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Evaluation of the Ability of a Feed Additive to Ameliorate the Adverse Effects of Aflatoxins in Broiler Chickens

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ABSTRACT

This study was designed to evaluate the efficacy of 3.5 g Humic acid (HA), 3.5g esterified glucomannan (EGM) and 5 g Hydrated Sodium Calcium Aluminosilicate (HSCAS) to counteract the toxic effects of 1 ppm aflatoxin in the broiler chicken. Three hundred 7-day-old broiler chicks were randomly divided into five 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 three groups 3 , 4 and 5 were AF fed contaminated basal diet supplemented with 3.5 g HA, 3.5 g GEM and 5 g HSCAS, respectively. All groups were kept under observation from 7-35 days of age. Results obtained can be summarized as follows: Feeding contaminated diets significantly decreased final body weight, BW gains and feed intake. Feed conversion rate (FCR) was lowest in the group consuming the aflatoxin diet ($P < 0.05$). Further, contaminated diet caused significant decreases in serum total protein, albumin, globulin , total lipids, calcium and phosphorus. Compared with the control, the contaminated diet with 1ppm AF significantly increased the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) , uric acid and creatinine . Also, there were impairment in liver and serum antioxidant enzyme activities ,antibodies titer against Newcastle Disease (ND) and aflatoxin B1 residual in the liver associated with aflatoxin toxicity. Increased relative weights of liver and decreased dressing % were observed in chicks fed the AF- diet. The results cleared that treatment of aflatoxicated birds with 3.5 g HA, 3.5 g GEM and 5 g HSCAS induced protection from the development of signs and lesions with significant ($p<0.05$) improvement of performance when compared with un-treated control group. HA, GEM and HSCAS treatment induced significant ($p<0.05$) amelioration of the measured organs body weights ratio, humoral immune response to Newcastle Disease (ND) and biochemical parameters in aflatoxicated chickens.

1. INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi that contaminate food or feeds in the field or during storage (Yiannikouris and Jouany, 2002). It was estimated that over 25% of food/feed crops worldwide are contaminated annually with mycotoxin-producing fungi (FAO, 2012), More than 500 different mycotoxins are known. Aflatoxins, one of the most toxic groups of mycotoxins mainly produced by strain of *Aspergillus flavus*, and *Aspergillus parasiticus*, are a major concern in the poultry production (Chand *et al*., 2011). Aflatoxin produce severe economic losses and health problems in the poultry industry because of their toxicity and frequency of occurrence in feedstuffs (Kubena *et al.*, 1993a). Feeding aflatoxin contaminated feeds to poultry affects animal health and production and causes changes in biochemical and hematological

parameters, changes in gene expression of liver enzymes, liver damage, kidney abnormalities, mortality, and immunosuppression, which may enhance susceptibility to infectious diseases (He *et al*., 2013 and Nemati *et al.,* 2014). The adverse effects of aflatoxins vary according to the dose, natural or pure aflatoxins, the duration of exposure, and animal factors such as age, sex, and level of stress (Whitlow *et al.,* 2005). Extensive research has been conducted to prevent mycotoxicoses that mainly include physical, chemical, nutritional or biological approaches. The use of adsorbing agents, which can trap the mycotoxin molecule by means of ion exchange and thereby hindering their absorption into blood from the gastrointestinal tract, has gained much attention in prevention of mycotoxins. Hydrated sodium calcium aluminosilicate (HSCAS) (Kubena *et al*., 1990; Huff *et al.,* 1992; Jindal *et al.,* 1993), bentonite (Santurio *et al.,* 1999), zeolite (Harvey *et al*., 1993), spent canola oil bleaching clays (Smith, 1984), activated charcoal (Edrington *et al.,* 1997), inorganic sorbents (Bailey *et al.,* 1998) and a blend of organic acids and aluminosilicates (Mahesh and Devegowda, 1996) have shown considerable promise in preventing aflatoxicosis. These clays, however, have some disadvantages like high inclusion rates (Devegowda *et al.,* 1998), possible interaction with the essential nutrients (Moshtaghian *et al.,* 1991) and lack of binding effect against many mycotoxins of practical importance (Chestnut *et al.,* 1992). Aluminosilicates are reported to selectively bind only those mycotoxin molecules that have polar function atomic groups.The beneficial effects of *Saccharomyces cereviciae* (SC) have been attributed to mannan in its cell wall. Esterified glucomannan (E-GM), a new generation biological toxin binder enzymatically extracted from the cell wall of certain strains of yeast SC has been identified as promising substance for mycotoxin neutralization. It has been successfully shown to alleviate the toxic effects of aflatoxin and other mycotoxins in broilers by research workers (Basmacioglu *et al*., 2005). EGM is porous in nature to provide more surface area for adsorption at low inclusion levels with none of the negative effects attributed to mineral clay binders. EGM showed considerably high binding ability (80–97%) with AF (Mahesh and Devegowda, 1996), and it has been preferred for detoxification of AF in poultry species. EGM was also used for detoxification of other mycotoxins such as zearalenone (Swamy *et al.,* 2002) and aurofusarin (Dvorska and Surai, 2004). Humic acids are ubiquitous and are found wherever matter is being decomposed or has been transposed, as in the case of sediments. Humic substances have demonstrated a strong affinity to bind various substances, such as heavy metals (Madronová *et al.,* 2001), herbicides (Nègre *et al.,* 2001), different mutagens (Cozzi *et al.,* 1993), monoaromatic (Nanny and Maza, 2001) and polycyclic aromatic compounds (Kollist- Siigur *et al.,* 2001), minerals (Elfarissi and Pefferkorn, 2000), and *Bacillus subtilis* bacteria (Moura *et al.,* 2007). In spite of its known binding characteristics, HA have been less evaluated previously as a mycotoxin adsorbent. In recent years, it has been observed that dietary intake of humates promotes growth in poultry (Kocabağli *et al.,* 2002; Rath *et al.,* 2006; Mirnawati and Marlida, 2013). Although there is not enough evidence to hypothesize how humates promote the growth in poultry, it is assumed that humates might increase the uptake of nitrogen, phosphorus, and other nutrients due to their chelating properties. The present trial was conducted to evaluate the efficacy of Humic acid, glucomannan-containing yeast product and HSCAS on performance, and serum immunological variables in broiler chickens exposed to aflatoxin.

