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GLUTATHIONE STIMULATES LIVER ANTI-OXIDANTS RELATED GENES AND REDUCES HEPATIC DYSFUNCTION INDUCED BY DIETARY MYCOTOXINS IN LAYING HENS

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ABSTRACT: This study was performed to evaluate the protective effects of feeding reduced glutathione (GSH) against hepatic dysfunctions related to dietary aflatoxicosis in Inshas chickens (a local Egyptian chicken strain). 5 ppm of GSH were used with 500 ppm of aflatoxin B1 (AFB1) /kg diet administrated. In total, 96 chickens were divided into 4 treatments with 3 replicates. T1 was the negative control. T2was the positive control group (AFB1). Groups T3 and T4 were administrated with GSH, and GSH+ AFB1, respectively. At the end of the experiment (28-38) wk of age, samples were collected for analysis. The results showed that AFB1 has significant potency for increasing aspartate transaminase (AST), creatinine in blood, thiobarbituric acid reactive substances (TBARS) in muscle , while reducing the plasma α-tocopherol content, antioxidants enzymes (glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) and total antioxidant capacity) in blood of chicken. In addition, the negative effects of AFB1 on mRNA for antioxidant enzymes (GPX, SOD and CAT), hepatosomatic index (HSI), lipid peroxidation on birds aflatoxicosis were recorded in this study, also the efficacy of GSH for ameliorating aflatoxicosis were recorded. Moreover, these results showed that the ability of GSH when use with aflatoxicosis to counteract the toxic effects of AFB1 on chickens. Additionally, the GSH-Px (T3) were enhanced the antioxidative status. Finally, the optimal levels of GSH and method of used for detoxification of aflatoxin effects need more studies.

Key Words: Aflatoxin B1- Glutathione- Antioxidants and Chickens.

INTRODUCTION

Chemical, biological, and toxicological properties of mycotoxins are diverse in animal production. Hence, their toxic effects are extremely variable, depending on type, the intake level, and duration of exposure, animal species, age, sex, physiological status, and eventual synergism between different types of mycotoxins simultaneously present in feed or foods. However, one of the toxic effects of aflatoxicosis is oxidative stress (Rawal et al., 2010), which is a common mechanism contributing to initiation and progression of hepatic damage. When animals or humans consume mycotoxin-contaminated foods, aflatoxin B1 is metabolized in the liver forming reactive intermediates AFB1-8,9-epoxide (AFBO) (Gallagher et al., 1996), that may, in part, be responsible for the carcinogenic activity of AFB1 (Shen et al., 1996).The mechanism of action of aflatoxin on the cell is mediated through the production of free radicals and reactive oxygen species (ROS) (Baynes, 1991: Van Dam et al., 1995).

The detoxification of AFB1-8,9-epoxide also occurs in liver through the conjugation or Phase II enzymes, where glutathione S-transferase-mediated conjugation with reduced glutathione (GSH) (Gallagher and Eaton,1995). AFB1-glutathione (GSH) conjugation is the major detoxification pathway of aflatoxin metabolites in the liver (Allameh et al., 2000). The resulting conjugate is often less toxic than the parent compound and its increased hydrophilicity can make it more readily extractable from the body. However, this process consumes a big amount of GSH, which is an intracellular antioxidant and thus indirectly accelerates the production of reactive oxygen species (ROS) in the cytosol. This AFB1-induced elevated ROS level also contributes to the hepatic cytotoxic and carcinogenic effects (Shen et al., 1994). Therefore, stimulation of the antioxidant defense system like reduced glutathione (GSH) may reduce the risk of AFB-mediated toxicity and carcinogenesis. Reduced glutathione is normally present in tissue at relatively high concentrations (Wu et al., 2004) working as a key component of antioxidant defense mechanisms (Sayeed et al., 2003; Shi et al., 2004). In addition, it is one of the important factors protecting from oxidative attacks by reactive oxygen species, because GSH acts as a reducing agent and free-radical trapper and is known to be a cofactor substrate for GSH-related enzymes (Verma et al., 2007), also, El-deep et al. (2014) highlighted that lipid peroxidation plays a major role in the toxicity of AFB1, a protective effect of antioxidants is possible. Antioxidants like vitamins (E, A and C), carotenoids, selenium and other antioxidants have an inhibitory action on biotransformation of AFB1 to their active epoxide derivatives, and have beneficial effects in ameliorating the adverse effects resulting from AFB1 (Surai, 2002).

The present work is targeting to clarify two points. The first one, the effect of supplemented dietary aflatoxin on antioxidant system of the liver in chickens. The second, evaluating the chemoprevention efficacy of glutathione (GSH) against aflatoxicosis- induced hepatic dysfunction in laying hens.

MATERIALS AND METHODS

The current study was carried out at the Sakha Poultry Research Station and the laboratories belonging to the Animal and Poultry Research Institute, Agricultural Research Center, Ministry of Agriculture.

