USING DIETARY GLUTATHIONE, ALUMINOSILICATE AND TAFLA AS AN ATTEMPT TO PREVENT AFLATOXICOSIS IN LAYING HENS

Qota, E.M.A.; E.H. El-Ganzory and R.A. Hassan
Poult. Nutrition Dept., Anim. Prod. Res. Institute, ARC, Giza , Egypt.

ABSTRACT

An experiment was conducted to evaluate effect of dietary tripeptide glutamate (reduced glutathione (GSH)) as antioxidant, tafla (TF), and hydrated sodium calcium aluminosilicate (HSCAS) as sorbent materials to prevent aflatoxicosis in laying hens. A total number of 371 (350 laying hens+21 cocks) thirty-wk's old El-Salam chickens were randomly divided into 7 groups; each group included 5 replicates of 10 hens each and reared in metallic batteries. The remaining 21 cocks were also divided into 7 groups of 3 cocks each and housed separately for semen collection. Birds were fed practical corn-soybean meal basal diet with or without 1 ppm aflatoxin B_1 (AFB$_1$) alone or plus either 5ppm GSH, 0.6% TF, 0.5% HSCAS, 0.6% TF+5ppm GSH or 0.5% HSCAS+ 5ppm GSH to form 7 diets fed from 30 to 38 wks old. Results show that contamination of basal diet with 1 ppm AFB$_1$ for 8 wks decreased (P<.01) feed intake (25.1%), egg production (42.8%), egg weight (22.3%), shell thickness (32.6%), fertility (21.9%), hatchability of fertile eggs (20%), economic efficiency (EE,38.5%), liver vitamin A (29.1%), blood hemoglobin (35.6%), serum albumin (68%) and total lipids (51%), increased relative liver weight (138.8%), liver lipids (141.9%), blood total leucocytes (WBC's) (28%) and lymphocytes (27.2%) counts, serum enzymatic activities of AST (64%) and ALT (69%), and deposited AFB$_1$ residues in livers (68 ng/g), egg yolk (52 ng/g) and muscles (36 ng/g) compared to the controls. Adding TF or HSCAS separately into AF diet recorded similar protection effects averaged 45-56% against aflatoxicosis for the studied traits. Including GSH alone into the AF diet resulted in a little protective effects against AF diet for the all studied traits, except AST and ALT activities that showed a significant protective effect (20-28%). However, GSH together with sorbent materials significantly negated the adverse effects of AF diet for all studied traits and improved EE by 81% for TF+GSH and 75% for HSCAS+GSH compared to AF-diet alone. There was mortality only in the both two groups fed basal diet with AFB$_1$ alone (10%) and AFB$_1$+GSH (6%). The present study revealed that TF (available product) presented similar safety protective effect for studied traits and EE as HSCAS (import product). Adding GSH as antioxidant together with TF or HSCAS, to AFB$_1$ contaminated diet significantly negated aflatoxicosis in the laying hens.
Keyword: Glutathione, aluminosilicate aflatoxin B_1, laying chickens, tafla, residues.

INTRODUCTION

Aflatoxin has elicited greatest public health concern of all mycotoxins because of its widespread occurrence in several grains as corn which comprises 50-60% of poultry diets (Phillips *et al.*,1988), in addition to the role of aflatoxins in the etiology of hepatocellular carcinoma that has been proved (Wild *et al.*,1990). The LD$_{50}$ values for AF (mg/kg body weight) were 6.5-16.5 in several chicken strains (Smith and Hamilton, 1970 and El-Samra, 1991). Depression by about 6-30% of chick growth (Edrington *et al.*,1997; Genedy *et*

al.,1999 and Qota, 2003), impairment of feed efficiency (Kubena et al., 1995; Qota, 1999 and Qota et al., 2005) and higher mortality rate (Abdelhamid et al.,1995[a]; Qota, 2003 and Ali et al., 2006) by 0.5-4ppm AF contaminated diet caused very high economic loses. Inhibition of metabolism and immunity system by 0.75-2ppm AF contaminated diet caused increasing liver fat (60% of dry weight) and liver size (2-3 times) and liver damage (Smith et al., 1993 and Abd El-Hamid et al., 1992). The same authors reported also that AFB_1 inhibits DNA synthesis in the liver and possibly prevents proteins synthesis. The HSCAS at 0.5% in the diets has been shown to reduce aflatoxicosis in chicken (Scheideler, 1993; Qota et al., 2005 and Ali et al., 2006). The HSCAS binds AFB_1 in vitro (Phillips et al., 1988 and Scheideler, 1993). Thus, the efficacy of sorbent materials as HSCAS or Tafla probably lies in their ability to bind AF in the intestine, rendering the toxin unavailable for absorption (Southern et al., 1994). Ingestion of HSCAS to broilers does not improve skin pigmentation (Brake, 1987). The sorbent additives have raised questions about their effects on minerals and vitamins status, although Chung and Baker (1990) with P, Chung et al. (1990) with riboflavin and Southern et al. (1994) and Qota (2003) with Ca and P, have reported that HSCAS does not impair the nutrient utilization. Glutathione (GSH) is a tripeptide which found almost in its reduced form. It contains an unusual peptide linkage between the amino group of cystine and the carboxyl group of the glutamate. It is an antioxidant, protects cells from toxins such as free radicals during the tissue-damaging peroxidation process and increases enzymatic detoxification in the liver (Wattenberg, 1976). Epoxidation of the 2,3-double bond has been emphasized as a metabolic activation step and recent results indicate that the 2,3-epoxide is a reactive metabolite responsible for reaction with cellular macromolecules, as nucleic acids, and thus may well be the ultimate carcinogen (Swenson et al.,1977). Most damaging epoxidation form is AFB_1 epoxide. The present study was designed to evaluate the effect of TF, HSCAS and/or GSH to prevent aflatoxicosis in laying hens.

