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INVESTIGATING THE POTENTIAL OF SILYMARIN AND/OR SPIRULINA PLATENSIS TO ATTENUATE THE DELETERIOUS CONSEQUENCES OF AFLATOXIN CONTAMINATION IN BROILERS' FEEDS

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ABSTRACT

The aim of this investigation was to establish the impact of silymarin and/or *Spirulina platensis* in mitigating the adverse effects of aflatoxin (AF) present in poultry feed. A total of 450 one-day-old, unsexed Ross 308 broiler chicks were allocated to a completely randomized design with five experimental groups. Each group consisted of six replicate, housing 15 chicks per replicate. The treatment groups comprised: 1) the negative control (NC) fed the standard diet lacking AF supplementation; 2) the positive control (PC) fed the standard diet contaminated with 1 mg AF/kg; 3) the silymarin (SIL) fed the PC diet + 0.6 g silymarin/kg feed; 4) the *Spirulina platensis* (SP) fed the PC diet + 1g /kg diet; 5) the silymarin +*Spirulina* fed the PC diet + 0.6 g SIL/kg + 1g SP/kg diet. Productive performance, serum biochemical profile (levels of AST, ALT, total cholesterol, HDL, uric acid, creatinine, and calcium), weight of lymphoid organs, levels of glutathione and malonaldehyde in the liver, antibody titers against NDV and IBD, concentration of cecal bacteria, nutrition composition of flesh, and level of remnants of AF in liver and flesh were studied. Our findings revealed a successful reversal of adverse effects caused by AF. Supplementation with either SIL and/or SP restored performance metrics to the levels observed in the non-contaminated (NC) fed control group. These results indicate that dietary inclusion of 0.6 g of SIL/kg and/or 1 g of SP/kg represents a suitable strategy to maintain broiler growth performance, immune function, serum composition, and meat quality in birds exposed to AF contamination at a concentration of 1 mg/kg of feed.

Keywords: aflatoxicosis, silymarin, *Spirulina platensis,* poultry, productive performance

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INTRODUCTION

Aflatoxins (AF) are a byproduct of metabolic processes of some fungi, including *Aspergillus parasiticus* and *Aspergillus flavus.* These toxins are thought to be the chief source of pollution for cereals, common foods, and feed ingredients, notably in Africa, Asia, and Latin America (Attia *et al.,* 2013; Ditta *et al.,* 2018). Several animal species, including poultry, are susceptible to the liver carcinogenic, hepatotoxic, teratogenic, and mutagenic effects of aflatoxins. The poultry industry has recently paid substantial attention to medicinal plants and their chemicals because they establish the groundwork for improved performance and feed quality. Aflatoxicosis, also known as aflatoxin poisoning, significantly lowers growth rate, feed intake, survival rate, and feed utilization in broilers (Kurniasih and Prakoso, 2019; Soltani *et al.,* 2019).

Aflatoxicosis can lead to anorexia, which may be the reason for the lower feed intake and thus weight gain of the animals. Trebak *et al.* (2015) demonstrated that AF decreased the manifestation of orexigenic and anorexigenic neuropeptides in the hypothalamus, causing a disbalance in appetite control in rats. Furthermore, AF interferes with energy metabolism due to the inhibition of the electron transport chain and destroys the normal

structure of mitochondria. Metabolites of AF react with various cells, inhibiting carbohydrate and lipid biochemical process, and protein synthesis (James *et al.,* 2021; Nabi *et al.,* 2022).

Although removing fungi from diet and feed is crucial, a high level of mycotoxins, notably aflatoxins, continues to be excepted. Manufacturers, researchers, and governments have been worried for a long time about using various methods to reduce the hazardous effects of aflatoxins (Oguz *et al.,* 2018). In this instance, it has been shown that natural substances like sodium bentonite, mannose oligosaccharide, and zeolite can counteract the harmful impacts of aflatoxin B1 (Attia *et al.,* 2016). However, mycotoxins in poultry diets could be counteracted by using biological techniques (Rasouli-Hiq *et al.,* 2017).

