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POSSIBLE PROTECTIVE EFFECTS OF GLUTATHIONE IN PREVENTING AFLATOXICOSIS IN EGYPTIAN LAYING HENS

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ABSTRACT:This study, glutathione (GSH) was used as feed supplements to ameliorate the deleterious effect of aflatoxin B1 (AFB1) on egg quality, semen quality, fertility and hatchability, immune response to Newcastle disease virus (NDV), and AFB1 residual in fresh meat , liver and egg yolk. A total number of 120 Inshas laying hens (Egyptian native strain) plus 24 cockerels from strain of 28 weeks of age were randomly distributed into 4 groups with 3 replicates each (10 hens + 1 cockeral). The remaining 12 cocks were also divided into four groups of 3 cocks each and reared separately for semen evaluation. The treatments were follow. 1: was the control basal diet which save all nutrients requirements of Inshas strain (control) ; 2: birds fed on control diet supplemented with (0.5 mg glutathion / kg diet, GSH); 3: birds fed on control diet contaminated with 1000 ppb of aflatoxin B1/ kg diet,AFB1, 4: birds fed on AFB1diet and supplemented with 0.5 mg glutathione / kg diet, AFB1+ GSH . The obtained results showed that feeding AFB1 diet significantly decreased egg shell weight%, egg shell-thickness, fertility, hatchability, moreover, the relative weights of liver, spleen, kidney and gizzard were significantly increased. In addition, emboryonic mortality percent was increased while, the overall semen quality measurements were depressed. Also AFB1 diet icreased the oxidative stress including depression of activities of antioxidant enzymes (GSH-Px, SOD and CAT) and increase MDA concentration in spleen extract. Addition of glutathione to AFB1 diet could make significant improvements of most of measurements including egg quality, semen quality, fertility, antioxidant status, immune status against NDV. Generally it could be recommend adding glutathione to decrease some of the adverse effects of aflatoxin B1on Inshas developed chicken strain.

Key words: Aflatoxin-B1- Glutathione - Laying hens.

INTRODUCTION

Aflatoxins are chemicals created by a few types of pathogenic fungi (Aspergillus flavus and Aspergillus parasiticus) generally known as molds. Aflatoxin B1 is a standout among the most toxic mycotoxin in the world; it has negative effects on poultry production and economic profits in aquaculture (Jantrarotai et al., 1990). Aflatoxin metabolites induce aflatoxicosis disease that can influence numerous species of poultry (Santacroce et al., 2008). Liver is the most affected organ by aflatoxicosis and is associated with biotransformation and detoxification processes. At first, AFB1 is metabolically biotransformed by the cytochrome P_{450} group of enzymes (phase I) to a reactive intermediate metabolite, AFB1–8, 9-epoxide (AFBO), (Gallagher et al., 1996) which interacts with cellular macromolecules (protein, lipid, DNA) to induce oxidative damage in cells and DNA leading to toxicity and carcinogenicity (Yang et al., 2000). Thus, the sensitivity of poultry to the hepatocarcinogen can often be clarified by variations in the biotransformation of AFB1 (Eaton and Gallagher, 1994 and Bailey et al., 1998). AFB1 toxicity shows clinical signs including loss of body weight, increased disease susceptibility, liver dysfunction and increased mortality (Santacroce et al., 2008). Moreover, the detoxification of AFBO occurs through the biotransformation enzymes, also in the liver (Manson et al., 1997). The pathway of biotransformation is mediated by phase I enzymes, such as cytochrome P450 (CYP) which plays a vital role in phase-I metabolism of many drugs or toxins and phase II (conjugation) as well, or sometimes even a combination of both (Brandon et al., 2003). Phase I reactions facilitate bioactivation (Sumit and Roger, 2010) whereas phase II biotransformation reactions have resulted in detoxification and excretion. Most poultry have a second group of biotransformation enzymes that are referred to as conjugation antioxidant defenses, such as glutathione (Clark et al., 1991). Such a conjugation has shown that it protects against the hepatocarcinogenic effects of AFB1 (Lotlikar et al., 1984) where the resulting conjugate is often less toxic than the parent compound. AFB1–glutathione conjugation is a key (segment) component of antioxidant defense mechanisms and the major detoxification pathway of aflatoxin metabolites (Shi et al., 2004). Liver is the major site of synthesis of glutathione, although it is synthesized in every cell of the body (Wu et al., 2004). Moreover, the spleen is the principal peripheral lymphoid organ which plays an important role in protective immune reactions (Cui et al., 2012). It is involved in humoral and cellular immune responses through its role in the generation, maturation and storage of lymphocytes (Sandford et al., 2011). Previous study revealed that AFB1 significantly affected the development of spleen in ducklings (Guo et al., 2012), and GSH could ameliorate the negative effects induced by AFB1 in Nile tilapia Oreochromis niloticus (El-Barbary, 2010). This study was carried out in order to investigate the effects of glutathione to reduce adverse effects of aflatoxin B1 on egg quality, fertility, hatchability, antioxidant status, immune status and residues of aflatoxin B1 in some organs of Inshas hens.

