EFFECT OF DIETARY GLUTATHIONE, ALUMINOSILICATE AND TAFLA ON LAYING HENS DURING AFLATOXICOSIS

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SUMMARY

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 reduce aflatoxicosis in chickens. A total number of 371 (350 laying hens+21 cocks) thirty-wk old El-Salam chickens were randomly divided into 7 groups; each group included 5 replicates of 10 hens each and housed 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 AFB1 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 TF or HSCAS significantly negated the adverse effects of AF diet for all studied traits. Supplementing sorbent materials plus GSH with AF-diet improved EE 80.97% for TF+GSH and 74.87% for HSCAS+GSH compared to AF-diet. There were mortalities only among two groups fed basal diet with AFB_1 alone (10%) and AFB_1+GSH (6%). The present study revealed that TF presented similar protective effect for studied traits and EE as HSCAS. Adding GSH as antioxidant together with TF or HSCAS, to AFB_1 contaminated diet significantly negated aflatoxicosis in the laying hens.

Keywords: Glutathione, aluminosilicate aflatoxicosis, laying hens, tafla, performance, 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 (Philips *et al.*,1988), in addition to the role of aflatoxins in the etiology

of hepatocellular carcinoma that has been proved (Wiled *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; El-Samra. 1991). Depression by about 6-30% of chick growth (Edrington *et al*.,1997; Genedy *et al*.,1999; Qota, 2003), impairment of feed efficiency (Kubena *et al*., 1995; Qota, 1999; Qota *et al*., 2005) and higher mortality rate (Abdelhamid *et al*.,1995a; Qota, 2003; 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; 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 chickens (Scheideler, 1993; Qota *et al*.,2005; Ali *et al.*, 2006). The HSCAS binds AFB1 *in vitro* (Philips *et al*., 1988; 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 he effect of TF, HSCAS and/or GSH to reduce aflatoxicosis in laying hens.

MATERIALS AND METHODS

The present study was conducted at Sakha Animal Research Station and Laboratories, APRI, ARC, Egypt during Feb.-May 2007 to study the effects 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-wks old El-Salam (Nicolas×Mamourah) chickens were divided into 7 similar (BW=1450±23 g) experimental groups (5 replicates of 10 hens each) and housed 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 tripeptide glutamate (reduced glutathione) (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 wks old. Aflatoxin 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 and ground to a fine powder which was analyzed for its AF content by method of 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 (tripeptide glutamate) were purchased (L.E. 12 /g) from Sigma.

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

Ingredient	%
Yellow corn	64.84
Soybean meal, 44%	24.60
Dicalcium Phosphate	1.70
Limestone	7.60
NaCl	0.30
Vit. + Min. Mix.[1]	0.30
Dl-Methionine	0.06
Clean sand	0.60
Calculated values[2]:	
ME, Kcal/Kg	2723
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
Crude fiber, %	3.22
Ether extract, %	2.66
Crude ash, %	9.75
Aflatoxin B_1, ppb	6.0
Price, LE/Kg	1.50

Composition[3]	HSCAS (%)	Tafla (%)
Silica	64.70	59.80
Aluminum	15.50	17.20
Iron	1.75	2.30
Calcium	1.26	1.90
Potassium	1.80	2.30
Sodium	2.55	2.90
Magnesium	1.54	1.90
Moisture	10.56	10.32
Price, LE/kg	15.0	0.50

(1) Vitamins and minerals premix provides per 3kg vit A 10 000 000 IU,vit D_3 2000 000IU, vit E 10000mg, vit $K_3$1000mg, vit B_1 1000mg, vit B_2 5000mg, vit B_6 1500, vit B_{12} 10mg, Pantothenic acid 10000mg, Niacin 30000mg, Biotin50mg, Folic acid 1000mg, Choline250 mg, Se 100mg, Cu 4000 mg, Fe 30000 mg, Mn 60000mg, Zn 50000mg, I 1000mg, Co 100mg and $CaCo_3$ to 3000g.
(2) According to Egyptian Feed Composition Tables (2001). (3)According to AOAC(1990).