Birds and management

A total number of 96 (12 cockerels and 84hens) chicks (Inshas local slow growing strain) at 28 wk of age with similar body weight (1310 ± 20g) , wing banded and randomly divided into 4 experimental groups of 24 (21hens+3 cockerels) birds each (three replicates for

each group) :T1- control (basal diet) ;T2- basal diet+AFB1 (500 ppm/kg diet); T3- basal diet+ 5 ppm GSH / kg diet and T4- basal diet+ 500 ppm AFB1 /kg diet+ 5 ppm GSH . The birds were given the experimental diets from (28-38) wk of age. The birds were housed in floor pens with light program 17 hours/day at laying period. The birds were placed in a room maintained at a constant temperature of 26±3ºC and a relative humidity from 50 to 70% throughout the experiment period. Feed and drinking water were always available ad libitum. The experimental diets were formulated using mainly ground yellow maize and a soybean meal, according to NRC (1994) as shown in Table 1. All the experimental procedures were done according to the institutional regulations concerning animal welfare.

Preparation of aflatoxin B1: Aflatoxin B1 was produced in liquid medium (potato dextrose) by Aspergillus parasiticus (NRRL. 2999) according to the procedure of Ready et al (1971). Aflatoxin B was dissolved in chloroform and quantitatively estimated by thin layer chromatography TLC (AOAC, 2000), and used with the level of (500 ppm/kg diet) to induced mycotoxicosis according to (Hassan, 2005).

Glutathione: Reduced GSH was obtained from Sigma Chemical Co. This antioxidant was used with the level of (5 ppm/kg diet), the GSH level was set according to (El-Barbary and Mohamed, 2014).

Sampling

Body weight was recorded every 7d, and feed intake was recorded daily during the experimental period. Egg production (EP) and egg weight (EW) were recorded daily to calculate egg mass (EM= EP × EW). At the end of the experimental period, 3 birds from each treatment were randomly chosen, weighted and slaughtered. Liver was weighted to calculate the hepatosomatic index (HSI) for each birds according to White and Fletcher (1985) HSI = liver weight (g)/body weight (g) x 100.

Liver and muscle samples were snap-frozen in liquid nitrogen and stored at -80ºC for gene expression analysis. Blood samples were collected into heparinised test-tubes, quickly centrifuged at 4000 g for 10 min at 4ºC to separate plasma, and stored at -20ºC until biochemical analysis.

Biochemical analysis

Lipid peroxidation: Concentration of breast muscle and liver thiobarbituric acid-reactive substances (TBARS) as indicator of lipid peroxidation were measured by the method of Ohkawa et al. (1979). Vitamin E content in plasma and muscles: The α -tocopherol concentration of the breast muscle and plasma were determined by the Shimadzu HPLC model LC6A with a Shim-Pack CLC-ODS column (6.0×150 mm) according to the method described by Faustman et al (1989).

Blood samples were analyzed for alanine aminotransferase, aspartate aminotransferase (AST), the concentrations of plasma total lipds and, antioxidant enzymes (GPX, CAT, SOD and total antioxidant capacity) were determined using calorimetric kits as described by manufacturer's protocol (Stanbio Laboratory Diagnostic, USA).

RNA extraction and real-time PCR. Total RNA was extracted from a piece of liver (about 50 mg) using an ISOGEN II Kit, according to the manufacturer's protocol. RNA concentration and purity were determined by NANODROP LITE Spectrophotometer. The ratio of A260/A280 for all samples was between 1·8 and 2·0. Complementary DNA was synthesized at 800 ng RNA/20 ml of the reaction solution with the PrimeScript ™ RT Master Mix Kit (Perfect Real Time; Takara) using the Program Temp Control System PC320 (Astec, Fukuoka, Japan) with the following protocol: reverse transcription at 37ºC for 15 min;

inactivation of RT at 85ºC for 5 s; refrigeration at 4ºC for 5 min. Real-time PCR primers were prepared as described by Nakashima et al. (2005 and 2006). Gene expression was measured by real-time PCR using the 7300 Real Time PCR system (Applied Biosystems) with the SYBR® Select Master Mix. The thermal cycle was as follows: one cycle at 50ºC for 2 min and 95ºC for 2 min and sixty cycles at 95ºC for 15 s, 60ºC for 15 s and at 72ºC for 1 min. The expression of 18s ribosomal RNA was used as an internal standard and was not significantly different between experimental groups. Results of gene expression are expressed as the percentage of the control value. Primers sequences for antioxdative genes GPX, SOD, CAT are described in Table (2).

Statistical analysis

The differences between the treatment groups and the control group were analyzed with a general linear model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Tukey's multiple comparison test was used to identify which treatment conditions were significantly different from each other at a significance level of $P \leq 0{\cdot}05$.

RESULTS AND DISCUSSION

Performance and egg production:

Data presented in Table (3), shows the effect of the GSH dietary treatments on live body weight (LBW), feed intake (FI), egg number, egg mass and hepatosomatic index.

