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AMELIORATIVE EFFECTS OF LYCOPENE ON AFLATOXIN-B1 TOXICITY IN BROILER CHICKENS

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ABSTRACT

The purpose of this study was to investigate the effects of adding varying amounts of lycopene (100, 200 and 400) mg/kg to broiler's diets contaminated with aflatoxin B1 (300 µg/kg) on growth and physiological performance. In a completely randomized design, three hundred unsexed, one-day-old Ross chicks were split into five treatments, each with six replicates (10 chicks per duplicate). All diets were created to meet the according to Ross Guide. Broilers were given unlimited access to feed and water. The results showed that adding lycopene to diets tainted with aflatoxin B1 significantly improved growth and cellular blood parameters, without negatively affecting the birds' health. Additionally, the results showed that lycopene supplementation decreased serum levels of low-density lipoproteins, triglycerides, cholesterol, and uric acid or creatinine in birds fed mycotoxin-contaminated diets. Moreover, increased high-density lipoprotein levels, which are thought to be a reliable predictor of blood lipid readings. The addition of lycopene enhanced the functions of the liver enzymes ALT and AST, as well as the concentrations of glucose, total protein, globulin, albumin, and antioxidant activities. The aflatoxin B1 residue in the liver and meat tissues of broiler chickens was also decreased. The present findings highlight the preventive role of lycopene supplementation in protecting against aflatoxin-contaminated diets and diminishing their harmful effect on the growth, physiological performance and health status of broiler chickens.

Keywords: Aflatoxin B1; Lycopene; broiler chickens; oxidative status

INTRODUCTION

In the feed industry, mycotoxin contamination is inevitable, and it poses a major risk to poultry health (Peng *et al*. 2018). Aflatoxins are derivatives of dihydrofurancoumarin, which are secondary metabolites of Aspergillus fungi, *Aspergillus parasitic* and *Aspergillus flavus*. The most common and harmful of the four major kinds of aflatoxins (B1, B2, G1, and G2) is aflatoxin B1 (AFB1), a hepatotoxic chemical compound (Saini and Kaur 2012). Aflatoxins were classified as Group I carcinogens by the WHO-affiliated International Agency for Research on Cancer (IARC). According to Diaz *et al*. (2010), cytochrome P450 (CYP450) isozymes activate AFB1 into the toxicant AFB1 -8, 9-epoxide (AFBO) during the phase I metabolic process. Inducing oxidative stress through the generation of

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reactive oxygen species (ROS) as intermediates during bioactivation is another significant avenue through which AFB1 might play hazardous functions (Marin and Taranu 2012). Glutathione-S-transferase (GST) catalyzes the conjugation of AFBO with reduced glutathione (GSH) in the phase II detoxification reaction, resulting in the non-toxic AFBO-GSH adduct (Rawal *et al.,* 2010). Research has indicated that by controlling phase I and phase II metabolic processes, some bioactive compounds included in food components may reduce the toxicity of AFB 1 (Yilmaz *et al*., 2018).

Lycopene is categorized as a natural antioxidant that guards against oxidative stress and free radical-induced cellular damage (Hidayat *et al*., 2023). Lycopene is one of the carotenoid family's most potent antioxidants, according to Karadas *et al*. (2016). It is more than 10 times more effective than β-carotene at reducing oxidative damage to tissues and cells, and it can scavenge reactive oxygen species (ROS) more effectively than α-tocopherol. It has anti-inflammatory qualities as well. According to Karaca (2021), lycopene also lowered hydrogen peroxide levels, increased the amounts of antioxidant enzymes like glutathione and catalase, and preserved the permeability of cell membranes. For these reasons, Lycopene can be regarded as a preventive factor against aflatoxin poisoning (Muhamme *et al*., 2023). According to Yilmaz *et al.* (2018), lycopene may help humans, mice, and rats recover from the harm produced by a variety of hazardous compounds. This might be due to, it regulates CYP450 isozymes and activates phase II detoxification and antioxidant mechanisms. Thus, the purpose of this study was to examine the effects of adding pure lycopene on lowering aflatoxin B1 contamination of feed, and its influence on the growth and physiological performance of broiler chickens.

MATERIALS AND METHODS

Experimental design, housing, management and tested diet

A trial was carried out at a private farm in Kafrelsheikh governorate, Egypt. The trial was designed according to a completely randomized design. The study used 300 one-day unsexed commercial Ross 308 strain broiler chickens. Chicks were randomly divided into five treatment groups. Using the density criterion of rearing 10 birds/m2, each treatment group had 60 chicks divided into six replicates, with 10 chicks per replication. The chicks were raised for 35 days in hygienic conditions in clean pens with deep litter. Thirty identically sized individual pens (1 m x 1.5 m) were set up in the experimental area and segregated by metal mesh partitions. The enclosures were equipped with manual plastic drinkers and feeders, and wood shavings were utilized as litter. The chicks were allowed unlimited access to food and water. After being maintained at 32°C for the first seven days of the trial, then gradually reduced to 24°C on day 21, and maintained there for the remainder of the experiment. The photoperiods were lowered to 23 hours per day for the remainder of the trial, after being set at 24 hours per day for the first week. The basal diet used in this study for the starter (0–10 days), grower (11–24 days), and finisher (25–35 days) stages was formulated as a mash to meet the total nutrient requirements of broilers (Table 1), as recommended in the Ross 308 Management Guide (Aviagen, 2019, New York, NY, USA). Treatments were as follows: Diets were prepared without addition of aflatoxin and lycopene as Control (CON); 300 µg/kg Aflatoxin B1(AFB1-diet); 300 µg/kg Aflatoxin B1+100 mg/kg of lycopene (LYC100); 300 µg/kg Aflatoxin B1+200 mg of lycopene (LYC200) and 300 µg/kg Aflatoxin B1+400 mg/kg of lycopene (LYC400). The feed was analyzed to ensure it was free of any mycotoxins.

