INFLUENCE OF TOMATO WASTE POWDER ON BIOCHEMICAL PARAMETERS, LYMPHOID ORGANS, OXIDATIVE STATUS, AND IMMUNE RESPONSE IN BROILERS DIET EXPOSED TO AFLATOXINS

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(Received 13/7/2024, accepted 27/7/2024)

SUMMARY

In the current study, biochemical parameters, oxidative status, lymphoid organs and immune response for broilers challenged with aflatoxins (AF) were examined to determine the preventive efficacy of food supplementation with tomato waste powder (TWP). A total of 300 Ross (308) broiler chicks one-day old were randomly distributed in five treatments and six replicate (ten birds per replicate); feed and water were provided ad libitum throughout the 35 days' experiment. Treatments were as follows: (T1) A basal diet containing neither AF nor TWP (positive control) and (T2) a basal diet containing 100 µg/kg AFB1 (Negative control). The other three treatment groups were supplemented with TWP at 2.5, 5.0 and 7.5 g/kg plus the 100 µg/kg AF. The results showed that Aflatoxicosis decreased total protein, albumin, and globulin concentrations and greater liver and kidney functions in broiler chickens as compared with the AFB1- group ($P<0.05$). As compared with the T2 group (Negative control), inclusion of 5 and 7.5 g TWP/kg increased serum total protein, albumin, and globulin and the TWP additive decreased liver and kidney functions of broiler chickens comparing with the control group. Lymphoid organs (thymus and bursa) relative weight, and immune response to NDV and IBD were decreased and increases were observed in interleukin 2 (IL-2), IL-6, and interferon γ (IFN-γ) in the T2 group, whereas spleen relative weight, heterophil percentage and heterophil-to-lymphocyte ratio were increased ($P<0.05$). Aflatoxicosis increased blood and hepatic malondialdehyde concentration, whereas TAC, SOD and GSH were decreased ($P<0.05$) in blood and hepatic. As compared with the T2 group (Negative control), the levels of TWP (5 and 7.5 g TWP/kg) improved liver and serum oxidative status concentration ($P<0.05$). Moreover, interleukin 2 (IL-2), IL-6, and interferon γ (IFN-γ) significantly ($P<0.05$) diminished in TWP + AFB1 groups when compared with the AFB1 group. It can be concluded that the levels of TWP (5 and 7.5 g TWP/kg) are capable to decrease the negative impact of AFB1 on broiler chickens' biochemical parameters, lymphoid organs, oxidative status, and immune response.

Keywords: *Aflatoxins; broiler chicks; tomato waste; biochemical parameters.*

INTRODUCTION

Mycotoxins are toxic substances that are typically produced by specific types of molds (organisms). Mycotoxin-delivering molds grow on a variety of foods, including cereals, nuts, dried fruits, and flavorings. Most mycotoxins survive nutrient preparation because they are chemically stable. Global estimates indicate that fungal mycotoxins infect about one-fourth of the cereals (Zhang *et al.,* 2022). Mycotoxins can be ingested through tainted cereals or residues in meat and eggs. Aflatoxin residues are produced by two fungal species, Aspergillus flavus and Aspergillus parasiticus, and they primarily deteriorate feed ingredients during storage, particularly in warm and humid climates (Ahmed *et al.,* 2022). Abdel-Daim *et al.* (2021) have reported that aflatoxin B1 (AFB1) can cause a number of health issues, such as hepatotoxicity, teratogenicity, mutagenicity, and carcinogenicity. Per the "International Agency for Research on Cancer (IARC)" (Williams *et al.,* 2004), AFB1 is classified as a Group I carcinogen for humans. The reactive metabolite (AFB1-exo-8,9- epoxide) produced from AFB1 biotransformation by cytochrome P450. The carcinogenic effect of AFB1 is caused by this metabolite's

strong binding to DNA adducts, and produced reactive oxygen species (Yilmaz and Bag, 2022). Indeed, in many nations, especially in Asia and Africa where cereals are more prone to be infected with aflatoxin due to favorable humid conditions, aflatoxin exposure has been connected to liver cancer (Abdel-Daim *et al.,* 2021). Antioxidants may be a useful strategy to slow the progression of the harmful effects connected to acute AFB1 toxicity, as evidenced by earlier research showing that oxidative stress, inflammation, and cell apoptosis are important factors in AFB1-induced toxicity (Wang *et al.,* 2022).

According to Mates (2000), antioxidants function as scavengers of free radicals, particularly superoxide, and they achieve this by activating proteins that are defensive or detoxifying. Due to their antioxidant properties, phytogenic compounds are known to prevent the biotransforming aflatoxin B1 into its hazardous forms (Lee *et al.,* 2001). It has been proposed that antioxidants can reduce the harmful and carcinogenic effects of mycotoxins (Sorrenti *et al.,* 2013). A well-researched species in the Solanaceae family is the tomato. Because of its anti-inflammatory and anti-cancer effects, both its production and use are rising (Rai *et al.,* 2021). According to Beutner et al. (2001), tomato components with the highest antioxidant capacity are lycopene (LYC), phenolic, flavonoids, vitamins C and E, and processed tomato products. Lycopene, a naturally occurring pigment derived from food that is part of the carotenoids utilized in food processing, is abundant in tomato powder and is primarily found in red fruits and vegetables (Liang *et al.,* 2019).

LYC can be used as a bioactive plant food material with vital activities, including antioxidant capacity, and has therapeutic potential against illnesses (Grabowska *et al.,* 2019). Owing to the significance of chicken farming, the goal of the research is to demonstrate how well tomato waste powder (TWP) works to reduce the harmful effects of aflatoxin in broilers using the following metrics: certain blood constituents, serum and hepatic oxidative status, and immunological status.