It is clearly evident that feeding birds on AFB1-supplemented diet dramatically ($P \leq 0.001$) suppressed live body weight and feed intake, while the other treatments onwards. At the end of study average live body weight of AFB1-treated birds hardly reached (8.54 %) of that for control ones. Generally, it could be seen that applied (GSH) significantly ameliorated the deleterious effect of AFB1 on LBW and FI. This results in agreement with (Verma et al., 2004; Hassan, 2005; Qota et al., 2005; Sayed et al., 2008).

Mechanisms suggested for the deleterious effect of AFB_1 on body weight include inhibition of ribonucleic acid (RNA) (Verma et al., 2004) and deoxyribonucleic acid (DNA) synthesis (Gursoy-Yuzugullu et al ., 2014), as well as decreased RNA polymerase activity (Verma et al., 2004). Consequences of partial inhibition of RNA and DNA synthesis involve reduced protein synthesis, which would depress growth and muscle breakdown as result of oxidative stress induced aflatoxicosis.

Results also clearly indicated that dietary AFB1 adversely affect ovarian development and onset of laying especially at early production period. The average egg number / hen for T2 was nearly half that for T1 (= 45.43%). The use of GSH (T4) significantly counteracted the adverse effect of AFB1 since it significantly accelerated sexual maturity and increased number of eggs produced / hen, in a parallel line egg mass was decreased by AF compared with other groups. This result is in general agreement with (Rizzi et al., 2003; Sayed et al., 2008). Garlich et al. (1973) suggested that the decrease in egg production observed in cases of aflatoxicosis in laying hens could be probably due to fatty liver syndrome caused by AFB1. On the other hand, adding GSH (T3) lead to increase LBW, egg number and egg mass significantly ($P \leq 0.001$) by 3.90, 11.67 and 21.18% , as compared with control group, this agree with El-barbary and Mohamed (2014) .

The fatty liver syndrome caused by aflatoxin rapidly causes a liver lesion indicated by rapid increase in serum alkaline phosphatese which is an indicator of liver disorders (Karl, 1968). The liver malfunction results in an increase of liver lipid and a decrease of plasma lipids and proteins which are produced in the liver

and are precursors of the yolk lipids and proteins (Zain, 2011). Moreover, fatty liver produces the characteristic, enlarged, friable, fatty liver associated with aflatoxicosis in broiler (Kubena et al., 1993; Ibrahim et al., 1998).

Like the response observed for performance, dietary AFB1 caused significant hepatomegaly throughout the experiment where the relative weight of the liver (HIS) in birds fed AFB1-contaminated diet (Sayed et al., 2008) reached to 216.67 % of control values Table (3). Our results are consistent with those reported by Ortatatli and Oguz (2001) and Verma et al. (2004) who concluded that AFB_1 caused increased liver relative weight .The beneficial effects of the (GSH) to overcome the negative effects of AFB1 were clear our results,with no significant differences among GSH (T3) and control group, also GSH (T3) was no significant with control group (T1).

Effects of dietary supplementation of glutathione (GSH) on antioxidant system

1- TBARS and α-tocopherol content in chickens

Effects of GSH on muscle and plasma α-tocopherol content and muscle TBRAS are shown in Fig. 1 A, B, and Fig. 2 . Muscle and plasma α-tocopherol content of AF group was significantly ($P \leq 0.01$) lower than the control, while addition GSH to AFB1- contaminated diets helped in overcoming this negative effect of AFB1, thus, muscle and plasma α-tocopherol were maintained at high level compared with AFB1-group (T2) than in GSH groups (T3 and T4) this indicate that the hens did not utilize big amounts of Vit E to overcome the oxidative stress condition induced by AFB1. On the other hand, lipid peroxide level was increased in liver homogenate and muscle of hens fed AFB1 (Fig. 2). Supplementation of the AFB1 diet with GSH reduced ($P \leq 0.05$) peroxidation levels. For that, muscle and plasma TBARS were decreased significantly ($P \leq 0.05$) in GSH groups in both condition when compared with positive and negative control respectively. Also, AFB1 group (T2) revealed the highest value in muscle and liver TBARS compared with other groups.

2- Gene expression encoding antioxidant enzymes in liver and antioxidative status in blood of hens

Fig. 3 shows the effect of GSH on the mRNA of antioxidant enzymes (Fig. 3 A) mRNA GPX, (Fig. 3 B) mRNA SOD and (Fig. 3 C) mRNA CAT. AFB1 group was the lowest value in mRNA GPX, mRNA SOD and mRNA CAT compared with other groups. In general, the antioxidant data suggest that glutathione (GSH) supplementation along with AFB1 (T4) stimulated the antioxidant system in the liver by trying to reach the control value for counteracting the oxidative damage caused by AFB1.

In this respect, glutathione (GSH) alone (T3) was increased significantly ($P \leq 0.01$) mRNA GPX, mRNA SOD and mRNA CAT when compared with control group (T1).