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 analyses for HSCAS and tafla (Table 1) were done (AOAC, 1990). Feed intake, egg number and egg weight were recorded weekly. Shell thickness using Micrometer were estimated 3 times (18 d intervals) using 50 eggs/group/time and the yolk was separated for analysis. Hens were artificially inseminated once a week. During the last 2 wks of the experimental period, eggs laid were collected, stored for 5d at 18°C then incubated to estimate fertility and hatchability. At the end of the experiment, five hens/group were slaughtered for

tissues analyses. Liver lipid was extracted (Folch *et al.,*1957). Liver vit. A (as retinol) 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). Blood hemoglobin (Hb) (Kampen and Zijlestra, 1961), total leucocytes (WBC's) and lymphocyte counts (Wintrobe, 1969), serum albumin (Doumas *et al*, 1977), total lipids (ChabrIol 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). Significant differences among treatment means were separated by Duncan's new multiple range test (Duncan, 1955) with 5% level of probability.

RESULTS AND DISCUSSIONS

Productive and reproductive performance and economic efficiency :

There was similar trend for treatments effect on the studied traits. Data presented in Table 2 show that contamination of basal diet with 1 ppm AFB_1 for 8 wks significantly ($P<.01$) 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) (38.47%) compared to the controls. Inclusion of 0.6% TF or 0.5% HSCAS separately with AF-diet recorded similar protection effects ($P<.01$) 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 80.97% for TF+GSH and 74.87% for HSCAS+GSH compared to AF-diet. There were mortalities only among 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; Ali *et al*.,2006) with different chicken strains and showed similar deterioration in laying performance traits by AF-diet. 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, 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 protective effect against aflatoxicosis as they contain similar chemical compounds (Table 1).

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).

Table 2. Productive and reproductive performance of El-Salam laying hens fed dietary treatments from 30 to 38 wks old

Dietary treatment[1]	Feed intake (g/b/d)	Egg prod. (%)	Egg wt. (g)	Shell thick. (mm)	Fert-ility (%)	Hatch-ability (%)	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	106.7[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=5 ppm Tripeptide glutamate (Glutathione). Values are means of five determinations.
*EE (Economic efficiency)= [Total revenue (number of newly healthy hatched chicks × its price (1.15 LE) + (useless eggs for incubation + unfertile eggs) × its price (0.50 LE)) per hen – Total feed cost (Total feed intake ×its price, LE/hen) ÷ Total feed cost] ×100.

Liver status and hematological traits:

Contaminating the basal diet with 1ppm AFB_1 significantly (P<.01) 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 blood hemoglobin contents (35.6%) 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, 51.8% for liver weight, liver lipids, liver vit. A contents, total leucocytes, lymphocytes and hemoglobin; 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 to the AF diet significantly negated aflatoxin effects on liver and hematological traits (Table 3). The present results confirmed those of Abdelhamid *et al* (1995b) with chickens and Qota (2003) with turkey who reported similar alterations in liver status by 0.5-2.5 ppm AF diet. Increasing liver weight in the present study may be due to increase in the 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 attributed 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. Zilva and pannall (1983) and Abdelhamid *et al* (1995b) refered 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).

Table 3. Liver status and hematological traits of El-Salam laying hens fed dietary treatments from 30 to 38 wks 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/100 ml
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=5 ppm Glutathione (Tripeptide glutamate). Values are means of five determinations.

Aflatoxin B_1 residues in fresh tissues and serum constituents:

Results of Table 4 show that birds fed basal diet contaminated with 1ppm AFB1 for 8 wks 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 AFB1 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 also by Rizk *et al.* (1993), Abdelhamid *et al.* (1995b) 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.,* 19999; Hassan, 2000; Qota *et al.*, 2005). The protective effect of sorbent materials against AF diet for AFB1 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).

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-epoxide, 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 concentration (Kim and Combs, 1992). From the results of the present study, it may be concluded that TF had a similar protective effect as HSCAS and adding GSH as antioxidant with TF or HSCAS, as sorbent materials to AF diet significantly negated aflatoxicosis in the laying hens.

Table 4. Aflatoxin B_1 residues in fresh tissues and serum constituents of El-salam laying hens fed dietary treatments during 30-38 wks old

Dietary treatment[1]	AFB_1 residues			Serum constituents			
	Yolk (ng/g)	Liver (ng/g)	Meat (ng/g)	Albumin. g/100ml	Lipid (g/l)	AST (IU/L)	ALT (IU/L)
Control	***	***	***	2.5^{a}	6.7^{a}	12.1^{e}	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 five determinations.
***=No detection of AFB1

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تأثير الجلوتاثيون والالومنيوم سيليكات والطفله في العلف على الدجاج البياض أثناء التسمم بالافلاتوكسينات