MATERIALS AND METHODS

The present study was conducted at Sakha Animal Production Research Station and Laboratories, APRI, ARC, Egypt during Feb.-May 2007 to study the effect of sorbent materials as TF or HSCAS and antioxidants as GSH on laying performance during aflatoxicosis. A total number of 371 (350 hens+21 cocks) thirty-wk's old El-Salam (Nicolas×Mamourah) chickens were divided into 7 similar (BW=1450±23 g) experimental groups (5 replicates of 10 hens each) and reared in metallic batteries. The remaining 25 cocks were also divided into 7 groups of 3 cocks each and housed separately for semen collection. Basal diet was formulated to cover nutrient requirements (Table 1) according to Egyptian Feed Composition Tables (2001). Birds were fed basal diet (control) without or with 1ppm AFB_1 alone (AF-diet) or plus either 5ppm GSH (AF+GSH), 0.6% Tafla (AF+TF), 0.5% HSCAS (AF+AS), 0.6% TF+ 5ppm GSH (AF+TF+GSH) or 0.5% HSCAS+ 5ppm GSH (AF+AS+ GSH) to form 7 experimental diets fed from 30 to 38 wk's old. The AF was produced

via fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as described by Shotwell *et al.* (1966) and modified by West *et al.* (1973). Fermented rice was autoclaved, dried, ground and analyzed for its AFB_1 content (Nabney and Nesbitt, 1965) as modified by Wiseman *et al.* (1967). The AFB_1 in the rice powder was extracted by chloroform then incorporated into basal diet and confirmed by HPLC to provide the desired level of 1 ppm. The GSH was provided from Sigma-Aldrich Quimica S.A. Madrid 28100, Spain. The HSCAS was purchased from Integrated World Enterprises Co.. Tafla (available natural substance) was washed, grounded to a fine powder. Chemical analysis for HSCAS and tafla (Table 1) were done (AOAC, 1990). Feed intake, egg number and egg weight were recorded weekly.

Table 1.Composition of the laying hen basal diet, HSCAS and Tafla.

Ingredient	%	Composition[3]	HSCAS (%)	Tafla (%)
Yellow corn	64.84			
Soybean meal, 44%	24.60	Silica	64.70	59.80
Dicalcium phosphate	1.70	Aluminum	15.50	17.20
Limestone	7.60	Iron	1.75	2.30
NaCl	0.30	Calcium	1.26	1.90
Vit. + Min. Mix.[1]	0.30	Potassium	1.80	2.30
DI-Methionine	0.06	Sodium	2.55	2.90
Clean sand	0.60	Magnesium	1.54	1.90
Calculated values[2]:		Moisture	10.56	10.32
ME, Kcal/Kg	2723	Price, LE/kg	15.0	0.50
Lysine, %	0.88			
Meth. + Cys., %	0.62			
Av. Phosphorus, %	0.46			
Calcium, %	3.30			
Determined analyses[3]:				
Dry matter, %	89.51			
Crude protein, %	16.55			
Ether extract, %	2.66			
Aflatoxin B_1, ppb	6.0			
Price, LE/Kg	1.55			

[1]Vitamins +Minerals Mixture provided /kg of diet: 6000 IU vit A. 2000 ICU cholecalciferol, 10 IU vit E, 2.5 mg vit K_3, 2.5 mg riboflavin, 12 mg nicotinic acid, 10 mg Ca pantothenate, 300 mg choline chloride, 4 μg cyanocobalamin, 5 mg pyridoxine, 3 mg thiamine, 0.5 mg folic acid, 0.2 mg biotin, Trace mineral (mg/kg of diet): 40 Mn, 40 Zn, 40 Fe, 4 Cu, 0.2 Se, 0.5 I.
[2]According to Egyptian Feed Composition Tables (2001).
[3]According to AOAC (1990).

Shell thickness using Micrometer were estimated 3 times (18-days intervals) using 50 eggs/group/time and the yolk was separated for analysis. Hens were artificially inseminated once a week. Eggs laid were collected daily, stored for 7days at 15°C and 70% RH then incubated to estimate fertility and hatchability of fertile eggs. At the end of the experiment, 5 hens /group were slaughtered for tissues analyses. Liver lipids were extracted (Folch *et al.*, 1957). Liver vit. A content was estimated (Thompson *et al.*, 1971). Also, AFB_1 residues in fresh meat (breast:thigh, 1:1), liver and egg yolk were measured (Stubblefield *et al.*, 1982). Economic efficiency was calculated based on feed cost. Blood hemoglobin (Kampen and Zijlestra, 1961), leucocytes (WBC's) and lymphocyte counts (Wintrobe, 1969), serum albumin (Doumas *et al.*, 1977), total lipids (Chabrlol and Charonnat, 1973) and alanine aminotransferase (ALT) and aspertate aminotransrerase (AST) enzymatic activities (Reitman and Frankel,1957) were measured using commercial kits. Data were statistically analyzed using one–way ANOVA of GLM procedure of

Statistical Analysis Software (SAS, 1994). Before analysis, all percentages were subjected to logarithmic transformation (log10x+1) to approximate normal distribution. Significant differences among treatment means were (p≤0.05) separated by Duncan's new multiple range test (Duncán, 1955).

