassein and Zghair,2012).** While in all other experimrntal groups blood urea, uric acid and creatnine return to normal levels So, addition of turmeric powder and ginger with or without HSCAS significally effective in the protection against AFB1 by preventing its toxic effects as it reflected by ameliorating alteration in serum total protein ,albumin,globulin,and decrease in serum urea ,creatnine and uric acid.Turmeric powder and ginger providing antioxidant protection and HSCAS decreasing the amount of AFB1 absorbed **(Kurkure et al.,2000 and Gowda et al,.2008)**. AST,ALTand ALP showed high significant increase in second group compared to control group which reflect liver tissue damage while other test groups showed non significant changes when compared to control group .These results in accordance with the study of **(Ali Rafiee et al.,2013)** and agree

with **(Emadi and Kerman Shahi 2007 and Wafaa et al.,2013)** who fed broiler chicks turmeric powder and found positive effects on liver enzymes reducing AST,ALT and ALP. Total cholesterol in second group showed high significant increase compared to control group1, while group 3 showed significant increase in total cholesterol compared to control group1, whereas groups (4,5,6,7,8) showed high significant decrease compared to control group1.**(El Fara 20008,Ali rafiee et al.,2013 and Zomrawi et al.,2013).Arafa (2005)** stated curcuma mixed with diet (0.5%v/v) decrease serum cholesterol by about 21% also it inhibit the aflatoxin production by Aspergillus flavus. **Gowda et al**.(2004) found that using ginger extract lowering serum cholesterol by interfering with intestinal sterol absorbtion .**ST-Ong et al.,(2000) and Abdollah et al.,(2014)** reported that supplementation of laying hen diet with ginger root at the rate of 0.5 or 0.75% diet positively influence egg production and plasma antioxidant status of laying hens and decrease concentration of egg yolk cholesterol with no adverse effect on egg weight and food conversion of hens.

Aflatoxin residues: results in table (3) indicated that the administration of aflatoxin B1 in broilers led to accumulation of AFB1 in liver, second group showed the highest level of AFB1 (0.78ppb), these results in agreement with that obtained by **(Bintvihok and Kositchaenkal 2006)** . Group (6) which treated with Curcuma longa, ginger and HSCAS showed the lowest amount of AFB1 residues (0.10) which reduced by (87.18%) **f**ollowed by groups (4,7,3,8,5) which reduced by (83.4%,80.1%,78.34%,,76.5%,76.3%) respectively.Addition of Curcuma longa and ginger with or without HSCAS reduce the AFB1 residues these results confirmed by **(Singh et al.,2002)** who reported the antifungal activity of turmeric volatile oil,about 90% reduction in aflatoxin at 5-10mg/ml concentration of turmeric,also these findings attributed to the antioxidant curcumin in turmeric **(Soni et al.,1992).**In addition of turmeric inhibited the spore count of Aspergillus flavus and enhancement the phagocytosis **(Gowda et al.,2004)**

Clinical finding:

Symptoms: No clinical signs were seen in birds of the negative control group (G1). The clinical signs in chickens fed on diet contaminated with AFB1 (G2) were depression, anorexia, ruffling feather, closed eye, purple discolored feet and leg and lameness. These signs were less pronounced in treated groups. No mortalities were recorded in any group during the study **(Bakshi et al., 2002).**

Macroscopical finding: The most observed gross lesions that detected in sacrificed chickens at 21 and 45 days of age were enlarged pale and friable liver with hemorrhagic patches on the surface. Enlarged and pale kidney with hemorrhagic patches and the intestine showed congestion and hemorrhage. The lesion severity decrease in the treatment groups than control positive group (G2), the lowest macroscopical changes were recorded

in (G6, G7&G8).

Microscopical finding:

Liver in G2 at 21 day showed congestion and intense lymphocytic exudate in hepatic tissue and heterophilic recruitment replacing hepatic tissue. At 45 day showed massive hemorrhage and intense heterophilic exudate in hepatic tissue, lymphohistiocytic aggregation replacing hepatic tissue and congestion in hepatic sinusoids (fig1) these results similar to that obtained by **(Rathod et al.,2013).** Liver in G3 at 21 day showed congestion and intense lymphocytic exudate in hepatic tissue and heterophilic recruitment replacing hepatic tissue. At 45 day showed congestion and mild thickening in the wall of portal vein (fig2) these results in accordance with**(Sapcota et al.,2009).** In G4 at 21 day liver showed mild vacuolar degeneration. At 45 day liver showed normal hepatocytes with normal radial arrangement around central vein (CV) (fig3) .In G5 liver at 21 day showed foci of lymphohistiocytic exudate replaced hepatic exudates. At 45 day showed mild congestion in central vein and mild necrosis of hepatocytes (fig 4). In G6 at 21 day and at 45 day liver showed normal hepatocytes with normal architecture (fig 5). In G7 at 21day liver showed mild vacuolar degeneration with radial arrangement around central vein. In G8 at 21 day liver showed mild vacuolar degeneration and mild congestion in portal vein. At 45 day liver in G7 and G8 showed normal hepatocytes with radial arrangement around central vein.these findings in agreement with **(Zahid Hussain et al.,2008 and Ahmed et al.,2009).**

Kidney in G2 at 21 day showed severe hemorrhage in interstitial tissue replacing renal tissue with necrosis in renal tubular epithelium. In 45 day kidney showed marked lymphohistiocytic exudate, hemorrhage in interstitial tissue and degenerative changes in renal tubular epithelium (fig6), these results agree with that obtained by **(Denli et al.,2009 and Yassein and Zghair 2012)** In G3 kidney at 21 day showed degenerative change in renal tubular epithelium and marked interstitial hemorrhage. At 45 day kidney showed hyperplasia in mesangial cells of glomeruli and mild necrosis in renal tubular epithelium (fig7). In G4 at 21 day kidney showed necrosis in renal tubular epithelium and intense hemorrhage in interstitial tissue. At 45 day kidney showed normal renal glomeruli and normal renal tubules with normal lining epithelium (fig8). In G5 at 21 day kidney showed diffuse hemorrhage in interstitial tissue. At 45 day kidney showed moderate increase cellularrity of glomerular tuft capillaries with mild degeneration in some renal tubules (fig9). In (G6) at 21day and at 45 day kidney showed normal renal glomeruli and normal renal tubules with normal lining epithelium (fig10). In G7 kidney showed cloudy swelling in some renal tubules. At 45 day showed normal renal glomeruli and normal renal tubules with normal lining epithelium. At 21day (G8) showed diffuse hemorrhage in interstitial tissue. At 45 day showed normal

glomeruli with mild degeneration in some renal tubules.So, using of cuecuma longa, ginger and HSCAS reduce the pathological lesions which induced by AFB1**(Zahid Hussain et al.,2008 and Ahmed et al., 2009).**