2. MATERIALS AND METHODS:

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

2. 1. Experimental Design, Bird and Data Collection:

A total of 400 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 finisher diet (20.0% CP, 3201Kcal ME/Kg diet) from 22 to 35 day (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, 300 chicks of similar weight were randomly divided to 20 clean pens in the same broiler house used for the adaptation period. The chicks were divided into 5 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 3.5 g Humic acid (FH = Farmagulator DRY^{TM} humate Yayalar Mah. Sanayi Cad. No. 29, Dolayoba, Pendik, Istanbul, Turkey), , 4) contaminated diet supplemented with 3.5 g esterified glucomannan (EGM) (Media Vet, Garlien, Egypt) and 5) contaminated diet supplemented with 5 g HSCAS (EL-NASR Co., Egypt). Humic acid contained 70 % HA, 14.50 % moisture, 3.6% crude protein, 0.05% crude fat, 17.35% total ash and 9.0% sodium. Hydrated Sodium Calcium Aluminosilicate (HSCAS) is a feed additive, adsorbent, anti-caking and toxin binder mixed with the ration at a rate of 5 g/kg (0.5%). HSCAS 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 ppm of AF/kg of diet. The detected levels of AF in the control diet were below the detection limits.

2.3. Growth performance determination

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 feed: gain ratios were calculated. Feed consumption and feed: gain was adjusted for mortalities when appropriate.

2.4. Blood parameters analysis

At the 35 day of age, three chickens from each treatment were randomly chosen individually weighed and then slaughtered according to the Islamic method. Liver was removed and weighed to the nearest 0.01 g. Data were expressed as relative to body weight. Three heparinized and three unheparinized blood samples were collected from wing vein per treatment at 35 day of age. Plasma and serum were obtained by centrifugation of blood at 1500 g for 20 min and stored at -18°C. Haemoglobin concentration (Hgb) was determined as g/dl by the cyanomethaemo globin procedure of Eilers (1967). Red blood cells (RBCs) and white blood cells (WBCs) were counted as 10^{6}/ µl and 10^{3}/ µl of blood using a bright line Hemocytometer (Sainty International Group, Jiangsu Yangzhou, China) at 400X magnification. Blood samples were diluted 200 times with physiological saline solution before counting RBCs. Blood samples were diluted 20 times with a diluter fluid (3 ml acetic acid glacial +97 ml distilled water + some Lush-man stain) for WBC determinant by Hepler (1966). Packed cell volume (PCV, %) was determined using Wintrobehaematocrit tubes (Jiangdu Sunflower Glass Instrument Factory, Jiangdu, China). Serum biochemical constituents were determined to monitor the changes in blood metabolites in relation to AF and additives using photometric methods. Concerning total proteins, it was measured colorimetrically according to the method of Doumas (1975), beside Albumin (A) was measured according to the method of Doumas *et al.* (1971) and the serum Globulin (G) levels were calculated as differences between total proteins and albumin (Varley, 1979). Serum creatinine was estimated by the method of Thomas (1992) and uric acid (Caraway, 1955). Liver enzymatic activities including Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities by Reitman and Frankle (1957) were measured colorimetrically using commercial diagnosing kits (Bio-Diagnostics® Egypt).

2.5. Antibody response against Newcastle virus

Antibody response against Newcastle disease virus was determined by Hemagglutination Inhibition (HI) test (Thayer and Beard, 1998). Blood samples from each of four birds of each group were collected on days 7, 14, 21, 28 and 35 of post-vaccination. Serum was separated and processed for HI test.

2.6.Determinations of nutrient metabolic rates

At the end of the 30 d feeding experiment, feed intake was recorded daily. Fresh feces were collected without contamination from each of 4 birds

in each group for 3 d, 3 collecting times daily. The fecal samples of each bird from 3 d collections were dried, ground and mixed. Crude protein (CP), crude fat (CF), calcium (Ca) and phosphorus (P) in diets and excreta were determined with Kjeldahl, ether extract, potassium permanganate ($KMnO_4$) and ammonium molybdate ($(NH4)_6Mo_7O_{24}$) protocols, respectively (Jurgens,1997). The calculation of nutrient metabolic rates was made as follows:

Nutrient metabolic rate = (nutrient content in diets - nutrient content in excreta)/nutrient content in diets.

2.7. Serum and Liver Antioxidant Enzyme Activities Assays

After the feeding experiment, three35-day-old broilers from each group were sacrificed. The serum and hepatic homogenates were prepared forthe assays of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA). SOD and GSH-Pxa ctivity were analyzed by monitoring inhibition rate of reducing nitrobluetetrazolium (NBT) (Shi *et al.,* 2006). MDA was based on measuring the fluorescence decay of R-Phycoerythrin induced by the peroxyl radicals obtained with thermal decomposition of azodiisobutylamidine (Debora *et al.,* 2010).

2.8. Analysis of AFB1 residues in liver

Three liver samples from each treatment were kept at −20°C for analyzing the residue of AFB1. Analysis of AFB1 residues were performed according to Tavcar-Kalcher *et al.* (2007). Briefly, 1 ground sample was mixed thoroughly with an aqueous solution of citric acid and diatomaceous earth. The mixture was extracted with dichloromethane. The filtered extract was dried, filtered again, and an aliquot was evaporated to dryness. The residue was dissolved in methanol and mixed with buffer and applied into an immune affinity column. Aflatoxin B1 was eluated from the column and the concentration of AFB1 in the final solution was determined by an HPLC method with fluorescence detection after derivatization with bromine in the Kobra cell (R-Biopharm Rhone Ltd.,Glasgow, UK).

2.9. Histopathological study

Specimens of all slaughtered birds were collected from liver, at day 35 from all groups, and rapidly fixed in 10% neutral buffered formalin solution for at least 24 h. The fixed specimens were processed through the conventional paraffin embedding technique. Paraffin blocks were prepared from which 5-lm-thick sections were obtained and stained by Meyer's Hematoxyline and Eosin (H&E), according to the method described by Culling (1983)**.** The sections were examined using light microscope equipped with digital camera using1000X power.

2.10. Statistical analysis:

The obtained data were analyzed using the liner model programs of SAS (2002).The difference among means were tested using Duncan Multiple range test (Duncan,1955). All percentage was transferred to their analogue arc sin before running the analyses.

The following model was used to study the effect of some feed additives (Humic acid, EGM and HSCAS with aflatoxin) on parameters investigated as follows:

Yij=μ+ Ti+eij

Where,

Yij= an observation

μ= overall mean

Ti= effect of the factor studied (I= 1,2…and 5)

eij=residual " random error"

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 2. Broilers fed 1ppm aflatoxin (T2) recorded significant ($P<0.05$) decrease in final body weight and cumulative weight gain throughout the trial period by (12.88% and 13.92% respectively in comparison with control group). Similar reduction in body weight gain was observed by Basmacioglu *et al.* (2005) in 21 days old broilers fed 2 ppm aflatoxin-B1. The deleterious effects of aflatoxin on body weight in broiler chicken have been well established from several studies at various levels of aflatoxin (Pimpukdee *et al.*, 2004; Churchil, *et al*., 2009). The adverse effect of aflatoxin on body weight gain could be associated with anorexia, listlessness and inhibition of protein synthesis and lipogenesis (Oguz and Kurtoglu, 2000). Impaired liver functions and protein/lipid utilization mechanisms might also have deleterious effects on growth performance and general health.