MATERIALS AND METHODS

The present study was carried out at Sakha Animal Research Station, Animal Production Research Institute, Ministry of Agriculture, Egypt. A total number of 120 Insha (Egyptian native strain) laying hens plus 24 cocks from Inshas strain of 28 weeks of age were randomly distributed into 4 groups with 3 replicates each (10

hens + 1 cocks). The remaining 12 cocks were also divided into four groups of 3 cocks each and reared separately for semen evaluation. The birds were reared under the same managerial conditions in open-sided house in floor pens (280 cm long X 220cm wide). The birds were exposed to 17 hrs photoperiod. All birds were provided with water and feed *ad libitum* and kept under similar conditions of management.

The experimental period was 12 weeks. Basal diet was formulated to save the nutrients requirements of birds (Table1) according to Egyptian Feed Composition Table (2001). Laying hens were allotted for the following treatments; (1) control, basal diet; (2) basal diet plus 0.5 mg glutathione /kg diet (GSH); (3) basal diet plus 1000 ppb aflatoxin B1 /kg diet, (AFB1); (4) AFB1- diet plus 0.5 mg glutathione /kg diet, (AFB1+GSH). The glutathione was provided from Sigma- Aldrich Quimica S. A. Madrid 28100, Spain.

Aflatoxin B1 Production:

The AFB1 was produced via fermentation of rice by *Aspergillus flavus*, strain NRRL 2999 available at the Institute of Animal Health, Egypt, as described by Shotwell, et al. (1966) and modified by West, et al. (1973). Fermented rice was autoclaved, dried then ground to fine powder which was analyzed sepectrophotometrically for its aflatoxin content by method of Nabney and Nesbitt, (1965) which modified by Wiseman, et al. (1967). Aflatoxin in the rice powder were extracted by chloroform then incorporated into the basal diet and confirmed by HPLC to provide the desired level 1ppm aflatoxin B1

Measurements:

Egg quality:

Thirty eggs / group were collected monthly (from the last three days of each month) throughout the experimental period to carry out egg quality measurements including shape index and yolk index were determined according to Romanoff and Romanoff, (1949) as follows:

$$\text{Shape index (\%)} = (\text{width/length}) \times 100$$

$$\text{Yolk index (\%)} = (\text{height/ diameter}) \times 100$$

Egg shell thickness, including shell membranes, was measured using a micrometer at the equator. The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the "1961, Roche Improved Yolk Color Fan".

Semen quality:

Semen was collected from cocks and artificially inseminated to hens (cock/5hens) two times per week. Also, monthly semen samples were individually collected by the massage method from all cocks. A small droplet from each cock semen was placed on a warm slide, covered with a cover slide and examined for sperm motility microscopically at 100x magnification **(**Melrose and Laing, 1970). Eosin-Nigrosine stain was used to determine the percent of morphologically dead spermatozoa (Lake and Stewart, 1978). Sperm-cell concentration was determined using the spectrophotometer density meter technique with diluted semen samples (1:250) as described by Lake and Stewart, (1978).

Fertility measurements:

Only nest eggs and clean floor eggs were selected for incubation. Dirty, misshaped, broken, cracked, excessively small, and double-yolk eggs were not incubated. Eggs were stored at room temperature (72°F) for up to a week and then placed in an incubator model PTO. The dry- and wet bulb temperatures for the incubators (setter and hatcher) were set at 37.6°C and 60% RH. On d 18, the eggs were candled to determine macroscopic fertility and early embryonic mortality before being transferred back to an incubator (hatcher) for hatching. Infertile eggs; early, late, and pipped embryonic mortalities; and hatching chick deaths were recorded as part of a hatchery residue analysis. All hatchability and mortality data were expressed as

percentages of fertile eggs set. On d 21, chicks were removed from the hatcher, and data on late embryonic mortality and chick weight were collected.

Some organs measurements:

At the end of experimental period, the chickens were fasted for 12 hours prior to slaughter without feed. Then 3 chicken from each treatment were randomly selected weighed and slaughtered to obtain organs weight after bleeding, Scalding, feather picking by hand and evisceration, different organs (liver, kidney, spleen, hart and gizzard), data expressed as a percentage of live body weight.

