Evaluation of the Efficacy of Feed Additives to Counteract the Toxic Effects of Aflatoxicosis in Broiler Chickens

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Abstract: This research was designed to evaluate the efficacy of Hydrated Sodium Calcium Aluminosilicate (HSCAS) and a phytobiotic (turmeric powder) to counteract the toxic effects of aflatoxin B_1 in broiler chickens. Five hundred, days old broiler chicks were divided equally into 5 groups. Birds of group 1 were fed on plain ration containing neither aflatoxin B_1 nor treatment while birds in groups 2-5 were fed on ration contaminated with aflatoxin B_1 at concentration of 2.5 ppm of ration from day old till the end of the experiment. Chickens in group 2 were given ration contaminated with aflatoxin B_1 only. Group 3 was treated with HSCAS at a concentration of 0.5% while group 4 was fed on ration containing turmeric powder in a dose of 80 mg kg^{-1} of the ration. Chickens in group 5 were given concomitant HSCAS and turmeric powder at the recommended doses. All groups were kept under observation till 5 weeks of age. The results cleared that treatment of aflatoxicated birds either with HSCAS or turmeric powder even their combination induced protection from the development of signs and lesions with significant ($p<0.05$) improvement of performance when compared with un-treated control group. Both HSCAS and turmeric powder 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.

Key words: Aflatoxicosis, hydrated sodium calcium aluminosilicate, poultry, turmeric powder, Egypt

INTRODUCTION

Aflatoxin, a class of mycotoxins which is ubiquitous in nature and continually encountered in feed ingredients (Manafi *et al.*, 2009b). Aflatoxin is a secondary toxic metabolite produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Smith *et al.*, 1995). Aflatoxin B_1 is the most toxic among all types of mycotoxins (Sweeney and Dobson, 1998) as it induces severe economic losses such as immunosuppression, poor growth and feed conversion, increased mortality, decreased egg production, leg problems, liver damage and carcass condemnations (Soliman *et al.*, 2008; Yarru *et al.*, 2009a). Added to that, potential mycotoxin residues were detected in tissues and eggs of birds (Pandey and Chauhan, 2007) and become particularly important as potential hazard for human health.

Feed safety is concerned since poultry feed worldwide is frequently contaminated by aflatoxins. Adsorbents like aluminosilicate binders have a potential to reduce aflatoxicosis in poultry (Kubena *et al.*, 1998;

Galvano *et al.*, 2001; Diaz *et al.*, 2003; Pimpukdee *et al.*, 2004; Dakovic *et al.*, 2005) as their antioxidant property prevents the damage of the cell membrane caused by free radicals generation or lipid peroxidation (Gowda and Ledoux, 2008).

Certain phytogenic compounds like coumarins, flavonoids and curcuminoids possess antioxidant property and inhibit the biotransformation of aflatoxin B_1 to their active epoxide derivatives (Lee *et al.*, 2001). Turmeric (*Curcuma longa*) is a tropical Asian plant. The main yellow biologically active substance extracted from rhizomes of Curcuma is curcumin (Osawa *et al.*, 1995). Curcumin possesses anti-inflammatory (Holt *et al.*, 2005), antibacterial (Araujo and Leon, 2001) antifungal (Wuthi-Udomler *et al.*, 2000), antioxidant (Iqbal *et al.*, 2003; Menon and Sudheer, 2007), antinematodal (Kiuchi *et al.*, 1993) and hypolipidaemic (Ramirez-Tortosa *et al.*, 1999) effects. Moreover, it has been studied that curcumin exhibits hepatoprotective, antitumor and antiviral activities (Duvoix *et al.*, 2005; Emadi and Kermanshahi, 2007). It is used in the treatment

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of respiratory and gastrointestinal problems (Gilani *et al.*, 2005). It was documented that turmeric powder has protective effects against aflatoxin B_1 (Manafi *et al.*, 2009a; Rangsaz and Ahangaran, 2011). The most recent, economical and promising approach to prevent the toxic effect of aflatoxins in poultry is the combined using of adsorbents and turmeric compound (Surai, 2002; Gowda *et al.*, 2008; Diaz *et al.*, 2009).

Hence, this research work was designed and conducted to investigate and evaluate the efficacy feed additives containing Hydrated Sodium Calcium Aluminosilicate (HSCAS) and a phytobiotic (turmeric powder) either singly or in combination to counteract the adverse toxic effects of aflatoxin B_1 in broiler chickens.