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أجريت تجربه لتقييم كفاءة كل من الجلوتاثيون (تراى ببتيد جلوتامات) كمضاد أكسده والطفلة والالومنيوم سيليكات كمواد ماصه بالعلف لخفض التسمم بالافلاتوكسينات وذلك باستخدام عدد ٣٧١ (٣٥٠ دجاجه بياضه + ٢١ ديك) طائر عمر ٣٠ أسبوع من سلاله السلام مقسمه عشوائيا إلى ٧ مجموعات بكل منها ٥ مكررات تشمل المكرره ١٠ دجاجات ربيت في بطاريات سلك و ٢١ ديك المتبقية قسمت أيضا الى ٧ مجاميع وكل مجموعة ٣ ديوك تم تسكينها فرديا لجمع السائل المنوى وغذيت الطيور إما على علف الأساس بدون إضافات أو مع أضافه 1 جزء في المليون أفلاتوكسين ب١فقط أو بالاضافه إلى ٥ جزء في المليون جلوتاثيون أو ٠.٦% طفله أو ٠.٥% الومنيوم سيليكات أو ٥ جزء في المليون جلوتاثيون + ٠.٦% طفله أو ٥ جزء في المليون جلوتاثيون + ٠.٥% الومنيوم سيليكات لتكون في النهاية ٧ علائق غذيت عليها الطيور من عمر ٣٠ إلى ٣٨ أسبوع. وأشارت النتائج المتحصل عليها إلى أن أضافه ١ جزء في المليون افلاتوكسين ب١ إلى علف الأساس لمده ٨ أسابيع تسببت (بالمقارنة بالكنترول) في نقص كل من كميه العلف 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 الاقتصاديه (٣٨.٤٧%) ومحتوى الكبد من فيتامين أ (٢٩.١%) وهيموجلوبين الدم (٣٥.٦%) ومحتوى السيرم من كل من الالبيومين (٦٨%) والدهون الكلية (٥١%) وأيضا زيادة كل من وزن الكبد (١٣٨.٨%) ودهون الكبد (١٤١.٩%) وعدد كرات الدم البيضاء (٢٨%) وعدد الكرات الليمفاوية(٢٧.٢%) ونشاط انزيمى AST و ALT (٦٤ و٦٩ % على التوالي) في السيرم كما أنها تسببت في احتجاز كميه من الافلاتوكسين ب١ في كل من انسجه الكبد (٦٨ نانوجرام/ جرام) وصفار البيض (٥٢ نانوجرام/ جرام) والعضلات الطازجة(٣٦ نانوجرام/ جرام). كما أدت أضافه اى من الطفلة أو الالومنيوم سيليكات بصورة منفردة إلى العلف الملوث بالافلاتوكسين ب١ إلى حدوث تأثير واقي متقارب يتراوح من ٤٥ الى٥٦ % للصفات المدروسه والكفتءه الاقتصاديه وذلك بالمقارنة بالمجموعة الملوثة بدون إضافات، وسجلت أضافه الجلوتاثيون منفردة إلى العلف الملوث تأثيرا واقيا 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 الملوث فقد سجلت أفضل التأثيرات وأزالت التأثير الضار للافلاتوكسين ب١ جوهريا في كل الصفات المدروسه كما انها الكفاءه الاقتصاديه بمقدار ٨٠.٧٩% (الطفله مع الجلوتاثيون) و ٧٤.٨٧% (الالومنيوم سيليكات مع الجلوتاثيون) مقارنه مع العلف الملوث. سجلت نسبه النفوق ١٠% في المجموعه المغذاه على علف ملوث بدون إضافات و٦% في المجموعه ذات العلف الملوث مضافا إليه الجلوتاثيون فقط. وتخلص الدراسة إلى نقطتين أولهما أن الطفله (المتوفره في البيئه المحليه) لها تأثير أمن

وواقي ضد الافلاتوكسين ب١ مساويا تماما فى جميع القياسات والكفاءه الاقتصاديه مع الالومنيوم سيليكات (المستوردة من الخارج) وثانيهما أن اضافه الجلوتاثيون كمضاد أكسده مع الطفلة أو الالومنيوم سيليكات على السواء كماده ماصه إلى العلف الملوث كانت افضل الاضافات و أزالت التأثير السام للافلاتوكسين ب١ في الدجاج البياض ويحتاج هذا الموضوع إلى مزيد من الدراسات تأكيديه.