MATERIALS AND METHODS

The purpose of this study was to ascertain whether supplementing tomato waste powder to the diets may mitigate the negative effects of aflatoxin on the immune system, oxidative status, and biochemical parameters in broiler chickens.

A total of 300 broiler chicks (ROSS 308), 1 day old, were acquired from commercial hatchery and raised for 35 days in deep litter system. They were distributed randomly into 5 treatments. Each group had 6 replicates of 60 broiler chicks.

Aflatoxicosis was induced by incorporating known amount of AF into the diet. TWP was added as an ameliorating agent to each of the toxin containing diets. The five treatment groups were as follows: T1 (Positive contr ol): basal diet without AF or TWP, T2 (Negative control): 100 µg AF / kg, T3: 100 µg AF plus 2.5 g TWP per kg feed, T4: 100 µg AF plus 5 g TWP per kg feed, and T5: 100 µg AF plus 7.5 g TWP per kg feed. The standard environmental and hygienic requirements were met when raising chicks in litter. From the first to the third day of the trial, the lighting system ran continuously for 24 hours. From that point on, it ran for 23 hours. For the first three days, the temperature was controlled at 33 ± 1◦C. After that, it was lowered by 3◦C per week until it reached 24◦C at the conclusion of the trial period. Throughout the experiment, the humidity was kept at roughly 60%. Feed and water were given ad libitum. At the appropriate dates, all birds received vaccinations against Newcastle disease and IBD disease. Corn-soybean-based diets were formulated according to NRC (1994) to meet the nutrient requirements from 1 to 21 days (starter) and 22–35 days (grower) experimental periods for broilers (Table 1). The basal diet contained 10 µg of AFB1/kg of diet, as determined by the HPLC techniques as described previously (Trucksess *et al.,* 1994).

Aflatoxin used in the current study was AFB1 which was obtained from the Animal Health Research Institute, Dokki, Giza, Egypt. The technique previously described (Shotwell *et al.,* 1995) was used to prepare and introduce AF into the basal diet. AF was produced by growing Aspergillus flavus on rice. The moldy rice was dried and ground to a final powder and determined using the HPLC method in the Central Lab of the Faculty of Veterinary Medicine at Assiut University. The rice powder contained 97.19% AFB1 and 2.8% AFB2. Crushed rice was supplemented to the basal diet to provide (100 ppb) according to Abdel-Sattar et al. (2019).

Preparation of tomato powder:

The tomato waste was obtained from commercial workstations (Egyptian International Co. For Food Products, the First Industrial zone, New Borg El Arab, Egypt). It was spread out on a plastic sheet and exposed to sunlight to dry. According to Yitbarek (2013) waste particle size is decreased by pounding with a stick and hand crushing.

Table (1): Composition and calculated analysis of the experimental starter and grower diets.

Ingredient	Starter (%)	Grower (%)
Corn grains	53.71	61.92
Soybean meal (44%)	33.42	28.05
Corn gluten meal (60%)	5.22	3.20
Soybean oil	3.32	2.94
Limestone	1.28	1.15
Dicalcium phosphate	1.84	1.68
DL-methionine	0.39	0.22
Vitamins and minerals premix*	0.30	0.30
L- lysine HCl	0.12	0.14
Salt (NaCl)	0.40	0.40
Total	100	100
Analyzed and calculated composition (NRC, 1994)		
Crude protein %	23	20
Metabolizable energy (Kcal/kg	3094	3142
Methionine %	0.80	0.58
Calcium %	1.00	0.90
Available phosphorous %	0.49	0.45
Lysine %	1.25	1.11

** Composition (per 3 kg): vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.*

Nutrient content of tomato waste samples was analyzed in accordance with AOAC (2011). Tomato waste contains crude protein (13.27%), crude fiber (31.20), ether extract (4.52, 0.52), and calcium (0.48%). While, analysis of tomato waste powder contents of active components by HPLC according to (Pataro *et al.,* 2018) were total carotenoids 185,55 µg/g, and estimated lycopene 146 µg/g.

Samples and measurements:

At the end of the experiment (35 days), 1 broiler chickens of each replicate were randomly selected and then slaughtered by Islamic method. Liver, spleen, bursa of fabricius and thymus weights were measured and relative weight to total body weight of broiler chickens were calculated. During slaughtering, at 35 days of age, blood samples were collected from 6 birds in each treatment. From one hand, blood samples were collected into a vacutainer (containing heparin) of 6 birds in each treatment. The haematological traits evaluated in present investigation to indicate general physiological status of birds consisted of the following: Erythrocyte counts (RBC) and leucocyte counts (WBC), haemoglobin concentration (Hb) and heterophil to lymphocyte (H / L) ratio.

From the other hand, during slaughtering, blood was collected in non-heparinised tubes from six birds (one bird per replicate) in each treatment. Blood samples were centrifuged at 3,000 rpm for 10 min and serum samples were separated and preserved at -20°C until analysis. Serum samples were analyzed individually for total protein, albumin, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and uric acid using a serum biochemical analyzer as per the recommendations of the manufacturer's kit (Spectrum, Cairo, Egypt). The glutathione peroxidase (GPX) activity, superoxide dismutase (SOD) activity, catalase (CAT) activity, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content in the sera were determined with assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

Immunity:

Serum immunoglobulin G (IgG), IgM, IgA, IL-2, IL-6, IFN-γ, anti-NDV antibody (NDV-Ab) titer, and anti-IBDV antibody (IBDV-Ab) titer were analyzed with enzyme-linked immunosorbent assay (ELISA) kits (Lair Biotechnology Co., Ltd., Hefei, China) according to the manufacturer's instructions.

