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Evaluation of Antimycotoxin Effects of Humate and Hydrated Sodium Calcium Aluminosilicate on Broilers Toxicated with Aflatoxin

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Abstract

The objective of this study was to investigate the protective effect of dietary natural sorbent-Humate (0.2%) and Hydrated sodium calcium aluminosilicate (HSCAS) (0.5%) on the prevention of aflatoxin (AF) toxicity in the broiler chicken 7-35 days of age. Two hundred and forty broiler chicks were randomly divided into four treatment groups of 4 replicates (each contained 15 chicks). While chicks in group 1 were fed a basal diet free of toxin (control), group 2 chicks were fed a basal diet contaminated with 1ppm AF, the other two groups 3 and 4 were fed contaminated basal diet supplemented with 0.2% Biofarm® Dry humate, and 0.5% HSCAS, respectively. Parameters evaluated were growth performance, some serum constituents, some organs weight, and immune response to Newcastle disease and infectious bursal disease. The results showed that, Aflatoxin (1 ppm/kg) significantly reduced feed intake, weight gain and feed efficiency. Further aflatoxin increased the relative weights of liver, kidney, gizzard and spleen, while the relative weights of thymus and bursa of Fabricius were decreased by AF. Aflatoxin toxin also reduced antibody titres against Newcastle disease and infectious bursal disease. Modified 0.2% Humate significantly ($P \leq 0.05$) improved body weight, feed intake, decreased relative organ weights and improved antibody titres, bursa of Fabricius and thymus weights. Hydrated sodium calcium aluminosilicate (0.5%) showed improvement against AF. The results of our experiment showed that 0.2% Humate and 0.5% HSCAS has a positive effect on the growth of broilers. And it could have been a suitable natural supplement for growing broilers against the adverse effects of aflatoxins.

1. INTRODUCTION

Aflatoxins are a group of naturally occurring, extremely toxic and biologically active metabolites produced by some strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species (Manafi *et al.*, 2011). Aflatoxin B1 (AFB1) is the most toxic among all aflatoxins (AFB1, AFB2, AFG1 and AFG2). Aflatoxin (AF) (as mean total aflatoxins) is a relatively low molecular-weight, lipophilic molecule that appears to be absorbed rapidly and completely (Kumagai, 1989) from the GIT (gastrointestinal tract). The harmful effects of these mycotoxins in animals are affected by some factors such as level of aflatoxin, species, duration of exposure, gender, age and general health status of animals (Manafi *et al.*, 2009b). In poultry, the economic losses associated with aflatoxin exposure include poor growth and feed conversion, increased mortality, leg problems, and carcass condemnations (Huff *et al.*, 1992).

A variety of physical, chemical and biological techniques for mycotoxin detoxification of feeds have been used, but they have had limited success (Pittet, 1998). Adsorbents used to prevent the gastrointestinal absorption of mycotoxins must form a strong complex and also have a high capacity to prevent saturation (Ramos, and Hernandez 1996). A hydrated sodium calcium aluminosilicate (HSCAS) , the in vitro experiments carried out with

radiolabelled aflatoxin B1 by Phillips et al. (1988) demonstrated that of 38 different sorbents tested (a variety of aluminas, zeolites, silicas, phyllosilicates and chemically modified phyllosilicates) HSCAS was the aflatoxin B1-adsorbing compound that was able to form the most stable complex with this mycotoxin (with a sorption of greater than 80% of aflatoxin Bl present in the medium). The complex was also stable in water at pH 2,7, and 10 and at temperatures of 25 and 37°C. The stability of the HSCAS-aflatoxin B1 sorption complex was evaluated by extraction with an elutropic series of solvents. As less than 10% of aflatoxin B1 was extracted, the authors suggested that the mechanism implicated in this process could be due to chemisorption by the formation of a strong bond between the molecules. Recently a more specific mechanism was proposed (Sarr et al., 1990), that of formation of a complex between the carbonyl system of the aflatoxin and the uncoordinated edge-site aluminum ions in the HSCAS.

The term 'humus' has been known to science for years; it is a transformation product of animal and plant organisms. Humate or humic acid (HA) is a class of compounds resulting from decomposition of organic matter and are natural constituents of drinking water, and solid and lignite disintegrated compounds particularly from plants. Humates are the salts of humic acid in which the exchange site is Ca^{+}, Na^{+}, Al^{+} and Fe^{+2} rather than hydrogen (HuminTech, 2004). Jansen van Rensburg et al. (2006) They reported that Humic substance able to inhibit bacterial and fungal growth, thus decreasing levels of mycotoxins in feed. Its beneficial effects include stress management, anti-inflammatory activity, antiviral properties, immune system prevention of intestinal diseases, mainly diarrhea in humans and animals as well as improved gut health for better nutrient utilization as well as improved the health status by working against pathogens developing immunity and improved growth of broilers by increasing the digestion of protein and improved trace element utilization. Research on humate includes humus, humic acid, fulvic acid, ulmic acid, and trace minerals and observed that humates included in the feed and water of poultry promoted growth (Islam, et al., 2005).. According to Jansen van Rensburg et al. (2006) humic acid was able to adsorb about 10.3, 7.4, and 11.9 mg of AFB1/g of oxihumate at pH 3, 5, and 7, respectively. In contrast, the maximum AFB1 adsorption capacity of sodium bentonite from southern Argentina was estimated to be 45 mg/g at pH 2 (Rosa, et al., 2001). A recent report by Jansen van Rensburg et al. (2006) described that humic acid, but not brewers'dried yeast, could alleviate some of the toxic effects of aflatoxin in growing broilers. Therefore, the objective of the present study was conducted to study the efficacy of 0.2% Humate and 0.5% HSCAS to eliminate the adverse effects of aflatoxin on growth performance (body weight, body weight gain, feed intake and feed conversion ratio) and physiological parameters as judged by percentage of organs, and serum biochemical constituents of Cobb broilers throughout the period from 7 to 35 d of age.