Table 1: Physical and chemical composition analysis of the experimental starter, grower and finisher diets (%, as-fed basis)

Ingredients %	Starter (0–10 d)	Grower (11–21 d)	Finisher (22 35 d)
Yellow corn	53.87	58.88	63.90
Soybean meal 44%	33.5	29.4	24.0
Corn gluten (60%)	5.0	5.0	5.0
Corn oil	2.0	2.65	3.15
Dicalcium phosphate	1.73	1.60	1.50
$CaCo_3$	1.35	1.00	1.00
Nacl	0.4	0.4	0.4
DL-Methionine*	0.15	0.12	0.10
Hcl-Lysine**	0.35	0.30	0.30
Premix***	0.3	0.3	0.3
Toxin binder	0.2	0.2	0.2
Sodium bicarbonate	0.1	0.1	0.1
Choline chloride	0.05	0.05	0.05
Total	100	100	100
Calculated composition			
ME, Kcal/kg diet	3005	3100	3195
CP %	23.0	21.5	19.5
Ca %	1.00	0.87	0.82
Avail. P %	0.47	0.44	0.41
Methionine %	0.56	0.51	0.47
Lysine %	1.44	1.29	1.14
Meth + Cyst.	0.93	0.86	0.78
Na %	0.2	0.2	0.2

ME = metabolizable energy, CP = crude protein, Av. (P) = Available phosphorous. * DL-methionine 99% feed grade China. ** L-lysine 99% feed grade. *** Vitamin and mineral premix (Hero mix) produced by Hero pharm and composed (per 3 kg) of vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 10,000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Aflatoxin-B1 (Purity ≥98%, HPLC) and LYC (Purity ≥80%, HPLC) were purchased from Shanghai Yuanye Biotechnology Co. Ltd. Previous research has shown that 300 μg/kg AFB1 in diets had clear negative effects on broilers, and appropriate concentrations of lycopene supplementation in the diet (100–400 mg/kg) could provide optimal protective impacts against aflatoxin broiler chickens (Liu *et al*., 2016; Peng *et al*., 2014; Sahin *et al*., 2016). On the basis of these data, an appropriate toxic concentration and dietary supplementation levels of lycopene were chosen.

Performance parameters

The chickens in each replicate were weighed at the start and each week during the study. Each replicate's total feed consumption (TFC), average weight gain (AWG), and the number of surviving birds in that replicate. FCR were calculated using the formulae, FCR = feed consumption (g) / body weight gain (g). The relative growth rate (RGR) was measured according to (Brody, 1945):

RGR= (final weight- initial weight) /[0.5 x (final weight + initial weight) x 100]

Blood collection and hemato-biochemical analyses:

At the conclusion of the 35-day experiment, six birds were chosen at random from the treatment group, fasted for 12 hours, and then slaughtered. Using commercial kits (Spinreact Co., Santa Coloma, Spain), to carry out chemical analyses of plasma, in order to quantitatively determine blood parameters (glucose, protein, albumin, Triglycerides, Cholesterol, High Density Lipoprotein, Low Density Lipoprotein, and Very Low Density Lipoprotein), following the same steps as described by.

Globulin was computed as the difference between albumin and total protein. The plasma concentrations of malonaldehyde, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were determined using additional commercially available assay kits (Institute

Co., Nanjing, Jiangsu, China) according to Placer *et al.* (1966), Sun *et al.* (1988), Cowell *et al.* (1994), and Habig *et al.* (1974). Complete blood count (CBC) in haematology is a test that measures haemoglobin level (Hb), red blood cells, white blood cells count, and packed cell volume, using an automated blood cell analyzer (MEK-5216K, Nihin Kohden, Tokyo, Japan). As a stress indicator, the heterophil to lymphocyte ratio was assessed.

Measurement of hepatic antioxidant status

Following blood collection, the birds (n=30) were slaughtered, painstakingly feathered, then disemboweled. Liver samples were collected, weighed, and expressed as the Relative Organ Weight. The bicinchoninic acid test was used to determine the liver homogenate's protein content after it was produced, in accordance with the instructions provided by the kits (Bainor *et al.* 2011). Following the instructions on the Nanjing Jiancheng Bioengineering Institute's (Nanjing, China) matching detection kits. A nanogram per gram of protein (ng/g protein) was used to represent the GSH content. The units per gram of protein (U/g protein) representation of GPx activity was used. Units of T-SOD activity per milligram of protein (U/mg protein) were used. The Shanghai YuBo Biotech Co., Ltd. (Shanghai, China) commercial ELISA kits were used to measure the levels of malondialdehyde (MDA), whose concentration was measured in nanomoles per gram of protein. The methods of Truckess *et al*. (1991) were used to extract and quantify the aflatoxin residue in the liver and muscle tissues of broilers.