Hepatic Oxidative status:

Liver (about 1 g) was removed and washed with normal saline, then snap frozen in liquid nitrogen and then stored at −80°C until analysis from 6 chicks in each treatment. Hepatic oxidative status was analyzed corresponding to the prior methodology (Khalil *et al.,* 2022). The levels of total protein, malondialdehyde (MDA) and glutathione (GSH), total antioxidant capacity (TAC) and total superoxide dismutase (T-SOD) in the liver samples were detected following the protocols of corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All findings were normalized against total protein level in each sample for inter-sample comparison. The level of MDA and TAC were expressed as nmol per milligrams of protein. The activity of GSH and T-SOD were expressed as units per milligrams of protein.

Statistical analysis:

Data were analyzed using the GLM procedure of SAS software (SAS, 2003) as a completely randomized design. Differences among treatments were assessed using Duncan (1955) multiple range tests ($P<0.05$). The statistical model performed was as follow:

$$Yik = \mu + Ti + eik$$

Where, Yik = An observation, μ = Overall mean, Ti = Effect of treatments (i = 1, 2….5), eik = Experimental random error

RESULTS

Relative organs weight:

The addition of TWP to aflatoxin containing diet (T3, T4 and T5) completely ameliorated the adverse effects of aflatoxin on liver and lymphoid organs (thymus, bursa of fabricius and spleen) as listed in Table (2).

Supplementation of TWP to the diet significantly ($p<0.05$) increased relative weight of thymus and bursa of fabricius and reduced relative weight of liver, kidney and spleen compared with the negative control (T2) group (Table 2). However, T5 group surpasses other treatments as regards all of these organs. On the other hand, T4 and T5 were in general superior to T3 group in relation with these organs.

Hematological parameters:

The addition of TWP to aflatoxin – containing diet (T3, T4 and T5) completely ameliorated the adverse effects of aflatoxin on haematological parameters included in this study.

Supplementation of TWP to the diet significantly ($p< 0.05$) increased RBC, Hb, PCV, and WBC and reduced the H/L ratio compared with T2 group or the negative control (Table 3). However, T5 group surpasses other treatments as regards all of these blood characteristics. On the other hand, T4 and T5 were in general superior to T3 group in relation with these traits.

Immunity:

The impacts of AFB1 exposure on immune response to NDV, IBD, immunglobuline (IgA, IgM and IgG) and cytokine levels (IL-2, IL-6, and IFN-γ) in the serum of the control and AFB1-treated chicks were examined (Table 5). The immune response to NDV, IBD and immunglobuline (IgA, IgM and IgG) were significantly ($P<0.05$) decreased in birds fed aflatoxin- contaminated diets. Results presented in Table 5 indicated that TWP addition to contaminated diets has an immune stimulator effect through the improvement of immune response against NDV and IBD, as well as, IgA, IgG and IgM with the best level was TWP (5 and 7.5g) compared with AFB1-negative control diet (T2) and other treatments and control group (Table 5).

The inflammatory activity is estimated by the production of pro-inflammatory cytokine in serum, as shown in Table 5. Notably, AFB1 treatment significantly increased serum cytokine levels (IL-2, IL-6, and IFN-γ) levels ($P < 0.05$), whereas TWP supplementation decreased the levels of these two

inflammatory markers in comparison with the AFB 1 birds (P < 0.05). The results proved that TWP is able to mitigate the inflammatory response induced by AFB 1.

Serum and hepatic oxidative status:

The effects of TWP on serum and hepatic antioxidant parameters of chicks challenged with aflatoxin are presented in Table 6.

Table (2): Effect of Tomato powder on relative organs weight of broilers at 35 day.

Items	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Liver %	2.44c	2.95a	2.71ab	2.63b	2.56bc	0.125	0.001
Lymphoid organs %							
Bursa	0.168a	0.126d	0.133c	0.154b	0.161ab	0.052	0.022
Thymus	0.544a	0.461c	0.488b	0.509ab	0.535a	0.122	0.031
spleen	0.119b	0.134a	0.128a	0.121b	0.120b	0.023	0.001

a,b,c values with different superscripts in rows were significantly different (P<0.05). SEM =Standard Error of the mean; T1: Basal diet (B); T2: B + AF (100µg/kg); T3: B + AF (100µg/kg) + TWP (2.5 g/kg); T4: B +AF (100µg/kg) + TWP (5.0 g/kg); T5: B + AF (100µg/kg) + TWP (7.5 g/kg).

Table (3): Effect of Tomato powder on some hematological parameters of broilers at 35 day.

Items	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Red blood cells (X 10^6/µl)	2.48a	2.06c	2.25b	2.38ab	2.40ab	0.185	0.024
White blood cells (X 10^3/µl)	0.168a	0.126d	0.133c	0.154b	0.161ab	0.052	0.022
Hemoglobin, g/dl	11.22a	9.18c	10.02b	10.63ab	11.08a	1.521	0.022
Heterophil, (H) %	32.15b	35.83a	33.98ab	32.88b	32.75b	3.641	0.002
Lymphocyte, (L) %	67.82a	64.15c	65.74b	66.25ab	67.05a	4.336	0.001
H/L ratio	0.474c	0.559a	0.517ab	0.496b	0.488c	0.038	0.003

a,b,c values with different superscripts in rows were significantly different (P<0.05). SEM =Standard Error of the mean; T1: Basal diet (B); T2: B + AF (100µg/kg); T3: B + AF (100µg/kg) + TWP (2.5 g/kg); T4: B +AF (100µg/kg) + TWP (5.0 g/kg); T5: B + AF (100µg/kg) + TWP (7.5 g/kg).

