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Diminution of Aflatoxicosis in Rabbits by addition of Glycyrrhizin in their polluted rations.

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ABSTRACT

This study was undertaken to evaluate the effectiveness of glycyrrhizin (GL) in alleviating the adverse effects of aflatoxin B₁ (AFB₁) in male New Zealand white rabbits. Forty-eight New Zealand white rabbits (1.5 ± 0.09 kg body weight and 7-9 weeks of age), were divided into four equal dietary treatment groups, namely T0 (basal diet), T1 (basal diet and GL powder, 500 ppm/kg diet), T2 (basal diet and AFB₁, 0.5 ppm/kg diet) and T3 (basal diet with AFB₁, 0.5 ppm/kg diet and GL, 500 ppm/kg diet). Parameters measured were average feed intake, weight gain, feed conversion ratio, nutrient digestibilities, as well as some biochemical parameters, antioxidants and carcass characteristics. Aflatoxin treatment significantly (P<0.05) increased the activities of serum aminotransferases, alkaline phosphatase as well as serum urea and creatinine concentrations were elevated. Total proteins, albumin levels were decreased; while serum cholesterol levels were not significantly affected by aflatoxin treatment. Levels of malonaldehyde and glutathione increased while superoxide dismutase levels were decreased in T2 and T3 compared to T0 group. Some carcass parameters and chemical composition of meat were closer to the standard values but others were significantly affected. Treatment with GL could diminish the adverse effects of AFB₁ on most of biochemical values, and enzymatic activities in rabbits. Finally, the results determined in the study might be important to demonstrate the effects of aflatoxicosis on some biochemical and antioxidant parameters in rabbits.

Keywords: Aflatoxins, Glycyrrhizin, rabbit, Antioxidant, Aflatoxicosis, Meat, carcass Parameters, Animal performance.

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INTRODUCTION

In recent years commercial rabbit production has been gaining much attention due to their high prolificacy, rapid growth rate, small body size and high meat yields, and can convert 20% of the protein they eat into edible meat which is higher than pigs (16-18%), beef (8-12%) [1]. But the global problem that low-cost rabbit feed constituents, such as maize-milling waste might be infected with moulds, mainly *Aspergillus* and *Penicillium spp.*, and consequently might contain mycotoxins such as aflatoxins [2].

Rabbits are considered of the most sensitive animals to aflatoxicosis. Ingestion of AFs by rabbits showed many effects including reduction of feed intake, poor efficiency of feed conversion and feed efficiency, poor growth, malabsorption of various nutrients, decreased tissues integrity, increased susceptibility to infection, vaccine and drug failure and increased sensitivity to temperature extremes [3].

The LD50 of aflatoxin B1 (AFB1) in rabbits was determined as single oral dose 300 µg kg⁻¹ b.w. [4]. Feeding diet naturally contaminated with 50 µg kg⁻¹ AFB1 has caused lesions in the liver, absence of lobular architecture [5]. However, AFB1 as low as 50 µg kg⁻¹ feed caused high level of morbidity and mortality [6]. Also, AFB1 toxicity caused damage of other tissues, such as kidney, testicles, brain and thyroids [7]. The teratogenic effects of AFB1 were described as enlarged eye sockets and enlarged liver of embryos [8]. The International Agency for Research on Cancer has classified AFB1 as a group I human carcinogen [9].

More attention has focused on the alleviation or prevention of aflatoxicosis by manipulation of dietary nutrients [10-15]. Herbal source has been used by mankind recently at very high rate due to their lower risk benefit ratio if compared to the modern allopathic medicines [16]. Licorice (*Glycyrrhiza glabra* L), a perennial leguminous plant, is the most popular herbal remedy in the Mediterranean and Middle East and it has a long history of medicinal use in both Eastern and Western systems of medicine (used in food and as medicine for thousands of years) [17]. Licorice contains a compound called glycyrrhizin (or glycyrrhizic acid). Licorice extract and its flavonoids were found to have potent hepatoprotective effect against a variety of hepatotoxic agents including carbon tetra chloride and aflatoxin B1 [18]. As the development of the natural medicine, the glycyrrhizin extracted from the licorice root was found to have potential therapeutic value in the treatment of several diseases, especially available for anti-SARS virus [19].

The present work, aims to study the antitoxic and antioxidant efficacy of a substance glycyrrhizin extracted from licorice roots against aflatoxin B1 toxicity at moderate level in New Zealand white rabbits through measuring some biochemical parameters and the antioxidant system, as well as the quality of the meat rabbits from the physical and chemical composition.

MATERIALS AND METHODS

Materials and chemicals

Standard and chemicals: All standards of aflatoxins were purchased from Sigma (USA). All Chemicals and solvents used were of ACS grade. Thin layer TLC aluminum plates recoated with 0.25 mm silica gel 60 (Merck). Source of glycyrrhizin: Licorice plant (*Glycyrrhiza glabra* L) was purchased from a company of medicinal plants (Harras Company, Cairo, Egypt).

Aflatoxin-producing strain: *Aspergillus parasiticus* (*A.parasiticus*) NRRL 2999 was obtained as lyophilized preparation from the Mycotoxin lab. National Research Center, Dokki, Giza, Egypt.

Experimental design and management

Depending on our previous results [11-15], concerning the antitoxic and antioxidant effects of herbs and medicinal plants, this study was achieved.