Statistical Analysis:

All data were analyzed by a one-way ANOVA using the GLM procedure of the SAS system (SAS, 2004) by performing the Duncan Multiple Range Test, to determine the differences between treatment means (Duncan, 1955). The chosen level of significance for all comparisons was set at $P<0.05$.

RESULTS

Growth performance

Table (2) shows the effects of dietary treatment on broiler growth performance. Broilers exposed to the AFB1 diet had diminished ($P<0.05$) AWG, RGR and raised FCR during the experimental trial compared to the control group. Broilers fed diet AFB1+LYC200 and AFB1+LYC400 significantly enhanced ($P<0.05$) the AWG, TFC and RGR in 1–35 days. Furthermore, compared to the AFB1 group, the FCR and mortality rate were decreased ($P<0.05$) after 1–35 days in the AFB1+LYC200 and AFB1+LYC400 groups.

Table 2: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B1 on the growth performance parameters

Items	Experimental diets					SEM	p-value
	CON	AFB1	AFB1+ LYC100	AFB1+LY C200	AFB1+ LYC400		
IBW,(g)	42	41.95	42.05	42	41.95	1.335	0.875
FBW, (g)	2200a	1970c	2010b	2100ab	2175a	18.36	0.0001
BWG, (g)	2158a	1928.1c	1967.9b	2058ab	2133.1a	17.52	0.0001
FI, (g)	3520a	3380b	3400b	3440ab	3500a	4.081	0.004
FCR, (g feed per g gain)	1.631b	1.753a	1.728ab	1.672b	1.641b	0.775	0.001
Viability %	98.33ab	93.33c	96.66b	100a	100a	3.254	0.002
Relative growth rate, %	192.51a	191.66b	191.8a	192.16a	192.43a	2.151	0.045

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error of mean; IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; FI: Feed intake; FCR: Feed conversion ratio.

Blood Biochemical parameters

The impacts of lycopene on some blood constituents of AFB1-exposed broiler chickens are shown in Table 3. AFB1-diet group exhibited a significant decrease (P<0.05) in protein, globulin, albumin, and glucose concentrations, compared to other treatments, which showed a significant increase. The birds in T2 (AFB1-diet) had the highest (P<0.05) plasma levels of ALT, AST, total cholesterol, triglycerides, uric acid and creatinine, compared to the control group. In addition, lycopene supplementation significantly (P<0.05) reduced the concentrations of triglycerides, total cholesterol, ALT, AST, uric acid and creatinine compared to the AFB1-group.

Birds in the AFB1 group had lower levels of TAC and GSH compared to the control group (P<0.05, Table 3). Compared to the AFB1 group, the GSH and TAC concentrations in the LYC100, LYC200, and LYC400 groups were greater (P<0.05). The AFB1 group's MDA concentrations were substantially higher than the control group (P<0.05, Table 3). However, compared to the AFB1 group, the MDA concentrations in the LYC100, LYC200, and LYC400 groups were lower (P<0.05).

Table 3: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B1 on some blood constituents and antioxidant parameters

Items	Experimental diets					SEM	p-value
	CON	AFB1	AFB1+ LYC100	AFB1+ LYC200	AFB1+ LYC400		
Total protein (mg/dl)	5.95a	5.04c	5.22b	5.72ab	5.85a	0.11	0.035
Albumin (mg/dl)	3.24a	2.85c	2.96c	3.03b	3.11ab	0.07	0.042
Globulin (mg/dl)	2.71a	2.19b	2.26b	2.69a	2.74a	0.06	0.030
Glucose (mg/dl)	165.3c	186.5a	177.2ab	170.3b	167.2c	2.33	0.001
Total cholesterol (mg/dl)	135.0d	205.8a	168.0b	158.0bc	146.0c	2.52	0.004
Triglycerides (mg/dl)	94.0c	145.0a	120.0b	104.0c	97.6c	1.30	0.044
HDL (mg/dl)	46.0a	33.0b	38.0b	45.0a	46.0a	1.58	0.026
LDL (mg/dl)	71.0c	94.8a	80.0b	78.10b	72.5c	0.70	0.002
Liver functions							
AST (U/L)	23.0c	68.0a	47.0b	32.0bc	27.0c	1.25	0.001
ALT (U/L)	15.8c	40.0a	30.9b	22.0bc	17.8c	0.66	0.005
Kidney functions							
Creatinine (mg/dl)	0.520[c]	0.780[a]	0.600[b]	0.580bc	0.530c	0.051	0.031
Uric acid (mg/dl)	3.25[b]	4.10[a]	3.08b	3.12b	3.20b	0.332	0.042
Antioxidant parameters							
SOD, U/mL	19.0a	15.36b	17.05ab	18.61a	18.93a	1.10	0.014
GSH-PX, U/mL	3.35a	2.89b	2.95b	3.00ab	3.21a	0.11	0.045
CAT, U/mL	11.58a	8.35c	9.57b	10.33ab	11.05a	1.36	0.036
MDA (nmol /mL)	3.65c	5.35a	4.12b	3.77c	3.52c	0.05	0.004

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error of the mean; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HDL cholesterol: High density lipoprotein-cholesterol; LDL cholesterol: Low density lipoprotein-cholesterol; SOD: Superoxide dismutase; GSH-PX: Glutathione peroxidase; CAT: Catalase; MDA: Malondialdehyde.

