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Efficacy of N-Acetylcysteine and Hydrated Sodium Calcium Aluminosilicate to Reduce the Effects of Aflatoxin B_1Intoxication in Broiler Chickens

Khalil M. Attia, Mohamed M.H. Asser*, F.A. Tawfeek, Hanaa A. Basuney
Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt

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***Corresponding to:**
M_hosny556@yahoo.co.uk

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ABSTRACT

This research was planned to determine the efficacy of N-acetylcysteine (NAC) and/or Hydrated Sodium Calcium Aluminosilicate (HSCAS) to combat aflatoxin B1 (AFB1) toxic effects in broiler chickens. A total of 720, day-old broiler chicks were distributed equally to eight experimental treatments with three 30 bird replicates each. The first treatment was fed basal diet and used as control. The other treatments (from 2 to 8) were fed the same basal diet and supplied with NAC alone (100 mg/kg diet), HSCAS alone (5 g/kg diet), NAC (100 mg/kg diet) plus HSCAS alone (5 g/kg diet), AFB1 alone in the diet (1 mg/kg of diet) for 42 d, AFB1 plus NAC (100 mg/kg diet) , AFB1 plus HSCAS (5 g/kg diet) and AFB1 plus NAC (100 mg/kg diet) plus HSCAS alone (5 g/kg diet, respectively).

Aflatoxin caused to a significant reduces in the activity of serum and hepatic catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH). However, serum malondialdehyde (MDA), liver and kidney function levels have increased. After the chicks diets were exposed to aflatoxin B1 pollutions, pancreatic enzyme activity showed a significant enhance in serum lipase and amylase activity. Moreover, aflatoxin B1 group had a reduce in levels of serum albumin, total protein, beta globulin, alpha globulin and gamma globulin together with A/G ratio. N-acetylcysteine and/or HSCAS administration resulted in decreased levels of malondialdehyde (MAD) and increased levels of CAT, GSH and SOD compared with the contaminated group. Therefore, due to the protective effects of N-acetylcysteine and/or HSCAS, the concentrations of AST, ALT, serum lipase and amylase are significantly decreased.

Broilers fed AFB1 plus NAC, HSCAS and NAC plus HSCAS were shown to be partially protected against adverse effects on BWG (68.38, 61.24 and 84.17 %), daily feed intake (76.92, 72.87and 86.96 %), feed conversion ratio (82.91, 73.64 and 99.65 %), serum total protein concentration, liver and kidney function, hepatic glutathione-Stransferase, and reduced glutathione liver concentration. Our findings suggest that NAC provided protection in broiler chickens against negative performance effects and biochemical changes caused by AFB1. Also assessed were the effects of NAC on chick performance alone. Adding NAC to diet (100 mg / kg diet) did not adversely affect feed intake, feed conversion ratio, or serum chemistry and did not result in structural changes in the liver or kidney.

1. INTRODUCTION

Aflatoxin is an omnipresent class of naturally occurring mycotoxins continuously included in feed ingredients (Manafi et al., 2009). Aflatoxin is a harmful secondary metabolite of the *Aspergillus flavus* and *Aspergillus parasiticus* fungi (Smith et al., 1995). Aflatoxin B1 is the most toxic of all forms of mycotoxin (Sweeney and Dobson, 1998), causing severe economic losses such as immunosuppression, poor growth and feed efficiency, increased mortality, decreased egg production, leg problems, hepatic damage and carcass condemnation (Soliman et al., 2008 and Yarru et al., 2009). However, possible residues of mycotoxin were found in birds ' tissues and eggs (Pandey and Chauhan, 2007) and are becoming

particularly important as a potential hazard to human health.

Extensive research was conducted to prevent mycotoxicosis, primarily involving physical, mechanical, dietary, or biological approaches. The use of adsorbent agents, which by exchange of ions will trap the mycotoxin molecule and thus escape its absorption from the gastrointestinal tract into the blood, has gained considerable interest in mycotoxin prevention.

Hydrated sodium calcium aluminosilicate (HSCAS) (Kubena *et al.*, 1990; Huff *et al*., 1992; Jindal *et al.*, 1993), bentonite (Santurio *et al*., 1999), activated charcoal (Edrington *et al*., 1997), zeolite ore compounds (Harvey *et al*., 1993), spent canola oil bleaching clays (Smith, 1984), 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.

Nevertheless, these clays have certain drawbacks, such as high inclusion rates (Devegowda et al., 1998), possible association with essential nutrients (Moshtaghian et al., 1991), and lack of practical binding effects on many mycotoxins (Chestnut et al., 1992). Aluminosilicates are said to selectively bind only those mycotoxin molecules which have atomic polar function groups.

The oxidase process of the hepatic mixed function biotransforms AFB1 and creates an aflatoxin or reactive epoxy metabolite. The conjugation of reduced glutathione inactivates this intermediate molecule: (π-glutamil-cysteinyl-glycine, GSH) (Eaton and Gallagher, 1994).Glutathione-S-transferase (GST) catalyzes this reaction to form a molecule released as mercapturic acid-AFB1 (8,9-dihydro-8-9-(S-cysteinyl-(N-acetyl))-9-hydroxy aflatoxin B1) or N-acetylcysteine (NAC) bound to AFB1 (Raney et al., 1992).When birds eat AFB1, they are swallowed by the intestine and spread throughout the body through the bloodstream; bile and renal secretion extract about 90% of AFB1 (Agacdelen and Acet, 1993). ^{14}C-AFB1 chicken delivery studies found that 6.2% of radioactivity was retained in breast and leg meats (Mabee and Chipley, 1973). Aflatoxin clearance intervals are 24 h for the muscle in laying hens and broiler chickens, and 8 d for the eggs (Agacdelen and Acet, 1993; Fernandez et al., 1995).