A well-known plant, *Silybum marianum* (SIL) commonly indicated as milk thistle, is most notable for its hepatoprotective extract known as slymarin (SIL). SIL contains several flavonolignans, including isosilibinin, silybinin, isosilychristin, silydianin, and silychristin, as well as a flavonoid called taxifolin, which together has anti-inflammatory, antioxidant, cell membrane-stabilizing, anti-lipid peroxidative, antifibrotic, and liver-regenerating properties. The primary active component of SIL is the silybin which exhibits antioxidant and hepatoprotective effects in both human and animal models (Federico *et al.,* 2017). SIL increases protein synthesis by metabolically stimulating liver cells and activating ribosomal RNA production (Vargas-Mendoza *et al.,* 2014). SIL has been demonstrated to enhance the activity of natural antioxidant enzymes in the livers of stressed laying hens (Pradeep *et al*., 2007). Additionally, it reduces lipid oxidation in broiler chickens (Alhidary *et al.,* 2017; Baradaran *et al.,* 2019). SIL has been associated with a decrease in DNA breakdown and cellular programmed death (Upadhyay *et al.,* 2010). Furthermore, it has been shown to mitigate hepatic damage induced by free radicals, leading to a reduction in the secretion of liver enzyme such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Amiridumari *et al.,* 2013).

Algae nutritional supplementation enhanced the intestinal and hepatic antioxidant capacity of aged laying hens and assisted in detoxifying diets contaminated with aflatoxin, according to Tufarelli *et al.* (2021). Due to its therapeutic benefits and its nutrient rich, *Spirulina platensis* (SP) has opened new avenues for study on various feed additives for animal diets. These algae may act as immunity or performance boosters, improving the nutritional value of the food consumed by animal species (Attia *et al.,* 2023). In a study conducted by Suwarno *et al.* (2019) demonstrated a significant reduction in aflatoxin production by *A. flavus* when exposed to commercially available carotenoids, particularly beta-carotene and beta-cryptoxanthin. For 38 Aspergillus genotypes isolated from maize, an in vitro investigation indicated that commercially available carotenoids suppressed aflatoxin production by >70%. This finding suggests a potential strategy to mitigate aflatoxin contamination in maize. Building on this initial discovery, the current study could explore the efficacy of dietary supplementation with carotenoid-rich sources, such as the microalga *Spirulina platensis* (SP), in reducing aflatoxin accumulation in broiler chickens. Such a study could evaluate the impact of supplementation on production performance, blood chemistry, lymphoid organ weights, immune response, and residual aflatoxin levels in the meat and liver tissues of birds consuming contaminated diets.

MATERIALS AND METHODS

Aflatoxin B1 production: Culture taken from reference strain *Aspergillus parasiticus* NRRL2999 were introduced into 250-mL Erlenmeyer flasks containing 25 g of sterilized rice and 10 mL of purified water. The aim was to generate aflatoxin B (AFB) following the method described by Shotwell *et al.* (1966).

The specimens were placed in a light-free environment at a temperature of 28 °C for a duration of 7 days, with the flasks being manually stirred forcefully for 1 minute once a day for the first 5 days to promote conidia diffusion in the rice. The cultures were sterilized after incubation. The flask contents were transferred onto a metal tray, covered with paper, and exposed to drying in a forced air oven at 60 °C, then pulverized in a laboratory mill. The amount of aflatoxin B1 in the resultant powder was established through.

High-Performance Liquid Chromatography (HPLC) was used as explained by Sharma and Marquez. (2001) for AFB1 determination. Therefore, the AFB1 was premixed to the control diet to have 60.0 ± 1.1 µg/g (positive control). The final concentration in the experimental diet was fixed at 1 mg of AFB1/kg of feed (positive control).

Birds and experimental design: The research adhered to the protocols outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the Animal Production Research Institute, ARDC, Ministry of Agriculture, Egypt. The approval, in accordance with animal welfare regulations (Decree No. 27, 1967), ensures the humane treatment of animals in general. This experiment was founded on a completely randomized design using 450, one-day-old Ross 308 unsexed broiler chicks with average weight 42.0 ± 2.1 g. Chicks were designed into five experimental groups as shown in Table (1) each containing six replicates of 15 chicks. The experimental diets (Table 2) were formulated according to (Aviagen, 2019), and broilers nutrients requirements using NRC (1994) feed stuffs composition tables.