2.MATERIALS AND METHODS:

This experiment was carried out at the Poultry Research Farm, Faculty of Agriculture, Kafrelsheikh University, Egypt, throughout the period from 2017.

2.1.Experimental Design, Bird and Data Collection:

A total of 300 one-day-old broiler chicks were adapted for a 7-day period before start of the experimental. During this period, the chicks were submitted to conventional broiler chicken management and housed in floor pens in an environmentally controlled broiler house with litter floors. They were fed a commercial starter-grower diet (based on corn and soybean meal, containing 23% CP, 3204 Kcal ME/Kg diet) up to 21 days of age and then switched to fininsher diet (20.0% CP, 3201 Kcal ME/Kg diet) from 22 to 35day (Table 1). Birds had access to feed and water *ad libitum* from one to 35 days of age. The basal diet was supplemented with amino acids, mineral and vitamins at the levels recommended by the National Research Council (NRC, 1994), and did not contain any antibiotics, coccidiostats, or growth promoters. As well as the basal diets used subsequently, was analyzed and tested negative for AF. In addition, birds were inspected daily and any health problem was recorded. Lighting was supplied for 23 h daily. At 7 days of age, 240 chicks of similar weight were randomly divided to 16 clean pens in the same broiler house used for the adaptation period. The chicks were divided into 4 treatment groups, with 4 replicates per treatment and 15 chicks per replicate: 1) basal diet (control), 2) diet contaminated with 1ppm AF, 3) contaminated diet supplemented with 0.2% Biofarm® Dry humate, and 4) contaminated diet supplemented with 0.5% HSCAS. Each kilogram of Humate contained 160 mg of polymeric polyhydroxy acid (humic, fulvic, ulmic and humatomelanic acids), 663.3 mg of SiO and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5

mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg; and Al, Na, K, Mg and P in trace amounts).
Hydrated Sodium Calcium Aluminosilicate (HSCAS) is a feed additive, adsorbent, anti-caking and toxin binder that was obtained from Trouw Nutrition International and mixed with the ration at a rate of 5 g/kg (0.5%). Hydrated sodium calcium aluminosilicate clay (HSCAS) is a chemical compound that contains Silicon oxide (64.7%), aluminum oxide (15.5%), oxides of iron, magnesium, calcium, sodium, potassium (8.9%) and moisture (10.9%).

2.2.Aflatoxins:
Aflatoxin (AF) was produced from *Aspergillus parasiticus* (NRRL 2999) was kindly provided by National Institute of animal Health, Dokki, Cairo, Egypt by culturing on rice using a modified method of Kubena *et al.* (1990) and modified by West *et al.* (1973). The fermented rice was autoclaved and ground to powder, and the AF content was measured by spectrophotometric analysis (Nabney, and Nesbitt. 1965) as modified by Wiseman *et al.* (1967). The AF within the rice powder consisted of 86.4% aflatoxin B1, 2.2% aflatoxin B2, 0% aflatoxin G1, and 11.4% aflatoxin G2. The rice powder was incorporated into the basal diet to provide the desired level of 1.0 mg of AF/kg of diet. The detected levels of AF in the control diet were below the detection limits.

2.3.Measurements:
Chicks were weighed individually, and feed consumption for each pen was measured weekly during the 5-wk experiment. Cumulative weight gain and feed consumption were determined, whereas weekly and cumulative gain:feed ratios were calculated. Feed consumption and gain:feed was adjusted for mortalities when appropriate.
Blood was collected at 35 d of age from 8 birds in each treatment in non-heparinised tubes by brachial vein puncture. Serum was obtained from these samples and analyzed for serum albumin, total protein, uric acid, total lipids and the activities of alanine amino transferase (ALT) and aspartate amino transferase (AST), the concentrations of alkaline phosphatase (ALP) in blood serum were analyzed by using commercial kits from Diamond Diagnostics Company, Egypt. Antibody titers against ND and IBD were determined employing ELISA technique using commercial test kits
On day 35 of age, 3 birds in each treatment were slaughtered and the liver, heart, proventriculus, spleen, bursa of Fabricius, thymus and gizzard were removed and weighed and calculated as a percentage of body weight. After that, different parts of the small intestine including the duodenum (from gizzard outlet to the end of pancreatic loop), jejunum (segment between pancreatic loop and Meckel's diverticulum) and ileum (segment between Meckel's diverticulum and ileocaecal junction) were removed and their weights and lengths were recorded. To measure villus height and crypt depth, 2 cm segments from the middle part of the duodenum, jejunum and ileum were removed, flushed with physiological saline and immediately put into a 10% buffered formalin solution until further processing. After embedding the samples in paraffin, a 5 μm section of each sample was placed on a glass slide and then stained, using haematoxylin and eosin, for measuring villus height and crypt depth. The distance from the tip of the villus to the villus crypt junction represents villus height, while crypt depth was defined as the depth of the invagination between adjacent villi. A villi and crypts per sample were measured using light microscope.