Extensive research was therefore performed to establish cost-effective and safe procedures and agents that would minimize AFB1's deleterious effects (Kubena et al., 1993).A variety of methods have been proposed to prevent contamination, decontaminate or treat feedstuffs (Bailey et al., 1998; McKenzie et al., 1998, and Ledoux et al., 1999).Recently, research has been centered on the use of chemoprotective agents that work against AFB1 to increase mycotoxin detoxification through drug-metabolizing enzymes in human and animal modeling (Wang et al., 1998 and 1999).

NAC, an acetylated form of L-cysteine amino acid ($C_5H_9NO_3S$), increased cytosolic and mitochondrial GSH levels, and reduced toxicity in patients with acquired immune deficiency syndrome and rats with varying xenobiotics (Raju et al., 1994 and Wispriyono et al., 1998). In several countries, this compound is already commercially available for human consumption; it is well tolerated in daily doses as high as 500 mg / kg per os and has been widely used as an antidote to various toxic agents and in several pathological disorders associated with GSH cell homeostasis (Kobrinsky et al., 1996). If NAC is given orally to healthy human volunteers, it is rapidly absorbed into the gut wall and liver and undergoes rapid and comprehensive metabolism.

Around 10 percent of the unchanged drug comes into the plasma and becomes cysteine and GSH (De Caro et al., 1989). NAC can have protective effects against AFB1 mutagenic attack by intracellular levels of glutathione in cells, blunting the activation effects of this mycotoxin, stimulating enzyme activity and interacting with AFB1 (De Flora et al., 1985).The purpose of this study was to establish whether dietary supplementation with NAC and/or HSCAS prevented the occurrence of negative effects in broiler chickens during AFB1 intoxication, as there is little literature for this therapeutic use of NAC.

2. MATERIALS AND METHODS

This experiment was conducted at the Poultry Research Farm, Sakha, Egypt with the goal of assessing the performance and immune response of broilers fed with aflatoxin B1 and NAC and/or HSCAS.

2.1. Aflatoxin B1 Production

Aspergilus parasiticus NRRL 2999 (source: National Institute of Animal Health, Dokki, Cairo, Egypt) developed aflatoxin through rice fermentation, as defined by Shotwell et al. (1966). The fermented rice was powdered and steamed. The quality of aflatoxins was calculated by Nabney's and Nesbitt's spectrophotometric analysis (1965). The rice powder was then incorporated into the basal diets of maize-

soybean meals to provide the desired level of aflatoxin B1.

2.2. Experimental chickens

A total of 720 one-day broiler chicks were adjusted for a period of 7 days before the experimental was launched. The chicks were submitted to conventional broiler chicken management during this period and housed in floor pens in an eco-controlled broiler house with litter floors. Birds have been held throughout the experimental period (42 days) on a 24-hour continuous light schedule. During the brooding time, temperature was kept to the necessary. At the age of 7, chickens are vaccinated against Newcastle Disease (ND) and infectious bronchietis disease using intraocular Hitchner B1+H120 vaccine and at the age of 9, using inactivated intramuscular H5N2 vaccine. At the age of 15, Gumboro (IBD vaccine, Infectious Bursal Disease) and ND are vaccinated using La Sota vaccines by intraocular route, respectively.

2.3. Ration

The basal diet was a meal diet of the commercial form of maize soybean. Chickens were fed on a commercial starter-grower diet (based on maize and soybean meal containing 23% CP, 3058Kcal ME / Kg diet) up to 21 days of age and then changed from 22 to 42 days to finisher diet (20.0% CP, 3207Kcal ME / Kg diet) (Table 1). The ration was not supplem ented with antibiotics, anticoccidium and antifungal medications. As recommended by the NRC (1994), the ration has been formulated to meet or exceed the nutritional requirements of chickens. Birds *ad libitum* are allowed to feed and drink. The basal diet was tested prior to feeding experimental chickens for potential residual mycotoxins such as aflatoxins, ochratoxins, zeralenone and fumins before feeding (Rottinghaus et al., 1982).

2.4. Experimental design

A completely randomized design was used with 720, day-old mixed-sex broiler chicks assigned to each of eight experimental groups, each group included 90 chicks, and each subdivided into three replicates with 30 chicks. The first treatment was fed basal diet and used as control. The other treatments (from 2 to 8) were fed the same basal diet and supplied with NAC alone (100 mg/kg diet), HSCAS alone (5 g/kg diet), NAC (100 mg/kg diet) plus HSCAS alone (5 g/kg diet), AFB1 alone in the diet (1 mg/kg of diet) for 42 d, AFB1 plus NAC (100 mg/kg diet) , AFB1 plus HSCAS (5 g/kg diet) and AFB1 plus NAC (100 mg/kg diet) plus HSCAS alone (5 g/kg diet, respectively).

2.5. NAC: It was purchased from Sigma Chemical Company. All groups were fully observed from day to 6 weeks of age (end of study).

2.6. Hydrated Sodium Calcium Aluminosilicate (HSCAS): HSCAS commercial product of the Integrated World Enterprises Co. USA was used. The chemical composition of HSCAS was detailed (analyzed by manufacturer) as follows: Silcon oxide (64.7%), Aluminum oxide (15.5%), Oxides of Iron, Magnesium, Calcium, Sodium, Potassium (8.89%) and moisture (10.9%). HSCAS was white crystals, fine powder and usually added to the diet (0.3 to 0.5 % as recommended by the manufacturer. In this study HSCAS was added at the rate of 0.5 %.

2.7. Data collection

2.7.1. Performance

Chicks were weighed individually, and each cell was assessed weekly for feed intake during the 6-wk test. Cumulative weight gain and feed intake were estimated while weekly and cumulative gain were calculated: feed ratios. Nutrition intake and gain: Food was optimized for mortality if necessary.

2.7.2. Serum bio-chemical variables:

By puncturing the brachial vein, blood was extracted from three birds (one bird per replicate) in non-heparinized tubes at the end of the experiment. The specimen was centrifuged for 10 min at 3,000 rpm and serum specimens were isolated and stored for examination at -20 ° C. The estimation of lipase and amylase levels in serum was done as recommended by Moss and Henderson (1999). The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured according to (Reitman and Frankel, 1957 and Tietz, 1996). While, serum uric acid was determined the method of Caraway, (1955), serum creatinine level after Henry (1974). Whereas, the estimation of serum total protein and electrophoretic pattern were carried out after Sonnenwirth and Jaret (1980) and Davis (1964), respectively.