Depression in final body weight and body weight gain caused by aflatoxin were significantly ($P<0.05$) improved by the 3.5 g HA, 3.5 g EGM and 5 g HSCAS supplementation throughout the experimental period . Addition of 3.5 g humic acid, 3.5 g EGM or 5 g HSCAS protections of final body weight at 35 day by (85.71%, 80.95 and 83.33% respectively), and body weight gain by (85.47% , 81.19 and 83.09% respectively) as compared with the

AF group (T2) . These results are in harmony with those obtained by Jansen van Rensburg *et al.* (2006) who found that , the decrease in BW gain caused by the addition of 2 mg of AFB1/kg of feed was diminished at 42 d of age by the addition of 3.5 g of oxihumate / kg of feed. The beneficial effects of yeast glucomannan on performance of broilers have been reported earlier by Raju and Devegowda (2000) studied the influence of modified glucomannan (Mycosorb) on performance in broilers exposed to aflatoxin (0.3 mg kg^{-1}) and reported that addition of Mycosorb (1 g kg-1 diet) increased body weight by 2.26%. Karaman *et al.* (2005) evaluate the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers and reported that decreasing the severity of pathological changes, slightly and moderately, respectively. Girish and Devegowda (2006) also evaluate the effects of modified glucomannan (Mycosorb) and hydrated sodium calcium aluminosilicates (HSCAS) to reduce the individual and combined toxicity of aflatoxin and T-2 toxin in broiler chickens and showed that supplementation of Mycosorb (1 kg ton^{-1} of feed) improved ($P<0.05$) live weight gain.

The cumulative feed intake at 7-35 day of broilers fed aflatoxin (1ppm) was significantly ($P<0.05$) lower than that of control throughout the experimental period (Table 2). The cumulative feed intake at 7-35 day showed significant ($P<0.05$) reduction in aflatoxin treated birds by (8.46 %) compared to the control group. Many researchers confirmed the deleterious effect of aflatoxin on feed intake in broiler chicken at various doses (Reddy *et al.,* 1982; Girish and Devegowda, 2006; Churchil *et al*., 2009; Manafi *et al.*,2012). The loss of appetite in aflatoxicosis might be due to the impaired digestion by reduced digestive secretions from liver and pancreas. On the other hand, the lower feed intake is attributed to increased blood ammonia concentrations following reduced glomerular filtration rate (Saei *et al.,* 2013).

The supplementation of HA, E-GM or HSCAS in aflatoxin containing diets showed protection for feed intake throughout the experimental period by (81.81%, 76.36% and 86.36 % respectively) as compared with the AF group (T2) . The depressed cumulative feed intake up to fifth week due to aflatoxin was also improved significantly ($P<0.05$) by EGM supplementation. These results agree with Raju and Devegowda (2000) reported that supplementation of glucomannan to the diet containing 0.3 mg aflatoxin kg-1 caused 1.6% increase in feed intake of broiler chicks. Similar result has been recorded by Girish and Devegowda (2006).

The feed efficiency of broilers receiving aflatoxin was poorer by (6.50%) than control group at the end of the study (Table 2). Majority of the earlier reports agreed with the deleterious effect of aflatoxin on feed efficiency at different dose levels in broilers (Girish and Devegowda, 2006; Churchil *et al*., 2009; Manafi *et al.,* 2012)**.** The poor feed efficiency in aflatoxin feeding could be attributed to hepatic cell damage and decreased pancreatic enzymes thereby affecting digestion and inhibition of protein synthesis. The chicks received HA, E-GM or HSCAS in aflatoxic diets improved feed conversion ratio in broilers at 35 days of age. Protection of feed efficiency by (91.32 , 88.79 or 81.44 % respectively) against 1 ppm AF/ kg diet. Similar effects were reported by Valarezo *et al.* (1998), Raju and Devegowda (2000), Aravind *et al*. (2003), Oguz and Parlat (2004) and Girish and Devegowda (2006). Raju and Devegowda (2000) reported that inclusion of 1 g kg-1 Mycosorb to the diet containing 0.3 mg aflatoxin kg-1 reduced feed conversion ratio by 2.63% and Valarezo *et al.* (1998) also recorded 2.95% reduction in feed conversion ratio.

The increase of 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)**, o**r, severely inhibiting the immune system of the birds (Pasha *et al.,* 2007). The significant decrease in mortality rate due 3.5g HA, 3.5 g EGM or 5 g 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)**.** This observation support earlier report of Girish and Devegowda (2004) and Basmacioglu *et al.* (2005) .

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: VitA 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 0.1gand carrier (CaCo3) to 3 kg.
**According to tables of NRC (1994).

Table (2): Effect of 3.5ghumic acid, 3.5 g GAM and 5g HSCAS on growth performance and nutrient metabolic rate for broiler chicks fed an aflatoxin contaminated diet from 7 to 35 days of age.

Items	Dietary treatments					SEM*	p-value
	Control	AF	AF + HA	AF + GEM	AF+ HSCAS		
Initial body weight at 7 d	124.5	124.5	125	124	124.2	1.40	0.856
Final body weight at 35 d	1630a	1420c	1600b	1590b	1595b	28.96	0.001
Body weight gain (g) (7-35d)	1505.5a	1296c	1475b	1466b	1470b	18.25	0.02
Feed intake (g/bird/period)	2820a	2600d	2780b	2768c	2790b	1.65	0.0001
Feed conversion (g:g)	1.87b	2.00a	1.88b	1.89b	1.90b	0.12	0.002
Mortality rate (%)	0.00	15.00[a]	3.33[b]	3.33[b]	0.00	0.02	0.0001
Nutrient metabolic rate							
Organic matter, (OM)	66.10a	55.21c	62.75b	62.30b	61.90b	2.352	0.042
Crude protein , (CP)	73.80a	40.80	70.62ab	68.90b	68.50b	1.582	0.001
Crude fiber, (CF)	85.16a	80.60c	83.20b	83.0b	82.60b	3.452	0.005
Calcium, (Ca)	23.80a	13.50c	22.52b	21.00b	20.00b	0.589	0.001
Phosphorus, (P)	12.00a	7.00d	11.20b	10.95b	10.52c	0.225	0.0001

a-d= Means with the same letter in each row are not significantly different at P≤0.05; AF: aflatoxin; HA: Humic acid (3.5 g/kg ration); EGM: Glucommanna (3.5 g/kg ration); HSCAS: Hydrated sodium calcium aluminosilicate (5 g/kg ration); SEM : Standard Error of the mean.