Animal grouping

In the present study, a total of Fourty eight healthy New Zealand white rabbits weighing 1.5 ± 0.09 kg each and between 7-9 weeks of age were selected from a local private farm of Giza province, Egypt and used

for the study that lasted for four weeks. Prior to the commencement of the experiment the rabbits were prophylactically treated against internal and external parasites by subcutaneous injection of broad spectrum antibiotics (Oxytetracycline L.A) at the rate of 0.2 ml/rabbit. Rabbits were weighed before the beginning of the study and randomly allocated to the four dietary treatment groups with 12 rabbits per treatment in a complete randomized design experiment with three replicates making a total of 12 experimental units. The three rabbits in each hutch were considered an experimental unit and the pooled data for the three rabbits was used in the analysis. Rabbits of each experimental unit were managed intensively and housed a well ventilated laboratory animal house and specially constructed metal cages measuring 150 x 120 x 150 cm with facilities for feeding, drinking and trays for the collection of feces and urine. The cages were kept in open sided house with asbestos roofing sheets. Temperature and humidity of laboratory animal house was adjusted daily at 0800 a.m. and 1600 p.m, for minimum and maximum values by using wet bulb and dry bulb thermometer. The rabbits received appropriate care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health. Rabbits were weighed at the beginning, in the morning before offering any feed or water, and live body weight changes and feed intakes were recorded at biweekly intervals to calculate an average daily weight gain.

Experimental diets: Basal diet

The commercial feed was served as a basal diet and composed of 5% maize, 29.0% of palm kernel cake, 16.0% cottonseed meal, 7.0% soybean meal, 20.5% wheat bran, 15.0% rice bran, 2.5% of oyster shell, 0.5% salt, 4.0% sawdust.

Glycyrrhizin

Glycyrrhizin (GL) powder was added to the basal diet to provide the desired level of 500 ppm/ kg diet.

Preparation of aflatoxin(s) - artificially contaminated ingredient.

Aflatoxins (AFs) which were used in this study were produced by a culture of *Aspergillus parasiticus* NRRL 2999 (obtained from the Mycotoxin Lab., N.R.C., Dokki, Giza, Egypt.) on wheat which was used as a basal material [20]. The infected medium was incubated for 17 days at 28 °C. Qualitative and quantitative assay for the presence of aflatoxins in the contaminated substrate has been carried out using HPLC (Agilent Technologies, Waldbronn, Germany) as recommended by AOAC methods [21]. The obtained data exhibited that each kg of the contaminated wheat material was proved to include AFs, B1; B2; G1 and G2 at levels of 167, 22.4, 75 and 15.5 mg /kg, for the four types, respectively. The AFs within the wheat material contaminated with aflatoxins incorporated into the basal diet in the ratio, 0.299% of the daily ration, providing the desired level of AFB1 used in this study (0.5ppm AFB1/kg diet). The diet containing AFs was analyzed and the presence of parent aflatoxins was confirmed by HPLC.

Preparation of glycyrrhizin (GL) from licorice roots

Glycyrrhizin prepared and extracted according to the procedure of Ong [22]. Briefly, 0.6g of sample was extracted with 20 ml of methanol/water mixture (70:30 v/v) at room temperature for 10 min and centrifuge at 2000 rpm for 10 min. The procedure was repeated three times. The extracts were combined; excess solvent was evaporated with the rotary evaporator and filtered. Total yield of glycyrrhizin is 3.11mg/g plant material.

Feeding system

A total of 100 g feed was supplied to each rabbit per day in two equal portions at rate of 50 g in the morning (0800 a.m) and 50 g in the evening (1600 p.m) to reduce wastage and to meet NRC nutritional requirements [23]. The Four equal groups were received the daily ration as shown in table 1:

Table 1: Ingredient composition and %, proximate composition of the New Zealand white rabbits experimental diet (% D.M.) during four week study.

| Parameters | Groups | | | |
|--|-----------------------|-----------------------|---------------------|------------------------------|
| | Negative Control (T0) | Positive Control (T1) | Aflatoxin only (T2) | Aflatoxin +Glycyrrhizin (T3) |
| <u>^aIngredient composition</u> | | | | |
| Commercial feed, g | 100 | 100 | 99.701 | 99.001 |
| Glycyrrhizin, ppm/kg diet | 0.0 | 500 | 0.0 | 500 |
| ^b Aflatoxins- contaminated material | 0.0 | 0.0 | 0.299 | 0.299 |
| <u>proximate composition</u> | | | | |
| Dry matter (g/100 g sample) | 89.92 | | | |
| Nutrient composition (g/100 g DM): | | | | |
| Organic matter (OM) | 84.12 | | | |
| Crude protein (CP) | 18 | | | |
| Crude fiber (CF) | 7.5 | | | |
| Ether extract (EE) | 5.3 | | | |
| Nitrogen free extract (NFE) | 53.32 | | | |

N.B. a- All ingredients except AFB1-contaminated material were aflatoxins- free.

b- aflatoxins-contaminated material was incorporated into the basal diet in the ratio 0.299% of the daily ration to provide the desired level of 0.838 mg total aflatoxins or 0.05 mg AFB1/ 100g diet.