Intestine in G2 at 21 day showed necrosis and desquamation of some intestinal villi and at 45 day showed destruction of some villi, with necrosis and sloughing of enterocytes, congestion and lymphocytic infiltrate in lamina propria, beside necrosis in enterocytes (fig11) these findings similar to that obtained by **(Yassein and Zaghair 2012)** . Intestine in G3 at 21 day showed necrosis in intestinal villi with heterophilic infiltrates. At 45 day showed normal intestinal villi and normal enterocytes with mild extravasation of erythrocytes in lamina propria (fig12). In G4 at 21 day intestine showed necrosis of enterocytes and histiocytic infiltrate in lamina propria. At 45days showed normal intestinal villi with mild lymphocytic infiltrate (fig 13). In G5 intestine at 21day showed necrosis and desqumation of enterocytes ,at 45 day showed normal intestinal villi with lymphohistiocytic exudate in lamina propria (fig14). In G6 at 21 day and 45 day intestine showed normal histological architecture of intestinal villi and normal enterocytes (fig15). In G7 and G8 at 21 day intestine showed necrosis of enterocytes and at 45 day intestine showed normal histological architecture of intestinal villi with normal enterocytes.the improvement of pathological lesions by using curcuma and ginger is attributed to their antioxidant property which inhibite the biotransformation of AFB1 to their active epoxide derivatives ,the affect of curcuma and ginger increase by using adsorbent agents as HSCAS **(Lee et al.,2001,Zahid Hussain et al.,2008, Ahmed et al.,2009 and Lafi et al., 2010).**

Table 1:Average of body weight of broilers feed on AFB1 and treated with HSCAS, turmeric powder (Curcuma longa) and ginger (Zingiber officinale)

group	DAY7	DAY14	DAY21	DAY28	DAY35	DAY45
G1	262.4^{a}±1.82	444.3^{ab}±3.17	712.2^{cd}±3.36	1066.3^{c} ±3.22	1352.2^{d} ±3.2	1620.0^{d}±3.6
G2	262.9^{a}±1.58	373.4^{c}±2.15	538.9^{e}±1.7	873.1^{d} ±2.4	1134.9^{e}±2.7	1327.50^{e}±3.2
G3	268.7^{a}±1.71	396.6^{bc}±2.49	685.0^{d}±3.94	1086.8^{c}±4.8	1512.0^{b} ±3.5	1820.0^{b}±3.6
G4	266.5^{a}±1.78	392^{c}±1.89	735.6^{bcd}±3.0	1071.8^{c}±3.8	1404.3^{cd}± 3.6	1775.0^{c}±3.6
G5	267.5^{a} ±1.89	369.2^{c}±1.92	824.7^{a}±1.80	1170.4^{abc}±2.2	1499.4^{b}±2.2	1824.5^{bc} ±2.2
G6	282.3^{a} ±1.81	404.4^{bc}±2.29	769.1^{abc}±2.41	1254.6^{ab}±3.5	1627.0^{a}±2.6	1968.0^{a}±2.5
G7	265.4^{a} ±1.87	477.4^{a}±2.07	805.4^{ab}±2.07	1145.8^{bc}±2.2	1486.5^{bc}±1.86	1803.8^{bc}±1.6
G8	290.6^{a}±2.26	490.4^{a}±1.92	766.6^{abc}±2.37	1261.6^{a}±3.8	1551.6^{ab} ±3.4	1868.0^{b} ±3.6

a-d= Means with the same letter in each column are not significantly different at P≤0.05

G1:controlG2:Fed on aflatoxin

G3: Fed on aflatoxin +HSCAS

G4: Fed on aflatoxin +Crucuma longa.

G5: Fed on aflatoxin +Zingiber officnale

G6: Fed on aflatoxin + HSCAS+ Crucuma longa + Zingiber officnale

G7: Fed on aflatoxin + HSCAS+ Crucuma longa

G8: Fed on aflatoxin + HSCAS+ Zingiber officnale

Table 2:The average body weight gain and FCR for broiler chicks feed on AFB1and treated with HSCAS, turmeric powder (Curcuma longa) and ginger(Zingiber officinale)

groups	Body weight gain	FCR
G1	1357.60^{c}±3.81	1.57^{c}
G2	1064^{d}±3.15	1.73^{a}
G3	1551.30^{b}±3.70	1.59^{c}
G4	1508.50^{b}±3.70	1.62^{b}
G5	1557^{b}±1.70	1.66^{b}
G6	1685.70^{a}±2.31	1.47^{d}
G7	1538.40^{b}±1.95	1.56^{c}
G8	1577.40^{b}±3.28	1.52^{c}

a-d= Means with the same letter in each column are not significantly different at P≤0.05

Table3: Effect of HSCAS, Curcuma longa and ginger on biochemical parameters of aflatoxicated broiler chickens (n=10 / group)