Similarly, antioxidative status in blood (GPX, SOD, CAT and total antioxidant capacity) were tested in the present study as shown in Table 4,were affected like mRNA of antioxidant enzymes by AFB1 when compared with control (T1), also GSH (T3) were enhanced the antioxidative status. The negative effects were an inhibited by addition GSH with AFB1 (T4) compared with control group (T1).

Aflatoxin B1 is known to cause lipid peroxidation in liver (Shen et al., 1994) and is a potent carcinogen that forms adducts with DNA and induces cellular oxidative damage (Imlay and Linn, 1988). An increase in peroxide level in liver due to feeding of AFB1 to rats was associated with decreased in activity of SOD, catalase, glutathione peroxidase, and reductase (Rastogi et al., 2001), and further supplementation of root extracts of

Picrorhiza kurroa and seeds of Silybum marianum ameliorated the effects of AFB1 and reversed peroxide and antioxidant enzymes to control levels. Rosamarinic acid, a phenolic compound which have antioxidant properties present in Boraginaceae species of plants (sage, basil, mint), reduced free radical oxygen formation and apoptosis of human hepatoma cells induced by AFB1 (Renzulli et al., 2004).

Biochemical parameters: Activities AST and total lipids were tested in the present study as shown in Table 5, the activity of AST which is usually used to evaluate the hepatic function, was dramatically significantly ($P \leq 0.05$) increased approximately in all aflatoxicosis hens whether with or without GSH comparing to the control group (T1). These results indicated that AFB1 group (T2) showed higher value of hepatic enzyme than the all control group (T1). On the other hand, ALT was no change in all groups. This confirms the previous report of Abou-El-Soud and El-Lakany (2001).

However, our results are disagree with previous reports by Sayed et al. (2008) who mentioned that the activity of different anabolic and catabolic enzymes as lactate dehydrogenase, ALT, sorbitol dehydrogenase and glutamic dehydrogenase were decreased in cases of aflatoxicosis (Fernandez et al., 1994). ALT has limited value for biochemical diagnosis in birds. Some authors report elevations in serum ALT activity in raptors, chickens, and ducks with hepatic insult. Others believe that ALT is not a useful diagnostic test for liver disease in birds (Lumeij, 1997).

Total lipids: The obtained results indicated that due to consuming AFB1-contaminated diet, serum total lipids content was significantly reduced being more or less equal half the normal values of control birds.

In this study, it has been observed that (GSH) significantly counteracted the effect of AFB1 as the values of serum total lipids in the fourth groups (T4) were insignificantly different from that of control group (T1). Similar results were reported by Oguz et al. (2000) and Ortatatli and Oguz (2001). This may be due to the interference of AFB1 with lipid metabolism as those reported by Hamilton et al. (1974) who explained that lipid transport is inhibited somehow by aflatoxicosis which could account for the accumulation of lipid in the liver and their decreases in the serum.

Role of anti-Oxidant (GSH) in reducing aflatoxicosis in chickens:

Confirmation protective effect of GSH in mycotoxicosis came from the results of Kang and Alexander (1996). Pig kidney cells pretreated with 0.1mM buthionine sulfoximine, A selective inhibitor of the enzyme Y-glutamylcysteine synthetase that catalyses the rate-limiting reaction in de novo GSH synthesis, for 12 hours were significantly sensitized to the FB1 cytotoxicity as determined by a long term survival assay. Therefore GSH may play a role in cytoprotection against FB1 toxicity.

Synthetic antioxidants can also be effective against mycotoxicoses. For example, addition of liver cytosolic fractions prepared from the rats pretreated with high doses of BHT to the cell free system caused a 47% inhibition in AFB1-DNA binding. In contrast, low dose BHT feeding (0.06% for 6 months) had little influence on GSH S-transferase activity (Allameh et al., 2000). Another synthetic antioxidant, BHA, may protect against the toxic effect of AFB1 by preventing the depletion of hepatic GSH (Monroe and Eaton, 1988). It is interesting that species specific differences in both bioactivation and bioinactivation of AFB1 exist. For example, the addition of mouse liver cytosol and reduced glutathione (GSH)

significantly reduced AFB1 dependent genotoxicity, whereas the addition of either human or rat cytosol (GSH) was without effect (Wilson et al., 1997). However, long term GSH treatment had no effect on the survival of AFB1pretreated male rats, on the incidence of liver tumours and on the activities of drug metabolisting systems (Wagner et al., 1985), probably reflecting an inability of GSH to be delivered to the cell directly.

Lipids peroxidation is associated with other forms of mycotoxicoses, including ochratoxicosis (Hoehler and Marquardt, 1996), fumanisin B1 toxicosis (Abado-Becognee et al., 1998) , aflatoxin B1 toxicosis (Harvey et al., 1994) and aurofusarintoxicosis (Surai and Dvorska, 2001).Since lipid peroxidation plays an important role in mycotoxin toxicity, it is expected that antioxidants in the diet will provide a protective effect. The results form a number of experiments in a variety of animal species provide support for this argument. For example, vitamin E supplementation ameliorated the prooxidative effects of ochratoxin A in chikens (Holehler and Maraquardt, 1996) and mice (Grosse et al., 1997). The protective effect of vitamin E was also found in T-2 toxicoses in rats (Rizzo et al., 1994), in mice (Atroshi et al., 1997) and in a cell-line in vito system (Shokri et al., 2000). Vitamin E can also protect against both fumonisin B1 (Mobio et al., 2000) and Aflatoxin B1 (El-Deep et al., 2009).