Water was provided ad libitum. Orts were collected and weighed daily in order to determine the daily feed intake. Commercial diet was in separate feeder. Any feed left over at the beginning of the next day was weighed and subtracted from that which had been fed the previous day to determine feed intake. Feed intake was recorded daily for each cage and pooled for 14 days.

Digestibility trial

At the end of the 3rd week of the treatment period, all animals from each group were taken to determine the digestibility and nutritive value of experimental diets. Samples of rations, refusal, feces and urine were taken daily, for seven days. Refusals were collected daily at 07.30h, weighed, sampled, and then stored. Total daily fecal output for each animal was also collected, weighed, homogenized and 10% of feces samples were dried at 70^o C for 24 hr, then blended and kept for aflatoxins (AFs) analysis according to AOAC methods [21]. Urine was collected from a sealed brown color bottle, which was also placed 1-day before the toxin administration. Different parameters of digestibility of the different four feeding treatments were measured as: dry matter intake (DMI), digestible crude protein intake (DCP, %), digestible crude fibre (DCF), digestible nitrogen-free energy (DNFE) and digestible crude ether extract (DCEE).

Digestibility coefficient = (Nutrient digested / Nutrient intake) x 100.

Feed conversion ratio/efficiency: Feed conversion efficiency is calculated as the quantity of feed that will produce 1kg weight gain. This was computed using the expression:

$$F.C.R = \text{Total feed intake (g)} / \text{Total weight gain (g)}.$$

Blood samples

Blood samples were collected from each rabbit from the marginal ear vein, at weekly intervals from day 0 up to week 4, early in the morning prior to feeding and watering. Sera were separated and frozen at -20 °C until it was analyzed for the biochemical analysis and aflatoxins and their metabolites (AFB 1, aflatoxin B2α and aflatoxicol).

Analytical methods

Aflatoxins analysis

Aflatoxin(s) B1, B2 G1 and G2 in feed and feces samples were extracted by B.F. method as described in AOAC methods [24]. Extracts were dissolved in chloroform, shaken in vortex; 20 μ l aliquot and 10 μ of the standards were stopped on TLC plates and developed in dark room with chloroform: acetone (90:10). After drying the spots were examined with U.V at A wave length of 365 nm.

Aflatoxin B2a and aflatoxicol were analyzed in the feces and urine samples according to method of Richarda and Lyona [25].

The chemical composition of the diet and feces

The chemical analysis of feeds and feces were carried out according to AOAC methods [24]. Feed samples were analyzed for determination of dry matter (DM), organic matter (OM), percentage of crude protein (CP), ether extract (EE), crude fibers (CF), and nitrogen-free extract (NFE) [21]. Feed and feces were successively ground in mills with 3- and 1-mm screens. Nitrogen was determined using the standard Kjeldahl procedure with K₂SO₄ and CuSO₄ as catalysts. The OM was determined by ashing at 550°C overnight.

Determination of several metabolic variables

The profiles of several metabolic variables were measured calorimetrically in the sera samples of each animal using spectrophotometer (Instruction Manual UV-1201, Shimadzu) and commercial kits. Urea [26], Creatinine [27], superoxide dismutase (SOD) [28], Alkaline Phosphatase (ALP) [29], Transaminases [30] (Alanine Transaminase, ALT and Aspartate Transaminase, AST), Cholesterol [31], total protein [32], albumin [33], malondialdehyde (MDA) [34] and glutathione (GSH) [35]; were measured using commercial Randox diagnostic kits, based on the manufacturer's procedure.

Carcass characteristics

Three animals from each group were slaughtered on day 28 of the experiment. Carcass parameters such as slaughter weight, total meat, and weight of offal's such as liver, heart, lungs with trachea were studied according to Gerrard [36].

Chemical composition of meat and bone were analyzed from the meat and bone collected. Protein and fat content in Longissimus dorsi muscle was analyzed. Calcium and phosphorous content of femur bone was analyzed according to AOAC methods [21].

Statistical analysis

All data generated were subjected to one-way analysis of variance (ANOVA) according to Steel and Torrie [37] and where significant differences were indicated, Duncan's multiple range test (Duncan) [38] was used to separate the means. All data were represented by means \pm standard error (SE). All differences were considered statistically significant at ($P < 0.05$).

RESULTS AND DISCUSSION

Efficacy of ingesting aflatoxin B1 (AFB1) and glycyrrhizin (GL) on the feed intake, growth rate, nutrient digestibilities and feed conversion ratio of treated rabbits:

Data represented in table 2 and figure. 1 clearly indicated that there were significant ($P < 0.05$) differences in the feed intake, growth rate, nutrient digestibilities and feed conversion ratio among the all tested groups. A lower ($P < 0.05$) in feed intake, growth rate and feed conversion ratio (Table 2); was observed in rabbits treated with aflatoxin only (T2) than other three experimental groups. There were however no differences in daily feed intake, feed conversion ratio observed in rabbits fed diet containing aflatoxins + glycyrrhizin, when compared with rabbit groups fed the control diet (T0 & T1). Averages of matter intake, daily gain, crude protein intake, and feed conversion ratio and energy intake of the New Zealand white rabbits are presented in table 2. Except for the aflatoxin only treated group, there were no significant differences in these parameters for the other three experimental groups. The decline in DM intake observed in this study for rabbits treated with aflatoxins only, may be as a result of physical barrier to feed intake. The total CP and

energy consumptions per day were also decreased as the voluntary feed intake decreased. The dry matter intake of the rabbit ranged from 77.06 g to 92.4 g/day/rabbit and this was higher in the range than those observed by Omole et al [39], and this may be due to difference in the experimental conditions such as feeding type..etc. The daily feed intake and feed conversion ratio obtained in our study tally with the values reported by Onifade and Tewe [40], who fed diets containing about 30 % of maize offal to growing rabbits.