Supplementation of 3.5 g HA, 3.5 g EGM or 5 g HSCAS adsorbents to the aflatoxicated diets effectively improved productive performance during the period from 7-35 day of age . The mechanism by which HA affect poultry performance is largely unknown. There are limited numbers of articles which show that HS promote growth by altering partitioning of nutrient metabolism (Abdel-Mageed, 2012; Taklimi *et al*., 2012). It is assumed that, due to the chemical compositions, proteins, water soluble vitamins, digestive enzyme and many other immune stimulating agents and antibacterial substances in HA, they will have significant role in productivity of birds.

This could be mainly attributed to their ability to change the gut microflora (by increasing the concentrations of beneficial bacteria) in the intestine (Schepetkin *et al.*, 2003). As reported earlier by Taklimi *et al.* (2012), HA had significant effect on crypt depth of villi in jejunum of treated birds compared to non-supplemented groups. It is obviously known that growth of villi is generally dependent on pH, microflora and toxic substances in the intestine, although HA have the ability to reduce pH and concentration of harmful bacteria in intestine. There is evidence that HA could have positive effect on poultry performances via digestive tract ecosystems (Taklimi *et al.*, 2012). 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).

Table (3): Effect of 3.5g humic acid, 3.5 g GAM and 5 g HSCAS on some blood constituents for broiler chicks fed an aflatoxin contaminated diet from 7 to 35 days of age.

Items	Dietary treatments					SEM*	p-value
	Control	AF	AF + HA	AF + GEM	AF+ HSCAS		
Total protein, (mg/dl)	4.49^{a}	3.52^{c}	4.33^{b}	4.31^{b}	4.3^{b}	0.22	0.022
Albumin, (mg/dl)	2.46^{a}	1.82^{c}	2.34^{b}	2.35^{b}	2.34^{b}	0.18	0.016
Globulin, (mg/dl)	2.03^{a}	1.7^{b}	1.99^{a}	1.96^{a}	1.96^{a}	0.14	0.002
Total lipids, (mg/dl)	423.25^{a}	260.8^{c}	396.25^{b}	390.15^{b}	395.22^{b}	22.5	0.001
Liver function							
AST,(IU/L)	125.23^{d}	250.6a	140.50c	145.61bc	150.65b	0.73	0.002
ALT,(IU/L)	42.26^{c}	89.35a	51.05b	52.25b	51.35b	0.47	0.025
ALP,(IU/L)	52.05^{c}	95.62a	61.25b	60.35b	61.55b	0.59	0.001
Kidney function							
Uric acid, (mg/dl)	3.20^{c}	7.80^{a}	4.00bc	4.21b	4.13b	0.23	0.012
Creatinine, (mg/dl)	0.72c	1.5^{a}	0.85^{b}	0.86^{b}	0.87b	0.18	0.002
Calcium, (mg/dl)	4.95a	3.15c	4.65ab	4.42ab	4.35b	0.245	0.001
Phosphorus, (mg/dl)	5.87a	3.45d	5.45b	5.16bc	5.20c	0.332	0.005

a-d= Means with the same letter in each row are not significantly different at P≤0.05; AF: aflatoxin;HA: Humic acid (3.5 g/kg ration); EGM: Glucommanna (3.5 g/kg ration); HSCAS: Hydrated sodium calcium aluminosilicate (5 g/kg ration); ALT: alanine transaminase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; SEM : Standard Error of the mean .

Table (4): Effect of 3.5g humic acid, 3.5 g GAM and 5g HSCAS on hematology and immune response to ND for broiler chicks fed an aflatoxin contaminated diet at 35 days of age.

Items	Dietary treatments					SEM*	p-value
	Control	AF	AF+ HA	AF+ GEM	AF+ HSCAS		
RBCs (x10^{6}/ml)	3.25^{a}	2.79^{c}	3.20^{a}	3.18^{ab}	3.14^{b}	0.14	0.002
WBCs (x10^{3}/ml)	25.22^{a}	20.12^{c}	24.00^{ab}	23.61^{b}	23.22^{b}	2.35	0.02
Hgb (g/dl)	15.88^{a}	9.35^{c}	14.81^{ab}	14.10^{b}	14.31^{b}	9.03	0.005
PCV (%)	39.21^{a}	32.50^{c}	38.32^{ab}	38.01^{b}	37.62^{b}	1.56	0.004
HI titers							
7th d	5.65	5.66	5.62	5.69	5.65	0.252	0.458
14th d	4.55	4.40	4.53	4.52	4.50	0.326	0.352
21th d	4.60	4.46	4.58	4.52	4.56	0.215	0.08
28th d	6.70^{a}	4.96^{c}	6.00^{ab}	5.68^{b}	5.70^{b}	0.225	0.02
35th d	6.30^{a}	4.38^{c}	6.05^{ab}	5.9^{b}	5.78^{b}	0.321	0.001

a-d= Means with the same letter in each row are not significantly different at P≤0.05; AF: aflatoxin; HA: Humic acid (3.5 g/kg ration); EGM: Glucommanna (3.5 g/kg ration); HSCAS: Hydrated sodium calcium aluminosilicate (5 g/kg ration) *SEM = Standard Error of the mean; RBCs: red blood cells; WBCS, white blood cells; Hgb, haemoglobin; PCV, packed cell volume

Table (5). Effect of 3.5g humic acid, 3.5 g GAM and 5g HSCAS on serum and liver antioxidant enzyme activities for broiler chicks fed an aflatoxin contaminated diet at 35 days of age.