Surai (2002) reported that in many cases membrane active properties of various mycotoxins determine their toxicity. Consequently, functional alterations in many biochemical pathways and changes in physiological functions including growth, development, reproduction etc. occur. An importance of lipid peroxidation in all these processes is confirmed by protective effects of natural antioxidants against mycotoxin toxicity. However, molecular mechanisms of mycotoxin-antioxidant interactions in vivo await investigation. In our results this was confirmed by reduced performance and lower antioxidative status in AFB1 treated birds and this was partially ameliorated by GSH supplementation.

El-barbary (2010) reported that, the positive effects of both of GSH and GSH-EH to overcome the toxic effects of AFB1 could be attributed to the anti-oxidative properties of these materials. Denzoin et al. (2013) showed that both free GSH (200 mg/kg) and niosomal GSH (14 mg/kg) treatments were highly effective in reducing both hepatotoxicity and hematotoxicity in cats intoxicated with a dose of 150 mg/kg acetaminophen, this are in harmony with the obtained results.

Conclusion, It could be concluding that dietary aflatoxin seriously ameliorate the antioxidative system of the laying hen. This could lead to sever reduction in the performance and eviontialy drop in physiological functions, especially at early stage of sexual maturity. Using dietary antioxidant like glutathione could have chemoprevention efficacy against aflatoxicosis induced hepatic dysfunction in laying hens.

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
Meth. + Cys., %	0.62
Determined analyses***	
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, 10mg; Vit.K_3,1mg; vit.B_1, 1mg; vit. B_2, 5mg; vit.B_6, 1.5mg; vit. B_{12}, 10mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50µg; Choline, 260mg; Copper, 4mg; Iron; 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1.3mg; Selenium, 0.1mg and Cobalt, 0.1mg.

** According to Egyptian Feed Composition Tables (2001)

***According to AOAC (1990)

Table (2): List of primers sequences used for qualitative real time polymerase chain reaction in chickens.

Gene1		2Sequence(5´-3´)	Position (5´-3´)	Size (BP)	Accession no.
GPX	Forward	TTGTAAACATCAGGGGCAAA	165-304	140	NM_001163245.1
	Reverse	TGGGCCAAGATCTTTCTGTAA			
SOD	Forward	AGGGGGTCATCCACTTCC	50-171	122	NM_205064.1
	Reverse	CCCATTTGTGTTGTCTCCAA			
CAT	Forward	GGGGAGCTGTTTACTGCAAG	819-956	138	AJ719360.1
	Reverse	CTTCCATTGGCTATGGCATT			
18s RNA	Forward	AAACGGCTACCACATCCAAG	333-486	154	KC433410.1
	Reverse	CCTCCAATGGATCCTCGTTA			

1Glutathione peroxidase (*GPX*), Cu/Zn-superoxide dismutase (*SOD*), catalase (*CAT*).
2 F = forward; R = reverse.

Table (3): Effect GSH on performance, some egg parametersand and hepatosomatic index of AFs-treated chickens (mean values ± SD).

Treatment	Initial BWT	BWT End	Feed consumption	Egg number	Egg Mass	hepatosomatic index
T1(control)	1301.8±28.7	1373.5±27.7^{a}	6361.0±27.7^{a}	10.19±0.27^{b}	5512.50±287.68^{b}	1.75±0.03^{bc}
T2(AF)	1321.9±11.1	1256.1±13.2^{b}	5727.1±13.2^{c}	5.56±0.22^{c}	1658.58±117.51^{c}	3.17±0.03^{a}
T3(GPX)	1299.4±18.1	1427.0±21.3^{a}	6105.7±21.3^{b}	11.38±0.25^{b}	6680.26±299.29^{b}	1.65±0.03^{c}
T4(AF+GPX)	1332.2±24.6	1401.8±25.5^{a}	5970.9±25.5^{b}	10.24±0.27^{a}	5548.17±317.84^{a}	1.78±0.02^{b}
Sig	N.S	***	***	***	***	***

a,b: Means in the same column followed by different letters are significantly different at $p \leq 0.05$

Table (4): Effect GSH on antioxidative status of AFs-treated chickens (mean values ± SD).

Treatment	GPX	SOD	CAT	TAC
T1(control)	1114.40±13.05^{b}	704.67±2.62^{b}	847.13±14.40^{b}	0.35±0.01^{b}
T2(AF)	950.47±25.29^{c}	616.43±3.01^{c}	812.87±6.25^{c}	0.23±0.03^{c}
T3(GPX)	1264.03±27.27^{a}	735.10±4.81^{a}	906.40±7.10^{a}	0.67±0.01^{a}
T4(AF+GPX)	1137.03±18.52^{b}	760.0±9.45^{a}	953.20±13.59^{a}	0.40±0.03^{b}
Sig	***	***	***	***

a, b: Means in the same column followed by different letters are significantly different at $p \leq 0.05$.