2.4. Pathological examination
Sample from liver, was taking and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%) cleared in xylene and embedded in paraffin, 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.
2.5.Detection of aflatoxin residues: liver was analyzed for quantification of aflatoxin residues using the method according to (AOAC, 2000).

2.6.Statistical analysis:
The obtained data were analyzed using the liner model programs of **SAS (2002)** at 5% level of significance using the following model:
$Y_{ij} = \mu + T_i + e_{ij}$
Where: Y_{ij} = An observation, μ = Overall mean, T_i = Treatments (i = 1, 2,…and 4) , e_{ij} = Experimental error

Table (1): Composition and calculated analysis of Starter and Finisher diets.

Ingredients	Starter-grower (1-21d)	Finisher (22-35d)
Yellow corn	54.40	62.00
Soybean meal, 44%	27.00	24.05
Corn Gluten meal, 60%	10.00	6.19
Soy bean oil	4.55	4.00
Limestone	1.10	1.00
Di-calcium phosphate	2.20	2.05
Vit &min. premix*	0.30	0.30
DL-Methionine	0.05	0.01
L-lysine (HCl)	0.15	0.15
Na Cl	0.25	0.25
Total	100	100
Calculated analysis: **		
CP, %	23.03	20.02
ME (Kcal/kg)	3204	3201
Calcium, %	1.05	0.97
Available phosphorus, %	0.45	0.42
Lysine, %	1.14	1.03
Methionine, %	0.52	0.41
TSAA, %	0.90	0.73

*Each 3kg contain: Vit A 12000000IU, Vit D3 2000 000 IU, Vit E 10g, Vit K3 2g,Vit B1 1g, Vit B2 5g, Vit B6 1.5g, Vit B12 10mg, Nicotinic acid 30g, Pantothenic acid 10g, Folic acid 1g, Biotin 50mg, Choline chloride 250g, Iron 30g, Copper 10g, Zinc 50g, Manganese 60g, Iodine 1g, Selenium 0.1g, Cobalt 0.1g and carrier (CaCo3) to 3 kg.
**According to tables of NRC (1994).

3. RESULTS AND DISCUSSIONS

3.1. Growth performance parameters:

The effects of dietary treatments on chick performance from day 7 to 35 are presented in Table 1. When compared with the control, feeding the contaminated diet with AF resulted in a significant decrease in final body weight, body weight gain and feed intake by (19.75%, 21.30 and 13.75 % respectively). When compared with control, feeding the contaminated diet with AF alone resulted in a significant increase in the feed:gain ratio (g of feed /g of gain) by (9.63 %) ($P<0.05$). The addition of 0.2% Humate or 0.5% HSCAS to the aflatoxicated diet significantly ameliorated the adverse effect of AF toxicty on the growth performance when compared to the group fed aflatoxic diet alone ($P<0.05$) (Table 1). Addition of 0.2% Humate or 0.5% HSCAS significantly increased final body weight at 35 day by (23.07% and 22.30% respectively), body weight gain by (25.37% and 24.53% respectively) and feed intake by (15.57% and 16.68% respectively) as compared with the aflatoxicated group. There were 7.8 and 6.3% improvement in feed conversion ratio for treatments fed AF-diet supplemented with 0.2% Humate and 0.5% HSCA respectively as compared with the AF-diet alone group.

The reduction in body weight, body weight gain and increase in FCR in aflatoxicated group (2) in agree with the results obtained by (Denli *et al.*, 2009 and Wafaa *et al.*,2013). The reduction in growth performance upon feeding aflatoxin might be attributed to reduced protein synthesis as citied by (Verma *et al.*,2002) reduce appetite by (Sharlin *et al.*,1980), increase lipid excreation in dropping (Osbrne and Hamilto,1981). Hassan *et al.* (2000) reported that the toxicity of AFB1 as characterized by reduction in body weight gain as interfere with normal metabolic pathway through 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.

This reduction in cumulative feed intake in AFB1 fed birds might have been due to impaired hepatic metabolism, interference of AFB1 with phosphenolpyruvate carboxylase, inhibition of elongation and/or termination of the translational process of protein synthesis and interference of AFB1 in consecutive steps in mitochondrial

respiratory chain. Further, Aflatoxin B1 accumulation in the liver and high content of microsomal cytochrome P-450 enzymes of hepatocytes favors the formation of DNA AFB1 adducts (Jay *et al*., 2007).

Table 2: Effect of 0.2% Humate and 0.5% HSCAS on growth performance for broiler chicks fed an aflatoxin contaminated diet from 7 to 35 days of age.