Items	Dietary treatments					SEM	p-value
	control	AF	AF+ HA	AF+ EGM	AF+ HSCAS		
Serum							
SOD (U/ mg protein)	120.32^{a}	96.8^{c}	116.55^{b}	115.35^{b}	115.86^{b}	2.95	0.005
GSH-Px (U/ mg protein)	490.25^{a}	415.35^{d}	480.2^{b}	481.25^{b}	477.50^{c}	6.685	0.027
MDA, (nmol/ mg protein)	16.01^{c}	24.25^{a}	17.25^{b}	18.11^{b}	18.69^{b}	1.038	0.001
Liver							
SOD, (U/ mg protein)	297.22^{a}	215.50^{d}	284.31^{b}	277.8^{c}	285.20^{b}	5.851	0.004
GSH-Px, (U/ mg protein)	74.25^{a}	52.49^{c}	68.25^{b}	70.26^{b}	69.25^{b}	2.632	0.025
MDA, (nmol/ mg protein)	0.205^{d}	0.425^{a}	0.233^{b}	0.228^{c}	0.240^{b}	0.005	0.003

a-d= Means with the same letter in each row are not significantly different at P≤0.05; AF: aflatoxin; HA: Humic acid (3.5 g/kg ration); EGM: Glucommanna (3.5 g/kg ration); HSCAS: Hydrated sodium calcium aluminosilicate (5 g/kg ration) ; SEM : Standard Error of the mean ; SOD : superoxide dismutase ; GSH-Px : glutathione peroxidase ; MDA : malondialdehyde

Table (6): Effect of 3.5g humic acid, 3.5 g GAM and 5g HSCAS on dressing % , relative liver weight % and AFB1 residual in the liver for broiler chicks fed an aflatoxin contaminated diet at 35 days of age.

Items	Dietary treatments					SEM	p-value
	Control	AF	AF+ HA	AF+ GEM	AF+ HSCAS		
Dressing (%)	72.5^{a}	63.52^{c}	71.05^{ab}	70.89^{b}	70.55^{b}	3.154	0.002
Relative liver weight (% of BW)	2.12^{c}	3.87^{a}	2.41^{b}	2.47^{b}	2.42^{b}	0.225	0.024
AFB1 contents, (µg/kg)	ND	2.38^{a}	0.445^{c}	0.455^{bc}	0.489^{b}	0.005	0.0001

a-d= Means with the same letter in each column are not significantly different at P≤0.05; AF: aflatoxin; HA: Humic acid (3.5 g/kg ration); EGM: Glucommanna (3.5 g/kg ration); HSCAS: Hydrated sodium calcium aluminosilicate (5 g/kg ration) ; ND: not detected (determination limit of the analytical method: 0.01 ug/kg for aflatoxin B1); SEM = Standard Error of the mean

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. According to Arvind and Churchil (2014) the possible mechanism of EGM in bringing about this beneficial effect could be attributed to the excretion of aflatoxin in an adsorbed form from the gut of the chicken, thereby minimizing its effects on liver and pancreas.

3.2. Nutrient metabolic rates

Results presented in Table (2) show that metabolic rates of organic matter (OM), crude protein (CP), crude fiber (CF), calcium and phosphorus were significantly reduced ($p < 0.05$) in birds fed diet contaminated with 1 ppm AF /kg diet by 19.72, 80.88 , 5.65, 76.29 and 71.42% respectively when compared with the control group.

Adding the studied additives decreased the severity of aflatoxin diets effects for nutrient metabolic rates. Protections of nutrient metabolic rates by HA were (82.55, 90.36 ,57.02, 87.57 and 84.00 %), EGM were (78.87, 85.15 , 52.63, 72.81 and 79.00 %) , while that by HSCAS were (75.21, 83.94 , 43.86, 63.11 and 70.40 %) for organic matter, CP, CF , Ca and P respectively, against 1ppm AF/kg diet.

The depression in nutrient metabolic rates upon feeding aflatoxin could be attributed to reduced protein synthesis Verma *et al.* (2002), increased lipid excretion in droppings, impaired nutrient absorption and reduced pancreatic digestive enzyme production Osborne and Hamilton (1981) and reduced appetite by Sharline *et al.* (1980). Hasan *et al.* (2000) stated that the toxicity of aflatoxin was characterized by reduction in body weight gain as aflatoxins interfere with normal metabolic pathway through the inhibition of protein synthesis and enzyme 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 reduces the ability of the bird to digest dry matter and amino acids and to utilize energy from aflatoxin contaminated ration. Dietary supplementation with HA, EGM or HSCAS improved the nutrient metabolic rates significantly ($p<0.05$), (Table 2). 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 adverse effects of aflatoxin on nutrient metabolic rates 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).

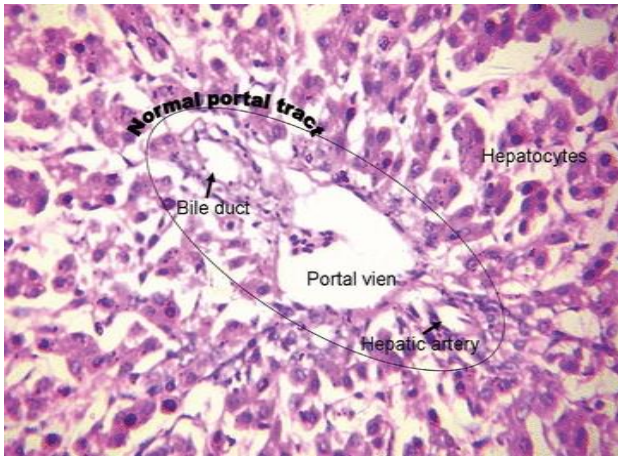

Fig 1 Liver of the control group of 35 days old broiler chickens showed normal hepatic architecture. H&E. X400.

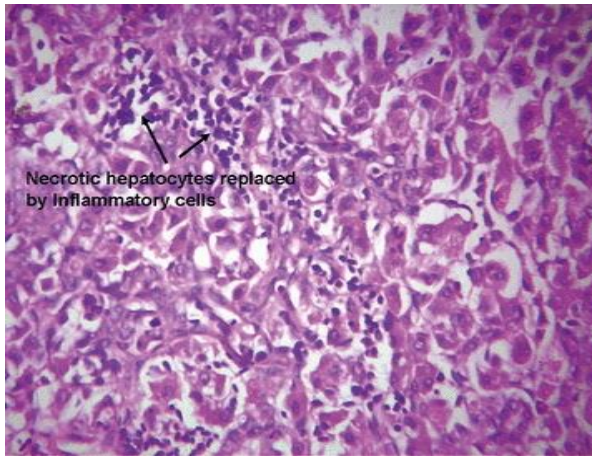

Fig 2 Liver of the AF group of 35 days old broiler chickens showed multifocal hepatic areas replaced by inflammatory cells. H&E. X400.

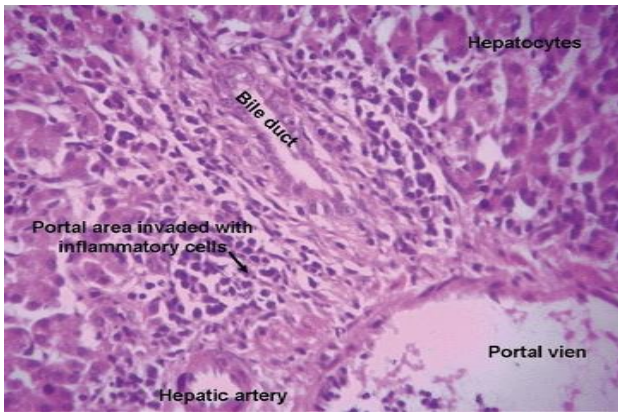

Fig3 Liver of the AF group of 35 days old broiler chickens showed severe inflammatory cells infiltration of the portal area. H&E. X400.