RESULTS AND DISCUSSIONS

Productive and reproductive performance and economic efficiency:

Data presented in Table (2) showed that there were similar trends for treatments effect on the studied traits. It is clear that AF-diet P≤0.05) impaired feed intake (25.1%), egg production (42.8%), egg weight (22.3%), shell thickness (32.6%) eggs fertility (21.9%), hatchability of fertile eggs (20%) and economic efficiency (EE) (38.5%) compared to the controls. Inclusion of 0.6% TF or 0.5% HSCAS separately with AF-diet recorded similar protection effects (P≤0.05) averaged 47-56% for productive and reproductive traits against aflatoxicosis. Adding 5 ppm GSH alone with AF-diet had little protective effects on laying performance traits studied. While GSH with TF or with HSCAS supplemented to AF-diet significantly prevented aflatoxicosis as assessed by performance traits (Table 2). Supplementing sorbent materials plus GSH with AF-diet improved EE by 81% for TF+GSH and 74.9% for HSCAS+GSH compared to AF-diet alone. There was mortality only in the both two groups fed basal diet with AFB_1 alone (10%) and AFB_1+GSH (6%). Many authors used 1-3 ppm AF (Edrington *et al.*, 1997; Genedy *et al.*, 1999 and Ali *et al.*, 2006) with different chicken strains and showed similar deterioration in laying performance traits by AF-diet.

Table 2. Productive and reproductive performance of El-salam laying hens fed dietary treatments from 30 to 38 wk's old.

Dietary treatment[1]	Feed intake (g/b/d)	Egg prod. (%)	Egg wt. (g)	Shell thick. (mm)	Fert- ility (%)	Hatchabi lity (%)	EE* (%)
Control	112.2[a]	66.8[a]	53.0[a]	0.371[a]	89.6[a]	84.0[a]	64.33[a]
AF-diet	84.1[c]	38.2[c]	41.2[c]	0.250[c]	70.0[c]	67.2[c]	39.58e
AF+GSH	92.0[bc]	45.4[bc]	44.1[bc]	0.301[bc]	74.7[bc]	71.4[bc]	45.36d
AF+AS	99.7[b]	52.3[b]	47.0[b]	0.324[b]	79.9[b]	75.4[b]	52.73c
AF+TF	100.1[b]	52.6[b]	46.9[b]	0.310[b]	80.3[b]	74.6[b]	53.61c
AF+AS+GSH	106.2[ab]	59.8[ab]	50.1[ab]	0.352[ab]	84.6[ab]	79.8[ab]	58.11[b]
AF+TF+GSH	106.1[ab]	60.1[ab]	49.9[ab]	0.343[ab]	85.0[ab]	79.6[ab]	59.62[b]
SEM	1.97	0.842	1.08	0.005	1.14	2.14	0.471
P value	0.007	0.002	0.004	0.003	0.006	0.005	0.014

[a-d] Values followed by different letters within columns are significantly different (P≤0.05).
[1]AF=1ppm Aflatoxin B1, TF=0.6% Tafla, AS=0.5% Hydrated sodium calcium aluminosilicate (HSCAS), GSH=5ppm Tripeptide glutamate (Glutathione). Values are means of 5 determinations.
*EE (Economic efficiency)= [Total revenue (number of newly healthy hatched chicks × its price (1.15 LE) + (useless eggs for incubation × its price (0.30 LE)) per hen − Total feed cost (Total feed intake ×its price, LE/hen) ÷ Total feed cost] ×100.

Lack of essential nutrients such as minerals and vitamins, as a result of feed intake decrease, and inhibition of metabolism and immunity system by aflatoxicosis may explain the present impairments of egg production, shell thickness and reproductive traits as those showed by Smith and Hamilton (1970). Regarding sorbent materials protection, the present results confirmed

those of Genedy *et al.* (1999) and Ali *et al.* (2006). They showed, by working on different chicken strains, that adding 0.5% HSCAS to basal diet contaminated with AF did diminished aflatoxicosis impact on productive and reproductive traits by about 50-60%. Tafla and HSCAS had similar (p≤0.05) protective effect against aflatoxicosis as they contain similar composition (Table1).They sorbed AFB_1 selectively during the digestive process, which rendered most of the AF unavailable for absorption from the gastrointestinal tract as those reported by Huff *et al.* (1992), Kubena *et al.* (1993) and Qota (2003).