Table 2: Average daily of dry matter intakes, body weight gain, energy intake, feed conversion ratio; and Mean nutrient digestibility coefficients of the four experimental groups during experiment (n = 3).

| Parameters | Diets | | | | LSD (p<0.05) |
|------------------------------------|-------------|-------------|-------------|-------------|--------------|
| | T0 | T1 | T2 | T3 | |
| DM intake | | | | | |
| g/day/rabbit | 92.4 ±7.13a | 91.80±4.63a | 77.06±7.13b | 86.92±8.36a | 7.56 |
| g/day/kg live weight | 83.1 ±4.06a | 82.56±6.18a | 69.30±3.11b | 78.15±5.17a | 7.11 |
| Daily gain: g/d/rabbit | | | | | |
| CP Intake g/day/rabbit | 17.87±2.01a | 18.53±2.15a | 10.13±1.08b | 14.66±2.16a | 2.89 |
| Feed Conversion Ratio (FCR) | 16.63±1.24a | 16.52±1.94a | 13.87±2.14b | 15.65±2.51a | 1.66 |
| Energy Intake Kcal/day | 5.42 ±0.96a | 5.65 ±1.02a | 3.68 ±0.05b | 4.72 ±2.66a | 0.99 |
| | 299.45a | 299.25a | 236.55b | 273.88a | 12.47 |

N.B. - a, b, c Means in a row with common letter do not differ (P > 0.05).

DMD = Dry matter digestibility, CP = Crude protein, CF = Crude fibre, EE = Ether extract.

DM, OM, CP, CF, EE and NFE; are means of dry matter, organic matter, crude protein, crude fiber, ether extract, and nitrogen free extract, respectively.

DE = 4.36-0.049×NDF = 28.924 + 0.657 (CF %) according to **Cheeke (1987)**.

There is a general agreement that dietary aflatoxins reduce weight gain, feed intake, and increase feed conversion ratio. Various reports on effects of aflatoxins on bird performance have been previously reviewed by some investigators. For instance, Raju and Devegowda [41] noted 21% decrease in final body weight at 35 days age in broilers fed on 0.3 mg AFB1/kg diet. In contrast, Tedesco et al [42] noted only 10% reduction in weight gain of broilers at 28 days of exposure to 0.8 mg AFB1/kg diet. Also, Zhao et al [43] found that levels of AFB1 of 1 mg/kg diet, after 21 days of exposure, caused 10% reduction in weight gain. While 15% reduction at 42 days exposure was noted by Denli et al [44]. On the other hand, Valdivia et al [45] noted higher levels of 3 mg AFB1/kg diet, only 11% reduction in weight gain at 21 days exposure. Similarly, Miazzo et al [46] found 11% reduction in weight gain when 2.5 mg AFB1/kg diet was fed to broilers from 21 to 42 days of age. From these reports, it is evident that both the level and length of AFB1 exposure affect the amount of reduction in weight gain of broilers. Furthermore, different type of and rations used in different studies make it impractical to generalize the dose-response relationship regarding weight gain.

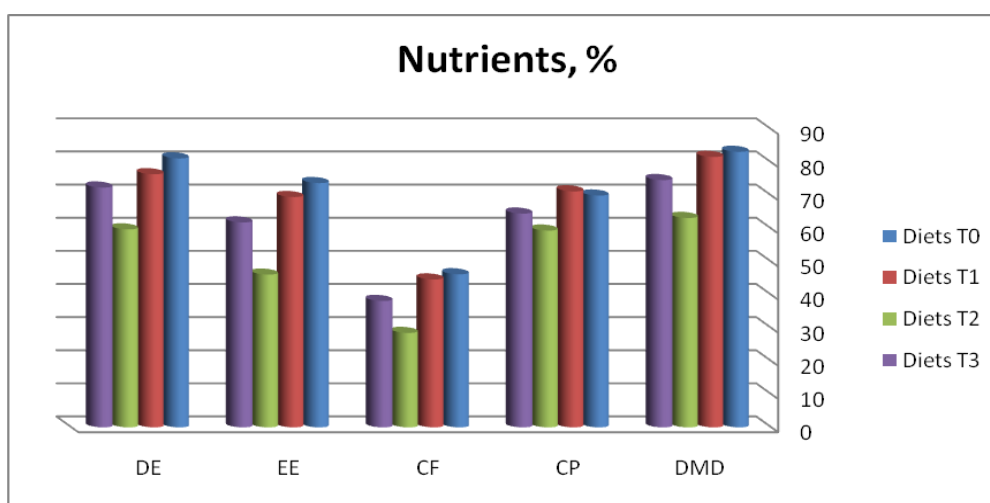


Figure 1: Mean nutrient digestibility coefficients of the four experimental groups during four weeks experiment, where: DE, EE, CF, CP and DM means digestibility coefficients for energy, ether extract, crude fibre, crude protein and dry matter; respectively.