Table (5): Effect GSH on biochemical parameters of AFs-treated chickens (mean values ± SD).

Treatment	lipids	AST
T1(control)	17.00±1.17^{a}	120.87±0.87^{c}
T2(AF)	8.2±1.352^{b}	135.24±0.54^{a}
T3(GPX)	10.67±1.00^{b}	129.46±0.34^{b}
T4(AF+GPX)	8.56±0.95^{b}	117.37±0.72^{d}
Sig	**	***

a, b: Means in the same column followed by different letters are significantly different at $p \leq 0.05$.

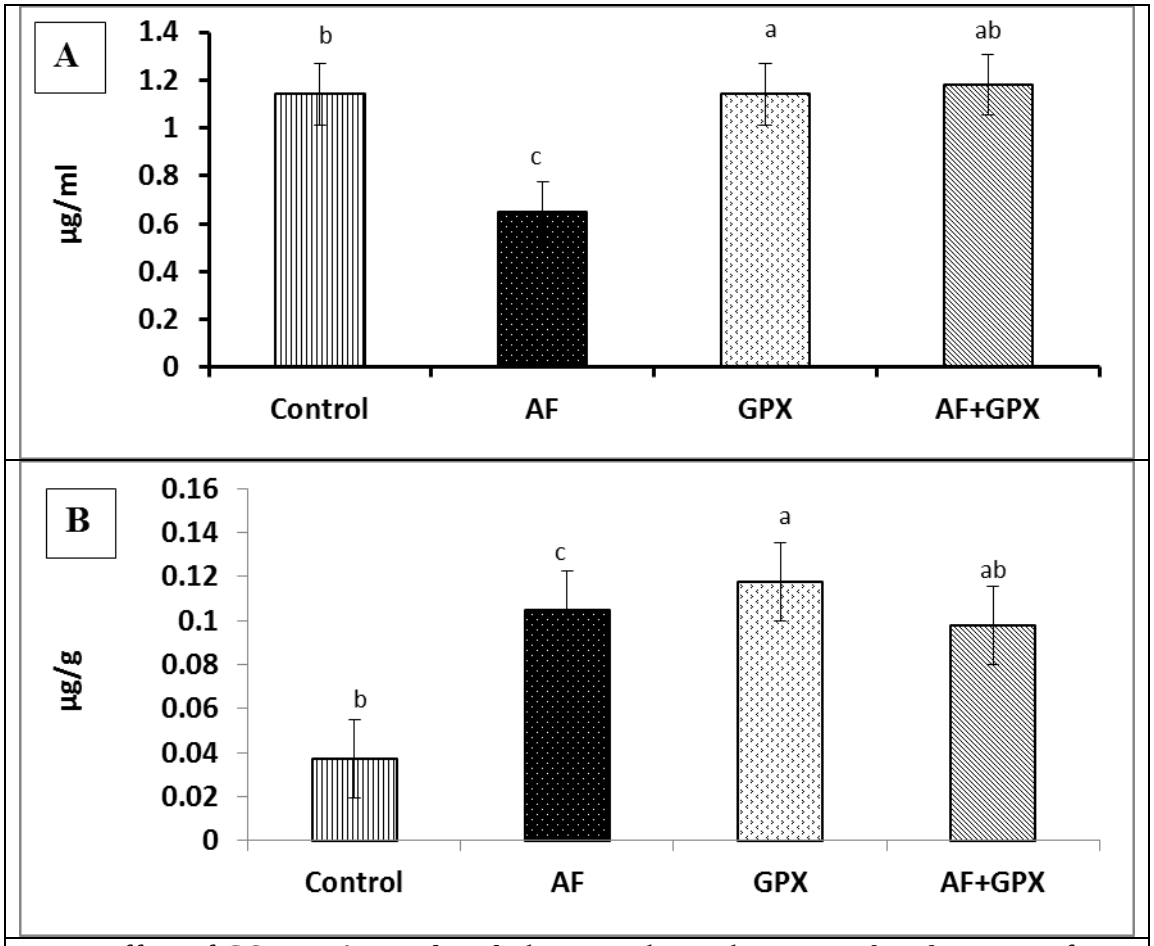

Fig 1: Effect of GSH on AFs-induced plasma and muscles α-tocopherol content of chickens.