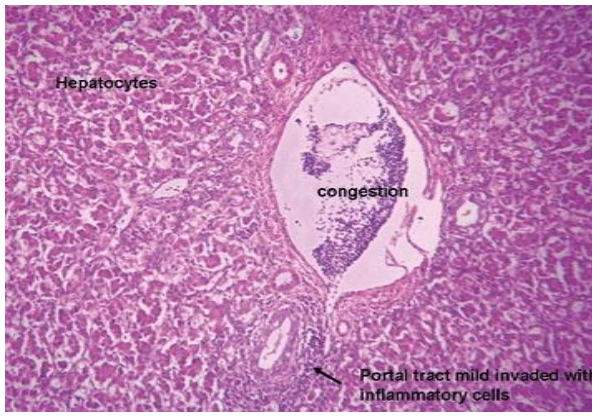

Fig 4 Liver of the AF+HSCAS group of 35 days old broiler chickens showed mild inflammatory cells infiltration of the portal area. H&E. X250.

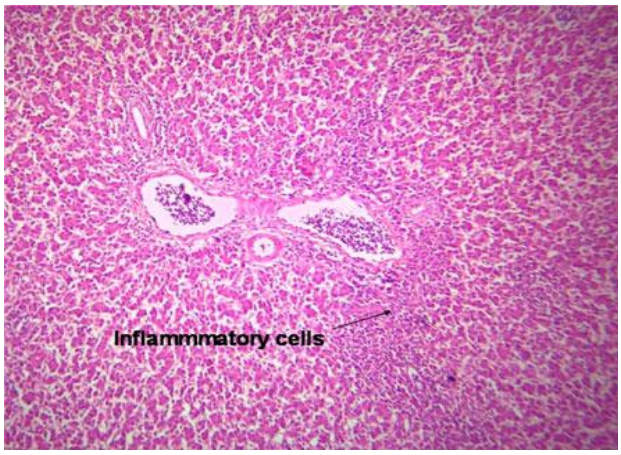

Figure 5. Liver of the +humic acid of 35-day-old broiler chickens showing moderate inflammatory cell infiltration of the portal area (H&E × 250).

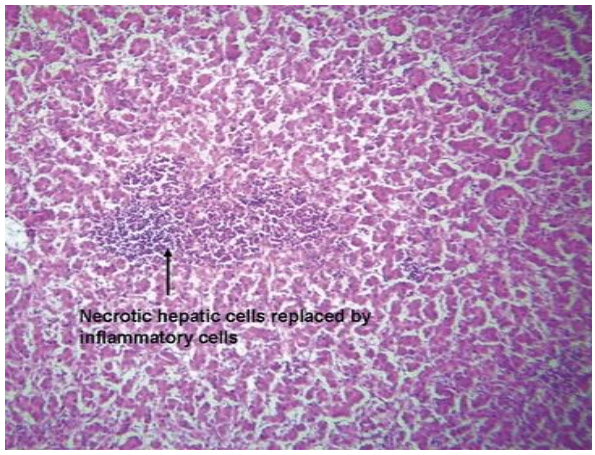

Fig 6 Liver of the AF+GEM, group of 35 days old broiler chickens showed focal necrotic hepatic area replaced by inflammatory cells. H&E. X250.

3.3. **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 27.55, 35.16, 19.41 and 62.28 % 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, creatinine and uric acid by (50.22,52.70, 45.56,58.97and 52.00 % 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 were reduced 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 decrease in the serum, but the three agents (HA, EGM or HSCAS) applied significantly counteracted the effect of AF. Protections of total protein, albumin, globulin and total lipids by 3.5 g HA were (83.50, 81.25, 87.87 and 83.37 %), 3.5 g EGM were (81.44, 82.81, 78.79 and 79.62 %) and 5 g HSCAS were (80.41, 81.25, 78.79 and 82.74) respectively , against 1 ppm AF/kg diet.

AST, ALT and ALP showed significant increase in afltoxicated group by 50.22, 52.70 and 45.56% respectively, compared to control group which reflect hepatic degeneration and subsequent leakage of enzymes into circulation. The addition of 3.5 g HA, 3.5 EMA and 5 g 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). Protections of AST, ALT and ALP by 3.5 g HA were (87.82, 81.33 and 78.88 %), 3.5 g EGM were (83.74, 78.78 and 80.95%) and 5 g HSCS were (79.72, 80.69 and 78.19%) respectively , against 1 ppm AF/kg diet. These results in agreement with (Jansen van Rensburg *et al.,* 2006**)** and agreed with (Hassan *et al.,* 2009) who fed local chicks Humate and HSCAS and found positive effects on liver enzymes reducing AST, ALT and ALP.

Serum uric acid and creatinine were significantly (p<0.05) increased in chickens fed AF-diet by 58.97 and 52.00 % respectively, compared with the control. The increased concentrations of uric acid and creatinine coupled with the observed kidney enlargement, may indicate some renal tissues damage due to aflatoxin. This significant alteration in kidney parameters in birds fed on aflatoxin treatments agreed with data reports of Denli *et al.* (2005) and Bintvihok and Kositcharoenkul (2006)**.** Adding 3.5 g HA, 3.5 EMA and 5 g HSCAS to AF-diets diminished the

sever effects of aflatoxin diets on uric acid and creatinine (Table, 3). Protections of uric acid and creatinine by 3.5 g HA were (82.60 and 83.33 %), 3.5 g EGM were (78.04 and 82.8182.05 %) and 5 g HSCS were (79.78 and 80.76 %) respectively , against 1 ppm AF/kg diet.

The effect of AF on the serum level of calcium and inorganic phosphorous are shown in Table (3). Data showed that serum level of calcium and inorganic phosphorous were significantly decreased by 57.14 and 70.14 % respectively, compared with the control group. The lower concentration of serum inorganic phosphorous may be due to reducing renal reabsorption or inhibition of tubular phosphate secretion and /or decreased intestinal absorption of phosphorus, meanwhile, the decrease in serum calcium level may be due to the impaired of gastrointestinal absorption and increased urinary excretion (EL-Sebai, 2005). Protections of Ca and P by 3.5 g HA were (83.33 and 82.64 %), 3.5 g EGM were (70.55 and 70.66 %) and 5 g HSCS were (66.66 and 72.31 %) respectively , against 1 ppm AF/kg diet.