Liver status and hematological traits:

Contaminating the basal diet with 1ppm AFB_1 significantly (P≤0.05) increased relative liver weight (138.8%), liver lipids (141.9%), blood total leucocytes (WBC's) (28%) and lymphocytes (27.2%), decreased liver vitamin A (29.1%), and hemoglobin (Hb) contents (35.1%) compared to the controls (Table 3). Supplementing 0.6% TF or 0.5% HSCAS separately to the AF diet resulted in a significant protection against aflatoxicosis by about 51.0, 50.7, 50.1, 53.3, 48.8 and 51.8% for liver weight, liver lipids, liver vitamin A, WBC's, lymphocytes and Hb, respectively, compared to the controls. Insignificant protective effects were recorded for liver status and hematological traits by adding GSH alone to the AF diet. Supplementing GSH with TF or HSCAS into the AF diet significantly negated AF effects on the liver and hematological traits (Table 3). The present results confirmed those of Abdelhamid *et al.* (1995[a]) with chickens and Qota (2003) with turkey who reported similar alterations in liver status by 0.5-2.5 ppm AF diets. Increasing liver weight in the present study may be due to increase the accumulation of fat as a result of interference of AF with lipid metabolism as reported by Smith and Hamilton (1970). In the same manner, Abd El-Hamid *et al.* (1992) reported that aflatoxicosis impaired fat transport which could attribute to inhibited RNA synthesis that caused a marked increase in liver fat content. Decrease liver vitamin A content caused by the AF diet may be due to maldigestion and malabsorbtion.

Table 3. Liver status and hematological traits of El-salam laying hens fed dietary treatments from 30 to 38 wk's old.

Dietary treatment[1]	Liver wt. (%)	Liver lipids (%)	Liver vit. A (µg/g)	Leuco-cytes $10^3/mm^3$	Lymph-ocytes $10^3/mm^3$	Hemo-globin mg/100ml
Control	3.12[c]	5.35[c]	21.47[a]	21.4[c]	14.83[c]	12.61[a]
AF-diet	7.45[a]	12.94[a]	15.22[c]	27.4[a]	18.87[a]	8.19[c]
AF+GSH	6.33[ab]	11.02[ab]	16.79[bc]	25.9[ab]	17.80[ab]	9.21[bc]
AF+AS	5.31[b]	9.18[b]	18.36[b]	24.5[b]	16.78[b]	10.31[b]
AF+TF	5.34[b]	9.22[b]	18.34[b]	24.6[b]	16.82[b]	10.33[b]
AF+AS+GSH	4.22[bc]	7.33[bc]	19.90[bc]	23.0[bc]	15.77[bc]	11.51[ab]
AF+TF+GSH	4.21[bc]	7.36[bc]	19.94[bc]	22.9[bc]	15.81[bc]	11.53[ab]
SEM	0.194	0.251	0.873	3.214	2.14	0.308
P value	0.001	0.003	0.006	0.006	0.005	0.011

[a-d] Values followed by different letters within columns are significantly different (P≤0.05).
[1]AF=1ppm Aflatoxin B1, TF=0.6% Tafla, AS=0.5% Hydrated sodium calcium aluminosilicate (HSCAS), GSH=5ppm Glutathione (Tripeptide glutamate). Values are means of 5 determinations.

Zilva and Pannall (1983) and Abdelhamid *et al.* (1995[b]) referred to presence of diseases or functional disorders in some organs, by aflatoxicosis, such as hepatitis, pancreatitis, nephrotic syndrome, anaemia, and carcinoma. The protection of sorbent materials against AF effects on liver and hematological traits was also observed by Kubena *et al.* (1993), Qota (2003) and Hassan (2006).

Aflatoxin B₁ residues in fresh tissues and serum constituents:

Results in Table (4) showed that birds fed basal diet contaminated with 1ppm AFB_1 for 8 wk's deposited AFB_1 residues by highest value in their fresh livers (68 ng/g) followed by egg yolk (52 ng/g) then muscles (36 ng/g), decreased serum albumin (68%) and total lipids (51%) and increased enzymatic activities of AST (64%) and ALT (69%). Adding TF or HSCAS to AF diet, similarly, reduced AFB_1 residues by 51, 48, and 50 % in the yolk, liver and meat; respectively, and diminished AF effects on albumin, lipids, AST and ALT by 56, 46, 49 and 51%; respectively. A significant alleviating effect for adding GSH separately to the AF diet was observed with AST and ALT. Moreover, adding GSH with TF or HSCAS to the AF diet significantly negated the adverse effect of the AF diet on serum constituents, and reduced the AFB_1 residues by 75, 74 and 75% in yolk, liver and meat; respectively. The present results confirmed those of Qota (2003), Ali *et al.* (2006) and Hassan (2006) who detected AFB_1 residues in tissues of birds fed contaminated diets. Increasing accumulation AFB_1 in the liver than other tissues, in the present study, was observed as showed by Rizk *et al.* (1993), Abdelhamid *et al.* (1995[b]) and Qota *et al.* (2005). Decreasing serum albumin and lipids, and increasing ALT and AST activities by aflatoxicosis were reported in many studies (Genedy *et al.*, 1999; Hassan, 2000 and Qota *et al.*, 2005). The protective effect of sorbent materials against AF diet for AFB_1 residues and serum constituents, in the present study, was observed also by Kubena *et al.* (1993), Genedy *et al.* (1999) and Qota *et al.* (2005).

Table 4. Aflatoxin B₁ residues in fresh tissues and serum constituents of El-salam laying hens fed dietary treatments during 30-38 wk's old.