MATERIALS AND METHODS

Aflatoxin B_1 production: Aflatoxin B_1 production was carried out according to Davis *et al.* (1966) using *Aspergillus flavus* standard toxigenic strain that was obtained from Poultry Diseases Department, Faculty of Veterinary Medicine, Cairo University. Determination of aflatoxin B_1 produced in liquid media after extraction was done by immuno-affinity chromatography according to Roos *et al.* (1997) compared with standard aflatoxin which was obtained from Sigma Chemical Company (B_1, B_2, G_1 and G_2). The 50 mg of aflatoxin B_1 (pure standard and toxigenic) were dissolved in 50 mL of benzene, the solution was added to 250 g of basal feed. After complete evaporation of the solvent under an exhaust fan overnight, this sample was added to 20 kg of the ration to obtain the right concentration of aflatoxin B_1 (2.5 ppm).

Hydrated Sodium Calcium Aluminosilicate (HSCAS): Hydrated Sodium Calcium Aluminosilicate (HSCAS) is a feed additive, adsorbent, anti-caking and toxin binder. It is a mineral silicates and organic acids that was obtained from Trouw Nutrition International and mixed with the ration at a rate of 5 g kg^{-1} (0.5%).

Tumeric powder (*Curcuma longa*): A phytobiotic feed additive turmeric powder (*Curcuma longa*) was added in a dose of 80 mg kg^{-1} of the ration.

Ration: The basal diet was a commercial type corn-soybean meal diet. Chickens were fed on a commercial starter, grower and finisher ration at 2, 2-4 and 4-6 weeks of age, respectively. No antibiotics, anticoccidial and antifungal drugs were added to the ration. The ration was formulated to meet or exceed the nutritional requirements of chickens as recommended by the NRC (1994). Feed and water were allowed for birds *ad libitum*. Before feeding experimental chickens, the basal diet was tested for possible residual mycotoxins like aflatoxins, ochratoxins, zeralenone and fuminsin before feeding (Rottinghaus *et al.*, 1982) but there were no detectable mycotoxins levels present except traces of aflatoxin B_1 was found at a level of 1.0 ppm.

Detection of aflatoxin B_1 in the ration: A statistically valid sample was drawn from the lot of the ration during the study to measure aflatoxin B_1 by immuno-affinity chromatography according to Schuller and Egmond (1981) and positive samples were estimated quantitatively by fluorometric method according to AOAC (2005).

Experimental chickens: Five hundred, day old (Cobb x Cobb) broiler chicks of mixed sex that used in this research were purchased from a commercial hatchery. The birds were kept in thoroughly cleaned and disinfected pens. Birds were maintained on a 24 h continuous light schedule throughout the experimental period (35 days). Temperature was kept to the required during brooding period. Chickens were vaccinated at the 7th day of age against Newcastle Disease (ND) and infectious bronchietis disease using Hitchner B1+H120 vaccine via intra-ocular route and at the 9th days of age against avian influenza disease using inactivated H5N2 vaccine via intramuscular route. At 15 days old, vaccination was done against infectious bursal disease and ND using 228E strain and La Sota vaccines, respectively by intra-ocular route.

Experimental design: Five hundred broiler chicks were randomly distributed into 5 equal groups (1-5) of 100 birds assigned to 2 replicates of 50 each. Birds of group 1 were fed on plain ration containing neither aflatoxin B_1 nor treatment (blank control negative) while birds in groups 2-5 were fed on ration contaminated with aflatoxin B_1 at concentration of 2.5 ppm of ration from day old till the end of the experiment (5 weeks). Chickens in group 2 were given ration contaminated with aflatoxin B_1 only (control positive). Group 3 was treated with HSCAS in the ration at a concentration of 0.5% while group 4 was fed on ration containing turmeric powder in a dose of 80 mg kg^{-1} of the ration. Chickens in group 5 were consumed ration containing combination of HSCAS and turmeric powder at the earlier recommended doses. All groups were kept under complete observation from day old till 5 weeks of age (end of the study). The experiment was carried out according to the National regulations on animal welfare and Institutional Animal Ethical Committee (IAEC).

Samples collection
Blood samples: Ten birds (2 replicates of 5 birds each) from each group were selected randomly and blood was collected via cardiac puncture for evaluation of humoral immunity and serum biochemical variables. For separation of the serum, blood was left to clot, centrifuged at 3000 rpm for 30 min and then the samples were preserved at -20°C till submission for further analysis.