2.7.3. Determination of antioxidant indexes, lipid peroxidation:

Three chicks from each class are sacrificed at the end of the test. For the screening of glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) and malondiadehyde (MDA), serum and hepatic homogenates was prepared. SOD activity was analyzed by monitoring of nitrobluetetrazolium (NBT) reduction inhibition level. The former method used to determine GSH-Px activity (Shi et al., 2006). The measurement of H_2O_2 decomposition at 240 nm

determined CAT activity. GR activity was measured using the Carlberg and Mannervik (1975) method by measuring the rate of oxidation of nicotinamide adenine dinucleotide phosphate at 340 nm with a spectrophotometer. Malondiadehyde (MDA) was based on reactive substances of thiobarbituric acid (TBARS) (Carlberg and Mannervik, 1975) calculation.
Analysis of AFB1 residues in liver and breast muscles: For the analysis of AFB1 residue, three samples of the liver and breast muscle from each patient are kept at −20 ° C. Tavcar-Kalcher et al. (2007) conducted an analysis of AFB1 residues.
Humoral immunity: Serum samples were collected regularly to measure Newcastle disease virus (ND) antibody titers. Hemagglutination inhibition (HI) test isolated and analyzed the blood serum (Thayer and Beard, 1998).
Statistics analysis: Data were statistically analyzed using SAS (2000). Duncan's multiple range test (Duncan 1955) was used the significance ($p \leq 0.05$) of differences among means.

3. RESULTS AND DISCUSSION

Data presented in Table 2 shows the effect of the experimental groups and alleviation of aflatoxicosis on daily body weight gain (BWG), daily feed intake (FI), and feed conversion ratio (FCR) during the experimental period. Feeding basal diet contaminated with AFB1 significantly ($p \leq 0.05$) suppressed BWG, FI and FCR from first 2 week onwards compared to negative control. Feeding the AFB1-diet decreased BWG by 21.47 % , FI by 13.79 % and poorer FCR by 9.77 % at the end of the experimental period. Our findings agree with those showing that AFB1 has a serious impact on chickens ' performance (Huff et al., 1988 and Bailey et al., 1998). Kubena et al. (1993) found that in broiler chickens, AF (3.5 mg / kg feed) had adverse effect on BW and feed conversion. We found that body weight reduced by 16 percent, and conversion of feed increased at 21 d of age (1.64 to 1.81). Anorexia, listlessness, protein synthesis inhibition and lipogenesis (Oguz and Kurtoglu, 2000 and Parlat et al., 2001) are the adverse effects of AFB1 on body weight, feed intake and FCR. Impaired liver functions and protein, lipid and carbohydrate metabolism may also have impaired growth efficiency and overall health (Miazzo et al., 2000, Ortatatli and Oguz, 2001).

In our research, the concurrent administration of NAC and/or HSCAS has minimized these changes, indicating a prevent effect of these additives on the adverse effects of feeding this aflatoxin. The addition NAC, HSCAS and NAC plus HSCAS protected BWG, FI and FCR of chicks against the adverse effect of AFB1 by (68.38 , 61.24 and 84.17 %), (76.92, 72.87 and 86.96 %) and (82.91, 73.64 and 99.65 %) respectively, at 6 weeks of age. NAC has been widely prescribed in several countries to humans, making it well-known for its health and pharmacological properties (De Caro et al., 1989). NAC is an excellent source of sulfhydryl groups and can promote GSH synthesis, promote detoxification and function as a free radical scavenger directly (De Flora et al., 1985; Lancet, 1991). NAC administration has been a mucolytic drug in a number of respiratory diseases; however, it also appears to have beneficial effects in conditions characterized by decreased GSH or oxidative stress, such as viral infection with human immunodeficiency, diabetes, heart disease and cigarette smoking. Apparently NAC is the center of the treatment for hepatotoxicity induced by acetaminophen. It also appears that this compound has some therapeutic utility in treating acute heavy metal poisoning (Kobrinsky et al., 1996; Kelly, 1998).

Diet which is supplemented with NAC, results in increase of GSH which conjugates with AFB1 and eliminated as conjugate of mercapturic acid. NAC's beneficial effects may be due to an improvement in the availability of GSH, created by increased pre-runner cystine affluence. The prevent effect of NAC could be attributed to the competitive binding with cystine, an intracellular derivative of AFB1 electrofilic metabolites. De Flora et al. (1985), Kelly (1998) and Valdivia et al. (2001) reported similar results. For these reasons, we conclude that NAC in broiler chickens is a useful and usable medication to control aflatoxicosis.

The growth performance of broilers fed in the AF diets improved with HSCAS supplementation ($p<0.05$). Such findings are consistent with earlier tests of HSCAS compound's protective effects (Ledoux et al., 1999).The beneficial effect of HSCAS can be due to the protective impact of HSCAS on the toxicity of aflatoxin alone. The results of this study are consistent with Kubena et al. (1990). A proposed mechanism for HSCAS AF chemisorptions is the creation of an AF B carbonyl system complex with uncoordinated ' edgesite ' aluminum ions in HSCAS that enables mycotoxin to pass through the animal harmlessly (Phillips et al., 1990).