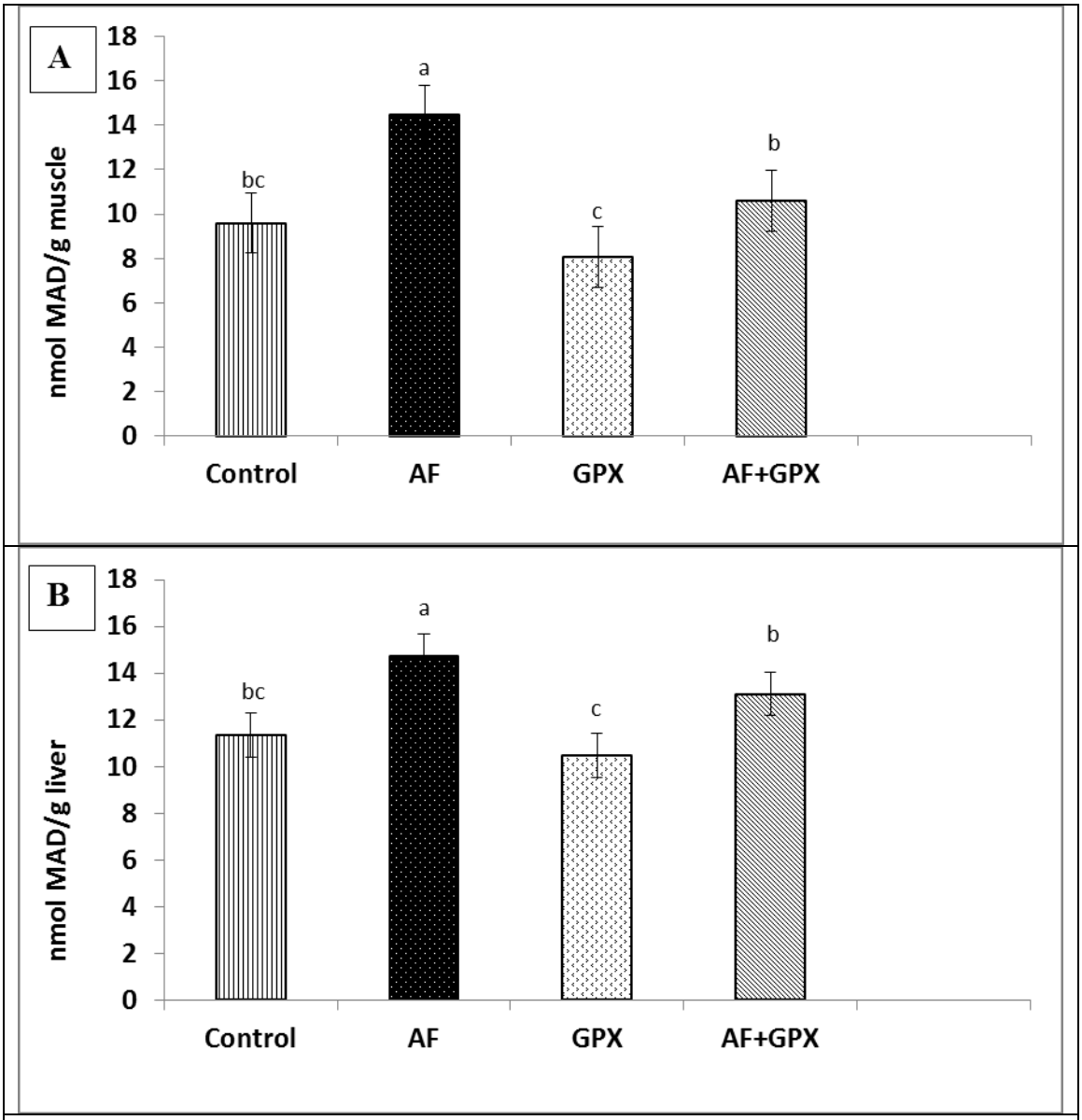

Fig 2: Effect of GSH on AFs-induced thiobarbituric acid reactive substance (TBARS) in muscle and liver of chickens.