Tissue samples: Liver, kidney, gizzard, spleen, thymus glands and bursae of Fabricus were collected from five weighed birds from each replicate for determination of relative organ weight expressed on a relative body weight basis.

Evaluation parameters
Symptoms, mortalities and gross lesions: Chickens in all groups were inspected daily and any health-related problems were observed. Mortalities as well as specific aflatoxins gross lesions were also recorded (Dhanasekaran *et al.*, 2009). At 5 weeks old, 5 chickens from each replicate were sacrificed and examined for any lesions concerning aflatoxicosis.

Zootechnical performance: Birds in each group were individually weighed on a weekly basis and feed consumption was recorded. Feed Conversion Rate (FCR) and European Production Efficiency Factor (EPEF) were determined (Bell and Weaver, 2002).

Absolute and relative organ weight: At 5 weeks of age, five birds from each replicate were selected randomly, weighed, slaughtered and the liver, kidney, gizzard, spleen, thymus glands and bursae of Fabricus were excised and weighed for determination of relative organ weight expressed on a relative body weight basis (organ to body weight index) as described by Montgomery *et al.* (1986).

Humoral immunity: Serum samples were collected weekly for estimation of antibody titers against ND. The blood serum was separated and analysed by Haemagglutination Inhibition (HI) Method as described by Sever (1962).

Serum bio-chemical variables: At the end of the study (5 weeks of age), serum samples were collected for detection of some bio-chemical parameters. 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.

Albumin/Globulin (A/G) ratio was calculated. Urease modified Berthelot reaction was the base for urea determination (Patton and Crouch, 1977), serum creatinine was estimated by the method of Thomas and uric acid (Caraway, 1955). Liver enzymatic activities including Aspartate Aminotransferase (AST) and Alanine aminotransferase (ALT) activities by Reitman and Frankel (1957) were measured colorimetrically.

Statistical analysis: Results data were statistically analyzed (Snedecor and Cochran, 1967). All statements of significance were based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Mycotoxicosis is the condition associated with fungal contamination of feed ingredients. Among mycotoxins, aflatoxin B_1 is the predominant and the major fungal toxin which is attributed to induction of potential problems in livestock feeds (Nilipour, 2002). In poultry, severe outbreaks of aflatoxin B_1, cause heavy economic losses in terms of health and production (Hussein and Brasel, 2001).

Due to the lack of practical solutions to totally preclude mycotoxin contamination in feeds (Anderson, 1983), binders like HSCAS has been proposed to sequestrate aflatoxins, bind with them and formed a more stable complex that prevent the toxins from being absorbed in the animal's digestive tract, thereby limiting their effect on animals and thus their transfer to edible animal products (Pasha *et al.*, 2007). But, it was found that adsorbents have limited efficacy against some mycotoxins, need to be incorporated at high levels and can have side effects on some dietary nutrients thus reducing the nutritional value of animal diets and they may also contain dioxins and heavy metals which is a huge limit for their use as feed additive (Jouany *et al.*, 2005).

Form abovementioned, alternative approaches have been studied to counteract the deleterious effects of aflatoxins in poultry field. Phytobiotics like turmeric powder (*Curcuma longa*) was used successfully for alleviation of the adverse effects of aflatoxins in poultry field (Soni *et al.*, 1992; Gowda *et al.*, 2009).

In this study, no clinical signs were seen in birds of the blank control negative (group 1). The observed clinical signs in chickens fed on diet contaminated with aflatoxin B_1 only (group 2) were severe depression, anorexia, ruffling and stunting and these signs were less pronounced in the other treated chickens groups (3-5).

No mortalities were recorded in any group during the course of the study while other studies (Huff *et al.*, 1983a;

Fukal *et al.*, 1989) showed increased death of the birds and these incomparable results may be related to the using of different aflatoxins B_1 doses.