Table (4): Effect of Tomato powder on some blood constituents of broilers at 35 day.

Items	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Total protein (g/dl)	4.08a	3.52c	3.70b	3.78ab	3.84a	0.247	0.024
Albumin (g/dl)	1.53a	1.13c	1.24bc	1.29b	1.32b	0.173	0.006
Globulin (g/dl)	2.55a	2.39b	2.46ab	2.49a	2.52a	0.189	0.021
A/G ratio	0.600a	0.472c	0.504bc	0.518b	0.524b	0.023	0.034
Cholesterol (mg/dl)	138.3a	112.6c	127.2b	131.0ab	136.5a	2.051	0.001
HDL (mg/dl)	89.5a	61.3c	77.8b	82.0ab	84.7a	2.341	0.035
LDL (mg/dl)	28.0c	45.8a	37.0b	32.5bc	29.0c	3.004	0.001
AST (u/l)	42.2c	71.8a	60.81b	51.17c	48.8c	2.345	0.028
ALT (u/l)	7.56c	11.86a	9.36b	8.20c	7.90c	1.003	0.025
Uric acid (mg/dl)	3.75c	4.99a	3.90b	3.86bc	3.65c	0.357	0.001
Creatinine (mg/dl)	0.475d	1.02a	0.685b	0.526c	0.508cd	0.044	0.002

a,b,c values with different superscripts in rows were significantly different (P<0.05). SEM =Standard Error of the mean; T1: Basal diet (B); T2: B + AF (100µg/kg); T3: B + AF (100µg/kg) + TWP (2.5 g/kg); T4: B +AF (100µg/kg) + TWP (5.0 g/kg); T5: B + AF (100µg/kg) + TWP (7.5 g/kg); AST= aspartate aminotransferase and ALT = alanine aminotransferase.

Table (5): Effect of Tomato powder on the immune factors content in serum of broilers at 35 day.

Items	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Immunoglobulin content in serum							
IgA (µg/mL)	1.92a	1.02c	1.10c	1.45b	1.76ab	0.025	0.004
IgG (µg/mL)	92.0a	63.0c	70.5c	77.8b	86.0ab	4.225	0.012
IgM (µg/mL)	4.0a	2.6c	2.9c	3.20b	3.6ab	0.125	0.001
The antibody content in serum							
NDV (ng/mL)	1300a	960d	1000c	1205b	1280a	12.354	0.025
IBDV (ng/mL)	15.0a	12.0c	12.8c	13.4b	14.0ab	1.002	0.001
pro-inflammatory cytokine in serum							
IL-2 (Pg/mL)	110d	310a	289b	208bc	165c	2.365	0.035
IL-6 (Pg/mL)	13.0d	22.0a	20.0b	18.6bc	15.0c	0.358	0.001
IFN-γ (ng/L)	136.0c	180.0a	168.0b	160.7b	140.2c	4.022	0.014

a,b,c values with different superscripts in rows were significantly different (P<0.05). SEM =Standard Error of the mean; T1: Basal diet (B); T2: B + AF (100µg/kg); T3: B + AF (100µg/kg) + TWP (2.5 g/kg); T4: B +AF (100µg/kg) + TWP (5.0 g/kg); T5: B + AF (100µg/kg) + TWP (7.5 g/kg).

Table (6): Effect of Tomato powder on serum and hepatic oxidative status of broilers at 35 day.

Items	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Antioxidant parameters of serum							
TAC (mmol/L)	0.59a	0.42c	0.45c	0.50b	0.60a	0.056	0.002
GSH-Px (U/ml)	1782.5a	1566.2d	1590.5c	1645.2b	1720.8ab	23.554	0.025
SOD (U/ml)	111.5a	100.6b	107.5b	109.3a	110.2a	4.361	0.001
MDA (nmol /ml)	3.16c	5.48a	5.11a	4.21b	3.75bc	0.258	0.032
Hepatic antioxidant parameters							
TAC(nmol/mgprot)	141.5a	110.2c	120.3c	129.2b	136.8ab	3.224	0.001
GSH-Px(U/mgprot)	58.35a	49.32c	51.62bc	54.65b	56.71a	5.321	0.032
SOD (U/mgprot)	1782.2a	1515.5c	1600.3c	1688.1b	1730.5ab	22.054	0.001
MDA (mol/mgprot)	2.01c	4.15a	3.15b	2.64c	2.38c	0.015	0.025

a,b,c values with different superscripts in rows were significantly different (P<0.05). SEM =Standard Error of the mean; T1: Basal diet (B); T2: B + AF (100µg/kg); T3: B + AF (100µg/kg) + TWP (2.5 g/kg); T4: B +AF (100µg/kg) + TWP (5.0 g/kg); T5: B + AF (100µg/kg) + TWP (7.5 g/kg). TAC, total antioxidant capacity; SOD, total superoxide dismutase; MDA; malondialdehyde.

Data showed that AFB1 administration (T2) increased serum and liver Malondialdehyde (MDA) content (p<0.05), this content of MDA was almost restored in AFB1+TWP group in comparison to the control level. The scavenging ability of antioxidant enzymes (SOD, GPx, and CAT) were decreased following exposure of chicks to AFB1 (P<0.05). Whereas TWP counteracts this effect in the liver of AFB1 treated group (P > 0.05). Co-administration of TWP and AFB1 relieved AFB1 -induced oxidative stress and lowered the MDA level.