Hematological parameters

The cellular blood properties of the various therapies show notable changes, according to Table 4's results. In comparison to T2 (AFB1-diet), it is clear that the red blood cells of birds in T5 (addition of 400 mg/kg feed of lycopene) had the highest blood hemoglobin value and a significant rise

(P<0.05). Table (4) includes blood hemoglobin values as well. AFB1-group, LYC200 and LYC400 had the highest values and most significant increases (P<0.05) in blood. The statistical analysis results indicated significant differences (P≤0.05) in white blood cell count, and T2 (AFB1-diet) achieved a significant increase compared to the control group. Furthermore, LYC200 and LYC400 recorded a significant decrease (P≤0.05) compared to AFB1-group. One sign of stress in birds, it is observed that birds in T2 (AFB1-diet) recorded the highest value of H/L ratio compared to the control group. The results indicated that the addition of lycopene at high concentrations resulted in the highest benefits, increasing the concentration of red blood cells and hemoglobin value and decreasing the H/L ratio.

Table 4: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B1 some blood constituents and antioxidant parameters

Items	Experimental diets					SEM	p-value
	CON	AFB1	AFB1+ LYC100	AFB1+L YC200	AFB1+ LYC400		
RBCs (10^6/µl)	3.70a	2.60b	2.96ab	3.50a	3.80a	0.18	0.001
Hb (g/dl)	7.88a	7.05b	7.26b	7.53ab	7.90a	0.25	0.034
MCV (%)	25.5a	22.8b	24.0ab	24.9a	25.3a	0.25	0.002
WBCs (10^3/µl)	28.80	29.06	28.25	28.00	28.00	0.60	0.054
H / L ratio	0.506c	0.781a	0.625b	0.500c	0.486c	0.02	0.001

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error of the mean; RBCs: Red blood cells; Hb: Hemoglobin; Hematocrit; MCV: WBCs: White blood cells; H/L: Heterophil/lymphocyte.

Hepatic antioxidant status

Birds in the AFB1 group had lower T-SOD and GPx activity than those in the control group (P<0.05, Table 5). In the LYC100, LYC200, and LYC400 groups, the T-SOD and GPx activities were greater than in the AFB1 group (P<0.05). The MDA concentrations in the AFB1 group were substantially higher than those in the control group (P<0.05, Table 5). The LYC100, LYC200, and LYC400 groups' MDA concentrations were lower than the AFB1 group's (P<0.05).

Table 5: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B1 on the relative weight of liver and hepatic oxidative status

Items	Experimental diets					SEM	p-value
	CON	AFB1	AFB1+ LYC100	AFB1+ LYC200	AFB1+ LYC400		
Relative weight of liver							
Liver %	3.05c	3.79a	3.46ab	3.32b	3.08c	0.174	0.001
Hepatic oxidative status							
T-SOD (U/mg protein)	23.0a	16.5c	18.0b	20.0ab	20.3ab	0.578	0.004
Gpx (U/ g protein)	102.0a	81.0c	87.1b	90.0ab	96.0ab	3.251	0.001
MAD (nmol/ g protein)	2.6c	3.9a	3.0ab	2.8b	2.6c	0.025	0.035

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error of mean; GPx: glutathione peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde.

Aflatoxin (AFB1) residues in liver and muscles tissues and muscles of broilers.

As shown in Table (6), AFB1 residues were detected in all AFB1 groups (groups 3, 4 and 5) in both liver and muscles tissues with higher concentrations in liver than muscles. Addition of lycopene significantly reduced liver and muscles AFB1 residues in comparison with the AFB1 group.

Table 6: Effect of lycopene supplementation to broiler diets contaminated with aflatoxin B1 on aflatoxin B1 (AFB1) residues in liver and muscles tissues (ppb), at 5 weeks.