Items	Dietary treatments				SEM*
	Control	AF	AF + 0.2% Humate	AF + 0.5% HSCAS	
Initial body weight at 7 d	115.5	116	115.6	115.5	1.40
Final body weight at 35 d	1620^{a}	1300^{c}	1600^{a}	1590^{b}	28.96
Body weight gain (g) (7-35d)	1504.5^{a}	1184^{c}	1484.4^{ab}	1474.5^{b}	18.25
Feed intake (g/bird/period)	2814^{ab}	2427^{c}	2805^{b}	2832^{a}	1.65
Feed conversion (g:g)	1.87^{b}	2.05^{a}	1.89^{b}	1.92^{ab}	0.12
Mortality rate (%)	0.00	15.00^{a}	3.33^{b}	3.33^{b}	0.02

a-d= Means with the same letter in each column are not significantly different at P≤0.05, *SEM = Standard Error of the mean

Table 3: Effect of 0.2% Humate and 0.5% HSCAS on serum constituents for broiler chicks fed an aflatoxin contaminated diet at 35 day of age.

Items	Dietary treatments				SEM*
	Control	AF	AF + 0.2% Humate	AF + 0.5% HSCAS	
Total protein, (mg/dl)	4.40^{a}	2.30^{b}	4.22^{a}	4.08^{a}	0.22
Albumin, (mg/dl)	2.52^{a}	1.65^{b}	2.36^{a}	2.30^{a}	0.18
Globulin, (mg/dl)	1.88^{a}	0.65^{b}	1.86^{a}	1.78^{a}	0.14
AST,(IU/L)	150.20^{d}	230.16^{a}	171.60^{b}	190.50^{c}	0.73
ALT,(IU/L)	40.81^{d}	95.00^{a}	55.25^{b}	60.76^{c}	0.47
ALP,(IU/L)	58.50^{c}	72.60^{a}	62.12^{b}	65.15^{b}	0.59
Uric acid, (mg/dl)	4.20^{c}	8.60^{a}	4.50^{c}	4.76^{b}	0.23
Creatinine, (mg/dl)	0.60^{c}	1.10^{a}	0.80^{b}	0.86^{b}	0.18
Total lipids, (mg/dl)	347.5^{a}	255.5^{c}	328.6^{b}	330.5^{b}	22.5
Antibody titer:					
ND	6.216^{a}	3.368^{c}	6.186^{a}	6.016^{b}	0.34
IBD	4.430^{a}	3.160^{c}	4.265^{a}	3.916^{b}	92.76

a-d= Means with the same letter in each column are not significantly different at P≤0.05, *SEM = Standard Error of the mean

Table 4: Effect of 0.2% Humate and 0.5% HSCAS on organ weights and AFB1 residues in liver for broiler chicks fed an aflatoxin contaminated diet 35 day of age.

Items	Dietary treatments				SEM*
	Control	AF	AF + 0.2% Humate	AF + 0.5% HSCAS	
Liver weight, %	1.85^{c}	2.66^{a}	1.96^{b}	2.00^{b}	0.19
Kidney weight, %	0.537^{c}	0.796^{a}	0.590^{b}	0.601^{b}	0.04
Gizzard weight, %	1.568^{d}	2.173^{a}	1.627^{c}	1.742^{b}	0.14
Spleen weight, %	0.141^{c}	0.242^{a}	0.144^{c}	0.170^{b}	0.001
Proventriculus weight, %	0.501	0.498	0.503	0.500	0.02
Heart %	0.591	0.600	0.595	0.590	0.044
Thymus weight, %	0.247^{a}	0.223^{b}	0.246^{a}	0.251^{a}	0.08
Bursa weight, %	0.215^{a}	0.203^{b}	0.211^{a}	0.220^{a}	0.02
AFB1(ng/g) residues:					
Liver	ND**	0.80^{a}	0.13^{b}	0.15^{b}	0.03

a-d= Means with the same letter in each column are not significantly different at P≤0.05, *SEM = Standard Error of the mean **ND: not detected (determination limit of the analytical method: 0.01 ug/kg for aflatoxin B1)

Table 5: Effect of 0.2% Humate and 0.5% HSCAS on Gut parameter for broiler chicks fed an aflatoxin contaminated diet at 35 day of age.

Items	Dietary treatments				SEM
	Control	AF	AF + 0.2% Humate	AF + 0.5% HSCAS	
Duodenum (mmx10^2)					
Villus height, (VH)	265^a	175^c	242^b	235^b	9.0
Crypt depth (CD)	30^c	48^a	38^c	40^b	2.2
Villus width	19^c	25^a	20^b	21^b	2.0
VH/CD	9^a	4^c	6^b	6^b	0.8
Ileum (mmx10^2)					
Villus height, (VH)	145^a	130^b	142^a	140^a	6.5
Crypt depth (CD)	25^a	20^b	23^a	22^a	1.8
Villus width	17^a	12^b	15^{ab}	16^a	0.9
VH/CD	6^b	7^a	6^b	6^b	0.8
Jejunum (mmx10^2)					
Villus height, (VH)	160^c	195^a	167^{bc}	170^b	6.0
Crypt depth (CD)	20	25	20	22	2.0
Villus width	19^a	18^b	17^b	18^b	1.8
VH/CD	8	8	8	8	0.4

a-d= Means with the same letter in each column are not significantly different at P≤0.05, SEM = Standard Error of the mean