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

Ingredients	Starter-grower (7-21d)	Finisher (22-42d)
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 different dietary treatments on productive performance, (1 -6 Weeks)

Treatments	BWG (g/day per bird)			Feed intake (g/day per bird)			FCR		
	7-28 days	28-42 days	7-42 days	7-28 days	28-42 days	7-42 days	7-28 days	28-42 days	7-42 days
G1	40.68^{a}	75.48^{a}	54.50^{a}	60.00^{a}	140.4^{a}	93.50^{a}	1.47	1.86^{c}	1.72^{b}
G2	40.70^{a}	75.60^{a}	55.0^{a}	60.50^{a}	141.0^{a}	94.50^{a}	1.48	1.87^{c}	1.72^{b}
G3	40.65^{a}	75.50^{a}	54.70^{a}	60.21^{a}	140.9^{a}	93.70^{a}	1.48	1.87^{c}	1.71^{b}
G4	40.73^{a}	75.96^{a}	55.10^{a}	60.00^{a}	140.5^{a}	93.80^{a}	1.47	1.85^{c}	1.71^{b}
G5	28.65^{c}	58.20^{d}	42.80^{d}	52.00^{b}	125.0^{c}	80.60^{c}	1.82	2.15^{a}	1.88^{a}
G6	33.60^{b}	63.76c	50.8^{c}	55.00^{b}	130.0^{b}	88.50^{b}	1.65	2.04^{ab}	1.74^{b}
G7	34.50^{b}	65.70^{bc}	51.8^{c}	54.60^{b}	129.60^{b}	90.00^{b}	1.58	1.97^{bc}	1.73^{b}
G8	36.70^{ab}	70.10^{b}	52.5^{b}	54.66^{b}	130.0^{b}	90.10^{b}	1.50	1.86^{c}	1.72^{bc}
SEM	0.976	1.411	1.451	0.770	1.371	1.000	0.034	0.026	0.043
Sig.	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.092	0.003	0.0001

$^{a, b}$ Means within the same column with different superscripts are significantly different (P≤0.05). SEM = standard error of the mean. G1) control group with basal diet, G2) NAC alone (100 mg/kg diet), G3) HSCAS alone (0.5%), G4) NAC (100 mg/kg diet) plus HSCAS (0.5%), G5) AFB1alone in the diet (1 mg/kg of feed) for 35 d, G6) AFB1 plus NAC (100 mg/kg diet), G7) AFB1 plus HSCAS (0.5%) and G8)AFB1 plus NAC (100 mg/kg diet) plus HSCAS (0.5%).

Table 3. Effect of different dietary treatments on levels of total protein and its fractionation (g/dl)

Parameters	Treatments								SEM	Sig.
	G1	G2	G3	G4	G5	G6	G7	G8		
T. protein	6.34^{a}	6.35^{a}	6.24^{a}	6.40^{a}	4.95^{d}	5.65^{bc}	5.55^{c}	5.83^{b}	0.102	0.0001
Albumin	2.35^{a}	2.34^{a}	2.40^{a}	2.37^{a}	1.80^{c}	2.25^{b}	2.15^{b}	2.27^{b}	0.054	0.0001
Globulin	3.99^{a}	4.01^{a}	3.84^{ab}	4.03^{a}	3.15^{d}	3.67^{c}	3.73^{b}	3.78^{b}	0.054	0.0001
A/G ratio	0.59^{ab}	0.58^{ab}	0.63^{a}	0.59^{ab}	0.57^{c}	0.61^{b}	0.58^{ab}	0.601^{b}	0.010	0.001
Alpha1	0.91^{a}	0.90^{a}	0.90^{a}	0.91^{a}	0.55^{d}	0.78^{c}	0.76^{c}	0.833^{b}	0.024	0.0001
Alpha2	0.40^{c}	0.40^{c}	0.39^{c}	0.41^{bc}	0.49^{a}	0.43^{b}	0.43^{b}	0.43^{b}	0.006	0.0001
Total alpha	1.30^{ab}	1.30^{ab}	1.29^{ab}	1.32^{a}	1.04^{d}	1.22^{c}	1.19^{c}	1.27^{b}	0.018	0.0001
Beta 1	0.75^{a}	0.76^{a}	0.75^{a}	0.76^{a}	0.43^{d}	0.62^{c}	0.63^{bc}	0.65^{b}	0.022	0.0001
Beta 2	0.59^{de}	0.61^{cd}	0.59^{e}	0.62^{c}	0.69^{a}	0.64^{b}	0.64^{b}	0.63^{bc}	0.006	0.0001
Total Beta	1.34^{b}	1.37^{a}	1.34^{b}	1.38^{a}	1.12^{d}	1.26^{c}	1.28^{c}	1.27^{c}	0.016	0.0001
Gamma 1	1.63^{ab}	1.65^{a}	1.62^{b}	1.64^{ab}	1.29^{e}	1.53^{d}	1.52^{d}	1.56^{c}	0.023	0.0001
Gamma 2	0.36^{c}	0.34^{c}	0.34^{c}	0.34^{c}	0.50^{a}	0.43^{b}	0.43^{b}	0.42^{b}	0.012	0.0001
T. gamma	1.99^{a}	1.99^{a}	1.97^{a}	1.98^{a}	1.79^{b}	1.95^{a}	1.95^{a}	1.99^{a}	0.013	0.0001

$^{a, b}$ Means within the same row with different superscripts are significantly different (P≤0.05). SEM = standard error of the mean. G1) control group with basal diet, G2) NAC alone (100 mg/kg diet), G3) HSCAS alone (0.5%), G4) NAC (100 mg/kg diet) plus HSCAS (0.5%), G5) AFB1alone in the diet (1 mg/kg of feed) for 35 d, G6) AFB1 plus NAC (100 mg/kg diet), G7) AFB1 plus HSCAS (0.5%) and G8)AFB1 plus NAC (100 mg/kg diet) plus HSCAS (0.5%).