Items	Experimental diets					SEM	p-value
	CON	AFB1	AFB1+ LYC100	AFB1+L YC200	AFB1+ LYC400		
AFB1 residual							
Liver	ND	3.2a	1.8b	1.0bc	0.8c	0.21	0.0001
Muscles	ND	2.1a	0.5ab	0.3b	0.1c	0.15	0.0001

[a-b]Means within the same row with different superscripts are significantly different (p<0:05); SEM: Standard error of mean; ND: no detected

DISCUSSION

Growth performance

Aflatoxin B1 (AFB1) is the most prevalent feed contaminant and the most prevalent danger metabolite in various regions of the world. The toxicity and clinical signs in animals are complex and varied when there are multiple mycotoxin types in the feed. An animal's reaction to a toxin may be influenced by its age, production status, species, rate of AFB1 contamination in its diet, period of exposure (acute or chronic), and toxin co-contamination (synergistic impact) (Yunus *et al.*, 2011). It is becoming more widely acknowledged that broiler growth and performance are negatively impacted by prolonged use of feed contaminated with AFB1. In the current investigation, AFB1 (300 µg/kg) raised the FCR during the entire trial period, while considerably lowering the body weight gain and feed consumption. Similar outcomes have been noted in earlier research, where AFB1 (50–300 µg/kg) could considerably reduce broiler body weight gain and FCR, resulting in economic losses (Farag *et al*., 2023). In broiler fed 0.3 mg AFB/kg feed, Raju and Devegowda (2000) observed a 21% reduction in ultimate body weight at 35 days of age. According to another study, ducks given diets tainted with 20 µg/kg of AFB1 showed a markedly higher FCR and a significantly lower ADG (Han *et al*., 2008).

The decline in performance brought on by toxins may be linked to a number of factors, including: (a) reduced feed intake; (b) altered activity of liver oxygenase enzymes, that are responsible for the metabolism of proteins, carbohydrates, and fats; (c) decreased absorption of essential nutrients in conjunction with patho-anatomical changes in vital organs, particularly the liver (Shabani *et al*., 2010); (d) toxic influence on the cellular contents through inhibition of protein, RNA, and DNA syntheses (Marai and Asker, 2008); (e) decreased digestibility of nitrogen free extract, ether extract, and dry matter; and (f) anorexia and lipogenesis (Oguz, and Kurtoglu, 2000). On the other hand, dietary LYC supplementation mitigated the negative effects of AFB1 on broiler growth. Previous research has demonstrated that broilers fed a diet supplemented with 100 mg/kg LYC increased their final live weight at 35 days of age (Sevcikova *et al*., 2008), that broilers fed a diet enriched with 5% LYC-enriched dried tomato pomace increased their body weight from 1 to 28 days of age

(Hosseini-Vashan *et al*., 2016), and that LYC supplementation improved the growth performance of broilers under heat stress (Sahin *et al*., 2016). These findings suggest that LYC or LYC-enriched materials may be advantageous for the growth of chickens. The current study found that adding LYC supplements at varying concentrations (100, 200, and 400 mg/kg) to the aflatoxicated diets improved the growth suppression induced by AFB1, as seen by noticeably higher body weight gain and lower FCR. Therefore, adding LYC to the broiler diet can counteract the harmful effect of AFB1 on growth performance.

Blood Biochemical test

Assessing alterations in serum biochemical and haematological markers prior to the onset of significant symptoms can help diagnose cases of chronic and subclinical aflatoxicosis (Yu *et al*., 2021). Sensitive markers of the harmful effects of AF on the target organs are these parameters. An extensively researched and well-known topic is the harmful consequences of AF on the body's biochemistry and hematology. The current investigation also made these harmful impacts quite evident. It is believed that AF targets the kidney and liver. According to Rosa *et al.* (2001), the hepatotoxic effects of AF, which include the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism, were the cause of increased AST, ALT, and MDA concentrations and decreased plasma total protein, albumin, total cholesterol, triglyceride, glucose, and antioxidant values ($P<0.05$; Table 3). One significant predictor of aflatoxicosis in broilers is the serum protein level (Tung *et al*., 1975). Serum TP concentrations were shown to be lower in broilers given 0.3 and 1 mg/kg AFB1 diet at 21 and 42 days of age (Safameher, 2008). Serum TP, ALB, and GLOB levels dropped when AFB1 was 30 μg/kg, but broiler production performance remained the same, suggesting that low AFB1 levels hinder protein synthesis, but had no effect on production performance.

The liver and kidneys are detoxifying organs that aid in the metabolism of mycotoxins. The primary indices for evaluating liver function are ALT and AST, which are released into the circulation when the liver is injured (Hu *et al*., 2014). Liver injury is indicated by elevated AST and ALT values (Yang *et al*., 2016). Aflatoxin-supplemented birds may have higher blood levels of the ALT and AST enzymes because of the damage to their enzyme-rich tissues. These tissues decomposition or death causes the cells to leak or become more permeable into the circulation. Notably, the liver is the primary producer of a number of enzymes, such as ALT and AST, and the activity of these enzymes is a sign of cellular stress or oxidative damage (Ali and Mousa, 2023). These results are consistent with Hussein and Ali (2018) research, which showed that lycopene supplementation at 250 and 500 mg/kg impacted the reduction of ALT and AST enzyme concentrations.