The increase in mortality rate due to aflatoxicosis at 35 days of age and in the entire period of experiment of this study was in agreement with that reported by Pasha et al. (2007). The increase of mortality rate due to aflatoxicosis at 35 days of age of this study may be attributed to reducing disease resistance, the gradually increase of toxic effects (Oguz et al., 2000), or, severely inhibiting the immune system of the birds (Pasha et al., 2007). The significant decrease in mortality rate due 0.2% Humate or 0.5% HSCAS supplementation, in the present study, was in agreement with previous investigations in broiler chicks fed aflatoxin contaminated diets with Humate or HSCAS (Jansen van Rensburg,et al., 2006 and Hassan et al., 2009).

Supplementation of 0.2% Humate or 0.5% HSCAS adsorbents to the aflatoxicated diets effectively improved body weight gain and feed efficiency. These effects of Humate might be attributed to mycotoxin adsorption, ability to block colonization of pathogens in the gastrointestinal tract, inhibitory effect on liver antioxidant depletion and growth promoter (Jansen van Rensburg,et al., 2006). Similar results were obtained by Sehu *et al.* (2007) and Zhao *et al*. (2010) who concluded that HSCAS at 5% concentration could significantly and completely ameliorate the growth-depressing effect of aflatoxin B1 as silica binders have been shown to bind the toxins in the digestive tract, making them unavailable for gut absorption and allowing the mycotoxin to pass harmlessly through the animal. The β-carbonyl portion of the aflatoxin molecule binds to the uncoordinated edge site of aluminum ions of the HSCAS, making the aflatoxin molecule unavailable for adsorption (Sarr *et al.*, 1990). On the other hand, Mabbett (2005) found that addition of binders at levels higher than 5% may have diluted the nutritional value of the formulated feed and ultimately reduced the growth performance of birds.

3.2. Biochemical parameters:

The effects of different dietary treatments on serum enzyme activities and biochemical indicators are shown in Table 3. AF caused decrease serum total protein, albumin, globulin and total lipids were significantly (P<0.05) lower by 47.7, 34.52, 65.42 and 26.47 % respectively, compared with the control group. When compared with controls, feeding the contaminated diet with AF caused a significant increase in serum AST, ALT, ALP activities, uric acid and creatinine by (53.23, 132.78, 24.10, 104.76 and 83.33 % respectively*)* at 35 days of age (P < 0.05).

The reduced levels of total protein and albumin are indicative of the toxic effect of aflatoxin on hepatic and renal tissues and are consistent with previous literature reporting aflatoxicosis (Kubena *et al.*, 1993b; Tejada-Castaneda *et al.*, 2008). The reduction in the total

serum protein in aflatoxin fed group could referred to impairment of amino acid transport and mRNA transcription by inhibiting DNA (Kubena *et al.,* 1993a) and is indicator of impaired protein synthesis (Kubena *et al.,* 1998). Serum total lipids was reduce when chicks fed aflatoxicated diet. Similar results were reported by Kubena *et al.* (1993a). This may be due to the interference of AF with lipid metabolism as those reported by Hamilton, and Garlich (1972) who explained that lipid transport is inhibited by aflatoxicosis which could account for the accumulation of lipid in the liver and their decreases in the serum, but the tow agents (0.2% Humate or 0.5% HSCAS) applied significantly counteracted the effect of AF. Serum creatinine and uric acid were significantly ($p<0.05$) increased in aflatoxin fed chickens. The increased concentrations of creatinine and uric acid coupled with the kidney enlargement observed, may indicate some renal tissues damage due to aflatoxin. This significant alteration in kidney parameters in birds fed on aflatoxin treatments agree with data reports of Denli *et al.* (2005) and Bintvihok and Kositcharoenkul (2006). The addition of 0.2% Humate or 0.5% HSCAS were significantly ($p<0.05$) effective in the protection against aflatoxin by preventing its toxic effect, as was reflected by ameliorating the alterations in serum biochemical parameters (increasing in serum total protein, albumin and total lipids and decreasing in serum creatinine and uric acid). 0.2% Humate or 0.5% HSCAS decreasing the amount of aflatoxin absorbed.

AST, ALT and ALP showed high significant increase in afltoxicated group compared to control group which reflect hepatic degeneration and subsequent leakage of enzymes into circulation. The addition of 0.2% Humate or 0.5% HSCAS to the aflatoxicated diet significantly ameliorated the adverse effect of AF toxicty on AST, ALT and ALP activites when compared to the group fed aflatoxic diet alone ($P<0.05$) (Table 3). These results in agreement with (Jansen van Rensburg *et al.,* 2006) and agree with (Hassan *et al.,* 2009) who fed local chicks Humate and HSCAS and found positive effects on liver enzymes reducing AST,ALT and ALP.