GROUP	G1	G2	G3	G4	G5	G6	G7	G8
Total protein gm/dl	4.43^{bc}± 0.20	2.72^{d}± 0.19	4.49^{b}± 0.20	4.34^{bc}± 0.16	4.07^{c}± 0.20	5.0^{a}± 0.22	4.31^{bc}± 0.21	4.37^{bc}± 0.17
Albumin gm/dl	1.77^{a}± 0.17	1.02^{c}± 0.17	1.36^{b}± 0.19	1.52^{ab}± 0.18	1.61^{ab}± 0.20	1.71^{a}± 0.19	1.35^{b}± 0.20	1.31^{bc}± 0.18
Globulin gm/dl	2.35^{c}± 0.14	1.78^{e}± 0.13	3.12^{a}± 0.20	2.78^{b}± 0.18	2.13^{cd}± 0.19	2.39^{c}± 0.16	2.01^{de}± 0.21	2.15^{cd}± 0.19
Urea mg/dl	4.95±^{bc} 0.22	12.3^{a}± 0.33	4.84^{bc}± 0.21	4. 63^{bc}± 0.23	4.96^{bc}± 0.24	5.14^{b}± 0.23	4.41^{c}± 0.26	4.66 ^{bc}± 0.22
Uric acid mg/dl	4.46^{b}± 0.21	8.91^{a} ±0.25	4.34^{b}± 0.21	4.5^{b}± 0.23	4.37^{b}± 0.19	3.16^{c}± 0.24	4.27^{b}± 0.23	4.15^{b}± 0.24
Creatinine mg/dl	1.1^{bc}± 0.18	2.54^{a}± 0.19	1.27^{b}± 0.16	0.93^{c}± 0.17	1.05^{bc}± 0.18	1.01^{bc}± 0.18	1.19^{bc}± 0.15	1.08^{bc}± 0.17
Alkaline phosphase Iu/I	58. 96^{b} ±0.0.60	68.42^{a}± 1.47	61.0^{ab}± 0.59	61.0^{ab}± 0.59	61.0^{ab}± 0.59	59.22^{b}± 0.61	58.96^{b}± 0.6	59.8 ^{b}± 0.64
AST Iu/I	156.0^{b}± 0.71	176.6^{a}± 0.79	154.1 ^{b}± 0.68	156.6 ^{b}±0.73	155.4 ^{b}±0.79	157.2 ^{b}±0.77	154.0^{b}± 0.62	154.2 ^{b}±0.62
ALT Iu/I	44.08^{b}± 0.41	64.33^{a}± 0.33	42.42^{bc}± 0.47	41.45^{b}± 0.36	45.4^{b}± 0.46	43.67^{b}± 0.33	45.4^{b}± 0.46	43.65^{b}± 0.33
Cholesterol mg/dl	198.2 ^{c}±0.92	217.2^{a}± 0.97	205.3^{b}± 0.79	184.3^{e}± 0.70	185.9^{e}± 0.75	190.2^{de}± 0.64	193.9^{de}± 0.68	198.4 ^{c}±0.92

a-d= Means with the same letter in each column are not significantly different at P≤0.05

Table4:AFB1 residues in liver of broilers and the effect of turmeric and ginger on its level

groups	G1	G2	G3	G4	5G	G6	G7	G8
AFB1	ND	0.78	0.16	0.13	0.19	0.10	0.15	0.18
% of reduction	-	-	78.34	83.4	76.3	87.18	80.1	80.1

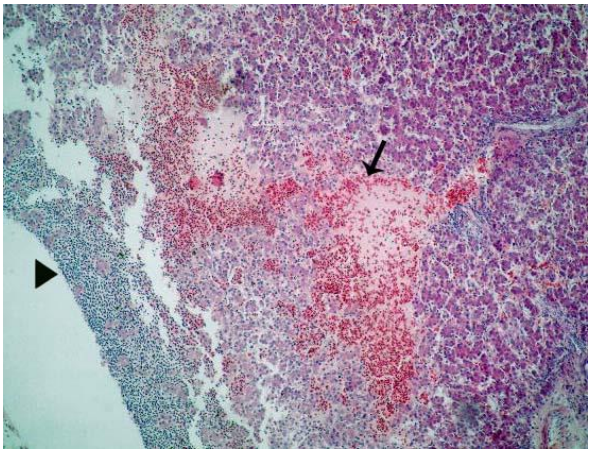

Fig 1: Liver in G2 showing lymphohistiocytic aggregation replacing hepatic tissue and massive hemorrhage (arrow) and congestion in hepatic sinusoids (arrow head) (HE, 100x)

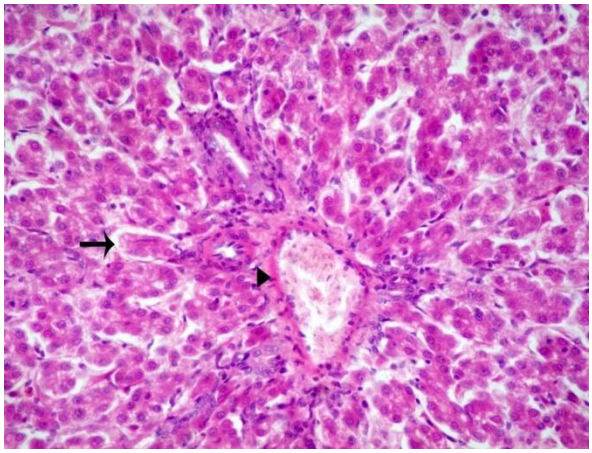

Fig 2: Liver G3 showing congestion and mild thickening in the wall of portal vein (arrow head) (HE, 400x).