Feed additives supplementation alleviated the adverse effect of aflatoxicosis on serum constituents. This could explain superior performance of these groups. These 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 the mean percentages of DNA fragmentation of liver cells, frequencies of micronucleated in bone marrow cells and the incidence of chromosomal aberrations.

3.4. Hematological parameters:

Aflatoxin also significantly decreased the RBCs and WBCs count as well as the Hgb concentration and the PCV of the AF groups (Table 4). Similarly; Fernandez *et al.* (1995) , Oguz *et al.* (2000) and Attia *et al.* (2013) found a decrease in broiler RBCs, haematocrit values, Hgb, lymphocytes, total WBCs and percentage heterophil because of AF treatment. Supplementation of HA improved ($P<0.05$) blood parameters as compared to basal diet containing AFB1, probably as a result of effective adsorption in the gut reducing the amount of aflatoxin absorption by the body. Ipek *et al.* (2008) reported that HA (0.04, 0.05 or 0.06%) increased red blood cell but did not have any effect on white blood cell in nine-week-old female Japanese quails.

3.5. Serum and Liver Antioxidant Enzyme Activities

Serum and liver antioxidant enzyme activities were affected ($p > 0.05$) by dietary treatments (Table 5). The serum activity of total superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities measured in chickens of aflatoxicated group was reduced by 24.29 and 18% respectively compared with the control but were enhanced with the supplementation of 3.5 g HA, 3.5 g EGM or 5 g HSCAS diets (Table 5). Protection of SOD and GSH-Px by 3.5 g HA were (83.97 and 86.58 %), 3.5 g EGM were (78.86 and 87.98%) and 5 g HSCAS were (81.03 and 82.97%) respectively, against 1 ppm AF/kg diet. While liver antioxidant enzyme activities , SOD and GSH-Px were significantly decreased in birds fed AF-diet by 37.92 and 41.42% respectively compared with the control but were enhanced with the supplementation of 3.5 g HA, 3.5 g EGM or 5 g HSCAS diets (Table 5). Protection of SOD and GSH-Px in the liver by 3.5 g HA were (84.18 and 72.41 %), 3.5g EGM were (76.23 and 81.65%) and 5 g HSCAS were (85.29 and 77.01%) respectively, against 1 ppm AF/kg diet.

The serum MDA content was increased by 33% in chickens fed diet aflatoxin compared with the control group. Supplementation of 3.5 g HA, 3.5 g EGM or 5 g HSCAS decreased serum MDA content. Protection of MDA by 3.5 g HA, 3.5 g EGM or 5 g HSCAS was (84.95, 74.51 and 67.47%) respectively, against 1 ppm AF/kg diet. Like the serum MDA level, the level of liver MDA in group aflatoxin was increased by 51.76% compared with the control group. Protection of MDA in the liver by 3.5 g HA, 3.5 g EGM or 5 g HSCAS was (87.27, 89.54 and 84.09%) respectively, against 1 ppm AF/kg diet.

Effect of AF on antioxidant enzyme activity is one of the major problems for animal health. Antioxidant capacity has direct effect on animal health and anti-tumor ability. SOD and GSH-Px are the main antioxidant enzymes in animal body, which scavenge the unwanted O^{2-} , H_2O_2 and ROOH produced by free radicals. SOD can catalyze superoxide radical and GSH-Px can decompose hydrogen peroxide (Wills, 1966). The decreased activity of these enzymes can induce high contents of free radicals, resulting in cell

damage. Shyamal *et al.* (2010) reported that the potent antioxidant properties would help to reduce cell damage caused by AFB1. Shi *et al.* (2006) reported that the diet containing 100 µ/kg AFB1 significantly decreased activities of the liver SOD and GSH-Px. In this study, SOD, GSH-Px activities in liver were decreased, and hepatic cells were damaged in chicks fed with AFB1 without additives, while addition of 3.5 g HA, 3.5 g EGM or 5 g HSCAS had the ability to keep liver's antioxidant capacity at the regular levels, and to keep liver from AFB1 damage to some extents. EGM is a toxin binder consisting of functional carbohydrates extracted from yeast cell walls of *Saccharomyces cervisiae*. It has a large surface area of 22,000 m^2 per 1 kg and contains a large number of pores of different sizes to trap a wide range of chemicals (Banlunara *et al.,* 2005). In the current study, addition of 3.5 g EGM to aflatoxic diet had partially ameliorated the changes of hematological and biochemical parameters, hepatic injury and hepatic antioxidative depletion. These results suggested that addition of 3.5 g EGM seemed to partially counteract the toxins and partially restored damages induced by mycotoxins, which may due to EGM partially traping the mycotoxin molecule in its glucomannan matrix and preventing toxin absorption from the gastrointestinal tract.

3.6. Liver weight and aflatoxin B1 residues in the liver of broiler chicks

As illustrated in Table (6) aflatoxin containing diets caused decrease in dressing % by 14.13 % while , liver weight % increased by 45.21% compared with the control group as well as AFB1 residues in the liver. The severity of aflatoxin effect on dressing % ,relative liver weight and AFB1 residues in the liver were decreased by adding the studied additives to aflatoxin diets. Protection of dressing % ,relative liver weight and AFB1 residues in the liver by 3.5 g HA were (83.85, 83.42 and 81.30%) , 3.5 g GEM were (82.07, 80.00 and 80.88%) and 5g HSCAS were (78.28, 82.85 and 78.41%) respectively , against 1 ppm AF/kg diet.

In poultry, the relative weight of the liver is increased by aflatoxin ingestion more than that of any other organ **(**Van Rensburg *et al.,* 2006**)**. 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 livers of these chicks also appeared to be friable and pale yellow as a result of fat accumulation in the cytoplasm of the hepatocytes. However, Magnoli *et al.* (2011) reported that the relative weight of livers remained unchanged when toxin levels were relatively low (50 µg/kg of dietary AFB1), at least for a broiler production period of 46 days. In the current study, supplementation of HA showed significant protective effects with respect to liver damage, as indicated by an inhibition of liver enlargement. Van Rensburg *et al.* (2006) showed a concentration-dependent increase in the rate of hemolysis, indicating AFB1-induced cytotoxicity, which could be due to lipid peroxidation of plasma membranes, permeability alterations, and cell lyses. These workers reported that additions of oxihumate (0.35%) inhibited this effect at the contamination level of 1000 µg of AFB1/kg of feed. The affected birds of basal diet containing AFB1 retained significantly higher residues of the AF in their livers as compared to other treatments. Humic acid has binding capacity for many molecules. In the current study, addition of 3.5 g HA as binding agent decreased ($P<0.05$) adverse effect of AFB1 on liver. These findings are in line with a study that evaluated oxihumate as AFB1 binder, *in vitro* and *in vivo* **(**Van Rensburg *et al.,* 2006**)**. who found that Oxihumate showed a high 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 pH3 proved to be considerably less than that of Oxihumate. 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. EGM-supplementation to aflatoxicated diet decrease the relative weight of liver .This observation supports the earlier works of Raju and Devegowda (2000), Aravind *et al.* (2003) and Girish and Devegowda (2006). Raju and Devegowda (2000) supplemented the diet containing 0.3 mg aflatoxin kg-1 by 1 g glucomannan and reported that weight of the liver decreased by 32.5%. However, Girish and