Table 1. The experiment groups tested herein.

Treatment	Aflatoxin	Silymarin	Spirulina	Silymarin+Spirulina
NC	0.0	0.0	0.0	0.0
PC	1 mg/kg	0.0	0.0	0.0
PC+SIL	1 mg/kg	0.6 g/kg	0.0	0.0
PC+SP	1 mg/kg	0.0	1 g/kg	0.0
PC+SIL+SP	1 mg/kg	0	0	0.6 g SIL+1 g SP

NC: Negative control; PC: Positive control; SL: Silymarin; SP: *Spirulina platensis*.

Table 2. Composition and calculated analysis of the negative control experimental starter, grower, and finisher diets for Ross 308 chicks.

Ingredients	Starter (1-10d)	Grower (11-21d)	Finisher (22−35 d)
Yellow corn	55.07	59.08	64.09
Soybean meal, 44%	33.5	29.4	24.0
Corn Gluten meal, 60%	5.00	5.00	5.00
Corn oil	2.00	2.65	3.15
Limestone	1.35	1.00	1.00
Di-calcium phosphate	1.73	1.60	1.50
Vit & min. premix*	0.30	0.30	0.30
DL-Methionine	0.15	0.12	0.10
L-lysine (HCl)	0.35	0.35	0.30
NaCl	0.40	0.40	0.40
Choline chloride	0.05	0.05	0.05
Sodium bicarbonate	0.10	0.10	0.10
Total	100	100	100
Calculated analysis			
Crude protein, %	23.00	21.50	19.50
Metabolizable energy (Kcal/kg)	3010	3105	3200
Calcium, %	1.00	0.87	0.82
Available phosphorus, %	0.47	0.44	0.41
Lysine, %	1.44	1.29	1.14
Methionine, %	0.56	0.51	0.47
Methionine+ Cystine	0.93	0.86	0.78

*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 2,000 mg, vitamin B1 1,000 mg, vitamin B2 5,000 mg, vitamin B6 1,500 mg, vitamin B12 10 mg, niacin 30,000 mg, biotin 50mg, folic acid 1,000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4,000 mg, iodine 300 mg, selenium 100mg, and cobalt 100 mg.

Before feeding the birds, the basal diet underwent examination to detect the presence, of residual aflatoxin and other mycotoxins. The results showed that the concentrations of these mycotoxins were not detectable according to the limits of the test, which were 2 µg/kg, 10 µg/kg, 50 µg/kg, 100 µg/kg, and 1.0 µg/kg for aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, and fumonisin B1, respectively using a method described by Sharma and Marquez (2001).

Mash feed and water were administered *ad libitum* from hatch to day 35. Experimental groups were reared on floor pens, each 1x 1.5 square meter, equipped with rice hulls as litter. The house temperature was kept at 32-33°C at the onset of the investigation, and by the conclusion of the initial week it declined progressively to 28-29°C and kept on the same thereafter. The illumination cycle was 23 h light: 1 h dark in the first week and 20 h light: 4 h dark beginning in the second week. Broilers were vaccinated via drinking water against Newcastle disease (NDV, Nobilis® ND Clone 30) at days 7 and 19 of age and Gumboro disease (IBD, Bursine® Plus) at days 14 of age, and subcutaneously injections against avian influenza and Newcastle disease (H9N1, CEVAC® NEW FLU H9 K) at day 7 of age. These vaccines were manufactured by MSD animal health Co., Egypt, Zoetis, US, and Ceva Animal Health, Egypt, respectively.

Silymarin and Spirulina platensis sources: Silymarin used in the trial was purchased from Samwon

International LTD. (Nanjing, China) and had a molecular weight and purity of 482.44 g/mol and 60%, respectively.

Desert Research Center of Egypt's "Utilization of marine algae for salt fodders, milk, meat, and fish production under saline conditions" project provided the powdered of *Spirulina platensis* with the following composition: 57% crude protein, 5.1% fat, 3.9% crude fiber, 13.6% ashes, 8.0% moisture, 1.2% Fe, 2.15% Ca, 1.6% Zn, 0.41% P, 0.8% Mg, 0.76% Mn, 9 mg/g ascorbic acid, 1.42 mg/g vitamin A, 7.82 mg/g chlorophyll A, 1 mg/g vitamin E, 4 mg/g chlorophyll B, and 7.05 mg/g total carotenoids (Desert Research Center, Mataria, Cairo, Egypt).