The most observed gross lesions that detected in sacrificed chickens at 5 weeks of age (end of the experiment) were enlarged, pale and friable liver with haemorrhagic patches on the surface, enlarged and pale kidneys, reduced size of spleen, thymus glands and bursae of Fabricus and various degrees of thigh and breast muscles haemorrhages. Severest lesions were recorded in birds of group 2 that fed on aflatoxin B_1 only. Necropsy lesions observed in the liver indicating mainly hepatotoxicity of aflatoxin B_1 as it produces 2, 3-dihydrodiol resulting in rapid onset of severe liver damage (TDRI, 1984). Damaged liver cells can account for most of the changes that observed in serum chemistries because less functional proteins are synthesized and secreted from damaged livers. Liver lesions herein are similar to those reported earlier (Eraslan *et al.*, 2006) however, kidney lesions are like to those observed previously (Randall and Reece, 1996). Ortatatli *et al.* (2005) reported that liver is more affected in aflatoxicosis with pin point hemorrhages and fat deposition, kidney enlargement, petechial or echymotic hemorrhages on thighs and bursal regression. The haemarrhages that observed on the muscles in the present study concurs with Huff *et al.* (1983b) who observed an increase in the susceptibility of broilers to bruising and what is called the bloody thigh syndrome during aflatoxicosis. These haemorrhages may be referred to impairment of blood coagulation and increasing in re-calicification time of clotted blood and prothrombin time that caused by aflatoxicosis.

The lesions severity decreased in the treated groups either by HSCAS (group 3) or turmeric powder (group 4) but lowest lesions severity was seen in chickens that treated with combination of HSCAS and turmeric powder (group 5). The decline in the severity of lesions in groups fed combinations of HSCAS and aflatoxin B_1 indicates that most of the aflatoxin B_1 was neutralized in the gut and not absorbed into the hepatic system. The addition of HSCAS to the aflatoxin B_1 diet largely prevented the aflatoxin B_1-associated pathology in the liver and completely prevented renal pathology (Ledoux *et al.*, 1999).

Comparable observations were also recorded by Soni *et al.* (1992) who found that curcumin reversed the aflatoxin induced liver damage produced by feeding aflatoxin B_1 (5 ug/day per 14 days) to ducklings where fatty changes, necrosis and biliary hyperplasia produced by aflatoxin B_1 were considerably reversed by this food additive. Mathuria and Verma (2007) detected that concurrent addition of aflatoxin (2.0 ug mL^{-1}) to extract of turmeric causing retardation in aflatoxin-induced hemolysis of red blood cells *in vitro*.

Table 1 shows the results of chicken's zootechnical performance (body weight, FCR and EPEF) of different groups during the course of the study (5 weeks). The results revealed that chickens of group 2 that fed on ration contaminated with aflatoxin B_1 only showed the significant ($p<0.05$) poorest performance compared with other groups (1-5). These obtained results are consistent with earlier reports on the performance depressing effects of aflatoxin B_1 (Santurio *et al.*, 1999; Tedesco *et al.*, 2004; Shi *et al.*, 2006; Denli *et al.*, 2009). The depression in growth upon feeding aflatoxin could be attributed to reduced protein synthesis as reported by Verma *et al.* (2002) increased lipid excretion in droppings, impaired nutrient absorption and reduced pancreatic digestive enzyme production by Osborne and Hamilton (1981) and reduced appetite by Sharlin *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. Feed consumption in broilers fed on aflatoxin B_1 was significantly ($p<0.05$) decreased and this is suggestive of reduced appetite during aflatoxicosis as a protection

Table 1: Effect of hydrated sodium calcium aluminosilicate and turmeric powder on zootechnical performance parameters of aflatoxicated broiler chickens

Treatments	Body weight/gram age/week					Cumulative FCR	EPEF
	1st	2nd	3rd	4th	5th		
Blank (control negative)	113.44±1.20[a]*	288.56±2.90[a]	590.57±4.2[a]	996.37±5.5[a]	1298.63±6.0[a]	1.64±0.10[a]	240.41±7.32[a]*
AFB1 (control positive)	80.54±1.00[b]	190.43±2.10[b]	301.29±3.3[b]	563.19±4.0[b]	716.29±5.3[b]	2.61±0.32[b]	68.10±1.50[b]
AFB1+HSCAS	90.85±1.10[bc]	240.78±3.60[c]	434.72±3.5[c]	871.86±4.2[c]	1009.75±6.9[c]	2.01±0.40[c]	199.18±3.74[c]
AFB1+TP	95.89±1.4[c]	254.81±2.00[c]	456.43±2.6[c]	895.61±6.4[c]	1107.23±7.9[c]	1.91±0.13[ac]	216.85±5.10[c]
AFB1+Concomitant HSCAS and TP	114.21±1.7[a]	281.643±2.5[a]	585.34±4.7[a]	990.71±5.1[a]	1200.73±6.0[a]	1.71±0.21[a]	233.24±7.12[a]

*Means with different superscripts, within age are significantly different ($p \leq 0.05$); AFB_1: Aflatoxin B1 contaminated ration (2.5 ppm); HSCAS: Hydrated Sodium Calcium Aluminosilicate (5 g kg^{-1} ration); TP: Turmeric Powder (80 mg kg^{-1} ration); FCR: Feed Conversion Rate; EPEF: The European Production Efficiency Factor (EPEF)

mechanism (Rauber et al., 2007) or due to impaired liver metabolism caused by the liver damage (Johri and Majumdar, 1990).