Antioxidant status:

Spleen was immediately collected for evaluating state of oxidative stress. Splenic tissue (1 g) was homogenized with normal saline buffer (9 mL) through a cell homogenizer in an ice bath and centrifuged at 3,000 r/min for 10 min to obtain a clear supernatant. After determining the amount of total protein in the supernatant of the splenic homogenate by the method of Bradford (1976), the GSH, MDA contents and GSH-Px, SOD, CAT activities in the splenic supernatant were measured by biochemical method as described by Li et al. (2010). GSH assays were based on the development of a yellow color when DTNB was added to compounds containing sulfhydryl groups. MDA assays were determined by the thiobarbituric acid (TBA) colorimetric method. GSH-Px activities were detected by the consumption of glutathione. SOD activities were determined by the xanthine oxidase method. CAT activities were determined by the H2O2 decomposition rate. The absorbance of the supernatants were measured by spectrophotometric assay at 532 nm for MDA, 412 nm for GSH and GSH-Px, 550 nm for SOD, and 240 nm for CAT, the values were expressed as nmol/mg protein for GSH and MDA, and units (U) per mg protein for GSH-Px, SOD, and CAT.

Serum measurements:

Calcium and phosphorus concentration in serum were determined colorimetrically by using sepectrophotometric and suitable commercial diagnostic kits according to methods of Gindler and King (1972) and El-Merzabani et al. (1977), respectively.

Immunity measurements:

Antibody titer against NDV was measured in samples from hens and progeny sera at the first day of chicks (at hatcher) and in yolk samples using method described by (Qureshi et al., 1998).

Residues of aflatoxin B1:

At the end of the experimental period, residues of aflatoxin in fresh meat samples (breast meat: thighmeat, 1:1), liver and egg yolk were determined according to (Stubblefield et al., 1982).

Statistical analysis:

Data were statistically analyzed using one-way ANOVA of GLM procedure of Statistical Analysis Software (SAS, 2000). Before analysis, all percentages were subjected to logarithmic transformation (log $10x^{+1}$) to approximate normal distribution. Significant differences among treatment means were ($p \leq 0.05$) separated by Duncan's Multiple Range Test (Duncan, 1955).

Model:

$X_{ij} = \mu + T_i + e_{ij}$

Where: X_{ij} = Any observation

μ = Overall mean

T_i = Treatments (i = 1, 2,…and 4)

e_{ij} = Experimental error

RESULTS AND DISCUSSION

Results regarding effect of experimental treatments on egg quality of laying hens are presented in Table (2). There were no effects due to treatments on shape index %, albumen %, yolk %, egg and yolk color. While, infection with aflatoxin B1 at level 1000ppb decreased egg shell %, egg shell-thickness and yolk cholesterol and total lipids compared with the control group, while supplementing hen diet with GSH in absence of AFB1 recorded improvement of egg quality parameters compared with control group. While incorporating GSH into the AFB1-contaminated diets partially ameliorated the adverse effects of AFB1on egg shell weight %, egg shell-thickness and yolk cholesterol and total lipids. Diet contamination with 1000ppb AFB1in laying hens resulted in reduction in shell thickness, egg shell weight % and egg weight (Table 2) may be due to decrease Ca and P concentration in serum (Table 5). The obtained results by Garlich et al. (1973) showed a decrease in plasma calcium of layers fed with AFB1 which may impair the normal egg shell calcification and thereby lowered shell thickness. While, aflatoxin contamination in boiler breeder diets insignificantly ($p \leq 0.05$) alter the Haugh unit scores and yolk color index of eggs.

As shown in Table (3) aflatoxin B1 contaminated diets resulted in ($p \leq 0.05$) decrease of semen volume, and depressed concentration, motility and live sperm while increased in both abnormalities and dead sperms %. These results are in agreement with those obtained by Clarke et al. (1986) and Abd El-Hamid et al. (1995). The significant decline in semen volume and its concentration due to feeding AF-diets was attributed to the impair spermatogenesis resulting from decreased feed intake which caused reduction in LH and therefore reduce testosterone level (Sharlin et al., 1981). Mohiddin (1982) have implied that AF caused degeneration and a decrease in germinal epithelial cells, disruption in spermatogenesis. The severity of aflatoxin effect on semen traits were decreased by adding GSH to aflatoxin diets (Table 3).

The reproduction data (fertility and hatchability percentage) and early (1 to 18 d) and late (18 to 21 d) embryonic mortality are presented in Table 4. Fertility levels, which were determined by breakout of candled eggs incubated for 18 d, were statistically significant among treatment groups ($p \leq 0.05$). Also, hatchability, which represents hatch of total eggs, showed considerable variation. Aflatoxin added to the layer diet had a detrimental effect on hatchability. Aflatoxin-treated layers produced a 70.8% hatch compared with the controls with a 80.5% hatch, a difference of 9.7%. The inclusion of GSH in the diet containing AF restored fertility, hatchability and chick weight at hatch. Addition of GSH to a control diet with no AF added to the diet produced no significant increases in fertility and hatchability as compared with controls. Chick weight taken immediately after hatching was decreased by AF.