Dietary treatment[1]	AFB₁ residues			Serum constituents			
	Yolk (ng/g)	Liver (ng/g)	Meat (ng/g)	Albumin g/100m	Lipid (g/l)	AST (IU/L)	ALT (IU/L)
Control	***	***	***	2.5[a]	6.7[a]	12.1[a]	7.52[e]
AF-diet	51.6[a]	67.8[a]	36.4[a]	0.8[c]	3.3[c]	19.8[a]	12.7[a]
AF+GSH	37.2[ab]	51.5[ab]	27.3[ab]	1.2[bc]	4.2[bc]	17.6[b]	11.4[b]
AF+AS	25.5[b]	34.3[b]	18.2[b]	1.6[b]	5.1[b]	15.8[c]	10.1[c]
AF+TF	24.9[b]	33.9[b]	18.4[b]	1.5[b]	5.2[b]	15.9[c]	10.2[c]
AF+AS+GSH	13.4[bc]	17.6[bc]	9.21[bc]	2.2[ab]	6.2[ab]	14.0[d]	8.79[d]
AF+TF+GSH	12.8[bc]	18.2[bc]	9.09[bc]	2.1[ab]	6.0[ab]	13.9[d]	8.81[d]
SEM	1.98	2.17	1.88	0.31	0.51	1.01	0.71
P value	0.003	0.001	0.004	0.002	0.01	0.01	0.01

[a-d] Values followed by different letters within columns are significantly different (P≤0.05).
[1]AF=1ppm Aflatoxin B1, TF=0.6% Tafla, AS=0.5% Hydrated sodium calcium aluminosilicate (HSCAS), GSH=5 ppm Glutathione (Tripeptide glutamate).
Values are means of 5 determinations. ***=No detection of AFB₁

Few studies have been carried on glutathione as detoxification of AF. Role of GSH comes after the absorption of AF and during its metabolism process in the liver. It, as an antioxidant, protects cells from toxins such as free radicals during the tissue-damaging peroxidation process and increases enzymatic detoxification in the liver (Wattenberg, 1976). Ehrich *et al.* (1984) and Ehrich and Larsen (1983) proved that detoxification enzyme systems in chickens could be increased by the administration of the antioxidants. Hsieh (1982) found that primary hepatic metabolites of AFB_1 may subjected to cytoplasmic reductase system producing aflatoxicol or to liver microsomal oxidase system producing AF: Q_1, M_1 and B_1-epoxide. Except for AFB_1-poxide, all metabolites contain hydroxyl groups are transformed into a water-soluble conjugate and to facilitate excretion. The transient B_1-epoxide can be conjugated by GSH to form another type of conjugate. A prospective action may be afforded by reaction of AFB_1 metabolite with GSH (Lotikar *et al.*, 1980). Presence of AFB_1-GSH conjugate in the bile of AF-treated rats, and its formation *in vitro* in liver-derived subcellular fractions, has been reported (Dengen and Neumann, 1978; Moss *et al.*, 1983). Some nutrients increased the activity of GSH for detoxification of AF in birds tissue such as Se is used as a cofactor for Se dependent GSH peroxidase (SeGSHpx) which is important in detoxification of hydrogen-px and lipid hydro-px, and increase GSH-px activity (Combs, 1981). Only Se increased the activity of Se GSH-Px in all tissues (Combs, 1981; Nahm, 1995). Also, Se enhanced the formation of water-soluble conjugated forms of AF which promotes the clearance of the toxin (Gregory and Edds, 1984). In the same manner methionine is a more distal precursor of GSH (Veltmann *et al.*, 1983). Vitamin C affected GSH metabolism at low level (Kim and Combs, 1992). From the present study, it may be concluded that TF (available product) had a similar safety protective effect for studied traits and EE as HSCAS (import product). Using GSH as antioxidant with TF or HSCAS, as sorbent materials into AF diet significantly negated aflatoxicosis in the laying hens.

REFERENCES

Abdelhamid, A.M.; T.M. Dorra and H.S.M. Arief (1995[a]). Effect of some dietary supplements to aflatoxic diets of chickens. 1. On the performance. J. Agric. Sci. Mansoura Univ., 20: 3208.

Abdelhamid, A.M.; H.S. Arief; F. El-Keraby and T.M. Dorra (1995[b]). Effect of some dietary supplements to aflatoxic diets of chickens. 2. On the tissue analysis. J. Agric. Sci. Mansoura Univ., 20: 3227.

Abd El-Hamid, H.S.; A.G.R. Shakshouk; M. Korshom; E.M. El-Manakhly and A.B.A. Bekhiet (1992). Effect of aflatoxin on broiler chickens. Egypt. Poult. Sci., 12: 443.

Ali, M.N.; E.M.A. Qota; R.A. Hassan and Abou-El maged (2006). Novel methods of detoxification of aflatoxin B_1 in contaminated local laying hen diets. Egypt. Poult. Sci., 26: 911-940.

Association of Official Analytical Chemists (1990). Official methods of analysis 15th ed. Association of Official Analytical Chemists, Washington, DC.

Brake, J. (1987). Field results on broiler chickens with a selected aluminosilicate. procedure symptom: On the Recent Developments in the Study of Mycotoxins, December 17, 1987, Rosemont, pp:F_1-F_{11}.