Table (4): Effect of different dietary treatments on some blood parameters

Parameters	**Treatments**								**SEM**	**Sign.**
	G1	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**		
Serum biochemical variables (6 weeks of age)										
ALT(IU/L)	67.3^{ef}	67.8^{e}	67.0^{f}	67.3^{ef}	102.1^{a}	75.6^{b}	70.5^{c}	68.8^{d}	2.337	0.0001
AST(IU/L)	198.2^{e}	198.0^{e}	198.2^{e}	198.2^{e}	301.8^{a}	225.2^{b}	216.7^{c}	205.9^{d}	6.915	0.0001
ALP(IU/L)	58.5^{d}	58.3^{d}	58.4^{d}	58.5^{d}	80.6^{a}	63.6^{b}	61.0^{c}	60.0^{c}	1.484	0.0001
Creatinine (mg/dl)	0.82^{d}	0.80^{d}	0.83^{d}	0.84c^{d}	1.23^{a}	0.91^{b}	0.89^{bc}	0.86^{bcd}	0.0276	0.0001
Uric acid, (mg/dl)	4.11^{c}	4.05^{c}	4.10^{c}	4.20^{c}	8.31^{a}	5.67^{b}	5.65^{b}	5.55^{b}	0.292	0.0001
Serum antioxidant indexes, lipid peroxidation										
MDA(nmole/l)	7.57^{bc}	6.43^{c}	6.23^{c}	5.63^{c}	14.20^{a}	9.45^{b}	9.15^{b}	8.77^{b}	0.622	0.0001
GSH(nmole/l)	281.9^{a}	283.3^{a}	280.5^{a}	285.0^{a}	160.9^{d}	250.5^{c}	260.5^{b}	265.8^{b}	9.698	0.001
Catalase (u/l)	180.8^{a}	181.5^{a}	180.9^{a}	183.5^{a}	116.9^{d}	165.8^{c}	166.3^{c}	170.2^{b}	5.050	0.0001
SOD (u/l)	7.45^{a}	7.60^{a}	7.76^{a}	8.26^{a}	4.50^{c}	6.90^{b}	6.80^{b}	7.16^{a}	0.285	0.014
serum pancreatic enzymes										
Amylase (u/l)	378.88^{e}	383.2^{e}	378.4^{e}	374.5^{c}	1065.8^{a}	658.5^{b}	533.3b^{c}	466.6^{d}	50.972	0.0001
Lipase (u/l)	34.9^{d}	34.5^{d}	35.1^{d}	34.8^{d}	150.3^{a}	50.8^{b}	54.9^{b}	47.3^{c}	7.661	0.0001
Liver parameters										
MDA(nmole/gprotein)	2.60^{c}	2.46^{d}	2.36^{d}	2.26^{c}	4.60^{a}	3.06^{b}	3.05^{b}	2.86^{c}	0.142	0.0001
SOD (u / g protein)	8.90^{b}	10.16^{a}	9.46^{ab}	11.06^{a}	4.50^{d}	7.87^{c}	7.80^{c}	8.05^{c}	0.469	0.0001
CAT (u/g protein)	243.7^{a}	245.5^{a}	243.4^{a}	244.3^{a}	185.6^{d}	232.5^{b}	230.5^{c}	235.6^{b}	4.378	0.0001
GSH (mg /g protein)	3.55^{a}	3.92^{a}	3.58^{a}	4.08^{a}	2.26^{c}	3.25^{b}	3.22^{b}	3.38^{a}	0.86	0.0001
Liver weight, %	2.67^{cde}	2.5^{e}	2.60^{de}	2.62^{de}	3.15^{a}	2.90^{b}	2.78^{c}	2.71^{cd}	0.039	0.0001
AFB1 residue in liver (µ/kg)	ND*	ND*	ND*	ND*	0.215^{a}	0.012^{b}	0.091^{c}	ND*	0.012	0.0001

$^{a, b}$ Means within the same row with different superscripts are significantly different (P≤0.05). SEM = standard error of the mean.. G1) control group with basal diet, G2) NAC alone (100 mg/kg diet), G3) HSCAS alone (0.5%), G4) NAC (100 mg/kg diet) plus HSCAS (0.5%), G5) AFB1alone in the diet (1 mg/kg of feed) for 35 d, G6) AFB1 plus NAC (100 mg/kg diet), G7) AFB1 plus HSCAS (0.5%) and G8)AFB1 plus NAC (100 mg/kg diet) plus HSCAS (0.5%).

*ND: not detected (determination limit of the analytical method: 0.01 ug/kg

Table 5: Effect of different dietary treatments on immune response to NDV

Treatments	Antibody titres ($log2^{10}$) /Age/Week					
	1st	2nd	3rd	4th	5th	6th
G1	5.00	4.20	6.80[a]	5.60[a]	6.10[a]	6.31[a]
G2	5.05	4.28	6.82[a]	5.70[a]	6.12[a]	6.35[a]
G3	5.10	4.18	6.85[a]	5.70[a]	6.00[a]	6.33[a]
G4	5.05	4.21	6.76[a]	5.63[a]	6.08[a]	6.36[a]
G5	5.00	4.00	4.61[c]	3.70[c]	3.90[b]	4.01[b]
G6	5.10	4.00	5.90[b]	4.85[b]	5.81[a]	6.00[a]
G7	5.00	4.16	6.00[b]	4.71[b]	5.73[a]	6.05[a]
G8	5.01	4.13	6.75[a]	5.10[b]	5.90[a]	6.11[a]
SEM	0.047	0.042	0.156	0.142	0.148	0.161
p-value	0.999	0.737	0.0001	0.0001	0.0001	0.0001

[a, b] Means within the same column with different superscripts are significantly different ($P \leq 0.05$). SEM = standard error of the mean. G1) control group with basal diet, G2) NAC alone (100 mg/kg diet), G3) HSCAS alone (0.5%), G4) NAC (100 mg/kg diet) plus HSCAS (0.5%), G5) AFB1alone in the diet (1 mg/kg of feed) for 35 d, G6) AFB1 plus NAC (100 mg/kg diet), G7) AFB1 plus HSCAS (0.5%) and G8)AFB1 plus NAC (100 mg/kg diet) plus HSCAS (0.5%).