DISCUSSION

Aflatoxin B1 is known to be the most harmful metabolite, predominantly in the sensitive species such as poultry particularly on their performance (Shareef, and Sito 2019), biochemical and immunological characteristics (Manegar *et al.,* 2010) and hematological (Oguz *et al.,* 2000). In the current study, the inclusion of 100 ppb of AFB1 to the diet of broilers had negative impact on the hematological, immunological and biochemical parameters confirmed by the other studies through the addition of AF at a rate of 250 and 500 ppb on body weight gain, feed intake, feed converion ratio and relative organ weights (Rosa *et al.,* 2001).

Liver and lymphoid organs weight:

The target organ for the bioconversion of AFB1 to AFB1 epoxide, which has the ability to bond with proteins, RNA, and DNA, is the liver. According to Shannon *et al.* (2017), this binding not only raises the relative weight of the liver but also causes the inactivation of antioxidant enzymes, which leads to the accumulation of peroxides. In agreement to our findings, it is reported that AFB1 elevated relative weight of liver and numerical increases in liver weight due to lipid accumulation in the liver, which results in hepatomegaly (Rajput *et al.,* 2017). Additionally, Magnoli *et al.* (2011) noted that AF impairs the liver by increasing fat and relative weight as well as decreasing the organ's total secretory capacity.

The current study found that the weight of the spleen was significantly higher ($P<0.05$) in the AF group (T2) when compared to the positive control group (T1). The weights of the thymus and bursa were significantly lower ($P<0.05$) in the AF group when compared to the control group (Table 2). The findings of (Valchev *et al.,* 2017; Nemati *et al.,* 2015; Shareef and Sito, 2019) were in agreement.

Conversely, the inclusion of AF in the diet increased the weight of the spleen while suppressing the relative weights of the lymphoid organs (bursa and thymus). These outcomes are consistent with research by Solcan *et al.* (2014) and Attia *et al.* (2016), which demonstrated that AF-contaminated diets caused a noticeable and progressive thymocyte depletion through the apoptotic process and decreased the relative weights of the thymus and bursa. In this connection, Campbell *et al.* (1983) found that the relative weight of the bursa of Fabricius, and thymus were decreased by AF. Hence, the current study's findings proved that AF has a negative impact on the immune system may account for the decreased relative weights of the thymus and bursa of Fabricius but increased spleen percentage when compared to the control group.

Lycopene-rich tomato waste powder also strengthened immune system performance and shielded lymphocytes from oxidative stress (Khalil *et al.,* 2022). Compared with other carotenoids like carotene, lutein, and zeaxanthin, LYC is the most effective singlet oxygen quencher (Palozza *et al.,* 2011). Sun *et al.* (2014) examined the beneficial impacts of lycopene on breeding hens exposed to lipopolysaccharide and found that feeding hen's lycopene (20–80 mg/kg) for 35 days improved the immune organ indices of the spleen, bursal and thymus.

Immunity parameters:

As an oxidative agent, aflatoxin may weaken the immune system and make animals more vulnerable to various illnesses (Wilasrusmee *et al.,* 2002). Aflatoxin was found to dramatically lower the titers of antibodies against vaccinations for Newcastle disease virus (ND), infectious bronchitis virus (IBV), and infectious bursal disease (IBDV) in an experiment conducted by Mamta et al. (2015).

The immunesuppressive effect of AFs has been linked to its direct inhibition of protein synthesis such as immunoglobulins IgA and IgG (Rajput *et al.,* 2017), reduction of the hemolytic activity of complement (Chen *et al.,* 2014), and reduction in the number of lymphocytes through its adverse impact on the bursa of Fabricius. This could be related to the fact that aflatoxins works as an inhibitor of protein synthesis and as resulted, destroy cells and tissues with a high protein turnover such as that found in the liver, immune system or gut epithelium, which is most susceptible to the adverse impacts of AF. Thus, it has been shown that AF exposure suppresses the immunological response in chickens (Hossein & Gurbuz, 2015). Moreover, aflatoxin can inhibit the development of the thymus gland or adversely effect on the relative weight of the bursa of Fabricius, which may result in in serious deficiency in both cellular and antibody responsiveness of the chicken immune system (Celik *et al.,* 2000). Inhibition of macrophage functions, T lymphocyte activity or cytokine expression by AF results in vaccine failure or pathogen persistence, as exemplified in numerous research by decreased immunoglobulin production (Yunus *et al.,* 2008).

When comparing the fourth and fifth groups of broilers fed on 5.0 or 7.5 g TWP/kg contaminated diets to the AF group (T2), data in Table (2) showed a substantial rise in the ND and IBD antibody titer level. The current findings showed that 7.5 g TWP/kg diet (T5) was the best level as an immune stimulator in broiler chicks.

Carotenoids, which act as natural antioxidants, are abundant in tomato by-products. Lycopene makes up around 80–90% of the total amount of carotenoids, while b-carotene makes up the remaining 7–10% (Nour *et al.,* 2018). The main carotenoid found in tomato waste, lycopene, has been shown to have an immunoprotective properties (Xu *et al.,* 2019). Tomato powder added up to 2% to the meals of growing rabbits improved the immune response by boosting phagocytosis, chemotaxis, and immunoglobulins (IgM, IgG, and IgA) (Elwan *et al.,* 2019). Furthermore, feeding chickens with lycopene up to 80 mg/kg improved their immunological organ index and reduced the stress caused by lipopolysaccharide (LPS)

(Sun *et al.,* 2014). According to Palozza *et al.* (2011), Lycopene mediates cell growth regulation, immune response, and modulation of phase I and II detoxifying enzymes and gene transcription.