Chabrol, E. and R. Charonnat (1973). Method for determination of serum total lipids. Press. Medical, 45:1713-1716.

Chung, T.K. and D.H. Baker (1990). Phosphorus utilization in chicks fed hydrated sodium calcium aluminosilicate. Anim. Sci., 68:1992-1998.

Chung, T.K.; J.W. Erdman and D.H. Baker (1990). Hydrated sodium calcium aluminosilicate: Effects on zinc, manganese, vitamin A and riboflavin utilization. Poult. Sci., 69: 1364-1370.

Combs, G.E. (1981) Influence of dietary vitamin E and selenium on the oxidant defense system of the chicks. Poult. Sci., 60: 2098-2105.

Dengen, G.H. and H.G. Neumann (1978). The major metabolite of aflatoxin B_1 in the rat is a glutathione conjugate. Chemicobiological Interactions, 22:239-243.

Doumas, B.T.; W.A. Watson and H.G. Biggs (1977). Albumin standards and the measurements of serum albumin with bromocrisol green. Clin. Chem. Acta., 31: 87.

Duncan, D.B (1955). Multiple range and multiple F-test. Biomet., 11:1-42.

Edrington, T.S.; L.F. Kubena; R.B. Harvey and G.E. Rotinghaus (1997) Influence of a superactivated charcoal on the toxic effects of aflatoxin or T-2 in growing broilers. Poult. Sci., 76: 1205.

Egyptian Feed Composition Tables for Animal and Poultry Feedstuffs (2001). Technical bulletin No.1, central lab. for feed and food, Ministry of Agric., Egypt.

Ehrich, M. and C. Larsen (1983). Drug metabolism in adult White Leghorn hens-response to enzyme inducers. Comprehensive Biochemistry and Physiology, 74c: 383-386.

Ehrich, M.; W.R. Huckle and C. Larsen (1984). Increase in glucuronide conjugation of aflatoxin P1: after pretreatment with microsomal enzyme inducers. Toxicology, 32: 145-152.

El-Samra, S.H. (1991). Aflatoxin and poultry nutrition. Proc. 3rd Sci. Symp. For Anim., Poult. and Fish Nutr., Sakha, Egypt, 26-28 November, pp:30.

Folch, J.; Lees, M.E. and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol.Chem., 226:407–409.

Genedy, S.G.K.; N.M. El-Naggar; N.S. Isshak and E.M.A. Qota (1999). Effect of aflatoxins contaminating agents on performance, blood constituents and some tissues of local poultry strains. Egypt. Poult. Sci., 19; 351-377.

Gregory, J.F. and G.T. Edds (1984). Effects of dietary selenium on the metabolism of aflatoxin of young turkeys and broiler chicken. Poult. Sci., 64: 1678-1684.

Hassan, R.A. (2000). Studies on the effect of certain feed-additives on the performance of broilers and layers fed aflatoxicated feed. Ph.D Thesis Fac. of Agric. Tanta Univ.

Hassan, R.A. (2006). Capability of mannan-oligosaccharide (Bio-mos®), organic selenium and hydrated sodium calcium aluminosilicate to detoxify aflatoxicosis for growing local chickens. 2. Lymphoid organs, immune response and residues in tissues. Egypt. Poult. Sci., 26:495.

Hsieh, P.H. Dennis (1982). Metablism and transmission of mycotoxins. Proc. Int. Sym. Mycotoxins, P:151.

Huff, W.E.; T.F. Kubena; R.B. Harvey and T.D. Phillips (1992). Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A. Poult. Sci., 71: 64.

Kampen, E.J. and W.G. Zijlestra (1961). Standarization of Hemoglobino-metry. II-The hemoglobin cyanidemethod. Clin. Chem. Acta. 61:538.

Kim, Y.S. and G.E. Combs (1992). Effects of selenium and vitamin E and C on glutathione and glutathione S-transferase in the chicks. Cornell Nutrition Conference, PP: 37-42.

Kubena, T.F.; R.B. Harvey, T.D. Phillips and B.A. Clement (1993). Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poult. Sci., 72: 651.

Kubena, T.F.; W.E. Huff; R.B. Harvey; A.G. Yersin; M.H. Elissable; D.A. Witzel; L.E. Giroir; T.D. Phillips and H.D. Peterson (1995). Effect of a Hydrated sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. Poult. Sci., 70: 1823.

Lotiker, P.D.; S.M. Setta; P.A. lyons and E.C. Jhee (1980). Inhibition of microsome-mediated binding of aflatoxin B1 to DNA by glutathione S-transferase. Cancer letters, 9:143-149.

Moss, E.J.; D.J. Judah; M. Przybylski and G.E. Neal (1983). Some mass-spectral and n.m.r. analytical studies of a glutathione conjugate of aflatoxin B1. Bioch. J., 210: 227-234.

Nabney, J. and B.F. Nesbitt (1965). A spectrophotometric method of determining the aflatoxin. Anaslyst, 90: 155-160.

Nahm, K.H. (1995) Prevention of aflatoxicosis by addition of antioxidants and hydrated sodium calcium aluminosilicate to the diet of young chicks. Japanese Poult. Sci., 32: 117-127.