As shown in figure. 1, Dry matter, crude protein, crude fibre and ether extract digestibility were significantly different ($P < 0.05$) among treatments. Rabbits of negative and positive control groups (T₀, T₁; respectively) showed a consistently higher nutrient digestibility values ($P < 0.05$) than the two other groups. However, the dry matter digestibility, crude fibre and ether extract digestibilities of groups: T₀, T₁, T₂; were not significantly different ($P > 0.05$).

Apparent nutrient digestibility showed in this study followed the same pattern mentioned above. The results in figure. 1 indicated that rabbits in groups fed diets of aflatoxin free (T₀, T₁) had better nutrient digestibilities than those fed either aflatoxin only or with glycyrrhizin. These results obtained here clearly emphasize the negative role of aflatoxins on the performance of rabbits, specially, if their diet contained this level of contamination. Generally, the coefficients decreased in value with the presence of aflatoxin contaminated diet in this experiment.

As general, the result from this part of the study showed that there is a great potential for glycyrrhizin to improvement feed intake, growth rate and nutrient digestibilities of New Zealand white rabbits.

Biochemical changes in different studied groups as affected by ingesting aflatoxins and glycyrrhizin

Oxidative damage mainly causes dysfunction of cellular components such as enzymes, nucleic acids, membranes and proteins [47]. In the present study, the liver functions were examined through the determination of alanine amino transferase (ALT) aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities which known as the most sensitive biomarkers enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage, therefore both enzymes are used as indicator for hepatic damage [48]. Data are shown in figure. 2 clearly indicate that treatment with aflatoxins only (T₂) significantly increased blood levels of ALT, AST and ALP activities. These finding, in part of ALT and AST activities, were in accordance with those obtained by Kececi et al [49] and Oguz et al [50] in intoxicated rabbits. The observed elevation in the enzymes (AST, ALT and ALP) in aflatoxin administered group (T₂) may be due to liver dysfunction and disturbance in the biosynthesis of these enzymes which all are indicative of liver damage and thus impaired liver function. Several studies on the mechanisms of aflatoxins induced liver injury have demonstrated that in animals fed diets contaminated with toxicants, the serum levels of these enzymes increased after liver damage because of increased membrane permeability or because of liver cell necrosis and cytosol leakage into the serum [10, 51,52]. Damage of cellular components may play an important role in death of liver cells [51], hence, ALT and AST may be released to serum levels of these enzymes would increase. The increase in the activity of ALP in blood might be due to the necrosis of liver, kidney and lung [53]. Adding glycyrrhizin to aflatoxin-contaminated diet (Group T₃) resulted in normalized activity in ALT, AST, ALP indicating improved liver function and protection against aflatoxin toxicity. This activity shows the hepatoprotective effect of glycyrrhizin in aflatoxin treated rabbits.

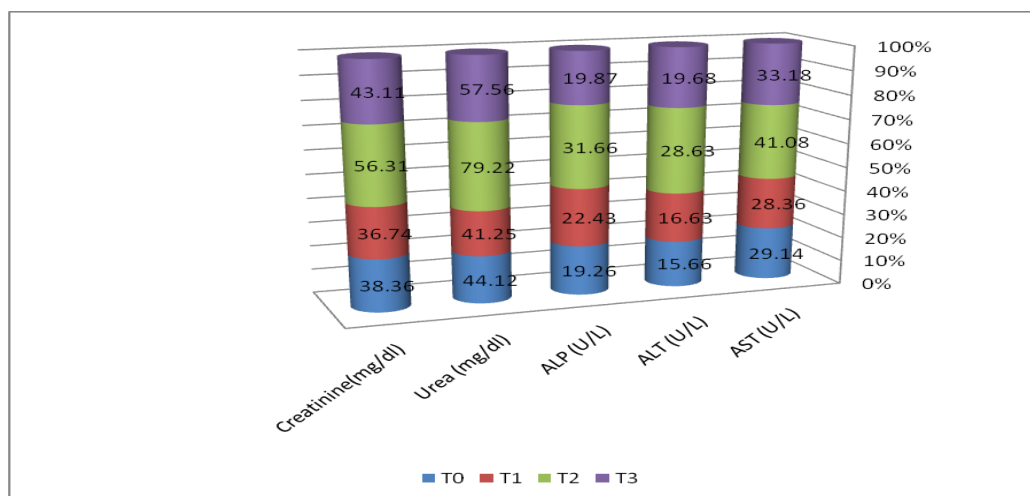


Figure 2: Effect of Glycyrrhizin and /or AFB1 treatments, on serum enzyme biomarkers, urea and creatinine levels of the experimental four groups during four weeks experiment.

The increase in the activities of these ALT, AST, and ALP enzymes and the decrease in the blood total protein and albumin levels (Table 3 & Figure 2), caused by aflatoxin B1 intoxication is in concordance with earlier reports of Yousef et al [54], who found that total proteins, albumin and glucose concentrations were decreased but ALT and AST activities in rabbits were increased with aflatoxicosis. On the other hand, Soliman et al⁵⁵ found an increase in AST, but no changes in serum total protein, albumin concentration in rabbits with aflatoxicosis. Furthermore Abdel-Fattah et al [11-15] studied aflatoxicosis in rats and described a decrease in total protein, albumin, cholesterol and triglyceride levels, but increased ALT and AST were determined. Increased ALT is indicative of liver damage and the change may also be due to aflatoxin B1-induced vascular changes leading to hepatic vascular congestion. Increased AST activity signifies muscular damage [56].