Feed additives supplementation alleviated the adverse effect of aflatoxicosis on serum constituents. This could explain superior performance of these groups. Thses results are in agreement with those of Jansen van Rensburg *et al.* (2006) and Hassan *et al.* (2009) who found that total protein, albumin, creatinine, cholesterol and uric acid were returned to control value, when Humate was used with aflatoxin exposed chicks. Addition of HSCAS to the aflatoxicated diet significantly decreased the adverse effects of aflatoxin on serum constituents (Hassan, 2000 and Qota *et al.,* 2005). Shebl *et al.* (2010) suggested that HSCAS had antigenotoxic effect against aflatoxin in poultry as monitored by significant decrease in the mean percentages of DNA fragmentation of liver cells, frequencies of micronucleated in bone marrow cells and the incidence of chromosomal aberrations.

3.3 Weights of some internal organs

Significant differences were found in the relative weights of liver, kidney, gizzard, thymus and bursa of Fabricius among the treatments (Table 4). AF caused significant increase in size of liver, kidney, gizzard and spleen (43.78%, 48.23%, 38.58% and 71.63 % respectively) when compared with the control group. Similar to our results those of Ortatatli *et al.* (2005) who found an increase in the absolute and relative weights of liver, kidney and gizzard of birds fed on ration containing aflatoxin indicating the hepto and nephrotoxicity of aflatoxins. Liver is considered the target organ for aflatoxin B1 because it is the organ where most aflatoxins are bioactivated to the reactive 8, 9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures and increasing liver weight (Bailey *et al.,* 2006; Pasha *et al.,* 2007). The increase in the liver weight could be attributed to increased lipid deposits in the liver due to impaired fat metabolism (Hsieh, 1979). The hepatic lipidosis is primarily mediated through inhibition of phospholipids synthesis and cholesterol. This in-turn affects the transportation of lipid from the liver (Manegar *et al.,* 2010). The increase in the gizzard weight in this study accords with Huff and Doerr (1981) who postulated that direct exposure of digestive organs to cytotoxins of aflatoxins during digestion process resulting in this response, while Hoerr *et al.* (1982) attributed this increase in weight to irritation properties of mycotoxins by direct contact with organs of upper alimentary tract. (Kubena *et al.,* 1990), which may be due to the results of severe inflammation and thickening of mucosal layer. Spleenomegaley due to dietary AF has been mentioned by Kubena *et al.* (1993a). The gross enlargement of the spleen may indicate the incidence of destruction of lymphocytes followed by edema within and around follicles and in some cases by associated hemorrhage. In addition an increase in germinal centers and pyroninophilic cells in the spleen is observed (Riddell, 1987).

Relative weight of thymus and bursa were significantly depressed in the AF (9.7% and 5.58 %

respectively). The aflatoxicated diet had no effect on the relative weight of the heart and proventriculus.

Supplementation of 0.2% Humate or 0.5% HSCAS significantly reduced the severity of aflatoxicosis on organs weight by (26.32 or 24.81 %), (25.87 or 24.50 %) (25.12 or 19.83 %) and (40.5 or 29.75 %) for liver, kidney, gizzard and spleen and respectively against 1ppm AF. These results agreed with those obtained by Jansen van Rensburg *et al.* (2006) who reported that Humate showed significant protective effects with respect to liver damage as indicated by an inhibition of liver enlargement. The results of Huff et al. (1992) and Hassan (2000) showed that adding 0.5%HSCAS to aflatoxicated diets alleviated the adverse effect of aflatoxicosis on liver damage. As well, Sehu *et al.* (2007) demonstrated microscopically that addition of HSCAS to quail feed partially decreased fat deposition caused by the aflatoxin in the liver and consequently reduced the liver's weight. Decreasing thymus and bursa glands may be attributed to the depletion of follicular lymphocytes (Abd El-Hamid *et al.*, 1992). Addition of 0.2% Humate or 0.5% HSCAS to diets contaminated with AF restored the weights of thymus and bursa of Fabricius.

3.4. Immune response to ND and IBD:

Antibody titers against ND and IBD were significantly ($p<0.05$) decreased in aflatoxicated diet (Table, 3). The aflatoxin causes low titer against the ND which may be attributed to the regression of bursa of Fabricius. This demonstrated immunosuppressive ability of aflatoxin (Dafalla *et al.*, 1987) which is may be due to inhibition of RNA polymerase *in-vivo* and subsequently limitation of protein synthesis (Lafarge and Frayssinet, 1970). In addition, this immune-suppression is claimed to functional inhibition of reticulo-endothelial system and bursa of Fabricus which is the efferent limb of the immunological system in chicken for antibody production (Co-Oper *et al.*, 1965). Aflatoxin increases the specific activity of lysosomal enzymes in the liver and muscles causing enhanced degradation of antibodies (Tung *et al.*, 1975). Mussaddeq *et al.* (2000) demonstrated that aflatoxin lowers resistance to diseases and interferes with vaccine-induced immunity in livestock. As well, Manegar *et al.* (2010) confirmed that aflatoxin causes bursal regression and suppress primary immune response for ND and Gumboro disease as evident by fall in ELISA titers. Enhancement of the humoral immune response after addition of binders like Humate or HSCAS in this investigation is in line with Ibrahim *et al.* (2000) who citied that supplementation of sodium bentonite binder was significantly effective in ameliorating the negative effect of aflatoxin on the percentage and mean of phagocytosis and HI-titer in chicks vaccinated against ND. It has been reported that Humate significantly improve antibody levels in broiler chickens, fed graded levels of AF (Swamy and Devegowda, 1998), multiple mycotoxins (Raju and Devegowda, 2002) and also it inhibited lipid peroxidation in liver of quails fed T-2 toxin (Dvorska and Surai, 2001). HSCAS inclusion to AF diets improved ($p<0.05$) the ND and IBD titers and it is in accordance with Barmase *et al.* (1990).