Phillips, T.D.; L.F. Kubena; R.B. Varvey; D.R. Taylar and N.D. Heidelbaugh (1988). Hydrated sodium calcium aluminosilicate a high affinity sorbent for aflatoxin. Poult. Sci., 67: 243-247.

Reitman, S. and S. Frankel (1957). Method for determination of amino transferase enzymatic activities. Amer. J. Clin. Path., 28: 56.

Rizk, R.E.; N.A. El-Sayed; G.A. Abd-Allah and S.A. El-Deeb (1993) The residue of low dietary aflatoxin B_1 and its effect on productivity and reproductivity of local chicken strains. Egypt. Poult. Sci., 13: 301.

Qota, E.M.A. (1999). Effectiveness of some feed additives for detoxification of mycotoxin contaminated local chicken diets. Ph.D. Thesis Fac. of Agric. Tanta Univ.

Qota, E.M.A. (2003). Hydrated sodium calcium aluminosilicate effects on ome mineral and vitamin status during aflatoxicosis in growing turkey. J. Agric. Sci. Mansoura Univ., 28(3): 1729-1743.

Qota, E.M.A.; M.A. Ali; R.A. Hassan and M.K. Abou-El maged (2005). Detoxification of aflatoxin contaminated local chicken diets using Aluminosilicate, sodium sulphate and peroxidase enzyme. 3rd International poultry conference 4-7 Apr. 2005 Hurgada, Egypt.

SAS Institute (1994). SAS/STAT user's guide:statistics, version 6 Edition. SAS Institute Inc. Cary., Nc, USA.

Scheideler, S.E. (1993). Effect of various types of Aluminosilicate and aflatoxin B1on aflatoxin toxicity, chicken performance and mineral status. Poult. Sci.,72: 282.

Shotwell, O.L.; C.W. Hesseltine; R.D. Stubbefield and W.G. Sorenson (1966). Production of aflatoxin on rice. Appl. Microbiol., 14: 425.

Smith, J.W. and P.B. Hamilton (1970). Aflatoxicosis in broiler chicken. Poult. Sci., 49: 207.

Smith, M.O.; D.S. Sachan and Y.S. Cha (1993). Effect of L-carnitine on aflatoxin toxicity in broilers. Poult. Sci. Abst., (136): 129.

Southern, L.L.; T.L. Ward; T.D. Bidner and L.G. Hebert (1994). Effect of sodium bentonite or hydrated sodium calcium aluminosilicate on growth performance and tibia mineral concentration in broiler chicks fed nutrient-deficient diets. Poult. Sci., 73: 848-854.

Stubblefield, R.D.; W.F. Kwolek and L. Stoloff (1982). Determination and thin layer chromatographic confirmation of identify of aflatoxin B1 and M1 in artificially contaminated beef livers. Collaborative Study. J. Assoc. of Anal. Chem., 65 (6): 1435.

Swenson, D.H.; J.K. Lin; E.C. Miller; J.A. Miller (1977). Aflatoxin B_1-2,3-oxide as a probable intermediate in the covalent bending of aflatoxin B_1 and B_2 to rat liver DNA and ribosomal RNA in *vivo*. Cancer Res., 37:172.

Thompson, J.N.; P.A. Erdody; R. Brien and T.K. Mussay (1971). Flurometric determination of vit. A in human blood and liver. Biochem. Med.,5:67.

Veltmann, J.R.; R.D. Wyatt; M.N. Voight and Z. shamsuddin (1983). Influnce of dietary sulfur amino acid levels on performance, free amino acids and biochemical parameters in plasma and hepatic glutathione of broiler chicks fed aflatoxin. Poult. Sci., 62: 1518-1519.

Wattenberg, L. (1976). Inhibition of chemical carcinogenesis by antioxidants and some additional compounds. In: Fundamentals in Cancer Prevention, Univ. of Tokyo Press, Tokyo, Japan: 153-166.

West, S.; R.D. Wyatt and P.B. Hamilton (1973). Improved yield of aflatoxin by incremental increases in temperature. Appl. Microbiol. 25: 1018.

Wild, C.P.; Y.Z. Jiang; G. Sabbioni; B. Chapot and R. Montesano (1990). Evaluation of methods for quantitation of aflatoxin albumin adducts and their application to human exposure assessment. Cancer. Res., 50: 245.

Wintrobe, M.M.(1969). Clinical Haematol., 6th Ed., Henery Kimpton Lond.

Wiseman, H.G.; W.C. Jacobsn and W.E. Harmeyer (1967). Note of removal of pigments from chloroform extracts of aflatoxin cultures with copper carbonate. J. Assoc. Agric. Chem., 50: 982.

Zilva, J.F. and P.R. Pannall (1983). Clinical chemistry in diagnosis and treatment. 3rd Ed. Loyd-Luke. (Medical Books) LTD, London.