Measurements

Growth performance: Upon the conclusion of the experimental period, the researchers assessed the feed intake (FI), measured the final body weight (FBW), and determined the body weight gain (BWG) of the chicks. Day to day mortality records were maintained. The feed conversion ratio (FCR) was computed using FI and BWG, adjusted as needed to account for mortality.

Blood profile: Upon completion of the experimental timeframe, six blood specimens, as 1 sample per replicate, were collected for each treatment during the chick's slaughter to determine the serum parameters. The sera were collected after 15 minutes of centrifuging blood at 3,000 rpm, stored at -20°C, and then submitted for biochemical evaluation. A spectrophotometric (Shimadzu UV 1601) method was used to evaluate each serum sample for aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), uric acid, creatinine, and calcium using commercially available kits by Stanbio Laboratory (Boerne, Texas, USA).

Oxidative status-related indices were evaluated on six samples of liver tissue per group. The samples were homogenized in cool physiological saline (PSA), filtered, and centrifuged with a fast-cooling rotator at 1,500 rpm for 20 min at 4° C. (Type 3K-30, Sigma, Osterodeam-Harz, Germany).

Malondialdehyde (MDA) and glutathione (GSH) amount were determined after storing the clear supernatant at -80°C. In line to Lowry *et al*. (1951), the amount of protein in the liquid portion was calculated as mg protein/g of wet tissue. The GSH and MDA were conducted following the procedures proposed by Nishikimi *et al.* (1972) and Uchiyama and Mihara (1978). The ratio between the MDA/GSH was calculated as an indicator of the antioxidant balance.

Immune indices: At day 35 of age, six birds from each treatment, as one bird per replicate, were randomly selected and slaughtered, and their liver and lymphoid organs (bursa, spleen, and thymus) were weighed and expressed as a share to of the alive body weight.

The ELISA assay was utilized to evaluate the immune reactions to NDV and IBD (Indical Bioscience-GmbH, Leipzig, Germany) (Elbaz *et al.,* 2021).

Intestinal microbial populations: At slaughtering 6 samples of cecal content per treatment were collected to evaluate the content of coliforms, *Salmonella* spp. and *E. coli*. About 1 g of cecal content of each bird was collected into an antiseptic receptacle. After correctly mixing, the samples were relocated to pH-7.2 in phosphate buffer (PBS) tubes. Coliforms were cultured on McConkey agar, *Salmonella* was cultured on Salmonella-Shigella agar (SS), and *E. coli* on eosin methylene blue agar (Darmstadt, Germany). Identical colonies with a minimum diameter of 1 mm were called colony-forming units (CFU) (Hosseintabar *et al.,* 2014).

Breast meat quality: The method of Anderson (2007) was used to evaluate breast meat's (six samples/treatment), total protein and fat content. The method defined by El-Medany and Reffaei (2015) was used for the determination of triacylglycerol and cholesterol contents.

Aflatoxin residual: The presence of aflatoxin in liver tissue and meat (6 samples/treatment) were evaluated applying liquid chromatography with high performance. The removals of aflatoxins from the tissues involved the use of immune-affinity columns (Aflatest ÒWB, Vicam USA), and their quantification was performed through an HPLC-fluorescent detector method with pre-column derivatization. A variation of the methods outlined by Chiavaro *et al.* (2005) and AOAC (2000) was applied, briefly described as follows.

Twenty-five grams of thawed sample were blended thoroughly with 5 grams of NaCl in 100 ml of methanol: water solution (80:20) for a duration of three minutes. After passing through a paper filter, a portion of 10 ml of the filtered liquid was mixed with 40 ml of PBS Wash Buffer, which contained 0.1% Tween-20, and then was filtered again through an immunoaffinity column. AFB1 was separated by washing it out using 1.0 ml methanol in a glass container and then dried almost completely using a gentle stream of nitrogen until it reached a nearly dry state. The process of pre-column derivatization was performed using trifluoroacetic acid (AOAC, 2000). Following this, a 20 ml segment of the derivatized extract was introduced into the HPLC system (Shimadzu LC-10 AS), which was outfitted with a reverse-phase column (Supelco SIL LC-18, measuring 15 cm x 4.6 cm ID) and a fluorescent detector (Shimadzu RF-530). The detector was set to wavelengths of 360 nm for excitation and 440 nm for emission. The mobile phase comprised water, acetonitrile, and methanol (60:20:20). The limit of detection (LOD) was 0.025 ng/ml, and the

recovery rate was 89%. Reference materials for aflatoxin B1, B2, G1, and G2 (Supelco®, Merk) were utilized in these analyses.