Feeding AF alone resulted in a significant increase in early and late embryonic deaths. Feeding GSH alone decreased early and late embryonic death equal to levels from eggs laid by hens provided the control diet. Addition of GSH to the diet containing AF significantly lowered early and late embryonic deaths and, therefore, significantly elevated the hatch from hens not provided AF. These results agree with those obtained by Qureshi et al. (1998) who found that AF dietary exposure resulted in embryonic mortality and reduction in hatchability compared to control and reported that developing embryos are very sensitive to AF. Also, Sur and Celik (2003) indicated that low concentration of AFB1 transferred into the fertilized eggs might be the cause of serious problems. The reduction in fertility rates in the present study may be

due to impaired semen characteristics in this study (Table, 3), while increasing total death of embryos during the hatching period may explain the reduction in hatchability of fertile eggs. This interference may be attributed to initial effects of aflatoxin upon the gonads (Abd El-Hamid et al., 1992). In addition, aflatoxins has direct effect on hatchability, another attractive hypothesis might be an inadequate or altered on egg for chemical composition. Alterations in the hen's protein, lipid and carbohydrate metabolism occur rapidly after feeding aflatoxin contaminated diet. Such alterations in metabolism could alter the composition of the egg and its subsequent hatchability. In the current study, the ability of dietary GSH to suppress toxicity of AFB1 was demonstrated in improved hatchability and decreased embryonic mortality when it was added to AFB1-treated diet. The high rate of embryonic death at both stages of incubation, producing low hatchability of eggs from the AF fed hens, could have been due to low calcium availability. Calcium is vital in the production of strong eggshell. Reduced blood serum calcium and phosphorus could have affected egg weight shell % and eggshell thickness in the AF-fed layers (Table 2). Poor eggshell could interfere with the oxygen-carbon dioxide ratio. Because developing embryos are very sensitive to reduced oxygen supply (Hargis and Van Elswyk, 1993), these changes caused by AF consumption might play a role in increasing the rate of embryonic death. The hens fed AF-diet recorded value of yolk cholesterol and total lipids which were found to be lower than the other treatments and these may be as a result to the inhibition of cholesterol biosynthesis (Table 8). In this respect, Pizzi et al. (2003) demonstrated for AF inhibitor of cholesterol biosynthesis in rats. Adding GSH to aflatoxin diet increased yolk cholesterol and total lipids. These results agree with Dvorska and Surai (2001) suggested that GSH prevents changes in fatty acid and antioxidant composition in the egg yolk.

The results indicated that the presence of AF alone in the diet caused a significant enlargement in the size of internal organs like liver, kidney, spleen, gizzard and heart while ovarian was decreased (Table 5) compared with recorded values of the rest of treatments. The recorded values of weights % of the mentioned organs by GSH group were not changed significantly than those of control group (with exception of liver weight %), but were lower than those recorded values by AFB1 group (with exception of ovarian weight %), significantly. While adding GSH to AFB1 contaminated diet at level 1000 ppb helped birds to record relative weights of liver, ovarian similar to those of control group, and enhance the relative weights % of the other studied organs (spleen, heart, and kidney). Feeding hens on AFB1 diet may cause relevant lesions in liver and in kidneys, heart and ovaries. The ovaries showed follicular atresia which has a detrimental effect on egg production (Del Bianchi et al., 2005 and Pandey and Chauhan, 2007).

Results in Table (5) showed the effect of the experimental treatments on concentrations of calcium and phosphorus in hen serum at age 40 wks (after 12 wks of treatments). The presented results showed that the serum concentrations of both calcium and phosphorus were decreased to the lowest determined levels in samples of AFB1 group compared to those of control and GSH groups. While using AFB1+GSH diet partially ameliorated the adverse effects of aflatoxin B1on Ca and P concentration and helped hens to record higher concentrations of Ca and P compared with those of AFB1 group.