3.2 Intestinal Morphology

Table (5) shows the results of intestinal morphology. Bouhet *et al.* (2004), found that, the gastrointestinal tract is the first organ to come in contact with chemicals, natural toxins and foods and such should be affected with greater potency compared to other organs. Aflatoxicated diet resulted in a decrease in the villus height of birds duodenum and ileum but an increase in the jejunum ($P<0.05$). Cavret and Lecoeur (2006) and Agence Française de Sécurité (2009) explained that about >80% of aflatoxins are absorbed at the duodenum part of the intestines. As such mycotoxins always compromise intestinal epithelium either before or throughout the entire intestines by non-absorbed toxins. The ratio of villus height to crypt depth in the duodenum decreased but experienced increase in the jejunum section. However, the supplementation of 0.2% Humate or 0.5% HSCAS to the AF- diets showed a significant ($P<0.05$) increase in the villus height of duodenum and ileum. The ratio of villus height to crypt depth of the three intestinal segments follows the same trend. It is clear that increasing the villus height increased surface area caused to greater absorption of available nutrients. The villus height to crypt depth ratio according to Caspary (1992) reflects differences in the digestion and absorption of the small intestines. Applegate *et al.* (2009) found that crypt depth of gut increases linearly with aflatoxin levels resulted in influencing the villus crypt ratio. A previous study by Girish and Smith (2008) reported that grains naturally contaminated with DON significantly reduced the height, width, and surface of villus in the duodenum and jejunum of broilers. The our results are in agreement with Yang *et al.* (2012) reported that there was a decrease significantly in villus height and the ratio of villus height to crypt depth when broilers birds were fed daily with diets contaminated with AFB1 and AFB2. Long-term exposure of AFB1 and AFB2 mainly would affect the morphology of the duodenum as a result of stimulating proximal

gastrointestinal tract Yang *et al.* (2012); the characteristics of the gut morphometric attributes to altering nutrients absorption. Girgris *et al.* (2010) observed an increase in the villus height of jejunum and ileum of birds fed a contaminated diet with Fusarium mycotoxins which is contrary to the present result. The authors suggested a compensation for the reduced surface area of the duodenum villi resulting from reduced villi heights in these birds.

3.3. Histopathological studies:

Examination of liver of control observed no histopathological lesions (fig.1). On the othe hand liver of chicks fed AF-diet alone showed kupffer cells proliferation as well as as portal infiltration with mononuclear inflammatory cells (Fig.2). Individual necrosis of hepatocytes (apoptosis), necrosis of epithelial lining the bile duct (Fig.3). However, apparent normal hepatocytes associated with slight kupffer cells activation (Fig. 4& 5) were observed in examined liver sections from groups fed 0.2% Humate and 0.5% HSCAS.

3.4. Aflatoxin residues:

Results in table (4) indicated that the administration of aflatoxin in broilers led to accumulation of AFB1 in liver, aflatoxicated- group showed the highest level of AFB1 (0.80ppb), these results in agreement with that obtained by (Bintvihok and Kositchaenkal 2006). Supplementation of 0.2% Humate or 0.5% HSCAS to aflatoxicated diets showed the lowest amount of AFB1 residues which reduced by (83.75 and 81.25%) respectively. These results agreement with Hassan *et al*. (2009).

In the present study, results indicated that adding HSCAS to broiler diets as a decontamination method to sorb aflatoxin selectively during the digestive process, was less effective compared to 0.2% Humate. This results agreement with Jansen van Rensburg *et al.*(2006*)* who found that Oxihumate showed ahigh affinity for AFB1, zearalenone, Ochratoxin A, ergosine, ergotamine, ergocryptine and ergocristine, but did not bind to vomitoxin. Also found that the binding capacity of Mycosorb (HSCAS*)* to AFB1 at pH_3 proved to be considerably less than that of Oxihumate.

4. 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 uric acid, creatinine and induced histopathological changes of liver, and intestine. Addition of 0.25 Humate or 0.5% HSCAS to ration were induced a protective effect against aflatoxicosis and improvement the growth performance parameters and histopathological picture at 35 day. So we advise to use 0.25 Humate or 0.5% HSCAS in poultry farms to counteract afltoxicosis and improve growth performance.