Assay of Spiking and Recovery of AFB1 in tissues: Retrieval tests were conducted in sets of three by introducing spikes of aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, and fumonisin B1 at levels 0.5, 2.0, 4.0, 8.0 ng/g in six samples per treatment. To attain a level of 40 µg of AFB1 per gram of tissue, 50 grams of finely ground tissue samples without aflatoxin B1 were measured into a 250 mL Erlenmeyer flask and subsequently enriched with a standard AFB1 solution. Two separate analyses were carried out on the fortified blank samples and the corresponding blanks. The above-described technique was followed in the retrieval, identification, and assessment of toxins from tissue specimens.

Statistical analysis: Conformity of the data and mistake allotment were examined through the Shapiro–Wilk test of Statistical Analysis Software (SAS, 2012). The random picking of broilers and samples ensures compliance with the four assumptions of analysis of variance (ANOVA). Homoscedasticity (variance homogeneity) was examined using Levene's test in SAS (2012). Statistical analysis and evaluation of data normality were conducted using Statistical Analysis Software (SAS, 2012). The statistical model used was as follows:

$Yij= \mu+Ti+eij,$

Where, Yij= the dependent variable; µ= the overall meaning; Ti= the effect of treatments; eij= the random error. The experimental unit was the replication for productive traits and/or sampled blood and analyzed measurements. The results were expressed as average value, and the significance level was set at ($p\leq0.05$). Mean comparisons were conducted out using the Tukey test, with significance set at $p \leq 0.05$.

RESULTS

Productive performance: The influences of the SIL and/or SP on the FBW, BWG, TFI, FCR and mortality rate of broiler chicks fed contaminated diets with AF are shown in Table 3. Broilers fed AF-contaminated diets had the worst productive performance ($p \leq 0.03$) with lower FBW, BWG, FI, and PI, as well as high values of FCR and mortality rate. The inclusion of SIL or SP in the diets resulted in better productive performance ($p \leq 0.03$) than the positive control (diets with AF). Contrasted with negative control, chickens fed diets with 0.6 mg SIL + 1 g SP/kg had similar growth performance.

Serum biochemical profile: Chickens fed contaminated diets without SIL and SP, showed higher values for AST, ALT, TC, LDL, uric acid, and creatinine, as well as lower values for HDL and calcium in serum ($p \leq 0.05$). The incorporation of SIL and/or SP in the contaminated diets improved ($p \leq 0.05$) the values at the same level as the obtained ones in the negative control group (Table 4).

Weight of liver and lymphoid organs, antibody titers, and levels of GSH and MDA in the liver: Aflatoxin-contaminated feed considerably raised the weight of the liver and spleen ($p \leq 0.04$), while decreasing the weight of the bursa and thymus ($p \leq 0.03$) and the antibody titers for Newcastle disease (NDV) and infection bursal disease (IBD) ($p \leq 0.004$). The addition of SIL and/or SP improved ($p \leq 0.04$) the values for the weight of liver and organ lymphoid as well as for antibody titers. The inclusion of AF in the diets lowered the quantity of GSH and increased the levels of MDA and MDA/GSH ratio ($p \leq 0.0001$), compared to the other treatments. SIL and/or SP inclusion reversed ($p \leq 0.0001$) the negative effect of AF on GSH and MDA and MDA/GSH ratio (Table 5).

Table 3. Productive performance of broilers exposed or not, to aflatoxin and treated with silymarin and/or *Spirulina platensis* (n= 6 replicates per treatment).