Urea and creatinine, which depend on glomerular filtration for their excretion, accumulate almost in proportion to the number of nephrons that have been destroyed and hence directly reflect the functional status of the kidneys. Aflatoxin treatment significantly ($p < 0.05$) increased serum concentrations of urea and creatinine in the T2 and T3 groups and there were significant ($p < 0.05$) differences between the two groups (Figure 2). Contrary, there were no significant changes observed for the two other control groups (T0, & T1). These results were in conformity with those reported by Abdel-Fattah et al [14, 15]. Treatment of the intoxicated rabbits with glycyrrhizin in our study resulted in significant improvement in kidney function as indicated by the marked decrease in serum urea and creatinine levels. These results were in agreement with those reported by Yokozawa et al [57] and Abdel-Fattah et al [15], who demonstrated that active component, saponin, in white ginseng roots could significantly reduce the blood urea nitrogen and creatinine levels in the blood of nephrectomized rats. Increased levels of urea and creatinine may indicate protein catabolism and/or renal dysfunction [11, 12, 14].

Earlier studies shown that feeding diet naturally contaminated with 50 $\mu\text{g kg}^{-1}$ AFB1 has caused lesions in the liver, absence of lobular architecture [5]. Also, AFB1 toxicity caused damage of other tissues, such as kidney, testicles, brain and thyroids [7].

Data presented in table (3) clearly indicate serum cholesterol levels were not significantly changed in this study. On the other hand, aflatoxin treatment decreased serum albumin and total protein. Albumin is considered the most abundant protein in plasma and synthesized in the liver. Little albumin is filtered through the kidney glomeruli and most of that is reabsorbed by proximal tubule cells and degraded by their lysosomal enzymes into fragment that are returned to the circulation [58].

The decreased serum total protein, albumin, and increased ALT, AST and ALP activities observed in our study (Table 3 & Figure 2), may be due to the hepatotoxic effect of AFB1 characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism. This finding may be in correct with that observed by Rastogi et al [47] and Basmacioglu et al [59], who found that oxidative damage mainly causes dysfunction of cellular components such as enzymes, nucleic acids, membranes and proteins.

Table 3: Effect of Glycyrrhizin and /or AFB1 treatments on some biochemical parameters of the experimental groups (n =3)

| Parameters | T0 | T1 | T2 | T3 | LSD ($p < 0.05$) |
|---------------------|-------------------------|------------------------|-------------|-------------|-----------------------|
| Cholesterol(mg/d) | 77.13±5.03a | 79.16±2.11a | 71.66±3.5a | 69.91±1.66a | 10.4 |
| Albumin (g/l) | 3.46± 0.17a | 3.42±0.53a | 3.08±0.4b | 3.30 ±0.43a | 0.096 |
| Total Protein (g/l) | 6.05 ±0.04 ^a | 6.08±0.08 ^a | 5.19±0.64b | 5.81 ±0.11a | 0.35 |
| MDA(nmol/l) | 4.01±0.14a | 4.11±0.45a | 3.01±0.16b | 3.45 ±0.03b | 0.041 |
| GSH (mg/dl) | 35.13±0.93a | 34.99±1.08a | 23.43±1.11b | 31.16±0.09a | 3.61 |
| SOD (U/ml) | 0.28±0.05a | 0.28±0.08a | 0.22±0.03b | 0.26±0.05a | 0.03 |

N.B: The same small letters in a row denotes no significant difference between treatments in the same row at ($P > 0.05$) and vice versa.

We suggest that glycyrrhizin has a potent protective action against AFB1-induced toxicity and may displays a pronounced hepatoprotective effect, assessed through the ALT, AST and ALP activities following hepatotoxicity in New Zealand white rabbits treated with aflatoxin contaminated diets.

In the present study, to describe cellular lipid damage caused by aflatoxin, MDA values were determined. Values of the sera MDA, GSH and SOD obtained from control and experimental groups were significantly affected by dietary treatment of glycyrrhizin (500 ppm/ kg diet) and / or aflatoxins added to rabbit

feed at level, 0. 5ppm / kg diet (Table 3). In the negative (T0) and positive (T1) control groups, the biochemical parameters and the antioxidant system were not significantly changed, compared with two other treated groups (T2, T3). The MDA level was higher in groups (T0 & T1), compared to the two other experimental groups (Table 3). GSH and SOD values decreased in aflatoxin treated groups, but the decrease found in the group T3 (fed aflatoxins + glycyrrhizin) was none significant, compared to the control groups (T0 & T1). These results were in accordance with those obtained by Choudhary and Verma [60], who studied aflatoxicosis in mice and found increased lipid peroxidation and decreased nonenzymatic antioxidants such as glutathione, ascorbic acid and enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase. Similarly, Rastogi et al [47] found that that aflatoxin causes reduction in SOD, glutathione- S-transferase, glutathione peroxidase and glutathione reductase activities. These findings supported the idea of reactive oxygen as a mean of cytotoxic effect of aflatoxin.

It is of interest to note that the effects of aflatoxin on both antioxidant system and biochemical parameters depend on the amount of aflatoxin, animal species and duration of toxicity. As a result, findings presented in this study contribute to the overall literature and also chronic intoxications can be diagnosed before clinical sings occurred.