Residues of aflatoxin B1 in samples of liver, meat, and egg yolk were determined at the end of experimental period (at 40 weeks of age) and showed superior concentrations in samples of AFB1 group and lower concentrations in

AFB1+GSH group, significantly. While no resudes detected in samples of either control or GSH groups. Generally the obtained results showed that although AFB1diet caused great accumulation of aflatoxin B1 in meat, liver and egg yolk, GSH supplementation at level 0.5mg/kg diet significantly decreased the accumulated concentrations. The feed : egg transmission for aflatoxin B1after 12 week feeding on contaminated diets at level 1000 ppb aflatoxin B1 was 3125:1. These results were similar to those reported by Oliveira et al. (2000) who found that residues of AFB1 were detected only in eggs of hens fed on 500 µ AFB1/kg feed at levels that ranged from 0.05 to 0.16 µ/kg and indicated that the feed : egg AFB1 transmission ratio was approximately equals to 3333: 1. Also, in previous reports of Ali et al. (2006) and Hassan et al. (2011) they found that hen fed on diet contained 1000 ppb AFB1 recorded 1.22 and 0.890ppb residue of aflatoxin B1 in the egg, respectively.

Concerning to antioxidants status in spleen tissue,(Table 7) the recorded results showed that compared with the control group, the glutathione contents of spleen were decreased significantly ($p \leq 0.05$) to the lowest value in spleen samples of AFB1. However, samples of hens fed on AFB1+GSH diet contained higher concentration of glutathione compared with AFB1 group, the highest significant glutathione were recorded in control and GSH samples. At the same time, feeding on AFB1 diet increased MDA concentration compared with samples of both GSH and AFB1+GSH group significantly ($p < 0.05$). As shown in Table (7), feeding on GSH diet caused significant increase of antioxidant enzyme activities including GSH-Px, SOD, and CAT compared with other treatments. While, feeding of AFB1 diet recorded the lowest level of each antioxidant enzyme activity. These results showed that feeding on AFB1 diet caused oxidative damages, which may be one of the underlining mechanisms for AFB1-induced cell injury and DNA damage, and eventually lead to tumorigenesis (Shen et al., 1994). As previous studies revealed, AFB1 induced oxidative stress, which included the decrease of the level of GSH and the activities of SOD and GSH-Px, and the increase of level of MDA in lymphocytes of human (Alpsoy et al., 2009 and Kotan et al., 2011), increased MDA and lipid hydroperoxide (LHP) in hepatocytes of rats (Farombi et al., 2005). Our results showed that 1000 ppb of aflatoxin B1 (AFB1 group), depressed the antioxidant status and increased MDA concentration which demonstrated an oxidative stress in spleen of layers. These results were confirmed by with previous studies.

AFB1 may cause reactive oxygen species (ROS) generation, lipid peroxidation and formation of 8-hydroxydeoxyguanosine (8-OHdG) *in vivo* and *in* vitro (Farombi et al., 2005). When the concentration of ROS exceeds the antioxidant capability of cells, oxidative stress occurs in a cell or tissue (Sies, 1991). The levels of enzymatic antioxidants and non-enzymatic antioxidants are the main determinants of the antioxidant defense mechanism of the cell (Verma and Nair, 2001). In the present study, the activities of antioxidative enzymes, including GSH-Px, SOD and CAT were all markedly decreased in AFB1 groups compared with those of the control group. These enzymatic antioxidants have been recognized to play an important role in the anti-oxidant mechanism of the body, which can eliminate ROS from cell, for instance, SOD converts $O2\cdot-$ into H2O2 and O2; CAT and GSH-Px reduces H2O2 into H2O and O2 (Liu et al., 2010). GSH, a non-enzymatic antioxidant, is also an early biological marker of the oxidative stress (Gagliano et al., 2006). It plays a role in the suppression of oxygen free-radical formation and the reduction in NO generation (Abdel-Aziema et al., 2011). As

well known, through the action of glutathione-S-transferase, the metabolites of AFB1 are mainly conjugated with GSH before to be excreted (Bernabucci et al., 2011). So, a decreased content of GSH was observed in AFB1 group in our study. The MDA is the end product of lipid peroxidation, considered as a late biomarker of oxidative stress and cellular damage (Dalel et al., 2011). In the present study, an increased level of MDA in the AFB1 group, which could result in extensive cell damage and death was found (El-Nekeety et al., 2011).

The present study showed that in GSH groups, the contents of GSH and the activities of GSH-Px, SOD and CAT were all increased when compared with AFB1 group, and the MDA content was decreased. It may be associated with increased antioxidative function resulting from an increase in activity of GSH-Px whose center is Se (Ding et al., 2010). As previous study revealed, Se can inhibit lipid peroxidation (Battin et al., 2006), our results suggested that adequate GSH levels could reduce ROS formation and protect against apoptosis. Moreover, Se can also prevent from oxidative damage to mitochondria DNA (Battin et al., 2006).