Dietary supplementation with HSCAS or turmeric powder alone improved the zootechnical performance parameters (group 3 and 4, respectively) at partial significant ($p<0.05$) however, the best significant ($p<0.05$) performance was recorded in chickens of group 5 that treated with HSCAS and turmeric powder mixture. 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 B_1 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. Mabbett 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. On the other side, the growth promoting effect exerted by turmeric (*Curcuma longa*) on aflatoxin B_1 was studied by Yarru et al. (2009b) and Rangsaz and Ahangaran (2011) as they demonstrated that supplement of ethanolic turmeric extract in a diet containing 3 ppm aflatoxin can significantly improve performance indices compared with the group that consumed aflatoxin alone. Curcumin, the major antioxidant ingredient of turmeric is known to inhibit the biotransformation of aflatoxin B_1 to aflatoxicol in liver (Lee et al., 2001) and is also responsible for its antimutagenic and anticarcinogenic action (Chun et al., 1999). Emadi and Kermanshahi (2007) fed broiler chicks turmeric powder (0.25, 0.5 and 0.75%) from hatch to 49 days of age and concluded that turmeric might have some positive effects on liver enzymes activities that directly or indirectly reflect a healthier liver status in the birds. Gowda et al. (2008) were partially agree with us as

they found that supplementation of chicks with turmeric powder 5% to the aflatoxin B_1 contaminated diet partially improved feed intake, body weight gain and feed conversion of chicks, suggesting antioxidant protection by turmeric powder while supplementation with of both HSCAS and turmeric powder to chicks did not result in any further benefits and they suggesting that the concentration of curcumnoids (74 mg kg^{-1}) supplied by the level of turmeric powder (0.5%) employed in their study was too low to exert a more potent antioxidant action.

The findings herein demonstrated that the absolute and relative weight of the liver, kidney and gizzard in control positive group 2 that fed on aflatoxin B_1 were significantly ($p<0.05$) different from blank control negative (group 1) and the other treated groups (3-5) while the weights of immune organs (spleen, thymus glands and bursa of Fabricus) of this group 2 were significantly ($p<0.05$) the lowest one (Table 2). An increase in organs weight and their relatives weight owing to reduction in body weight. Parallel to the 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 B_1 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 (Miazzo et al., 2005; 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

Table 2: Effect of hydrated sodium calcium aluminosilicate and turmeric powder on absolute and relative organs weights in aflatoxicated broiler chickens

Treatment	Liver Absolute Weight	Liver Relative Weight	Kidney Absolute Weight	Kidney Relative Weight	Gizzard Absolute Weight	Gizzard Relative Weight	Spleen Absolute Weight	Spleen Relative Weight	Thymus glands Absolute Weight	Thymus glands Relative Weight	Bursa of Fabricus Absolute Weight	Bursa of Fabricus Relative Weight
Blank (Control negative)	19.15±0.6ᵃ*	1.9±0.20ᵃ	8.15±0.4ᵃ	0.99±0.1ᵃ	22.15±0.2ᵃ	3.11±0.1ᵃ	0.87±0.01ᵃ	0.187±0.030ᵃ	2.98±0.05ᵃ	0.30±0.02ᵃ	2.79±0.06ᵃ	0.29±0.04ᵃ
AFB1 (Control positive)	16.87±0.3ᶜ	2.87±0.1ᶜ	6.32±0.3ᶜ	1.53±0.1ᵈ	19.11±0.3ᶜ	4.02±0.1ᵇ	0.16±0.02ᵇ	0.035±0.002ᵇ	0.63±0.01ᵇ	0.10±0.04ᵇ	1.01±0.04ᵇ	0.12±0.10ᵇ
AFB1+HSCAS	13.92±0.5ᵇ	2.19±0.2ᶜ	5.76±0.1ᵇᶜ	1.0±0.40ᵃ	18.20±0.1ᶜ	3.23±0.1ᵃ	0.29±0.02ᵇ	0.065±0.010ᶜ	1.09±0.02ᵇᶜ	0.13±0.03ᵇ	1.87±0.05ᶜ	0.18±0.11ᶜ
AFB1+TP	14.76±0.6ᵇᶜ	1.81±0.3ᵃ	5.41±0.2ᵇᶜ	0.60±0.2ᶜ	18.64±0.1ᶜ	3.02±0.2ᵃ	0.56±0.01ᶜ	0.086±0.010ᶜ	1.87±0.03ᵃ	0.20±0.05ᵃ	2.05±0.01ᵃᶜ	0.21±0.12ᵃ
AFB1+ Concomitant HSCAS and TP	13.54±0.4ᵇ	1.44±0.2ᵇ	4.97±0.2ᵇ	0.23±0.3ᵇ	17.98±0.0ᵇ	2.95±0.1ᵃ	0.78±0.03ᵃ	0.110±0.020ᵃ	2.76±0.04ᵃ	0.29±0.01ᵃ	2.57±0.07ᵃ	0.25±0.01ᵃ