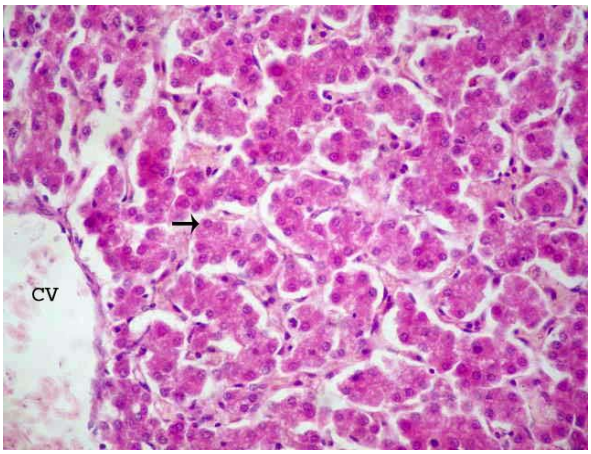

Fig 3: Liver G4 showing normal hepatocytes (arrow) with normal radial arrangement around central vein (CV) .(HE, 100x)

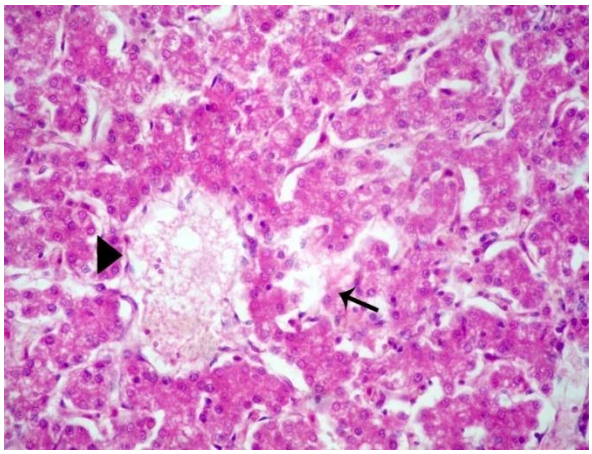

Fig 4: Liver G5 showing mild congestion in central vein (arrow head) and mild necrosis of hepatocytes (arrow).(HE, 400x)

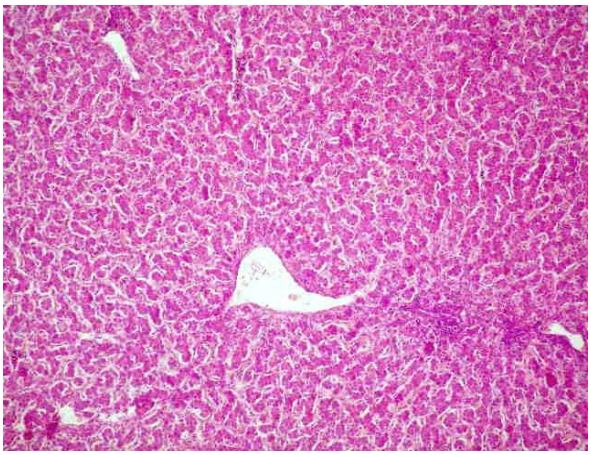

Fig 5: liver in G6 showing normal hepatocytes with normal hepatic architecture.(HE, 100x)

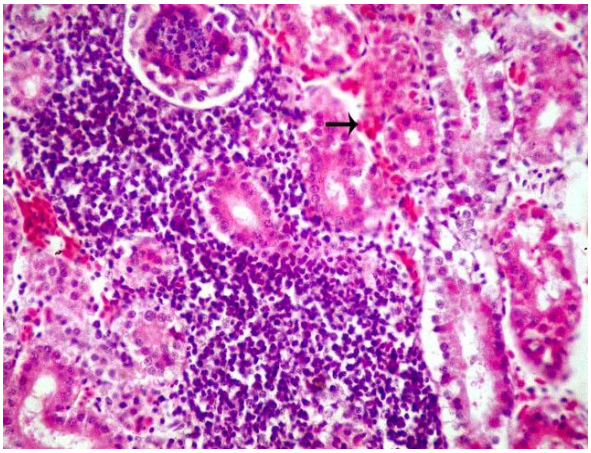

Fig 6: Kidney in G2 at 45 day showing marked lymphohistiocytic exudate, hemorrhage in interstitial tissue (arrow), and degenerative changes in renal tubular epithelium (HE, 400x)

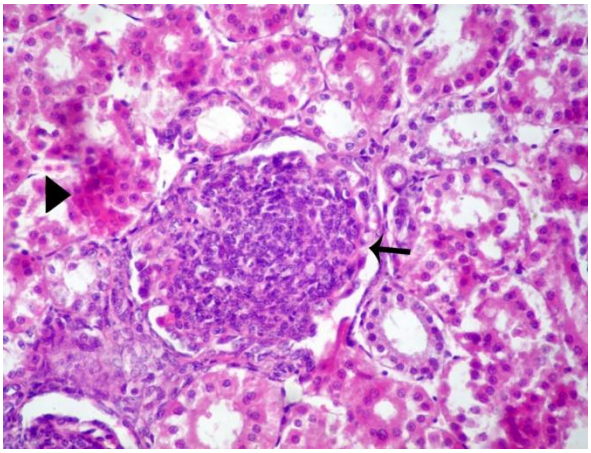

Fig 7: Kidney in G3 45 day showing hyperplasia in mesangial cells of glomeruli (arrow) and mild necrosis in renal tubular epithelium and degenerative changes (arrow head).(HE, 400x)

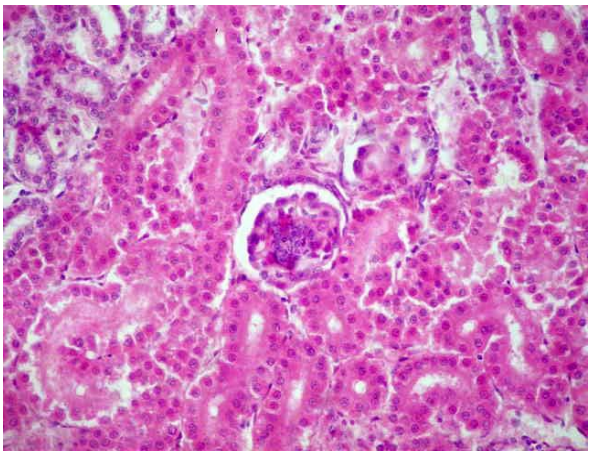

Fig 8: Kidney in G4 at 45 day showing normal renal glomeruli and normal renal tubules with normal lining epithelium. (HE, 200x)

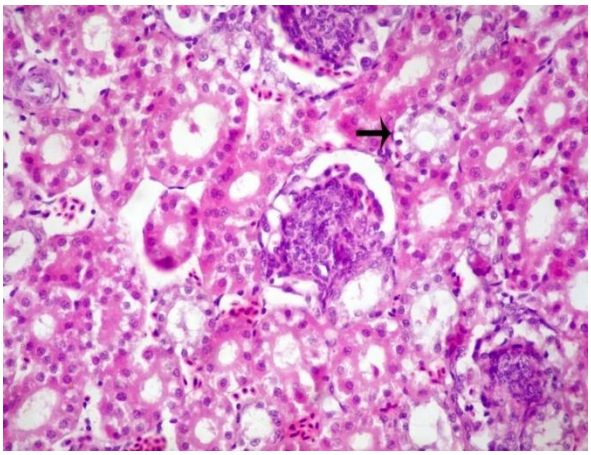

Fig 9: Kidney in G5 at 45 day the glomeruli showed hyperplasia, increase cellularity, vaculation of tuft capillaries (arrow) with mild degeneration of some renal tubules. (HE, 200x).