The effects of experimental treatments on immune response of Inshas hen to NDV are presented in Table 8. The determined values of antibodies titer against NDV showed that consuming AFB1 contaminated diet resulted in significant reduction in antibody titers against NDV in either hen serum, egg yolk, or posthatch chick as compared to the control and GSH groups. The addition of GSH to the aflatoxin contaminating diet significantly ameliorated the harmful effect of aflatoxin on immune response to NDV in all examined samples. Table (8) showed that results of antibodies titer against NDV of egg yolk showed the same trend of serum samples these results suggested that maybe we can use yolk to measure the antibodies titer and get real immune status which will be more easy and applicable since the eggs collection are more easily than collection of serum samples. Furthermore the presented results showed that post-hatch chicks showed the same trend of immune response of hen, but values of antibodies titer against NDV were higher in egg yolk followed by hen serum and the lowest values recorded in post-hatch chicks of each treatment. Adding GSH to contaminated diet decreased the severity of aflatoxin B1 effects on NDV antibodies and increased titer values compared with AFB1 group. This reduction in titer levels of immune response to NDV is cleared inducement of immune depression effects of aflatoxin on humoral antibody response. These finding confirmed that reorted with previous authors (Gupta et al., 2003). In previous study authors mentioned that the reduction of antibody titer may be resulted by inhibition of DNA and protein synthesis by aflatoxin B1 through impairment of mRNA transcription and amino acid transport resulting in low level of antibody (Gupta et al., 2003). Also aflatoxin causes reduction of bursa fabricius thence the low of antibody titer against NDV and Gumboro disease may be attributed to the depression of this immune organ (Thaxton et al., 1974). The results of this study showed significant increase of antibody titer against NDV at 40 wks old Inshas hen when GSH was supplemented to AFB1 diet compared with determined values by hens fed on AFB1 diet.

The improvement in NDV titer in group fed AFB1+GSH diet could be attributed to the role of glutathione in reducing aflatoxin effects by changing the toxic metabolic: increasing or activation the glutathione peroxidase (GSH-PH) (Bottin et al., 2006). Aflatoxin is inactivated by conjugation with glutathione-s-transferase (GST) to the aflatoxin glutathione and excreted through urine and bile (Kotan et al., 2011).

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) proved that detoxification enzyme systems in chickens could be increased by the administration of the antioxidants. Hsieh (1982) found that primary hepatic metabolites of AFB1 may subjected to cytoplamic reductase system producing aflatoxicol or to liver microsomal oxidase system producing AF: Q1, M1 and B1-epoxide. Except for AFB1-poxide, all metabolites contain hydroxyl groups are transformed into a water –soluble conjugate and to facilitate excretion. The transient B1-epoxide can be conjugated by GSH to form another type of conjugate. A prospective action may be afforded by reaction of AFB1 metabolite with GSH (Lotlikar et al., 1980). Presence of AFB1-GSH conjugate in the bile of AF-treated rats, and its formation in vitro in liver-derived subcellular fractions, has been reported (Moss et al., 1983).

Based on the overall recorded results of this study, it could be concluded that administrated dietary glutathione (GSH) can partially alleviate of the harmful effects of 1000 ppb aflatoxin B1 diet including fertility, hatchability, some external egg quality traits, antioxidants status of spleen and immunity status of Inshas hens at week of age.

Table (1): Chemical composition and calculated analysis of the basal experimental diet.

Ingredients	%
Yellow corn	64.84
Soybean meal (44%)	24.60
Limestone	7.60
Di-calcium phosphate	1.70
Sodium chloride	0.30
Vit.& Min. Mixture*	0.30
DL.Methionine	0.06
Clean sand	0.60
Calculated values**	
Metabolizable energy (kcal/kg)	2723
Crude Protein, %	16.43
Calcium, %	3.30
Available phosphate, %	0.46
Lysine, %	0.88
Methionine, %	0.45
Methioine + Cystine, %	0.62
Determined values***	
Dry matter, %	89.51
Crude Protein, %	16.55
Ether extract, %	2.66
Crude fiber, %	3.20
Aflatoxin B1, ppb	5.00

*Supplied per kg of diet: vit. A, 10000 IU; D_3, 2000 IU; Vit. E, 10 mg; Vit. K_3, 1 mg; vit. B_1, 1 mg; vit. B_2, 5 mg; vit. B_6, 1.5 mg; vit. B_{12}, 10 mcg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1mg; Biotin, 50 µg; Choline, 260 mg; Copper, 4 mg; Iron, 30 mg; Manganese, 60 mg; Zinc, 50 mg; Iodine, 1.3 mg; Selenium, 0.1 mg and Cobalt, 0.1 mg.
** According to Egyptian Feed Composition Tables (2001)
***According to AOAC (1990)

Table (2): Effect of using supplemental glutathione in contaminated and uncontaminated layer diet with aflatoxin B1 on parameters of egg quality of Inshas laying hens during the experimental period (28-40 wks of age).