3.2. Serum constituents:

Data in Table 3 demonstrate the effect of AFB1 on total protein and its fractionation at 6 weeks. The results clearly indicated that serum T. protein, albumin and globulin concentrations in AFB1-fed chickens were lower (21.92, 23.40 and 21.05 % ; $P < 0.05$) than in the control group (Table 3). The addition of NAC to AFB1-diet decreased the severity of AFB1 on serum T. protein, albumin and globulin by 69.78, 81.82 and 61.90 %, while HSCAS gave protection 66.90, 63.64 and 69.05%, furthermore, NAC plus HSCAS resulted in the greatest protection showing 79.14, 85.45 and 75.00 % respectively, compared with AFB1-diet. While serum activity of ALT AST and ALP enzymes in AFB1-fed chickens were higher (51.68, 52.27 and 37.79 %) than in the control group (Table 4). The addition of NAC, HSCAS and NAC plus HSCAS to contaminated diet with AFB1 gave protection by (76.08, 73.91 and 76.93 %), (91.00, 82.11and 88.69 %) and (95.75, 92.54 and 93.22 %) for ALT AST and ALP enzymes respectively, compared to AFB1-diet.

The kidney function data (Table 4) showed significant increase in uric acid and creatinine by AFB1 by 50.00 and 51.34 % respectively, compared to the control group. The supplementation of NAC, HSCAS and NAC plus HSCAS to contaminated diet with AFB1 gave protection by (78.05 and 73.46 %), (82.93 and 93.36 %) and (90.24 and 91.94 %) for uric acid and creatinine respectively, compared to AFB1-diet.

The present data in (Table, 3), showed that aflatoxin B1 group had a reduce in levels of serum , albumin, total protein, beta, alpha and gamma globulin together with A/G ratio. Aflatoxin B1 has been associated with other shifts in the sub-fractions of the serum protein. In aflatoxicated chicks, the significant increase in alpha-2, beta-2 and gamma-2 globulins was observed and a significant decrease in alpha-1, beta-1 and gamma-1 globulins. Some studies recorded that aflatoxin had an electrophilic reactivity at the carbonyl carbon atom and could probably adduce amines, imidazoles and sulfhydryl groups on proteins and enzymes via the Michael carbonyl condensation reaction that could cause conformational changes that interfere with their work (Lee et al., 2010 and Rawal et al., 2010). Whereas, decreased total serum protein and complement activity may cause AFB1 to induce immunotoxicity (Azzam and Gabal, 1998). The increased uric acid level and decreased albumin and total protein (TP) levels suggested protein synthesis inhibition, increased protein catabolism, and/or renal dysfunction (Jindal et al., 1994, and Mogda et al., 2014). Similarly, Yunus, et al. (2011) suggested that AFB1's immunotoxicity could be attributed to inhibiting the production of antibodies through the effects of the toxin on lymphocytes, resulting in increased serum antibodies turnover and consequently reduced half-life of the antibody. While, the alteration of globulin sub fractions alpha-2, beta-2 and gamma-2 globulins and increase in alpha-2 macroglobulin and beta-2 lipoprotein may results of inflammatory tissue (Kaneko et al., 1997).

Alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST), serum activities were recognized as important serological markers in hepatic tissue impairment and biliary system impairment (Abdel-Wahhab and Aly, 2003). The increase in serum AST and ALT activity

indicates initial hepato-cellular damage as a result of AFB1 treatments (Abdel-Wahab et al., 2006). Whereas the hepato-biliary dysfunction is associated with ALP increases. Increased uric acid and creatinine levels in the current AFB1-treated group study clearly indicated the detrimental and traumatic impact on renal tissue (Abdel-Wahhab and Aly, 2003). Such results clearly suggested that AFs had stressful effects on the hepatic and renal tissues, as stated in the aflatoxicosis literature (Sun et al., 2015).

3.3. Activities of antioxidant biomarkers:

In view of oxidative stress, the present results indicated that the activities of antioxidant biomarkers (GSH-Px, CAT and SOD) in broiler serum were significantly decreased by 42.94, 39.60 and 35.35 % and liver by 36.34, 49.44 and 23.83 % respectively, when compared to the control group , while serum and hepatic Malondialdehyde (MDA) was significantly increased by 87.58 and 76.92 % when broiler chicks fed contaminated diets compared with control group (Table, 4). Table 4 shows that serum and hepatic GSH-Px, CAT and SOD activities in treatment fed diets containing NAC and /or HSCAS were significantly increased compared with birds fed on diet contaminated with 1 ppm AFB1 ($p < 0.05$). Supplementation of NAC and /or HSCAS decreased the severity of AFB1 on GSH-Px, SOD, CAT and MAD . NAC decreased the severity of AFB1 on serum GSH-Px, SOD, CAT and MAD by 74.06, 81.36, 76.56, and 71.64 %, while HSCAS showed 82.32, 77.97 , 77.34 and 76.17 % , furthermore, NAC plus HSCAS resulted in the greatest protection showing 86.70, 90.17, 83.44 and 81.90 % respectively. While ,the addition of NAC , HSCAS and NAC plus HSCAS to contaminated diet with AFB1 gave protection by (76.74, 76.59, 80.73 and 77.0 %), (74.42,75.0, 77.28 and 77.50 %) and (86.82, 80.68, 86.07 and 87.00%) for hepatic GSH-Px, SOD, CAT and MAD respectively, compared to AFB1-diet.

Eraslan et al. (2005) examined a significant increase in the amount of malondialdehyde (MDA) shown in broilers receiving AFB1 alone. Malondialdehyde is known to be the most important indicator of membrane lipid peroxidation resulting from reactive oxygen species (ROS) interaction with cell membranes. Upon ingestion of aflatoxins, the induced rise in lipid peroxidation may be due to the fact that the initiation of lipid peroxidation results in the gradual accumulation of lipid hydroperoxides in plasma membranes, which then decomposes into MDA under pressure or toxic conditions (Kandeil and Abu El-Saad, 2005). AFs will reduce the absorption of vitamins and lower their body levels, thereby weakening the antioxidant defense mechanism (Decoudu et al., 1992). This may be one of the reasons why the frequency of MDA has increased.