Inflammation is also an essential factor for AFB1 hepatotoxicity. A number of danger signals linked to AFB1 metabolites, such as high ROS and toxins, contribute to the inflammatory cascade that causes cell damage. Pro-inflammatory cytokine levels in serum are thought to be indicators of cellular immunity. In the current study, AFB1 group (T2) showed a significant elevation in serum pro-inflammatory cytokines such as (IL-2, IL-6, and IFN-γ). These findings are consistent with recent studies suggesting that AFB 1 may cause inflammation and modifying the immune system (Hassan *et al.,* 2020). However, the addition of tomato waste powder considerably reduced the elevated levels of IL-2, IL-6, and IFN-γ in the serum of chicks fed AFB1.

According to Camara *et al.* (2013), fresh tomatoes have lycopene concentrations ranging from 0.594 to 03.09 mg/100 g. Lycopene has been demonstrated to have significant prooxidant and antioxidant capabilities (Selim *et al.,* 2013). However, because of its conjugated structure generated from a double bond system, lycopene has shown an extraordinary capacity to absorb reactive oxygen species. Accordingly, antioxidants have the potential to drastically alter the high levels of inflammatory cytokines in broiler blood subsequent to AFB1 diet consumption, as observed by Rajput *et al.* (2017) and Rajput *et al.* (2019). All these findings also validated the anti-inflammatory and antioxidant action of TWP that could be responsible for its decisiveness effect.

Hematological parameters:

In broiler chicks, feeding aflatoxin alone (T2) resulted in a significant ($P<0.05$) drop in RBC, Hb, PCV, and WBC, and a significant increase in the H/L ratio (Tables 3). These results are consistent with other publications that explain aflatoxin's suppressive effects on immunological response and hematopoiesis (Oğuz *et al.,* 2003). When broiler chicks are fed 2.5 to 3.5 g aflatoxin/kg feed, they exhibit lower PCV, Hb, and lymphocyte and monocyte counts (Scheideler, 1993); also, heterophils are increased (Oğuz *et al.,* 2000). Aflatoxin may have impacted immune system and hemopoietic tissue, which could have impacted cell production. Numerous investigations have revealed that aflatoxin reduced PCV and RBC levels, while aflatoxisis resulted in lymphocytopenia and heterophilia in broiler chicks (Safameher *et al.,* 2004). Nonetheless, Gross and Siegel (1983) found that the H/L ratio is a reliable measure of physiological stress and that there is a positive association between plasma corticosterone and H/L. Consequently, a rising H/L ratio suggested that the birds were experiencing extreme stress. When comparing the fourth and fifth groups of broilers fed on 5.0 or 7.5 g TWP/kg contaminated diets with AF to the AF group, data in Table (4) showed significantly improve in the hematological parameters. Moreover, the antioxidant activity of lycopene may be responsible for the rise in white blood cells, particularly the percentage of lymphoid cells, and the fall in the fraction of developed lymphoid cells (Ried and Fakler, 2011).

Serum biochemical parameters:

The significant rise in the concentration of liver enzymes being AST and ALT (Table 4) in AFB1 group (T2) was in agreement with findings of (Denli *et al.,* 2009; Naseem *et al.,* 2018). Liver is the primary organ of AF accumulation and metabolism, liver is also the main site where AF is metabolized and where the metabolites bind with nucleic acids and proteins (Fouad *et al.,* 2019). ALT is particularly useful in measuring hepatic necrosis, as it is a key cytoplasmic enzyme present in liver and other cells. Because AST and ALT are found in the cytoplasm and are released into the bloodstream following cellular injury, their elevated serum levels have been linked to the liver's damaged structural integrity (Ahsan *et al.,* 2009). Thus, elevated levels of AST and ALT in the blood suggest altered metabolism, increased membrane permeability, and cellular injury (Ramazzotto and Carlin, 1978).

At the end of the trial period, there is a significant reduce in AST and ALT levels in group treated with TWP in comparison with AF group (T2) and that current finding agrees with (Ţigu *et al.,* 2016). There is substantial evidence that lycopene (primary ingredient of tomato) is capable of decreasing serum ALT and AST (Eze *et al.,* 2016). These findings are consistent with earlier researches, suggesting that lycopene may have protective benefitst against liver damage induced by toxic compounds including AFB1, ochratoxin A, atrazine in rats and mice (Aydin *et al.,* 2013; Xia *et al.,* 2016).

The primary regulatory mechanism for preserving bodily homeostasis is the kidneys. Renal function is determined by the plasma concentrations of uric acid and creatinine, which is why they are regarded as biomarkers for kidney disease (Levey *et al.,* 1999). According to Eaton and Pooler (2004), the kidney is in charge of preserving the extracellular medium's homeostasis, electrolyte balance, and eliminating

waste products from the body's metabolism. The majority of aflatoxin residues are found in the kidneys, which also participate in the detoxification of aflatoxins (Fernandez *et al.,* 1994).