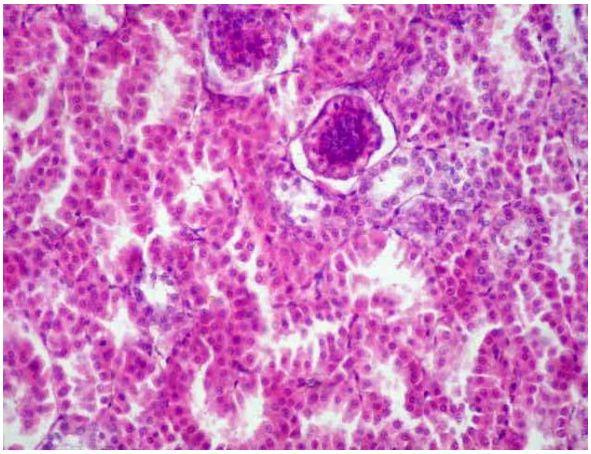

Fig10: kidney in G6 at 45 day showing normal renal glomeruli and normal renal tubules with normal lining epithelium. (HE, 200x).

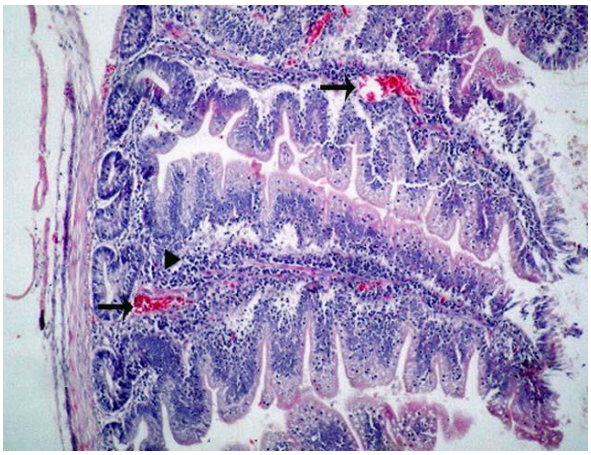

Fig 11: Intestine of G2 at 45 day showing congestion (arrow) and lymphocytic infiltrate (arrow head) in lamina propria, beside destruction of some intestinal villi. (HE,100x)

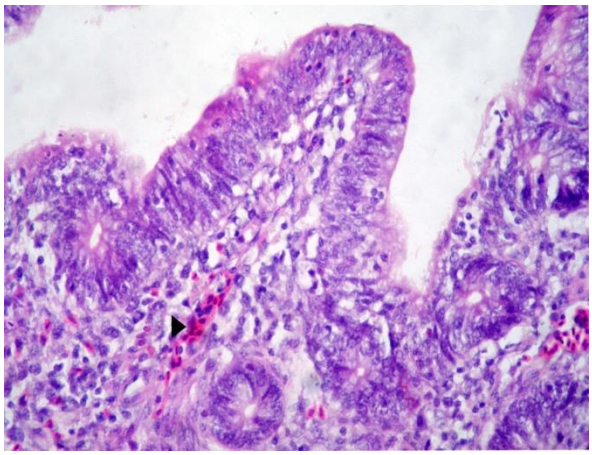

Fig 12: Intestine in G3 at 45 day showing normal intestinal villi and normal enterocytes with mild extravasation of erythrocytes in lamina propria (arrow head) (HE, 200x).

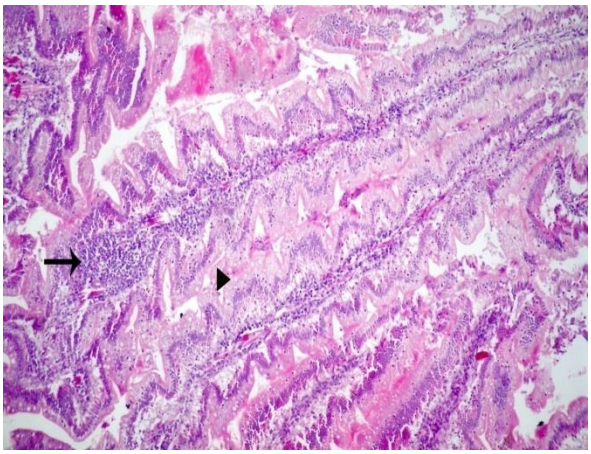

Fig13: Intestine of G4 showing normal intestinal villi with mild lymphocytic infiltrate (arrow) (HE, 200x).