The results of Rashidi *et al*. (2018) were consistent with the current findings of a markedly raised serum creatinine. Aflatoxin's accelerated rate of protein catabolism and nephrotoxic impact, which is demonstrated by pathomorphological alterations in the kidneys, may be the cause of an elevated creatinine level. According to Eaton and Pooler (2004), the kidney is in charge of preserving electrolyte balance, extracellular medium homeostasis, and eliminating waste products from the body's metabolism. Kidneys also take part in detoxification of aflatoxins and are also among the organs where most aflatoxin residues are detected (Fernandez *et al*., 1994). The primary regulatory system preserving homeostasis in the body is the kidney. According to Levey *et al.* (1999), creatinine plasma concentrations are thought to be biomarkers for kidney disease since they determine renal

function. Sato *et al*. (2005), proantho-cyanidins' notable capacity to scavenge radicals may be the mechanism behind their nephroprotective effects. According to Yilmaz *et al*. (2018), lycopene's effects are linked to preventing oxidative stress, which boosts the body's antioxidant capacity and preserves cell membrane permeability.

The protective effect of lycopene on the liver and pancreas against damage from free radicals resulting from high oxidation or aflatoxin contamination, particularly on the β-cells in the pancreas that secrete the hormone insulin, which is responsible for blood sugar regulation, may be the reason for the decrease in blood glucose levels. Additionally, because lycopene has a strong potential to lower blood lipids and cholesterol, excessive amounts of fat and cholesterol induce insulin receptors to close, which impacts glucose metabolism and representation (Wu, 2018). This outcome is in line with Sahin *et al.* (2006) findings of lycopene's potent capacity to lower cholesterol and blood sugar levels. However, lycopene has been shown by a number of researchers to protect the pancreas and its cells from oxidation, free radicals, and maintaining liver cells during glucose metabolism and storage of excess glucose as glycogen (Rao and Shen, 2002). Lycopene also aids in keeping blood glucose levels within the usual range, which guarantees a steady supply of glucose, the building block of cellular energy. As noted by Noaman (2017), the data support the idea that adding lycopene to broiler feed at concentrations of 200, 150, and 100 mg/kg significantly lowered blood glucose levels. Additionally, the current outcomes are in agreement with research showing that lycopene at a dose of 400 mg/kg lowered blood glucose levels and protected liver cells from oxidative stress-induced damage (Albrahim, 2022). In relation to protein concentration, it is noted that the addition of lycopene has positively impacted raising the total protein values. Lycopene stimulates the liver to produce and increase the concentration of insulin-like growth factor (IGF-1), which is responsible for increasing protein (Alina *et al.,* 2007). These results are consistent with the findings of Rao and Shen (2002), who found that increasing lycopene concentration in the blood led to an increase in blood protein concentration.

The findings also revealed a significant decrease in the liver enzymes ALT and AST ($P \leq 0.05$), when lycopene was added to feeds. This decrease was directly correlated with the increased lycopene levels. Additionally, the decline could be explained by the fact that lycopene, a fat-soluble antioxidant found in cell membranes, helps shield cell walls from peroxide-induced damage. The researchers ascribed the decline to lycopene's capacity to lessen oxidative damage in birds brought on by stress and oxidation. The findings are consistent with those of Al-Dawoodi (2022), who found that birds fed meals contaminated with aflatoxin had significantly higher levels of ALT and AST than birds provided diets supplemented with 10 g/kg of lycopene. The study suggested that mycotoxins induced liver damage or cell death, which increased cell membrane permeability and allowed ALT and AST enzymes from the liver to escape into the circulation. They linked the decrease to the role of lycopene in protecting liver cells (Ahsan *et al.,* 2009). Lycopene can accumulate in liver and lungs, and it remains in tissues, especially liver, for a longer period, compared to carotenoids, which makes it more effective in protecting these tissues from damage (Rao, and Shen, 2002).

Lycopene supplementation has improved the lipid profile of birds' serum that tainted by aflatoxin. As a strong antioxidant, lycopene scavenges free radicals produced by lipid oxidation and keeps the lipids transported to the liver, where they are represented (Agarwal and Rao, 1998). The

results of the previous Table demonstrate this impact, with lycopene's addition helping lower the harm caused by aflatoxin in treatments T2, T3, T4, and T5. Additionally, it increased the concentration of high-density lipoproteins (HDL) while decreasing triglycerides, cholesterol, and low-density lipoproteins (LDL). These effects significantly affect general health, which enhances liver function and has a positive impact on productivity. These results align with Bende *et al.* (2016), who demonstrated the role of lycopene in lowering cholesterol and triglyceride levels in birds by inhibiting or blocking their production. The findings of our study are in line with those of AL-Dawoodi (2022), who found that adding tomato powder high in lycopene improved the lipid profile of broilers fed aflatoxin-contaminated feed.

Hematological parameters

Hematological results showed that broiler chickens exposed to aflatoxin B1 had significantly lower hemoglobin content and erythrocyte count (Table 4). This decrease is associated with malnutrition, disruptions in protein metabolism, and decreased feed intake, particularly decreased protein intake. Protein synthesis was generally inhibited by aflatoxins, because they impair nuclear DNA template function and inhibit DNA-dependent RNA polymerase (Yu, 1977). Furthermore, according to Sun *et al*. (2018), bucks fed a high level of AFB1 had lower RBCs than bucks fed a low level of AFB1 or the control group. This decrease is exemplified by anemia, which results from afla-toxicosis, which may be caused by altered protein metabolism and a decrease in serum iron. The results of the current investigations are consistent with those of Donmez *et al.* (2012), who observed a decrease in hemoglobin, hematocrit, and erythrocyte count in the aflatoxin-treated group. Previous studies have also reported a significant decrease in these parameters (Oguz *et al.* 2000), which is indicative of the detrimental effects of AFB 1 on immune systems (Oguz, 1997) and hemopoietic tissue (Mohiuddin *et al.,* 1986).