The current results of significantly elevated serum creatinine were in consistent with those of (Hashem & Mohamed 2009; Rashidi *et al.,* 2018). Elevated level of creatinine may be attributed to the accelerated rate of protein catabolism and nephrotoxic impact of aflatoxin evident by pathomorphological changes in kidneys. Furthermore, Gowda *et al.* (2009) found that elevated uric acid and creatinine concentrations in 2 and 6-weeks old broilers fed 3 mg/kg AFB1 contaminated feed was related to inflammatory and dystrophic processes in the renal tubules. These findings are acknowledged as a suggestion that AFB1 exposure may lead to degenerative changes in the kidney, leading to a reduce in the function of this organ.

The mechanism underlying proanthocyanidins nephroprotection may be due to the marked radical scavenging ability of proanthocyanidins (Sato *et al.,* 2005). These impacts of lycopene are linked to the prevention of the oxidative stress, which raised the antioxidant capacity of the body and maintenance of the permeability of the cell membrane (Yilmaz *et al.,* 2018).

Serum total protein and albumin were significantly reduced at the conclusion of the trial in the aflatoxin administrated group (T2) when compared to the positive control group (T1). This is indicative of the adverse impact of aflatoxin B1 on the liver and kidneys and is an indicator of decreased protein synthesis (Hussain *et al.,* 2016). These findings were in consistent with that reported by (Arafat *et al.,* 2017); (Subhani *et al.,* 2018) and (Cruz *et al.,* 2019). Aflatoxin in broilers is thought to be best detected by measuring the serum protein level (Tung *et al.,* 1975). Serum total protein concentrations were shown to be lower in broilers given 0.5 and 1 mg/kg AFB1 diet at 21 and 42 days of age (Safameher 2008). When AFB1 was 30 µg/kg, serum total protein, albumin, and globulin levels dropped, but the growth performance of broilers was unaffected, suggesting that low AFB1 concentrations do not affect growth performance but do hinder protein synthesis (Cruz *et al.,* 2019).

Generally, the addition of tomato waste powder showed a significant improve in serum protein, albumin and globulin at the end of the trial when compared to the aflatoxicated group (T2). Our findings are consistent with those of Cruz et al. (2019) who discovered the addition of plant antioxidants to AF contaminated diets and showed a significant rise in serum protein and albumin at the conclusion of the trial when compared to the AF group (T2).

The current study's results demonstrated that, when compared to the AF group (T2), the TWP supplemented plus AF groups had significantly lower serum concentrations of cholesterol, triglycerides and LDL. In contrast, the same groups had significantly higher levels of HDL ($P<0.05$).

It was anticipated that tomato powder, which contains lycopene, would act as an antioxidant and lower the overall cholesterol level of broiler chicks exposed to aflatoxin. According to Palozza *et al.* (2012), there are three mechanisms by which antioxidants lower cholesterol: 1) block the activity of HMG-CoA reductase, which lowers mevalonate synthesis, the basis for cholesterol formation; 2) Inhibit the cholesterol acyl transferase which will reduce the storage of cholesterol ester in the tissue; and 3) increase the activity of LDL receptor, which lowers LDL cholesterol in blood.

Hepatic and serum antioxidant status:

In the AF group of broilers exposed to aflatoxin-contaminated diet (T2), the current study found a significant increase in MDA level in Table (6) and a significant decrease in TAC, GSH, and SOD enzymes. These findings were consistent with those of earlier studies (Sharma *et al.,* 2011; Gowda *et al.,* 2009). Radical oxygen species (ROS) are produced when GPX and SOD are reduced, which is the hepatotoxic mechanism caused by AF. Oxidative stress can be brought on by ROS that are not counterbalanced by antioxidants (Wang *et al.,* 2005). The endoplasmic reticulum, mitochondria, and cell membrane will all sustain damage from free radicals. The phospholipase, protease, endonuclease, and ATPase enzymes are activated in this state, leading to a drop in phospholipids, disruption of membrane proteins, DNA fragmentation, and decreased ATP, ultimately causing necrosis in hepatocytes (Sulistyowati *et al.,* 2013).

There are two types of antioxidants: enzymatic and nonenzymatic (Palozza *et al.,* 2011). Glutathione reductase (GSH-r), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) are examples of enzymatic antioxidants. Enzymatic antioxidants, on the other hand, act as scavenge free radicals from both intracellular and extracellular origin, preventing lipid peroxidation of plasma membranes. Whereas, the nonenzymatic antioxidants found in plant-based foods are carotenoids, which also include vitamins C and E and phenolic compounds can be used to mitigate the adverse consequences of environmental origin stress (Wang *et al.,* 2020).

The fact that AF is metabolized via the cellular cytochrome P450 enzyme system, which causes lipid peroxidation and cellular injury (Stresser *et al.,* 1994), may be the cause of the increase in hepatic MDA levels (Kheir Eldin *et al.,* 2008), and may be because of a notable decline in the actions of enzymatic antioxidants. According to Chen et al. (2014), the primary enzymes of the antioxidant system, SOD and Gpx, are able to prevent oxidative damage, scavenge free radicals produced by oxidant stress, and preserve cell integrity.

Conversely, table (5) in the AF & TWP groups demonstrated a large rise in TAC, GSH, and SOD enzymes and a significant drop in MDA levels (Table 6). Similar findings were reported by Sahin et al. (2016), who found that in heat-stressed broilers, dietary lycopene concentrations of 20, 50, and 100 mg/kg food linearly increased serum activity of SOD and GSH-Px and decreased MDA concentration. Additionally, Li *et al.* (2017) found that at weeks two and four, broilers' levels of low density lipoprotein (LDL) and thiobarbituric acid-reactive substances (TBARS) decreased when fed tomato paste containing 10, 20, and 17 g/kg of lycopene.