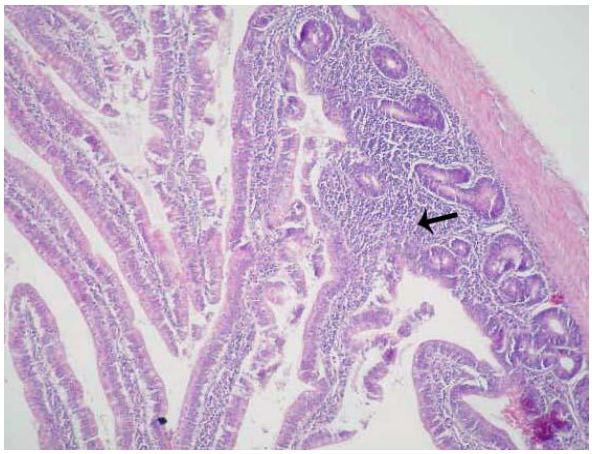

Fig14: Intestine of G5 showing normal intestinal villi with lymphohistiocytic exudates in lamina propria(HE, 50)

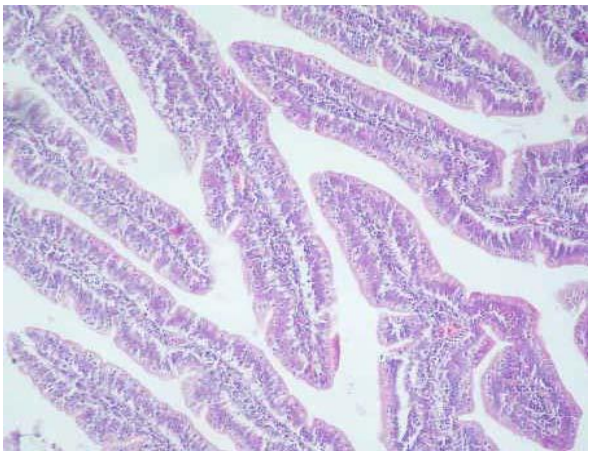

Fig15: intestine of G6 showing normal histological architecture of intestinal villi and normal enterocytes.(HE,100x)

Conclusion

From the obtained results it can concluded that aflatoxin has hepatotoxic effects through decrease total protein, albumin and globulin. Moreover increase ALT,AST,ALP and nephrotoxic effects through increase urea, uric acid, creatinine and induced histopathological changes of liver, kidney and intestine. Addition of turmeric, ginger and HSCAS to ration were induced a protective effect against aflatoxicosis and improvement the growth performance parameters and histopathological picture at 45 day. So we advise to use turmeric powder ,ginger and HSCAS in compination in poultry farms to counteract afltoxicosis and improve growth performance.

References

AbdollahAk.,AbolghsemGO.,Ardashi SH. And HosseinMoravej(2014): Effects of ginger root(Zingiber officinale) on egg yolk cholesterol ,antioxidant status and performance of laying hens. J. Of applied Animal research 39:1, 19-21

Abo-GhanemaII, El-Nasharty MA, El-Far AH, Hanan AG, (2012): Effect of ginger and L-carnitin on the reproductive performance of male rats. World academy of science, Engineering and technology, 64, 04-22.

Ahmed M.A.E.,RavikanthK.Rekhe D. and Maini S.(2009): Histopathological alteration in aflatoxicity and its amelioration with heromineral toxin binder in broilers .Veterinary world ,2(10);390-392.

Ali Rafiee,Yaser R.,Farshad Z. and Fatemeh A.(2013): Ginger(Zingiberofficinale) and thymus(Thumus vulgaris)extract on performance and hematological parameters on broiler

chicks .Sci.Agri.4(1):20-25.

Al-yahyaMA, (1998): phytochemical studies of the plants used in traditional medicine of Saudi Arabia. Fitoterapia., 57:179-18 .

Anto RJ,.Kuttan G., Dinesh Babbuk K.V. Rajasekharank N.and Baba KDF(1998): Anti inflammatory activity of nature synthetic curcuminoids. Pharma. Pharmacol. Commun.4(2):103.

Arafa H.R. (2005): Curcumin attenuates diet induced hypercholesterolemia in rats Med.Sci. Monit., 11(7):BR228-234.

AravindKL,PatilVS,Devegowda G, Umakantha B and Ganpule SP, (2003): Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally contaminated feed on performance and serum biochemical and hematological parameters in broilers. Poultry science, 82, 4,571-576.

Azza M. mohammed and Nadia S. Metwally. (2009): Anti aflatoxigenic activites of some plant aqueous extracts against aflatoxin B1induced renal and cardiac damage.J. prnal of Pharmacology and Toxicology ,4 (1) 1-16.

Bakshi, C. S. A. Sikdar, T. S. Johri, Meenakshi Malik and R. K. Singh.(2000): Effect of grade dietary levels of aflatoxin on humoral immune response in commercial broilers. Ind.J. Comp. Microbiol. Immunol. Infect. Dis. 21:163-164

Bancroft J. D. and M. Gamble (2007): Theory and practice of histological techniques (5^{th} Ed), Churchill Livingstone London 125-138.

Batt , B.M. , T. Talat and R. Sadar (2001) : Estimation of aflatoxin B1 in feed ingredients and compound poultry feed pak. Vet. J. 21 : 57-60

Bintvihok,A. and Kositcharoenkul (2006): Effect of dietary calcium propionate on performance hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low level of aflatoxinB1 .Toxicol.,47:41-49.

Cavin C,Marin –kuan M. and Schilter B. (2008): Induction of Nrf2- mediated cellular defenses and alteration of phase 1 activites as mechanism of chemoprotective effects of coffee in the liver. Food and Chemical Toxicology 46:1239-1248.

Davis N.D.,Dionber U.l. and Dridge (1966): Production of aflatoxin B1 by Aspergillus flavus in a semi synthetic medium .APPl.Microbiol.,14:378-380.

Dawoud A.S.,Doaa A.H. EL Matary and Nagwa A. Said(2002): Effect of microbial phytase enzyme in correlation of calcium, phosphorus and biochemical serum conistituents in broilers fed on mycotoxiccated mycotoxin controlled ration.SCVMJ,(2) 599-610.

Demir E.,Ozcan MA. andSuicmez M**.,(2003**): The use of natural feed additives as alternative for an antibiotic growth promoter in broiler diet Br. Poultry Sci.44:44-45.

Denli M.,OkanF.,Doran F. and inal T.C.(2005): Effect of dietary conjucated Linoeic acid (CLD) on carcass quality ,serum lipid variable and histophathological changes of broiler

chickens infected with aflatoxin B1 .S.ArF.J.Anim.Sci.,35:109-116.