Changes in Carcass parameters and meat composition as affected by ingesting aflatoxins and glycyrrhizin

The results of carcass parameters and meat composition are shown in table 4. In this study, except for the aflatoxin only treated group (T2), the results of carcass parameters showed no significant difference between the other three test groups.

The results of meat composition such as dry matter ranged from 26.14 % to 24.36 % and crude protein (21.84 -18.15) are in concurrence with Kiran [17], who reported the dry matter content of 26.05 and crude protein content of 21.52 in the meat of broiler rabbits. Whereas total ash ranged from 48.06 % to 47.33 %, calcium (19.23-17.89) and phosphorous (7.96-7.01) in the bone and were higher than the values of total ash (43.59), calcium (16.27) and phosphorous (6.17) in the bone if compared to the results obtained by Kiran [17] and differences may be a result of differences in experimental conditions.

Table 4: Effect of Glycyrrhizin and /or AFB1 treatment on carcass parameters and meat composition of the experimental groups (n=3).

| Item | T0 | T1 | T2 | T3 | LSD (p<0.05) |
|----------------------------------|---------------|---------------|---------------|---------------|--------------|
| Live weight (g) | 1968±56.45a | 1989±88.43a | 1657±56.77b | 1895±61.13a | 155.32 |
| Carcass weight (g) | 877.8±56.02a | 869.04±38.25a | 816.62±43.11b | 869.25±32.99a | 36.42 |
| Offal's weight (g) | 56.72±9.15a | 53.87±4.50a | 51.45±10.4a | 53.02±5.77a | 4.55 |
| Carcass-offal's weight (g) | 821.08±74.2a | 815.17±51.22a | 765.17±36.23b | 816.23±35.78a | 38.25 |
| Meat (g) | 504.73±31.22a | 500.57±36.15a | 458.37±43.2b | 494.51±28.19a | 28.99 |
| Bone (g) | 61.44±8.56a | 59.04±11.05a | 57.12±6.23a | 59.11±8.11a | 5.3 |
| Meat chemical composition | | | | | |
| Dry matter | 26.14±8.31a | 26.03±9.12a | 24.36±4.58b | 25.16±5.44a | 1.01 |
| Crude protein | 21.84±4.63a | 21.17±3.21a | 18.15±4.25b | 21.33±4.11a | 2.75 |
| Fat | 1.69±0.03a | 1.67±0.01a | 1.35±0.2b | 1.66±0.09a | 0.09 |
| Ash (bone) | 48.06±7.05a | 48.16±5.12a | 47.88±6.44a | 47.33±9.12a | 3.11 |
| Acid insoluble ash (bone) | 0.42±0.03a | 0.42±0.03a | .043±0.02a | 0.41±0.05a | 0.05 |
| Calcium (bone) | 19.23±2.11a | 18.66±4.62a | 18.75±3.11a | 17.89±4.18a | 1.66 |
| Phosphorous(bone) | 7.96±1.33a | 7.53±2.13a | 7.15±0.96a | 7.01±1.45a | 1.54 |

N.B: - The same small litters in a row denotes no significant difference between treatments in the same raw at (P > 0.05) and vice versa.

The results of meat composition did not differ significantly between the groups T0, T1, T2 and T3, and were in accordance with Dal Bosco et al [61], who did not find significant effect on composition of rabbit meat due to the addition of antioxidant. Pla, et al. [62] reported that chemical composition of rabbit meat varies extremely with lipid composition ranging from 3.6-8%. These results clearly indicate that treatment with AFB1 and / or glycyrrhizin had no significant changes in meat chemical composition except for DM, CP and fat. However, the chemical composition of meat is higher compared to those obtained by Kalita et al [63] and higher levels obtained in our study may be due to higher energy intake, higher body weight gain. Dersjant et al [64] in their review concluded that AFB1 has the capability to reduce broiler performance and increase the

incidence of bruising in carcass when present at levels of more than 0.5 mg/kg diet. Also, they reported that each mg of AFB1/kg diet would decrease the growth performance of broilers by 5% However, data published during last decade regarding effect of low doses of AFB1 on weight gain is not consistent with this generalization.

Residues of aflatoxin B1 and its corresponding metabolites (aflatoxicol, AFB_{2α}) of different studied animal groups fed aflatoxins with or without glycyrrhizin for four weeks.

In the present study the aflatoxin residue in the breast muscle was not observed in the all aflatoxin treated groups. It is of interest to mention that aflatoxin residue was decreased with increasing age of rabbits (Table 5). Tebeb and Fakhry [65] reported that in breast muscle, aflatoxin residue was related to concentration of aflatoxin in the diet. Similarly, Bonomi et al [66], mentioned that residues of AF B1 were obtained only in liver, kidneys and longissimus dorsi muscle of white male pigs fed diets containing 500, 650 or 800 ppb aflatoxins B1+G1. Values were directly related to the level of contamination.