High levels of lycopene in tomato powder give it strong antioxidant properties. In order to avoid cell damage, antioxidants function by providing electrons to inhibit the reactivity of free radicals, preventing the oxidation of lipids and proteins (Purnama *et al.,* 2020). Because of its acyclic structure, multiple conjugated double bonds, and high hydrophobicity, lycopene has a strong antioxidant defense system. This defense system protects and stabilizes biomolecules like DNA, proteins, lipids, and lipoproteins, preventing the onset of carcinogenesis and atherogenesis processes. When it comes to quenching singlet oxygen, lycopene the primary carotenoid found in tomato products has the highest capacity among the other carotenoids. Additionally, it scavenges the free radicals by means of three distinct pathways, including hydrogen atom transfer, electron transfer, and adduct formation. In this respect, Yilmaz *et al.*, (2017) found that torulene and lycopene are more reactive than β-carotene at scavenging peroxide radicals. Aflatoxicosis may be prevented by lycopene, a potent antioxidant (Juan et al. 2008). The acte of Lycopene was clear in reducing the damage of aflatoxin by activating the stage 2 detoxification and AFB-NAC production and blocks phase 1 metabolism of AFB1 and lowering AFB-N7 that adduct in liver DNA (Tang *et al.,* 2017; Reddy *et al.,* 2006). The hepatic activity of cytochrome P450 2A6 (CYP2A6) and P450 1A1 (CYP1A1) were significantly reduced in an experiment by Wan *et al.* (2022) when broilers fed AFB1-contaminated diets for 42 days and given 100–400 mg/kg lycopene. Lycopene has shown resistance to oxidative damage caused by aflatoxin B1 (AFB1), as evidenced by inhibition of Cyp450 isozymes and raised mRNA expression of the NRF2 signaling pathway (Wan *et al.,* 2022; Sarker *et al.,* 2021).

CONCLUSION

In conclusion, the broiler's immunological response, oxidative status, lymphoid organs, and metabolic parameters are all negatively impacted by the addition of 100 µg/kg AFB1 to the feed. Supplementation of tomato waste powder at levels (5.0 and 7.5 g/kg) improved the metabolic parameters, lymphoid organs, oxidative status, and immunological response in broiler chickens challenged with AFB1. The results of this study also suggested that supplementing broiler diets with tomato powder would be a useful strategy to mitigate the detrimental effects of AFB1 on broiler output.

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تأثير مسحوق مخلفات الطماطم على المعايير الكيموحيوية والأعضاء اللمفاوية والحالة التأكسدية والاستجابة المناعية في علائق دجاج التسمين المعرضة للأفلاتوكسينات

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في الدراسة الحالية، تم فحص المعلمات البيوكيميائية، وحالة الأكسدة، والأعضاء اللمفاوية والاستجابة المناعية لدجاج التسمين المتحدى بالأفلاتوكسين (AF) لتحديد الفعالية الوقائية للمكملات الغذائية مع مسحوق مخلفات الطماطم. تم توزيع 300 كتكوت لحم روس (308) بعمر يوم واحد عشوائياً في خمس معاملات وكل معامله تتكون من ستة مكررات (عشرة طيور لكل مكرره)؛ تم توفير العلف والماء للأستهلاك الحر طوال التجربة التي استمرت 35 يومًا. كانت المعاملات على النحو التالي: (T1) نظام غذائي أساسي لا يحتوي على افلاتوكسين اومخلفات طماطم (كنترول موجب) و (T2) نظام غذائي أساسي يحتوي على 100 ميكروجرام افلاتوكسين /كجم علف (كنترول سالب). تم استكمال المعاملات الثلاثة الأخرى بمخلفات الطماطم عند 2.5 و5.0 و7.5 جم/كجم علف في وجود 100 ميكروجرام افلاتوكسين /كجم علف على التوالي. أظهرت النتائج أن وجود الأفلاتوكسين أدى إلى انخفاض تركيز البروتين الكلي والألبومين والجلوبيولين وزيادة وظائف الكبد والكلى في دجاج التسمين مقارنة بمجموعة الكنترول الموجب. بالمقارنة مع مجموعة الكنترول السالب (T2)، أدى إدراج 5 و7.5 جم مخلفات طماطم /كجم علف الى زيادة البروتين الكلي في المصل والألبومين والجلوبيولين كما أدت إضافة مخلفات طماطم إلى انخفاض وظائف الكبد والكلى في الدجاج التسمين مقارنة مع مجموعة الكنترول. انخفض الوزن النسبي للأعضاء اللمفاوية (الغدة الصعترية والجراب) والاستجابة المناعية لـ NDV وIBD في مجموعة الكنترول السالب (T2)، في حين زاد الوزن النسبي للطحال ونسبة التغاير ونسبة الخلايا اللمفاوية المتغايرة ($P < 0.05$). أدى وجود الأفلاتوكسين إلى زيادة تركيز المالونديالدهيد في الدم والكبد، في حين انخفض تركيز TAC وSOD وGSH في الدم والكبد. بالمقارنة مع مجموعة الكنترول السالب (T2)، فإن مستويات 5 و 7.5 جم مخلفات طماطم / كجم) حسنت تركيز حالة الأكسدة في الكبد والمصل ($P < 0.05$). يمكن الاستنتاج أن مستويات 5 و 7.5 جم مخلفات طماطم / كجم) قادرة على تقليل التأثير السلبي للأفلاتوكسين على الخصائص البيوكيميائية لدجاج التسمين، والأعضاء اللمفاوية، والحالة التأكسدية، والاستجابة المناعية.