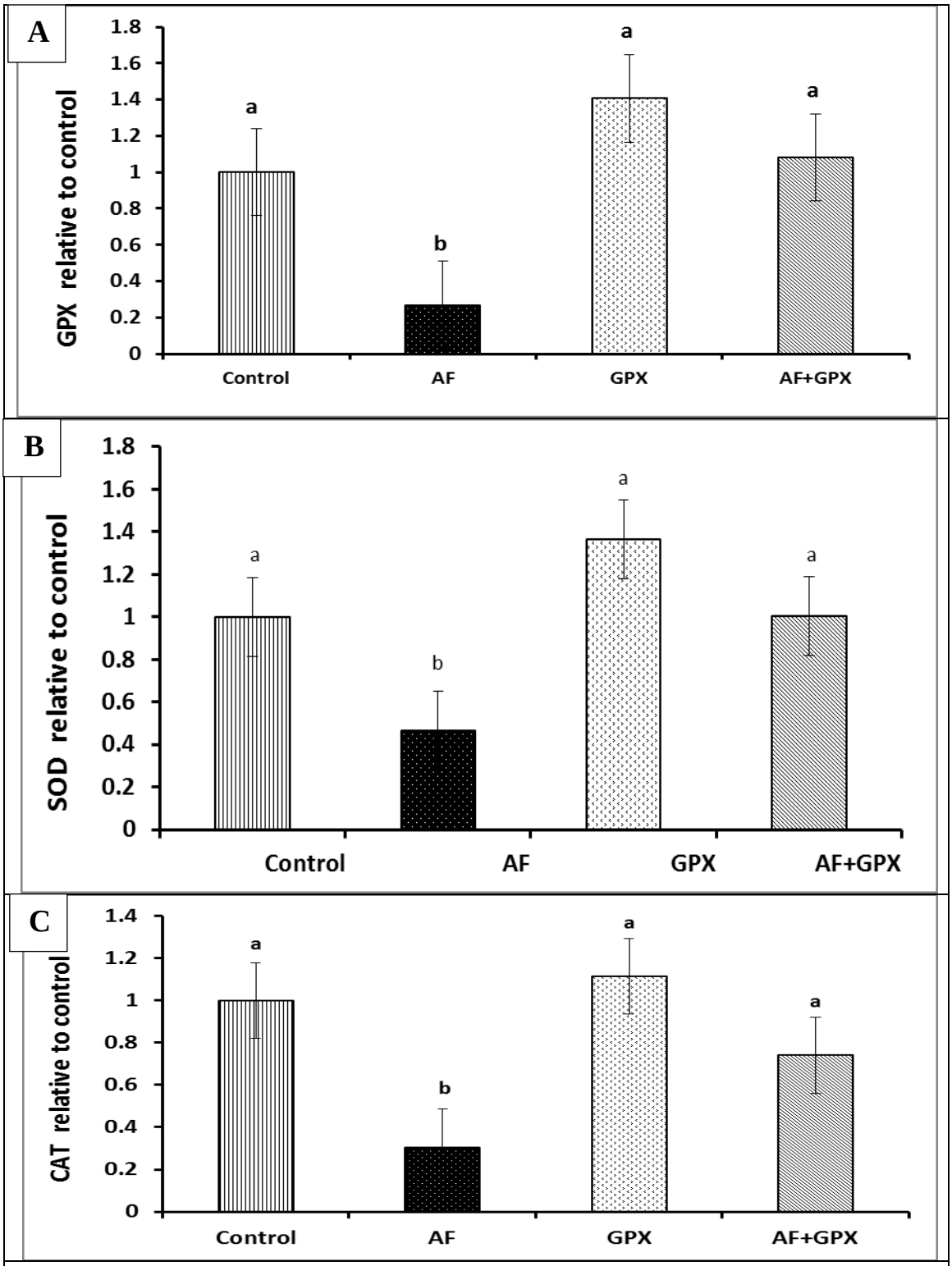

Fig 3: Effect of GSH on AFs-induced antioxidative defense mechanisms {mRNA GPX (A) , mRNA SOD (B) and mRNA CAT(C)} of chickens.

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الملخص العربي

تحفيز الجلوتاثيون للجينات المتعلقه بمضادات الاكسده الكبديه وتقليل من القصور الكبدى الناتج من تناول السموم الفطريه فى علف الدجاج البياض

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أجريت هذه الدراسة لتقييم التأثيرات الواقية من تغذية الجلوتاثيون المختزل (GSH) ضد القصور الكبدي المتعلق بالتسمم ألافلاتوكسيني في الدجاج من سلاله انشاص. واستخدمت PPM٥٠٠ من GSH مع ppm٥٠٠ / كجم عليقة من الأفلاتوكسين(B1 (AFB1) . و تم تقسيم ٩٦ دجاجة إلى ٤ معاملات كل معامله بها ٣ مكررات. كانت T1 كنترول سالب. T2 كنترول موجب (AFB1) و كانت المجموعات T3 و T4 من GSH وAFB1 + GSH على التوالي. وكانت التجربة من عمر (٢٨-٣٨) أسبوع ، وقد تم جمع عينات فى نهايه التحربه لتحليلها. وأظهرت النتائج أن AFB1 ادت الى زياده معنويه فى AST و كرياتينين في الدم و كذلك TBARS في العضلات بينما تم خفض محتوى البلازما α-توكوفيرول، ومضادات الأكسدة enzyms (GSH) و (CAT) و (SOD) و ايضا (total antioxidant capacity) في دم الدجاج. وبالإضافة إلى ذلك، سجلت الآثار السلبية لAFB1 على mRNA لكل من انزيمات(GPX)و (SOD) و(CAT) و كذاك(HSI) وتاكسد الدهون للطيور المسممه بأفلاتوكسين في هذه الدراسة و أظهرت النتائج قدرة GSH عند استخدامه مع تسمم أفلاتوكسيني لمواجهة الآثار السامة لAFB1 الدجاج. بالإضافة إلى ذلك ادى GSH-Px (T3)الى تحسين وضع وحاله مضادات الأكسدة فى الدجاج. وأخيرا، فإن المستويات المثلى من GSH وطريقة استخدامها لإزالة السموم من آثار الأفلاتوكسين بحاجة الى مزيد من الدراسات.