According to Mozos *et al.* (2018), lycopene helps shield red blood cells from oxidation, extending their life and preventing deterioration. This is clear from the current study's findings, which show that red blood cell parameters are directly correlated with increased lycopene supplementation. Due to lycopene's ability to fight free radicals and lessen lipid oxidation in cell membranes, the birds in T5 and T4 had the highest values for packed cell volume and blood hemoglobin. The low H/L values in the Table demonstrated that treatments with lycopene supplementation did not exhibit any symptoms of stress, which is clear from the H/L ratio results (Table 4). It is significant that birds in T2 (AFB1-diet) recorded the highest H/L ratio, which is a measure of stress in birds and can be brought on by heat, oxidative stress, or disease. This may be because, unlike the other treatments, which were exposed to the same degree of contamination, but had lycopene added to their diets, the birds were exposed to aflatoxin B1 toxins without the presence of antioxidants or detoxifying agents. The findings are in line with Zeweil *et al.* (2016), who noted that rabbits raised in the summer had improved blood profiles. The findings are also consistent with Ogundeji *et al.* (2023), who showed that supplementing broiler diets with 10 mg of lycopene/kg reduces stress and enhances the health of the birds, as evidenced by the lower H/L values compared to the control group. However, the findings contradict those of Skevchenko *et al.* (2021), who found no impact of lycopene supplementation on laying hens' blood hemoglobin, packed cell volume, or red blood cell concentration.

Hepatic antioxidant status

Furthermore, the liver is essential for the metabolism and uptake of proteins, amino

acids, and lipids (Fouad *et al*., 2019). Abnormal liver size may be associated with liver dysfunction. AF can cause an imbalance in lipid metabolism, which can encourage lipid deposition in the enlarged liver, as well as inhibit the activity of antioxidant enzymes and anti-inflammatory cytokines (Siloto *et al.,* 2013). Additionally, it can raise pro-inflammatory cytokines, lipid peroxidation, and hepatocyte apoptosis (Wang *et al.,* 2019).

According to Frankic *et al*. (2008), oxidative stress in animals can be caused by dietary factors, such as the presence of fungal toxins in feed. In an uncontrollable condition, aflatoxin caused lethally injured hepatocytes, leading to denaturation of structural proteins, inhibition of enzymes, and mitochondrial dysfunction. Because AF is metabolized by the cellular cytochrome P450 enzyme system (Kheir-Eldin *et al.,* 2008), lipid peroxidation and cellular damage result (Stresser *et al.,* 1994). This may be the cause of the rise in hepatic MDA levels. may also be the result of the current study's notable decrease in the activities of enzymatic antioxidants like Gpx and SOD. According to Chen *et al.* (2014), SOD and Gpx are the two main enzymes of the antioxidant system that can scavenge free radicals produced by oxidative stress, lessen oxidative damage, and preserve cell structure.

One indicator of lipid peroxidation is the concentration of MDA. AFB1 has been shown to raise the levels of oxidative damage products, such as MDA, in tissues (Marin and Taranu, 2012). Previous studies have shown that broilers exposed to AFB1 have higher concentrations of MDA and lower GSH concentrations, and activities of GST, SOD, CAT, and GPx (Sun *et al.,* 2015 and Zhang *et al.*, 2016). Similarly, the study found that the liver of broiler chickens exposed to AFB1 had higher concentrations of oxidative damage products (MDA), and decreased various antioxidant enzyme activities, all of which confirmed the presence of oxidative stress.

A tomato powder with a high lycopene content has strong antioxidant properties. Antioxidants prevent cell damage by giving electrons to halt the reaction of free radicals, which stops the oxidation of proteins and fats (Purnama *et al.,* 2020). The well-known antioxidant lycopene has a great capacity to scavenge free radicals. Previous research showed that lycopene enhanced antioxidant capacity in growing rabbits and heat-stressed broilers (Sahin *et al.* 2016; Casamassima *et al.* 2017), raised GSH concentration, and enhanced antioxidase activities in AFB1 and zearalenone challenged rats and mice (Xu *et al.*, 2017; Yilmaz *et al.,* 2018). Mycotoxin-induced oxidative stress and the anti-stress properties of plant-derived additives in animals have been recognized (Sridhar *et al.,* 2015; Zhang *et al.,* 2016). Thus, it is evident that lycopene can both improve the enzymatic and non-enzymatic antioxidant systems, thereby reducing oxidative damage in broilers exposed to mycotoxins.

Aflatoxin B1 (AFB1) residues in liver tissues.