Devegowda (2004) tested two dietary levels of aflatoxin (0 and 2 mg kg-1 diet) and reported that aflatoxin increased the relative weight of liver by 21.72%, but supplementation of glucomannan (1 g kg-1 diet) restored the weight of the liver. Aravind *et al.* (2003) also recorded higher relative weight of liver in birds given a naturally contaminated diet containing 168 µg aflatoxin kg-1. The mode of action of EGM in decreasing the liver weight is not clear. It is thought to trap the mycotoxin molecule in its glucomannan matrix, which prevents its absorption from gastrointestinal tract and the subsequent toxin-induced tissue changes. The reductions caused by HSCAS in the liver weight was earlier reported by Kubena *et al.* (1998). The basic mechanism for protection against the toxicity of AF appears to involve sequestration of AF in the gastrointestinal tract and chemisorption of AF (Phillips *et al.*, 1990).

3.7. Immune response to ND:

The effects of dietary treatments on antibody production against NDV in broilers from day 7 to 35 are presented in Table 4. On the 7th day of the study, there was no difference among antibody titer of experimental groups. The feeding of AF reduced the antibody production against ND in broilers from 28 to 35 days of age ($P<0.05$).The antibody production against ND at the end of the experimental period (35 day) was decreased with AF-diet by 43.83% compared with the control group. The supplementation of HA, EGM or HSCAS improved the antibody production against NDV in broilers from 28 to 35 days of age ($P<0.05$). Protection of antibody production against ND was by 3.5 g HA were (86.97%) , 3.5 g EGM was (79.16%) and 5g HSCAS was (72.92%), respectively , against 1 ppm AF/kg diet. The present results showed that the feeding of AF reduced the antibody production against ND in broilers. This was similar to the results in other studies (Ghosh and Chauhan, 1991; Hegazy *et al*., 1991; Gabal and Azzam, 1998) showing the immunotoxic effects of AF with 100 to 2500 µg AF/kg in the diet. The immunosuppressive effect of AF has been related to its direct inhibition of protein synthesis **(**Oğuz *et al.*, 2000) including those with specific function such as immunoglobulin G (IgG) and A (IgA), inhibition of migration of microphages (Ibrahim *et al.*, 2000)**,** interference with the hemolytic activity of complement, reduction of number of lymphocytes (Ghosh and Chauhan, 1991) through its toxic effect on the bursa of Fabricius (Ortatatli *et al.*, 2002) and impairment of cytokines formation by lymphocytes (Gabal and Azzam, 1998). This study agrees with previous findings by Oğuz *et al.* (2003), who reported that ND titers were significantly lower ($P<0.05$) in 100 µgAF/kg fed chicks, while no significant differences were seen in 50 µg AF/kg group compared to the control group ($P<0.05$).In the current study, addition of HA to the AFB1-containing diet ameliorated the adverse effect of AF on antibody production. Similarly, Hasan *et al.* (2010) reported that addition of 0.4, 0.6, 0.8 and 1.0 % of HA to the AF-containing diet, significantly ameliorated the adverse effect of AF on antibody production against ND in broilers from 28 to 35 days of age ($P<0.01$). This effect could be attributed to the role of HA as binding agent to the AF molecules in gastrointestinal tract and precluding their absorption that can alleviate the toxicity of AF in poultry (Van Rensburg *et al.*,2006). They further reported that HA was able to absorb (*in vitro*) about 10.3, 7.4and 11.9 mg of AFB1/g of oxihumate at pH 3, 5 and 7, respectively. These results clearly demonstrate that 100 µg of AFB1/kg diet-treatment significantly affects the HI against ND and simultaneous addition of HA (0.1 to 0.3%) to the AFB1 containing diet provides significant reduction to the immunotoxic effects of AF.GEM significantly diminished the adverse effect of AF on antibody titers against ND. Similar improvements in immune response with mannanoligosaccharide supplementation were recorded earlier (Savage *et al.*, 1996). This might have been due to its mycotoxin binding ability and for its indirect effects on cellular immunity through activation of B cells, T cells and macrophages (Lyons, 1994). It has been reported that GEM 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). Its mycotoxin binding ability might have been primarily responsible for these beneficial effects noted on immune competence. HSCAS inclusion to AF diets improved ($p<0.05$) the ND titers compared with the AF-diet alone, and it is in accordance with Barmase *et al.* (1990).

3.8. **Histopathological studies:**

Liver of the control group showed normal histology (Figure 1). Aflatoxin caused an increase in inflammatory cells in the portal area of the liver and hepatic cell necrosis (Figures 2 and 3). HA, EGM or HSCAS reduced the toxin's effect on the liver (Figure 5, 6 and 4 respectively) .

The results of liver histology (Fig. 2) show a Liver exhibited an inflammatory in the hepatic cells in the portal area of the liver, and necrosis of hepatic cells was observed. Similarly, Kubena *et al.* (1990) found diffused hepatocellular lipoidosis accompanied by hyperplasia of bile duct and early periportal fibrosis. In addition, Tessari *et al.* (2006) observed changes in broiler livers including vascular degeneration and cell proliferation of bile ducts in the liver. Moreover, Kumar and Blachandran (2009) revealed vacuolar degeneration, macrovesicular fatty degeneration and ballooning degeneration of hepatocytes in broiler chickens fed diets containing 1 ppm AF from day 1to 21 of age. The hepatocytes showed fatty degeneration and fatty cysts in some areas. Congestion, bile ducts hyperplasia, focal infiltration of heterophils, perivascular infiltration of mononuclear cells and heterophil and fibrosis were also noticed in liver. The present results indicated that AF negatively affected humoral and cell-mediated immune system of broiler chickens.

4. Conclusion

In conclusion, HA induced the highest recovery in growth performance, serum constituents , serum and liver antioxidant enzyme activities, relative liver weight, dressing %, AFB1 residual in liver, and immune response to ND. GEM also improved the productive traits of broilers but was less effective than HA and more effective than HSCAS.

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