Denli M.Blaandon J.C.Salado S. and Perez J.F.(2009): Effects of dietry aflatoxin on performance, serum biochemistry, histobahological changes and aflatoxin residues in broilers exposed to aflatoxin B1.Poult.Sci.. 88(7); 1444-51.

Dewegoda G and Murthy TNK, (2005): Mycotoxins: Effects on poultry, performance and health. The mycotoxin Blue Book, Diaz D.E. (ed.), Nottingham University Press, Nottingham, PP, 25-56.

Diaz G.J.,cortes A and Botero L. (2009): Evaluation of the ability of a feed additive to ameliorate the adverse effects of aflatoxin in turkey poults. Br.Poult. Sci.50 (2):240-250.

Duncan D.B.(1955): Multible range and multible F-Test Biometrics 11-1.

El-Fara.H. (2008): The biochemical protective role of some herbs against aflatoxicosis in duckling. PhD.ThesisFac.Vet.Med.Alex.Univ.

Elmakki AM, Abdelatti AK,DousaMB, Elagib AAH, Malik EEH,Elamin MK, (**2013):** Effect of treated Cowpea Seeds on Broiler Chicken, Global Jornal of Animal Scientific Research, 1(1), 61-68.

Emadi,M.and H. Kermanshahi (2007):Effect of turmeric rhizome powder on the activity of some blood in broiler chickens. Int.J.Poult.Sci. 6:48-51.

Gowda N.K.,MalathiNV. And Suganthi R.U. (2004): Effect of some chemical and herbal compounds on growth of Aspergillus parasiticus and aflatoxin production .Animal Feed Science and Technology 116(3-4):281-291.

Gowda N.K.Ledouxa D.R..,Rottighausa A.J.and Chena Y.C.(2008): Afficacy of turmeric (Curcumin longa),containing a known level of curcumin and hydrated sodium calcium aluminosilicate to ameliorate the adverse effect of aflatoxinin broiler chicks .Poult. Sci., 87:1125-1130

Gowda N.K.S.,Ledouxa D.R.,Rottighausa A.J. and Chena Y.C.(2009): Antioxidant efficacy of curcuminoids from turmeric (Curcuma longa L) powder in broiler chickens fed diets containing aflatoxin B1.Br.J.Nutr.,102(11):1629-1634.

Hassan, A.A., R. Shahid, H.S. Tassawar and A. Iqbal, (2000): Effect of sodium bentonite as aflatoxin binder in broiler feeds containing fungal infected grains. Pak. J. Agri. Sci., 37(3-4): 163-165.

Iqbal Z.,Nadeem OK., and Waraich FN.(2011): In vitro anthelmintic activity of Allium sativum ,Zingiber officinale, Curcurbita mexicana and Ficus religiosa **.**International J. of Agriculture Biology,3:454-457.

Kalorey, D.R., Kurkure, N.V., Ramgoankar, J.S., Sakhare, P.S., ShubhangiWarkeandNagot, N.K. (2005): Effect of polyherbal feed supplement "Growell" during induced aflatoxicosis, ochratoxicosis and combined mycotoxicosis in broilers. Asian Australian Journal of Animal Sciences. 18(3): 375-383.

Kurkure, N. V., S. P. Pawar, S. M. Kognole, A. G. Bhandarkar, A. G. Ganorkarand D. R. Kalorey (2000): Ameliorative effect ofturmeric (Curcuma longa) in induced aflatoxicosis in cockrels. Ind. J. Vet. Pathol. 24:26-28.

Lafi et al. (2010). Histopathology of the liver affected with aflatoxins in broiler chicks. *Al-Anbar Journal Veterinary Science*, *3*, 1.

Lee, S.E., B.C. Campbell, R.J. Molyneux, S. Hasegawa and H.S. Lee, (2001): Inhibitory effects of naturally occurring compounds on aflatoxin B_1 biotransformation. J. Agric. Food Chem., 49: 5171-5177.

Mabbett, T.,(2005):. Integrated management of mycotoxins. Poultry Int., 44(8): 10-14.

Malekizadeh M. , M.M. Moeinil , Sh. Ghazi ,(2012) : The Effects of different levels of Ginger (Zingiber officiale Rosc) and Turemic (Curcuma longa Linn) Rhizomes Powder on Some Blood Metabolites and Production Performance Characteristics of Laying Hens , J. Agr. Sci. Tech. , Vol. 14:127-34 .

Maurice DV,Bodine AB and RehrerNJ. (1983): Metabolic effects of low aflatoxin B level on broiler chicks. Applied and Environmental Microbiology, 45, 3,980-984.

Najafi S. and Taherpour K., (2014): Effect of Dietary Ginger (Zingiber oficinale), Cinnamon (cinnamomum), Synbiotoc and Antibiotic Supplementation on Performance of Broilers. J AnimSCi .Adv. 40: 658-667.

NayakS.and SashidharR.B. (2010): Metabolic intervention of aflatoxinB1toxicity by curcuamin .J.Ethnopharmachol. 17:127(3)641-644.

Nelson, T.S., Z. Johnson, L.K. Kirby and J.N. Beasly, (1982). Digestion of dry matter and amino acids and energy utilization by chicks fed mold corn containing mycotoxins. Poultry Sci., 61: 584-585.

Nisarani K. S. Gowda, David R. Ledoux, George E. Rottinghaus, AlexJ.Bermudez and Yin C Chen(2009): Antioxidant efficacy of curcuminodis from turmeric (Curcuma longa L.) powder in broiler chickens fed diets containing aflatoxin B1. British Journal of Nutrition 102, 1629-1.

Osborne D. and HamiltonP.B. (1981): Decreased pancreatic digestive ezymes during aflatoxicosis. Poultry Sci., 60:1818-1821.

Pasha T.N.,Farooq M.U.,JabbarM.A.and Khan A.D.i (2007): Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbent in diet for broiler chickens. Anim.Feed .Sci.Technol. 132:103-110.

Ramadevi, N. R., R. Gopal Naidu and P. Ravikumar (2000): An assessment of the protective effect of bentonite on ochratoxicosis in broiler with reference to certain haematological profile. Ind. Vet. J. 77:303-306.