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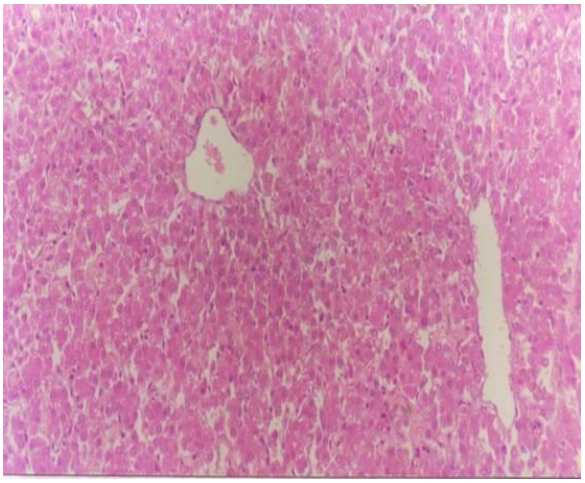

Fig. (1): Liver of control chicken showing no histopathological changes *(*H and E x 200)

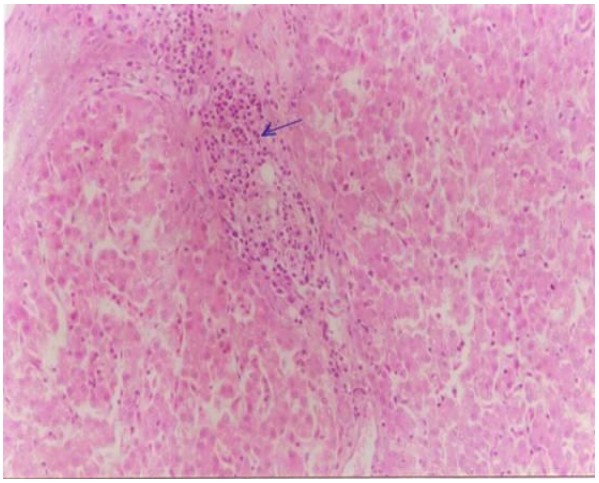

Fig. (2): Liver of chicken from group fed AF-diet showing kupffer cells proliferation as well as portal infiltration with mononuclear inflammatory cells (H and E × 200)

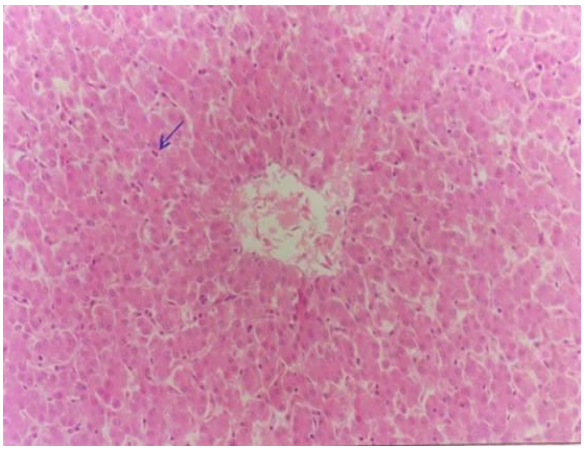

Fig. (3): Liver of chicken from group fed AF-diet showing individual necrosis of hepatocytes and necrosis of epithelial lining the bile duct (arrow) (H and E×400)

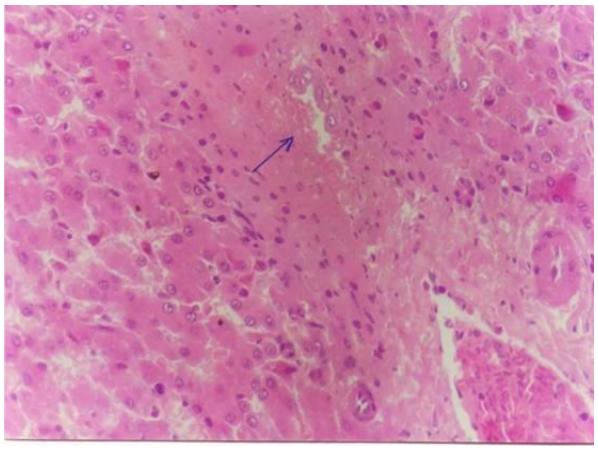

Fig. (4): Liver of chicken from group fed AF with 0.2% Humate showing apparent mormal hepatocytes and kuppffer cells activation (arrow) (H and E ×200).

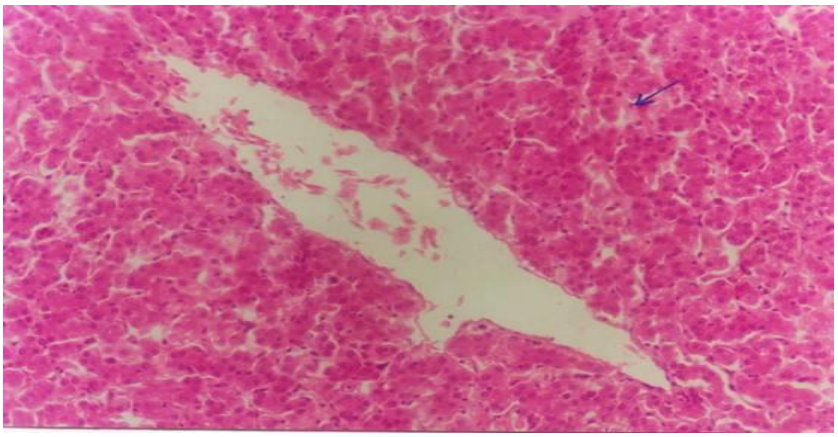

Fig.(5) Liver of chicks from group fed AF with 0.5% HSCAS showing apparent normal Hepatocytes and slight activation of kupffer cells (H and E ×200).

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