Treatment	External egg quality				Internal egg quality			
	Egg weight (g)	Shell weight %	Shell thickness (mm)	Shape index %	Albumin weight %	Yolk index %	Yolk weight %	Yolk color score
Control	50.58^{a}	11.46^{ab}	0.362^{a}	76.43	55.84	46.86	32.68	5.60
GSH	50.47^{a}	11.55^{a}	0.369^{a}	76.80	55.83	46.35	32.61	5.66
AFB1	48.68^{c}	10.80c	0.310^{c}	75.00	55.82	45.60	33.38	5.66
AFB1+ GSH	48.00^{b}	11.32^{b}	0.352^{b}	75.46	55.59	45.81	33.09	5.60
SEM	0.091	0.030	0.002	0.241	0.072	0.188	0.089	0.054

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

Table (3): Effect of using supplemental glutathione to contaminated and uncontaminated layer diet with aflatoxin B1on some semen quality traits of Inshas chockerles at 40 wks of age.

Treatments	Semen volume, ml	Concentration of sperm, 10^9/ml	Sperm motility, %	Life sperm, %	Abnormal sperm, %	Dead sperm, %
Control (C)	0.350^{a}	2.18^{a}	81.65^{b}	87.0^{a}	4.0^{c}	9.0^{b}
GSH	0.352^{a}	2.20^{a}	82.55^{a}	87.5^{a}	3.8^{c}	8.7^{c}
AFB1	0.340^{b}	2.15^{b}	80.35^{c}	85.0^{c}	5.0^{a}	10.0^{a}
AFB1+ GSH	0.348^{ab}	2.17^{ab}	81.25^{b}	86.0^{b}	4.8^{b}	9.2^{b}
SEM	0.112	0.624	0.048	0.166	0.836	0.253

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

Table (4): Effect of using supplemental glutathione in contaminated and uncontaminated layer diet with aflatoxin B1on parameters of fertility and hatchability of Inshas laying hens during the experimental period (28-40 Wks of age).

Treatment	Fertility and Hatchability				Embroyonic mortality %	
	Fertility%	Hatchability%	Hatchability of fertile eggs%	BW of hatched chicks (g)	Early death	Late death
Control (C)	87.04^{a}	80.5^{a}	86.9^{a}	34.8^{a}	7.1^{b}	8.3^{b}
GSH	88.5^{a}	81.6^{a}	88.8^{a}	34.9^{a}	5.7^{c}	7.5^{c}
AFB1	76.8^{c}	70.8^{c}	72.6^{c}	33.2^{c}	9.0^{a}	15.0^{a}
AFB1+ GSH	80.8^{b}	78.9^{b}	80.6^{b}	33.8^{b}	7.7^{b}	9.1^{b}
SEM	3.25	3.58	2.05	3.33	2.95	2.79

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

Table (5): Effect of using supplemental glutathione in contaminated and uncontaminated layer diet with aflatoxin B1on some organs weights of and some serum contents of Inshas laying hens at 40 Wks of age.

Treatment	Organs weight %						Serum	
	Liver	Spleen	Ovarian	Gizzard	Heart	Kidney	Ca, g/100ml	P, g/100ml
Control (C)	2.10^{b}	0.085^{bc}	1.133^{a}	1.310^{bc}	0.400b^{c}	1.00b^{c}	11.05a	5.60^{a}
GSH	2.04^{c}	0.075^{c}	1.170^{a}	1.293^{c}	0.340^{c}	0.973^{c}	11.08a	5.65^{a}
AFB1	2.70^{a}	0.176^{a}	0.777^{b}	1.513^{a}	0.556^{a}	1.150^{a}	9.12c	4.25^{c}
AFB1+ GSH	2.15^{bc}	0.100^{b}	1.085^{ab}	1.346^{b}	0.490^{b}	1.040^{b}	10.45^{b}	5.04^{b}
SEM	0.706	0.010	0.038	0.031	0.020	0.017	0.52	0.352

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

Table (6): Effect of using supplemental glutathione in contaminated and uncontaminated layer diet with aflatoxin B1on residual concentration of aflatoxin B1 in liver, meat and egg yolk of Inshas laying hens at 40 Wks of age.

Treatment	Aflatoxin B1 (ng/g)		
	Liver	Meat	Egg yolk
Control	ND*	ND	ND
GSH	ND	ND	ND
AFB1	8.83[a]	3.50[a]	0.32[a]
AFB1+GSH	1.40[b]	1 .00[b]	0.08[b]
SEM	1.390	0.868	0.258

a-b Means in the same column with different letters, differ significantly ($p \leq 0.05$).
*ND: not detected (determination limit of the analytical method: 0.01 ug/kg for aflatoxin B1)

Table (7): Effect of using supplemental glutathione in contaminated and uncontaminated laying diet with aflatoxin B1on antioxidant status of Inshas laying hens during the experimental period (28-40 Wks of age).