استخدام الجلوتاثيون والالومنيوم سيليكات والطفله فى العلـــف كمحاولـــه لازالـــه التسمم بالافلاتوكسينات فى الدجاج البياض

الشحات محمد عبد الحليم قوطة ، النبوي حامد الجنزورى و رضا على حسن

قسم بحوث تغذية الدواجن– معهد بحوث الإنتاج الحيواني–مركز البحوث الزراعية– جيزة–مصر

أجرى هذا البحث لتقييم كفاءة كل من الجلوتاثيون (تراى ببتيد جلوتامات) كمضاد أكسده والطفلة والالومنيوم سيليكات كمواد ماصه بالعلف كمحاوله لازاله التسمم بالافلاتوكسينات فى الدجاج البياض وذلـك بمحطه بحوث الانتاج الحيوانى بسخا– معهد بحوث الانتاج الحيوانى باستخدام عـدد ٣٧١ دجاجـه بياضه + ٢١ ديك) طائر عمر ٣٠ أسبوع من سلاله السلام مقسمه عشوائيا إلى ٧ مجموعات بكل منهـا ٥ مكررات تشمل المكرره ١٠ دجاجات ربيت فى بطاريات سلك و ٢١ ديك المتبقيـة قسمت أيضـا الـى ٧ مجاميع وكل مجموعة ٣ ديوك تم تسكينها فرديا لجمع السائل المنوى وغذيت الطيور اما على علـف الأسـاس بدون إضافات أو مع أضافه ١ جزء فى المليون أفلاتوكسين ب، فقط أو بالاضافه إلى ٥ جزء فـى المليـون جلوتاثيون أو ٠,٦% طفله أو ٠,٥% الومنيوم سيليكات أو ٥ جزء فى المليون جلوتاثيون + ٠,٦% طفلـه أو ٥ جزء فى المليون جلوتاثيون + ٠,٥% الومنيوم سيليكات لتكون فى النهاية ٧ علائق غذيت عليها الطيـور من عمر ٣٠ إلى ٣٨ أسبوع. وأشارت النتائج المتحصل عليها الـى أن أضـافه ١ جـزء فـى المليـون أفلاتوكسين ب، إلى علف الأساس لمده ٨ أسابيع تسببت (بالمقارنة بالكنترول) فى نقص كل من كميه العلـف المأكول (٢٥,١%) وإنتاج البيض (٤٢,٨%) ووزن البيضة (٢٢,٣%) وسمك القشرة (٣٢,٦%) ونسبه البيض المخصب (٢١,٩%) ونسبه التفريخ من البيض المخصب (٢٠%) والكفاءه الاقتـصاديه (٣٨,٤٧%) ومحتوى الكبد من فيتامين أ (٢٩,١%) وهيموجلوبين الدم (٣٥,٦%) ومحتوى السيرم من كل من الاليومين (٦٨%) والدهون الكلية (٥١%) وأيضا زيادة كل من وزن الكبد (١٣٨,٨%) ودهـون الكبـد (١٤١,٩%) وعدد كرات الدم البيضاء (٢٨%) وعدد الكرات الليمفاويه(٢٧,٢%) ونشاط انزيمـى AST و ALT (٦٤ و ٦٩ % على التوالي) فى السيرم كما أنها تسببت فى احتجاز كميه من الافلاتوكسين ب، فى كل من انـسجه الكبد (٦٨ نانوجرام/ جرام) وصفار البيض (٥٢ نانوجرام/ جرام) والعـضلات الطازجـة(٣٦ نـانوجرام/ جرام). كما أدت أضافه اى من الطفلة أو الالومنيـوم سـيليكات بـصورة منفـردة إلـى العلـف الملـوث بالافلاتوكسين ب، إلى حدوث تأثير واقي متقارب يتراوح من ٤٥ الى٥٦ % للصفات المدروسه والكفـتءه الاقتصاديه وذلك بالمقارنة بالمجموعة الملوثة بدون إضافات، وسجلت أضافه الجلوتاثيون منفردة إلى العلـف الملوث تأثيرا واقيا غير قليلا غير معنوي للصفات المدروسه ماعدا نشاط انزيمى AST و ALT فقد كان التأثير الواقي (٢٠-٢٨%) معنويا، أما أضافه الجلوتاثيون مع الطفله أو مع الالومنيوم سيليكات إلى العلف الملـوث فقد سجلت أفضل التأثيرات وأزالت التأثير الضار للافلاتوكسين ب، جوهريا فى كل الصفات المدروسه كمـا انها حسنت الكفاءه الاقتصاديه بمقدار ٨١% (الطفله مع الجلوتاثيون) و ٧٥% (الالومنيـوم سـيليكات مـع الجلوتاثيون) مقارنه مع العلف الملوث. نسبه النفوق ١٠% فى المجموعه المغذاه على علـف ملـوث بـدون إضافات و٦% فى المجموعه ذات العلف الملوث مضافا اليه الجلوتاثيون اما باقى المجـاميع لـم تـسجل اى نفوق. وتخلص الدراسة إلى نقطتين أولهما أن الطفله (المتوفره فى البيئه المحليه) لها تأثير أمن وواقي ضـد الافلاتوكسين ب، مساويا تماما فى جميع القياسات والكفاءه الاقتصاديه مع الالومنيوم سيليكات (المستوردة من الخارج) وثانيهما أن أضافه الجلوتاثيون كمضاد أكسده مع الطفلة أو الالومنيوم سيليكات على السواء كمـاده ماصه إلى العلف الملوث كانت افضل الاضافات و أزالت التأثير السام للافلاتوكسين ب، فى الدجاج البيـاض ويحتاج هذا الموضوع إلى مزيد من الدراسات لتأكيد هذه النتائج.