Nevertheless, the decrease in superoxide dismutase (SOD) activity observed for AF administration may be correlated with the ingestion of highly active components during conversion to H_2O_2 due to the effect of AFs (Gokhan, et al; 2005 and Abdel-Wahhab, and Aly, 2003).Catalase (CAT) activity was significantly decreased in broilers received AFB1 alone compared to the other groups, this result was in agreement with many researches carried out on this subject in different animal species Verma and Nair (2001) and Rastogi et al. (2001) Who indicated that catalase in testicles and liver should be reduced by AFs. Responsibility for increased lipid peroxidation observed during aflatoxicosis could be the significant reduction in CAT and SOD activities.

CAT and SOD are the body's main antioxidant enzymes which scavenge excess free radical nacent oxygen, H_2O_2, and OH. The decreased activity of the enzyme and increased levels of MAD provided by AFB1 (Table, 4) may be due to lower tissue capacity to scavenge free radicals and prevent lipid peroxidation (Amresh et al., 2007).

Concerning the activity of pancreatic enzymes, following exposure of broiler chicks to aflatoxin B1, the current data in (Table, 4) showed a significantly increased in serum lipase and amylase activities. Similar findings to our research have been documented in their layer chickens studies (Richardson and Hamilton, 1987 and Matur et al., 2010), where AFB1 therapy increases pancreatic chymotrypsin, amylase, and lipase production. On the other hand, when broilers were exposed to AFB1, Osborne and Hamilton (1981) observed a lower pancreatic amylase, trypsin, lipase, RNase, and DNase activity. However, Osborne et al. (1982) proposed that ingestion of AFs would affect various digestive enzymatic activities causing steatorrhea, hypocarotenoidemia, and bile, pancreatic lipase, trypsin, and amylase syndrome. Other studies have shown that ROS degradation of polyunsaturated fatty acids in cell membranes has resulted in membrane destruction and MDA formation, an indication of ROS generation (Dabrowski et al., 1999). In addition to the cytoskeleton disruption in aflatoxicated animals, Jungermann et al. (1995) reported disruption of the intracellular transport of digestive enzymes. The large quantities of ROS and activated pancreatic enzymes

leaked from the capillary endothelium injury cells improved capillary permeability and edema (Dabrowski et al., 1999). Aflatoxin B1 is suspected to have induced hepatocyte injury by inducing hepatocyte apoptosis as demonstrated by Barlas et al. (2004), where ROS plays an important role in the pathogenesis of hepatic damage caused by pancreatitis.

At present, N-acetylcysteine administration has resulted in a reduction in malondialdehyde concentrations and an inhance in GSH, SOD, GPx and CAT relative to the aflatoxicated band. Similar findings have been found to suppress apoptosis caused by oxidative radical pressure, growth restriction or a number of cytotoxic drugs (Skrzydlewska and Farbiszewski, 2004). Other study suggested that N-acetylcysteine may have the potential protective agent to prevent changes in the negative oxidative parameter linked to oxidative stress in lysated RBCs caused by aflatoxin B1(Nayak and Sashidhar, 2010) and act as a reducing agent by decreasing its strength and stimulating the synthesis of significant cellular GSH and increased GSH activity (Moldeus and Cotgreave, 1994 and Tylicki et al., 2003).On the other hand, NAC could inhibit and reduced the expression of inflammatory cytokines which caused the difference of serum ALT , AST and ALP levels, that considered to be one metric to assess the severity of liver injury (Wang et al., 2015). The reduction in levels of AST, ALT and ALP could be attributed to the protective effects of N-acetylcysteinefor hepatocyte (James et al., 2003) and also significantly decreased plasma amylase and lipase levels, hence, the use of N-acetlycysteine as feed additive significantly reduced histopathological damage score in both liver and pancreas (Eşrefoğlu et al., 2006). Therefore, treatment with 100 mg of NAC can interfere with normal hepatic metabolic functions and impair the hepatotoxicity of aflatoxin B1 in the liver (Yang, et al., 2009). Regarding, the administration of HSCAS to aflatoxicated chicks, the results indicated that it had antioxidant activity and protective effect against dietary aflatoxin which come in parallel with that findings reported by(Chen et al., 2013).

3.4. Liver weight and AFB1 Residues

Table 4 indicates the relative weight of liver and concentration of AFB1 in the liver. Feeding AFB1-contaminated diet without NAC and/or HSCAS caused the relative weight of the liver to increase significantly. Increased liver weight was expected to result in impaired fat metabolism due to increased fat deposition. Fatty liver is primarily mediated by phospholipid and cholesterol inhibited synthesis, hence fat transport through the liver (Manegar et al., 2010).

In this study, separate addition of NAC (G6) and HSCAS (G7) to the AFB1 diet significantly prevented the enhance in liver weight in chicks ingested aflatoxin B1 alone ($p \leq 0.05$). In addition, after feeding on concomitant NAC and HSCAS (G8), substantial prevention or improvement of changes in the liver weight of chicks was obtained. Gowda et al (2008) showed that the introduction of HSCAS to the diet could counteract an increase in organ weights induced by aflatoxin B1 in broiler chickens. Sehu et al. (2007) also microscopically demonstrated that adding HSCAS to quail diet partly reduced the accumulation of fat caused by aflatoxin in the liver and subsequently decreased the weight of the liver.

In this study, Treatments did not detect AFB1 residues in the breast muscle (analytical procedure detection limit: 0.05 µg / kg). The residues of aflatoxin found in the livers are shown in Table 4. There were no detectable AFB1 residues in the livers of broilers fed the diets of Groups G1, G2, G3, G4 and G8. AFB1 residues was the highest in Group 5 at 0.215µ/kg. Contaminated diets supplemented with NAC (G6) and HSCAS (G7) found significant reduces in residues of AFB1 (0.012 and 0.091 µ/kg), while supplemented NAC plus HSCAS (G8) no AFB1 residues was detected in liver when compared to the contaminated Group G5 ($P < 0.05$).