Aflatoxin residues and their metabolites may be found in meat, dairy products, eggs, and other products of animals fed aflatoxins contaminated rations (Hussain *et al.,* 2010). These residues may cause health issues for humans (Pandey and Chauhan, 2007; Denli *et al.,* 2009). The maximum tolerance level of AFB1 in human food products is 2 µg/kg in many countries (Zhang *et al.,* 2017). AFB1 residues were found in both the liver and the muscle tissues of the AFB1 groups, compared to the control group, with the concentrations in the liver being higher than those in the muscle, as indicated in Table (6). Lycopene considerably decreased AF residues in the muscle and liver. According to several studies, the livers of broilers fed diets containing 50

and 100 µg of AFB1/kg for 42 days had residue levels of AFB1 (0.05 and 0.13 µg/kg) (Bintvihok and Kositcharoenkul, 2006). Additionally, laying hens fed a 2.5 mg/kg AFB1 diet for four weeks showed AFB1 residue in their livers (Zaghini *et al.,* 2005). The livers of ducks fed the aflatoxin-contaminated diet for 21 days retained low levels of AFB1 (0.12 µg/Kg). According to Zhang *et al*. (2017), the type of bird and diet, the concentrations of AFB1, and the length of exposure can all affect the residue levels. Similar to Hussein and Atiyah (2020), the current study found that dietary Lycopene treatment reduced the amount of Aflatoxin B1 residue in both the T4 and T5 groups. The effects of Lycopene were dose dependent, with higher doses reducing the influence of the Aflatoxin B1 effect. This could lead to the conclusion that lycopene is an antioxidant compound that helps prevent the toxicity of aflatoxin (Juan *et al.,* 2008). Lycopene plays a crucial role in reducing the negative effects of aflatoxin by promoting stage 2 detoxification and the synthesis of AFB-NAC. It also works to stop the phase 1 metabolism of AFB1 and reduces AFB-N7, which causes an adduct in liver DNA. This agreed with Tang *et al.* (2017), Reddy *et al.* (2006) and Wang *et al.* (1999).

CONCLUSION

The detrimental biochemical effects of aflatoxin toxicity at 300 µg/kg dose in broilers are lessened by dietary addition of lycopene at 100, 200 or 400 mg/kg diet, whereas the 400 mg was more effective than 100 or 200 mg/kg diet. The addition of lycopene to chicken rations may therefore be more beneficial to both human and chicken health than the use of chemical feed additives, because of its contributions to antioxidant status and other metabolite activities.

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التأثيرات المحسّنة لليكوبين على سمية الأفلاتوكسين-ب١ في دجاج التسمين

رضا على حسن ، محمد عبد العظيم موسى ، عبير ربيع خشت ، ابراهيم حمدان سالم، أحمد العراقي شمس الدين ، أحمد صبري عرفة ، ماجد عبد النبي الديب ، حمدي محمد الكومي ، سامية مصطفى مبارز

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كان الغرض من هذه الدراسة هو التحقيق في تأثيرات إضافة كميات مختلفه من الليكوبين (١٠٠ و٢٠٠ و٤٠٠) ملجم/كجم من العلف إلى علائق دجاج التسمين الملوثة بالأفلاتوكسين-ب١ (٣٠٠ ميكروجرام/كجم) على النمو والأداء الفسيولوجي. في تصميم عشوائي كامل، تم تقسيم ثلاثمائة كتكوت غير محدد الجنس وعمرها يوم واحد إلى خمس معاملات، كل منها بستة مكررات (١٠ كتكوت لكل مكرره). تم إنشاء جميع الأنظمة الغذائية لتلبية نفس المتطلبات. تم منح دجاج التسمين إمكانية الوصول غير المحدود إلى الغذاء والماء. أظهرت النتائج أن إضافة الليكوبين إلى الأنظمة الغذائية الملوثة بالأفلاتوكسين-ب١ أدى إلى تحسين النمو ومعايير الدم الخلوية بشكل ملحوظ دون التأثير سلبًا على صحة الطيور. بالإضافة إلى ذلك، أظهرت النتائج أن مكملات الليكوبين قللت من مستويات البروتينات الدهنية منخفضة الكثافة والدهون الثلاثية والكوليسترول وحمض اليوريك أو الكرياتينين في مصل الطيور التي تتغذى على علائق ملوثة بالسموم الفطرية. علاوة على ذلك، ارتفعت مستويات البروتينات الدهنية عالية الكثافة، والتي يُعتقد أنها مؤشر موثوق لقراءات الدهون في الدم. أدى إضافة الليكوبين إلى تحسين وظائف إنزيمات الكبد ALT وAST بالإضافة إلى تركيزات الجلوكوز والبروتين الكلي والجلوبيولين والألبومين ومضادات الأكسدة. كما انخفضت بقايا الأفلاتوكسين B1 في أنسجة الكبد واللحوم في دجاج التسمين. تسلط النتائج الحالية الضوء على الدور الوقائي لمكملات الليكوبين في الحماية من الوجبات الملوثة بالأفلاتوكسين وتقليل تأثيرها الضار على نمو وأداء فسيولوجي وحالة صحية لدجاج التسمين.