*Means with different superscripts are significantly different ($p \leq 0.05$); AFB$_1$: Aflatoxin B1 contaminated ration (2.5 ppm); HSCAS: Hydrated Sodium Calcium Aluminosilicate (5 g kg^{-1} ration)

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. Moreover, the reduction of weight of immunological competence organs caused by aflatoxin B_1 is a strong indication of the immune suppression (Kubena *et al.*, 1990).

In the present study, separate addition of HSCAS (group 3) and turmeric powder (group 4) to the aflatoxin B_1 diet prevented significantly (p<0.05) the increasing in organs weights observed in chickens consumed aflatoxin B_1 alone. Furthermore, significant (p<0.05) prevention or amelioration of the changes in organs weights of chickens was obtained after feeding on concomitant HSCAS and turmeric powder (group 5). Kubena *et al.* (1991), Ledoux *et al.* (1999) and Gowda *et al.* (2008) showed that an increase of the organs weights caused by aflatoxin B_1 in broiler chickens could be counteracted by addition of HSCAS to the diet. 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. As regard to the protection afforded by turmeric powder, the study of Gowda *et al.* (2008) and Yarru *et al.* (2009b) found liver's weight in chicks fed the combination of turmeric powder and aflatoxin B_1 diet was not significant compared with liver weight of chicks fed aflatoxin B_1 alone indicating partial hepato-protection due to the feeding of turmeric powder.

Regarding the results of humoral immune response of chickens to ND vaccination using HI test, Table 3 indicates that the antibody titers of chickens group 2 that fed on diet contaminated with aflatoxin B_1 alone were significantly (p<0.05) the lowest during all intervals compared with blank control negative (group 1). Whilst feed addition of either HSCAS or turmeric powder and their combination to aflatoxin B_1 contaminated ration in groups (3-5) significantly (p<0.05) enhanced the immune response of chickens. This result indicates that aflatoxin severely inhibited the immune system of the birds to ND vaccine resulted to reduce the titer of ND. Comparable results were obtained by Otim *et al.* (2005) who detected

significant (p<0.05) reduction in the HI of ND antibody titer following initial priming with Hitchner B1 and subsequent booster with La Sota vaccines and a delayed hypersensitivity test following sensitization with dinitrochlorobenzene showed aflatoxin to be a more potent immunosuppressant than infectious bursal disease virus also, Tessari *et al.* (2006) who record that broilers received aflatoxin B_1-treated ration showed reduced geometrical mean antibody titers against ND. 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.*, 1966). 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 a flatoxin 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 HSCAS in in this investigation is in line with Ibrahim *et al.* (2000) who detected that addition 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. Moreover, the effect of HSCAS on the humoral immune response of quails fed on aflatoxin B_1 contaminated ration was studied by Sehu *et al.* (2007) and found decrease in the antibody titer induced by ND vaccine due to aflatoxins was relatively prevented.