Table 5: Proportional urinary (ng/ml) and fecal excretion of aflatoxin B1, and its corresponding metabolites (aflatoxicol, AFB_{2α}) of different studied animal groups fed aflatoxins with or without glycyrrhizin for four weeks (n = 3).

| Items | Groups | | | | LSD (p<0.05) |
|--|-----------------------|-----------------------|----------------|----------------|--------------|
| | T0 Means± | T1 Means±SE | T2 Means±SE | T3 Means±SE | |
| Average daily DM intake, g | 92.4±8.6 ^a | 91.8±6.5 ^a | 77.06±5.2a | 86.90±6.3a | 5.14 |
| 2-Average daily intake as AFB1(µg /head) | | | | | |
| 3- Average daily excreted in feces (ng/ml/head) | 0.0a | 0.0a | 38.53±2.3b | 43.45±3.8b | 3.89 |
| AFB1 excreted | | | | | |
| Aflatoxicol excreted | 0.0a | 0.0a | 289.26±11.7b | 342.67±15.4c | 26.4 |
| AFB _{2α} excreted | 0.0a | 0.0a | 0.0a | 0.0a | - |
| 4- Average daily excreted in urine(ng/ml/head) | 0.0a | 0.0a | 0.0a | 0.0a | - |
| AFB1excreted | | | | | |
| Aflatoxicol excreted | 0.0a | 0.0a | 103.24±8.6b | 209.12±11.4c | 41.03 |
| AFB _{2α} excreted | 0.0a | 0.0a | 77.16±7.1b | 246.97±13.5c | 39.17 |
| 5- Total average daily as AFB1 (µg /head/day). | 0.0 | 0.0 | 0.0 | 0.0 | - |
| In feces | | | | | |
| In urine | 0.0a | 0.0a | 7.81±0.9b | 10.28±1.4c | 1.96 |
| Total | 0.0a | 0.0a | 8.86±0.07b | 22.30±3.7c | 11.04 |
| 6- %, recovery in feces and urine of total excreted | 0.0a | 0.0a | 16.67±4.6b | 32.58±3.11c | 10.25 |
| In feces | | | | | |
| In urine | 0.0a | 0.0a | 46.85±6.5b | 31.55±3.8c | 9.53 |
| | 0.0a | 0.0a | 53.15±3.9b | 68.45±7.2c | 12.46 |
| Total recovery,% | 0.0a | 0.0a | 43.26b | 74.98c | 8.96 |

N.B: 1- The same small letters in columns denotes no significant difference between treatments in the same row at (p ≤ 0.05) and vice versa.

2- Excretion of aflatoxicol expressed in terms of AFB1 equivalents as calculated from the molecular B. weight of AFB1 /molecular weight of aflatoxicol (412/436) x µg of Aflatoxicol.

3- Total recovery = (AFB1 intake / AFB1 excreted) x 100.

Represented data in table (5) showed that the proportional excretions of AFB₁ and its metabolites (AFB_{2α} and aflatoxicol) via feces and urine were varied according to dietary treatment. The cumulative excretion of AFB_{2α} is expressed in terms of AFB₁ equivalents. AFB1 excreted in urine was found to be 53.61 %, 68.93 %, for T2 and T3 groups, respectively. Whereas total amounts of AFB1 excreted in feces were recorded 46.39 % and 31.07 %, for the same groups, respectively. These findings clearly indicate that urine was the main route for AFB1 excretion. Our results were in the same accordance with those obtained by Abdel-Fattah et al [11], on rats.

Results shown in table (5) clearly indicate that approximately 77.16 and 246.79 ng/ ml, was found as aflatoxicol in urine, for T2 and T3 groups, respectively. Whereas no amount was found as aflatoxicol in feces and this indicate that treatment with glycyrrhizin in group treated with dietary aflatoxin (T3), had affected the route of aflatoxin excretion and metabolism where major the most of the excreted AFB₁ was found as the metabolite aflatoxicol in the urine. No AFB_{2α} amount was detected in feces or urine samples tested. These results indicate that the major excretory route was found to be the urine. Treatment with glycyrrhizin improved the AF excretion via feces and urine in identifiable forms, mainly aflatoxicol and unchanged AFB₁ without any detectable amount of AFB_{2α}. In this respect, our results were in contrast with those observed by Richarda and Lyona [25] in pigs and were in accordance with our previous results [11].

Fecal excretion of aflatoxicol reduced significantly by the glycyrrhizin treatment, this might be explained by a more pronounced renal elimination, which in turn might result in lower biliary secretion of aflatoxicol or AFB_{2α} in these groups. Our results were in the same trend with those observed by (Bennett et al [67]), who found that the lower toxicities of AFB₁ and AFB_{2α} in mammals are mainly as a result of a faster rate of clearance via urine and feces compared with that of AFB₁. Similarly, our results indicated that AFB₁ metabolites are cleared at a much faster rate than AFB₁. Hence, the rate of AFB₁ biotransformation represents the main mechanism through which detoxification occurs.

CONCLUSION

In conclusion, our results may lead us to suggest that aflatoxin of 0.5 ppm / kg diet could increase the liver enzyme levels and affect some hematological parameters and cause liver and kidney alterations in New Zealand white rabbits. Increase in these parameters may occur due to peroxidation reactions, arising in aflatoxin biotransformation, and these reactions may inflict oxidative injury to cellular components. Administration of glycyrrhizin at level 500 ppm /diet in this study resulted in a significant improvement in all hematological and biochemical parameters of the liver and kidney and as well as improvement in feed intake, growth rate and nutrient digetibilities. In the light of these results, the effectiveness of glycyrrhizin at this dose is believed to be important to induce the potent protective action in New Zealand white rabbits without any adverse effect.

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