Treatment	Antioxidant activities of spleen				
	GSH nmol/mg of protein	MDA nmol/mg of protein	GSH-Px U/mg of protein	SOD U/mg of protein	CAT U/mg of Protein
Control (C)	1.38[a]	3.0[ab]	17.0[b]	290[b]	28.5[a]
GSH	1.40[a]	2.46[c]	18.0[a]	300[a]	29.3[a]
AFB1	1.20[c]	3.80[a]	15.5[c]	250[c]	22.3[c]
AFB1+ GSH	1.30[b]	3.2[b]	16.4[ab]	285[b]	27.5[b]
SEM	0.706	0.010	0.038	0.031	0.020

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

Table (8): Effect of using supplemental glutathione in contaminated and uncontaminated laying diet with aflatoxin B1on antibodies titer against NDV in hen serum, yolk and progeny at a day of hatch, of Inshas strain and some lipid compounds in egg yolk at 40 Wks of age.

Treatment	Antibodies titer against NDV			Egg yolk	
	Serum hen at 40 wks	Egg yolk at 40 wks	Post-hatch chick	Cholesterol mg /g	Total lipids mg/g
Control (C)	7.50[a]	9.45[a]	6.00[a]	14.25[a]	230.5[a]
GSH	7.76[a]	9.82[a]	6.10[a]	14.28[a]	230.8[a]
AFB1	5.40[c]	7.15[b]	5.20[b]	11.95[c]	210.4[c]
AFB1+ GSH	6.81[b]	8.15[ab]	5.73[ab]	12.96[b]	220.8[b]
SEM	0.706	0.010	0.038	0.031	0.020

a-d Means in the same column with different letters, differ significantly ($p \leq 0.05$).

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الملخص العربى

التأثيرات الوقائية الممكنة للجلوتاثيون للوقاية من الأفلاتوكسين ب١ فى الدجاج البياض المصرى المحلى.

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فى هذة الدراسة تم أستخدام الجلوتاثيون كأضافة للعلف لتقليل التأثيرات الضارة للأفلاتوكسين-ب١ على جودة البيض- جودة السائل المنوى – نسبة الفقس والخصوبة – الأستجابة المناعية لمرض النيوكاسل والمتبقى من الأفلاتوكسين ب١ فى اللحم والكبد وصفار البيض. تم أستخدام عدد ١٢٠ دجاجة أنشاص و ٢٤ ديك انشاص عمر ٢٨ أسبوع وتم توزيعها عشوائيا الى اربعة مجاميع و كل مجموعة تتكون من ٣ مكررات وكل مكررة تتكون من (١٠ دجاجات + ديك) والديوك الباقية (١٢ ديك) تم تقسيمها ايضا الى ٤ مجاميع كل مجموعة تتكون من ٣ ديوك وتم تسكينها فى اقفاص فردية لزوم جمع السائل المنوى . المعاملات كالأتى ١- عليقة تحتوى على جميع الأحتياجات الغذائية لدجاج سلالة انشاص (كنترول). ٢- تغذية الطيور على عليقة الكنترول مع اضافة ٠,٥ مليجرام جلوتاثيون / كجم علف. ٣- تغذت الطيور على عليقة ملوثة بالأفلاتوكسين ب١ (١٠٠٠ جزء فى البليون / كجم علف). ٤- تغذت الطيور على عليقة ملوثة بالأفلاتوكسين ب١ مع أضافة ٠,٥ مليجرام جلوتاثيون /كجم علف. أظهرت النتائج ان التغذية على علف ملوث بالأفلاتوكسين ب١ قلل معنويا وزن قشرة البيضة % و سمك قشرة البيضة ونسبة الفقس والخصوبة. فى حين زادت معنويا الأوزان النسبية للكبد والطحال والكلية والقونصة. بالأضافة الى زيادة نسبة النفوق الجنينى بينما انخفضت قياسات السائل المنوى. أيضا تسبب الأفلاتوكسين ب١ الى زيادة الأجهاد التأكسدى ويشمل نقص نشاط انزيمات الأكسدة (GSH-Px, SOD, and CAT) وزيادة تركيز المالونالدهيد (MDA) فى الطحال . ادت أضافة الجلوتاثيون الى العليقة الملوثة بالأفلاتوكسين ب١ الى تحسن معنوى لمعظم القياسات والتى تشمل جودة البيضة وصفات السائل المنوى ونسبة الفقس ومضادات الأكسدة والأستجابة المناعية لمرض النيوكاسل . عموما نوصى بأضافة الجلوتاثيون لتقليل التأثيرات الضارة للأفلاتوكسين لدجاج انشاص.