Rangsaz,N. and M.G.Ahangaran (2011): Evaluation of turmeric extract on performance indices impressed by induced aflatoxicosis in broiler chickens.Toxicol.Ind.Health,27:956-

960.

RathodP.R.,Kulkarni G.B. and Gangane G.(2013): Pathological effect of low afltoxicity in broilers. The Biasean 8(30):1115-1118.

Rosa, C.A.R., Miazzo, R... Magnoli, C., Salvano, M.,Chiacchiera, S. M., Ferrero, S., Saenz, M., Carvalho,E.C.Q. and Dalcero, A. (2001). Evaluation of the Efficacy of Bentonite from the South of Argentina to Ameliorate the Toxic Effects of Aflatoxin in Broilers. *Poultry Science* 80:139–144.

Sandhu B.S.Singh H. and Sngh B.(2005): Pathological studies on broiler chicks fed aflatoxin or ochratoxin and inoculated with inclusion body hepatitis virus singly and in concurrence Vet. Research Communication:19(1) :27-37.

Sapcota D.,Gogoi R.,Gohain A. (2009): Economic advantages of supplementation of curcuma longa during induced aflatoxicosis in broilers. Indian Journal of Animal Sciencs. 79 (9), 942-944.

SAS Institute Inc.(1990): SAS/STAT User's Guide. Version 6,4 ed. Vol.2 SAS.Inst.,Cary,NC.

Senthil K.D.,SugunaRao,PradeepK.P.andKarikalan M/(2014): Ameliorative efficacy of citrus fruit oil in aflatoxicosis in broilers:a growth and biochemical study.Turk Vet.Anim.Sci.38:207-211.

Sharline K.S.,Howarth B.J. and Wyat R,D.(1980): Effect of dietary aflatoxin on reproductive performance of mature white leghorn. Poult .Sci. 59:1311-1315.

Shi Y.H.Xu J.L. and wang C.Z. (2006): Efficacy of modified montmorrillonite nanocompositeto reduce the toxicity of aflatoxin in broiler chicks Anm. Feed.Sci. Technol, 129:138-148.

Singh, G., Singh, O. P. and Maurya, S (2002): Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species, Progress in Crystal Growth and Characterization of materials, 75-81.

Smith JE, Solomons G, Lewis C, et al. (1995): Role of mycotoxins in human and animal nutrition and health. Nat toxins 3,187-192.

Soni, K. B., A. Ranjan and R. Kuttan (1992): Reversal of aflatoxin induced liver damage by turmeric and curcumin. Cancer Letters. 66:115-121

St-Onge MP, Farnworth ER, Jones PJH. (2000): Consumption of fermented and non fermented dairy products: effects on cholesterol concentrations and metabolism. The American Journal of Clinical Nutrition 71:674_ 681.

Sur E. Celik (2003): Effect of aflatoxin B1 on the development of bursa of fabricius and blood lymphocytes acid phosphatase of chicken. British poultry Science 44:558-566.

Surai , P. F.(2001) : Natural Antioxidants in Avian Nutrition and Reproduction . 1st Edition, Nottingham University Press,UK, PP. 81-91.

Tedesco D.,S.Stodler,S.Galletti M.Taneni ,O.Sonzogni and L.Ravarotto(2004): Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxinB1 in broiler chicks .Poultry Sci.,83:1839-1843.

Tejada-Castaneda, Z.I., E.A. Vila-Gonzalez, M.T. Casaubon-Huguenin, R.A. Cervantes-Olivares, C. Va-Pelaez, E.M. Hernandez-Baumgarten and E. Moreno-Martınez, (2008): Biodetoxification of aflatoxin-contaminated chick feed. Poultry Sci., 87: 1569-1576.

Verma, J.; Swain, B.K.; Johri, T.S. (2002): Effect of various levels of aflatoxin and ochratoxin A and combinations of there on protein and energy utilisation in broilers. *J. Sci. Food Agric., 82,* 1412–1417.

Wafaa A. Abdel-Ghany, M.E. Hatem andM.Ismail (2013): Evaluation of the efficacy of additives to counteract the toxic effects of aflatxicosis in broiler chickens .International J.Animal and Veterinary Advances 5(5):171-182.

Wild C.P., YinF,.CheminA.,Kirk G. D. and Hall A.J.(2000): Enviromental and genetics determination of aflatoxin albumins adducts in the Gambia .International J. of Cancer,86:1-8.

Yarru ,L .P., R. S. Settivari ,N. K. S .Gowda ,E .Antoniou, D.R.Led,oux and G.E.Rottinghaus(2009): Effects of turmeric (Curcuma longa) on the expression of hepaticgenes associated with biotransformation, antioxidant and immune systems in broiler chicks fed aflatoxin .Poult .Sci.,88:2620-2627 .

Yassein SH. N.andZghair Z.R,(2012): Study of toxicity and Pathogenicity of aflatoxin B1 and G1 in mice. Al –Anbar J. 5(1)22-31.

Zahidhussain, mohammadzargham khan and zahoorulhassan (2008): Production of aflatoxins from Aspergillus flavus and acute Aflatoxicosis in young broiler chicks. *Pak. J. Agri. Sci.,* 45(1) 233-236.

Zhang GF Yang ZB Jiang SZ. And Gia GS. (2009): Effects of Ginger root (Zingiber officinale) processed to different particle sizes on growth performance, antioxidant, status, and serum metabolites of broiler chickens .Poultry Sci.88:2195-2166.

Zhao, J.; Shirley, R.B.; Dibner, J.D.; Uraizee, F.; Officer, M.; Kitchell, M.; Vazquez-Anon, M.; Knight, C.D.(2010**):** Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler **chicks. *Poult. Sci, 89,* 2147–2156.**

ZomrawiW.B.,AbdelAtti KHN.,Dousa B M. and Mahla AG.(2013): The effectof ginger root powder (Zingiber officinale) on broiler chick performance, carcass characteristic and serum conistituents .J. of Animal Science Advances3,42-47.