Table 3: Effect of hydrated sodium calcium aluminosilicate and turmeric powder on Haemagglutination Inhibition (HI) antibody titers against Newcastle disease vaccine in aflatoxicated broiler chickens

| Treatments | HI titers/Age/Week | | | | |
	1st	2nd	3rd	4th	5th
Blank (control negative)	5.0±0.21[a]*	4.20±0.20[a]	6.80±0.18[a]	5.6±0.16[a]	6.1±0.11[a]
AFB1 (control positive)	2.1±0.50[b]	1.98±0.27[b]	4.61±0.21[b]	3.7±0.17[b]	3.9±0.18[b]
AFB1+HSCAS	3.2±0.33[bc]	2.90±0.27[c]	5.90±0.26[a]	4.0±0.33[c]	5.1±0.36[c]
AFB1+TP	4.0±0.24[c]	3.80±0.27[a]	6.00±0.36[a]	4.2±0.20[c]	5.7±0.19[a]
AFB1+Concomitant HSCAS and TP	4.8±0.43[a]	3.90±0.21[a]	6.70±0.23[a]	5.1±0.29[a]	5.9±0.31[a]

*Means with different superscripts are significantly different (p≤0.05); AFB₁: Aflatoxin B1 contaminated ration (2.5 ppm); HSCAS: Hydrated Sodium Calcium Aluminosilicate (5 g kg⁻¹ ration); TP: Tumeric Powder (80 mg kg⁻¹ ration)

Table 4: Effect of hydrated sodium calcium aluminosilicate and turmeric powder on serum biochemical variables of aflatoxicated broiler chickens

Treatments	Total protein	Albumin (A) (mg dL^{-1})	Globulin (G) (mg dL^{-1})	A/G ratio	Urease (mg dL^{-1})	Creatinine (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
						Serum biochemical variables (5 weeks of age)		
Blank (control negative)	3.46±0.05[a]*	1.53±0.03[a]	1.93±0.02[a]	0.79±0.050[ac]	15.11±0.13[a]	0.42±0.03[a]	198.17±1.08[a]	67.32±3.05[a]
AFB1 (control positive)	1.27±0.07[b]	0.67±0.05[b]	0.60±0.02[b]	1.11±0.020[b]	24.31±0.10[b]	0.84±0.02[b]	301.76±1.14[b]	102.11±2.39[b]
AFB1+HSCAS	3.40±0.03[a]	1.60±0.02[a]	1.80±0.01[a]	0.88±0.040[c]	21.67±0.31[bc]	0.71±0.04[c]	235.82±1.20[c]	75.64±4.11[c]
AFB1+TP	2.81±0.08[c]	1.30±0.07[a]	1.51±0.01[c]	0.86±0.030[c]	19.62±0.23[c]	0.59±0.06[ac]	226.71±1.32[c]	70.45±6.01[c]
AFB1+Concomitant HSCAS and TP	3.15±0.09[a]	1.10±0.06[a]	2.05±0.03[a]	0.53±0.043[a]	16.89±0.14[a]	0.46±0.01[a]	205.99±0.10[a]	60.75±1.68[a]

*Means with different superscripts are significantly different ($p \leq 0.05$); AFB$_1$: Aflatoxin B1 contaminated ration (2.5 ppm); HSCAS: Hydrated Sodium Calcium Aluminosilicate (5 g kg^{-1} ration); TP: Tumeric Powder (80 mg kg^{-1} ration); AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase

Chronic mycotoxicosis could be diagnosed by determining serum biochemical alterations even before major clinical symptoms appear (Oguz et al., 2000). The effects of dietary supplementation of aflatoxin B$_1$ on serum biochemical parameters (Table 4) clears that serum total protein, albumin and globulin levels were significantly ($p < 0.05$) decreased. The reduced levels of total protein and albumin are indicative of the toxic effect of aflatoxin B$_1$ on hepatic and renal tissues and are consistent with previous literature reporting aflatoxicosis (Kubena et al., 1993; 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., 1993) and is indicator of impaired protein synthesis (Kubena et al., 1989). The reduction of A/G ratio was due to decreased albumin concentrations. Serum urea, creatinine and uric acid were significantly ($p < 0.05$) elevated in aflatoxin B$_1$ fed chickens. The increased concentrations of urea, creatinine and uric acid coupled with the kidney enlargement observed may indicate some renal tissues damage due to aflatoxin B$_1$. This significant alteration in kidney parameters in birds fed on aflatoxin B$_1$ treatments agree with data reports of Denli et al. (2005) and Bintvihok and Kositcharoenkul (2006).