Nevertheless, the production of aflatoxins in animal products is the most concern for people. In our research low levels of AFB1 (0.215 µg / kg) are held in contaminated diets fed by broiler liver. After 42 days of feeding 1000 µg / kg AFB1, Denli et al. (2009) discovered 0.166 µg / kg AFB1 in broiler liver tissue. Residues of AFB1 (0.05 and 0.13 µg / kg) were also observed for 42 days in broiler livers given the 50 and 100 µg AFB1/kg feed (Bintvihok and Kositcharoenkul, 2006).The reasons for these variations may be the differences in bird species and diet, concentrations of AFB1 and exposure period. In many countries the mean tolerance for AFB1 for human food products is 2 µg / kg. Although a small amount of aflatoxin residues in food broilers pose a very low risk to consumer safety, it is important to examine aflatoxin residues in different bird tissues in order to safeguard the quality control of poultry products for public health. This study's results on aflatoxin residues are consistent with previous reports that concentrations of AFB1 in the liver are higher than in muscle tissues (Bintvihok et al., 1998).

The integration of NAC and/or HSCAS into the diet during the time of AFB1 exposure caused to reduce in the liver residue of AFB1. This finding suggests that HSCAS ' protective effects may be due to its ability to direct AFB1 chemisorption in the gastrointestinal tract, which decreases bioabilities of AFB1. Similarly, Bintvihok and Kositcharoenkul (2006) stated that the residues of AFB1 in broiler's muscle and liver were reduced by incorporating calcium propionate in the diet that was used as a detoxifier.

3.5. Immunity

Table 5 describes the effects of dietary treatments on the development of ND antibodies in broilers from day 7 to 42. There was no difference between the experimental group antibody titer on the 7th day of the study. AFB1 feeding decreased the development of antibody against ND in broilers between the ages of 3 and 6 weeks ($P<0.05$). NAC and/or HSCAS supplementation increased the development of antibodies against ND in broilers between 3 and 6 weeks of age ($P<0.05$).

The immune system's responsiveness to mycotoxin-induced immunosuppression reflects the vulnerability of constantly proliferating and differentiating immune cells involved in immune response and controlling complex interaction between cellular and humoral immunity components (Pestka and Bondy, 1994). Immunosuppressive effects of aflatoxins are associated with protein synthesis inhibition, including specific proteins such as immunoglobulins IgG and IgA, inhibited macrophage migration, and reduced haemolytic function, lower lymphocyte counts in Fabricius bursa, and lower lymphocyte synthesis levels (Ibrahim et al., 2000). Higher titers of antibodies are due to inhibition of protein and DNA synthesis. *In vitro*, aflatoxin unevenly inhibits RNA polymerase and disturbs the synthesis of albumin, globulins and immunoglobulins (Makinia, 2014). When aflatoxins in immunocompetent organs decrease the density of lymphoid follicles, Newcastle disease antibody titers and infectious bursitis decrease (Ali, 2004).Similar results have also been shown in this experiment with ND vaccinated turkeys. Aflatoxin improves the specific activity of lysosomal enzymes in the liver and muscles, leading to increased antibody degradation (Tung et al., 1975). Mussaddeq et al. (2000) have shown that aflatoxin enhances disease resistance and interferes with immunity in livestock caused by the vaccine. Manegar et al. (2010) reported that aflatoxin induces bursal regression, and suppresses primary immune response to ND and Gumboro disease as evident from dropping ELISA titers. Improving the humoral immune response after binders such as HSCAS were added in this investigation is consistent with Ibrahim et al. (2000) who found that the introduction of sodium bentonite binder was significantly successful in reducing the negative effect of aflatoxin on the percentage and mean of phagocytosis and HI-titer in ND-vaccinated chicks. In addition, Sehu et al. (2007) tested the effect of HSCAS on the humoral immune response of quails fed to the infected aflatoxin B1 ration and observed a decrease in the ND vaccine-induced antibody titer due to aflatoxins.

HSCAS, a smectite class phyllosilicate clay, is shown to bind tightly aflatoxins in animals and to avoid aflatoxicosis (Abbès et al., 2006). In the current study, the introduction of 0.5% HSCAS to the AFB1-contaminated diet partially improved biochemical parameters and hepatic antioxidant status with the exception of HSCAS partially counteracts AFB1 which may be due to the high adsorptive properties of HSCAS. Similarly, Gowda et al. (2008) suggested that HSCAS enhanced the adverse effects of AFB1 on some parameters of serum chemistry, increased antioxidant potential in the liver, and decreased the extent of AFB1-related hepatic microscopic lesions. Abbès et al. (2006) stated that the addition of HSCAS contributed to the re-establishment of haematological parameters, serum biochemical enzyme activity, and hepatic and kidney histology. Nevertheless, Watts et al. (2003) confirmed that applying HSCAS to diets containing multiple mycotoxins did not prevent the chicks and poultry from having negative effects. Huwig et al. (2001) confirmed that HSCAS was active in the prevention of aflatoxicosis, but limited its efficacy against zearalenone, ochratoxin, and trichothecenes.

N-acetylcysteine may be useful to boost the effects of aflatoxicosis in broilers alone or in complementary form with other methods. Previous studies have shown that aluminosilicate use in broiler chicks can be effective in reducing the deleterious effects of aflatoxins; the protective effect of these adsorbents was attributed to the gastrointestinal tract's sequestration of aflatoxins, which reduces their bioavailability (Kubena et al., 1993; Bailey et al., 1988; Ledoux et al., 1999).Whereas NAC appears to be an agent able to protect the liver and kidney from damage and to bind this compound or its intracellular tiol derivatives to AFB1 metabolites (De Flora et al., 1985; Lancet, 1991; Kelly, 1998).

CONCLUSION

There appears to be a direct association between dietary intake of aflatoxin and the incidence of liver cancer in developing countries. It also resulted in significant animal health casualties. Today's results indicate that N-acetylcysteine and/or HSCAS could be a possible protective agent to avoid changes in the negative oxidative parameter caused by aflatoxin B1. Hence, regular animal feed testing program and other environmental factors for the contamination of fungi and mycotoxins and the use of feed additives such as HSCAS and N- acetylcystein are a vital demand today for healthy animal and human health.

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