The addition of HSCAS and turmeric powder were significantly ($p < 0.05$) effective in the protection against aflatoxin B$_1$ by preventing its toxic effect as was reflected by ameliorating the alterations in serum biochemical parameters (increasing in serum total protein and albumin and decreasing in serum urea, creatinine and uric acid). Tumeric powder providing antioxidant protection and HSCAS decreasing the amount of aflatoxin B$_1$ absorbed. Kubena et al. (1990), Ledoux et al. (1999) and Gowda et al. (2008) recorded that addition of such additives to the aflatoxin B$_1$ diet prevented the decrease in these aflatoxin-sensitive serum proteins. Supplementation of plant extracts of cumin (Nigella sativa) and clove (Syzygium aromaticum) to rat diets containing aflatoxin B$_1$ overcame the negative effect of aflatoxin B$_1$ on serum chemistry (Abdel-Wahhab and Aly, 2005).

In comparison with chickens fed on only aflatoxin B$_1$ (group 2), liver enzymatic activities including (AST) and (ALT) showed significantly ($p < 0.05$) the lowest values in group (5) that treated with combination of HSCAS and turmeric powder while these values were relatively low in chickens either treated with HSCAS (group 3) or turmeric powder (group 4). An increase in liver's enzyme profile in aflatoxicated ration is most likely reflects liver tissue damage, alternated hepatocyte membrane integrity with leakage of enzymes into the blood (Duncan and Prasse, 1986). The results herein are in accordance with the findings of Aravind et al. (2003) and Denli et al. (2009) who reported an increase in AST and ALT activities upon feeding diet contaminated with different doses of aflatoxin. Van Zytveld et al. (1970) detected transfer of toxin into the liver tissue when aflatoxin was fed directly into the crop at very high doses. On the contrary, Stanley et al. (1993) detected a decrease of 17-42% in ALT activity in aflatoxin B$_1$-intoxicated chickens and Fernandez et al. (1994) found decrease of 36% in plasma ALT activity in laying hens treated with 2.5 mg kg^{-1} of aflatoxin B$_1$. Manegar et al. (2010) could not record any significant alteration in liver's enzyme profile upon feeding birds with aflatoxin. The discrepancies in enzyme profile as reported by several researchers indicate that AST and ALT levels may not suggest the extent of liver damage or could be a true indicator during aflatoxicosis.

Modulation of the liver enzymes after treatment of aflatoxin contaminated diet with HSCAS was successfully studied by Abdel-Wahhab et al. (1998) on the other hand, Emadi and Kermanshahi (2007) fed broiler chicks turmeric powder (0.25, 0.5 and 0.75%) from hatch to 49 days and concluded that turmeric might have some positive effects on liver enzymes by reducing alanine aminotransferase and alkaline phosphatase activities that directly or indirectly reflect a healthier liver status in the birds.

Reduction in the serum chemistry data due to HSCAS and turmeric powder supplementation of the aflatoxin B$_1$ diet is consistent with the severity of lesions due the antioxidant status in the liver. 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 while Gowda *et al.* (2009) concluded that the addition of 222 mg kg^{-1} total curcuminoids to the 1.0 mg kg^{-1} aflatoxin B_1 contaminated diet demonstrated maximum antioxidant activity against aflatoxin B_1. The mode of action of curcumin as an antioxidant is explained by its strong inhibitory action on superoxide anion generation (Iqbal *et al.*, 2003) and biotransformation of aflatoxin B_1 to aflatoxicol in liver (Lee *et al.*, 2001). Supplementation of turmeric is known to reduce aflatoxin B_1-DNA adducts formation through modulation of cytochrome P 450 function (Soni *et al.*, 1997).

The data from the present study showed that aflatoxin B_1 caused severe adverse toxic side effects on broiler chickens this damage was obvious from the results of clinical view (signs and lesions), zootechnical performance parameters, relative organs weights, immune response and serum biochemical variables. Dietary addition of HSCAS or turmeric powder either separately or concomitantly significantly diminished the toxicity resulting from aflatoxin B_1. So, it can be concluded that HSCAS and/or turmeric powder can be considered an integrated approach for the control of aflatoxicosis in broiler chickens.

Notwithstanding, it is necessary to point out that mycotoxins are complex organic compounds and each of them has different functional groups thus the binding capacity of an adsorbent depends on its chemical and physical properties and its relation with the physical structure of the target mycotoxins. Thus, the physicochemical differences among the adsorbents used in the studies mentioned above could explain the higher or lower efficacy among them.

CONCLUSION

Addition of HSCAS and or turmeric powder can be considered an integrated approach for the control of aflatoxicosis in broiler chickens.

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