Treatments	FBW (g)	BWG (g)	FI (g)	FCR	Mortality (%)	PI (%)
NC	2400^{a}	2358^{a}	3560^{a}	1.51^{b}	2.22^{c}	159^{a}
PC	1920^{c}	1878^{c}	3400^{b}	1.81^{a}	11.11^{a}	106^{c}
PC+SIL	2325^{b}	2283^{b}	3500^{a}	1.53^{c}	3.33^{bc}	152^{b}
PC+SP	2370^{b}	2328^{b}	3550^{a}	1.52^{c}	4.44^{b}	155^{ab}
PC+SIL+SP	2385^{ab}	2343^{ab}	3540^{a}	1.51^{bc}	3.33^{bc}	158^{a}
SEM	50.22	45.85	60.15	0.03	0.025	4.88
p-value	0.0001	0.0001	0.025	0.001	0.0001	0.002

$^{a–c}$Means with different superscripts in each column are significantly different ($p \leq 0.05$).

NC: Negative control; PC: Positive control; SL: Silymarin; SP: *Spirulina platensis*; SEM standard error of the mean; IBW: Initial body weight; FBW: Final body weight; BWG: body weight gain; TFI: total feed intake; FCR: feed conversion ratio; PI: performance index.

Table 4. Serum biochemical profiles of broilers exposed or not, to aflatoxin and treated with silymarin and/or *Spirulina platensis* (n= 6 samples per treatment).

Treatments	AST U/L	ALT U/L	TC mmol/L	HDL mmol/L	LDL mmol/L	Uric acid mmol/L	Creatine µmol/L	Calcium mmol/L
NC	13.6^{b}	26.0^{b}	205.5^{b}	52.0^{a}	110.5^{b}	5.82^{b}	0.685^{c}	9.70^{a}
PC	32.0^{a}	48.0^{a}	236.0^{a}	41.5^{b}	125.6^{a}	7.60^{a}	1.851^{a}	9.00^{b}
PC+SIL	13.8^{b}	27.0^{b}	210.0^{b}	51.8^{a}	113.0^{b}	5.80^{b}	0.858^{b}	9.60^{a}
PC+SP	13.5^{b}	26.8^{b}	215.0^{b}	51.6^{a}	110.8^{b}	5.90^{b}	0.756^{bc}	9.70^{a}
PC+SIL+SP	13.4^{b}	26.2^{b}	208.0^{b}	51.7^{a}	110.6^{b}	5.70^{b}	0.705^{bc}	10.00^{a}
SEM	0.525	1.362	15.48	1.226	5.458	0.258	0.054	1.365
p-value	0.0001	0.0001	0.0001	0.025	0.001	0.004	0.045	0.001

$^{a–c}$Means with different superscripts in each column are significantly different ($p \leq 0.05$).
NC: Negative control; PC: Positive control; SL: Silymarin; SP: *Spirulina platensis*; SEM standard error of the mean; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TC: total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

Table 5. Weight of liver and lymphoid organs, antibody titers, and levels of glutathione (GSH) and malondialdehyde (MDA) and MDA/GSH ratio in the liver of broilers exposed or not, to aflatoxin and treated with silymarin and/or *Spirulina platensis* (n= 6 samples per treatment)

Treatments	LW (g/100g BW)	Lymphoid organs weight (g/100 g BW)			Spleen/ Bursa ratio	Antibody titers (log10 GMT)		GSH (nmol/g)	MDA (µmol/g)	MDA/GSH
		Bursa	Thymus	Spleen		NDV	IBD			
NC	2.16^{b}	0.228^{a}	0.260^{a}	0.151^{b}	0.662^{c}	6.52^{a}	335.8^{a}	50^{b}	0.82^{a}	61.0^{b}
PC	3.80^{a}	0.205^{b}	0.200^{b}	0.182^{a}	0.887^{a}	3.36^{c}	302.0^{c}	120^{a}	0.60^{c}	200.0^{a}
PC+SIL	2.36^{b}	0.222^{a}	0.253^{a}	0.163^{ab}	0.734^{b}	5.85^{b}	326.5^{b}	56^{b}	0.73^{b}	76.7^{b}
PC+SP	2.40^{b}	0.219^{a}	0.250^{a}	0.160^{ab}	0.730^{b}	5.76^{b}	320.1^{b}	55^{b}	0.75^{b}	73.3^{b}
PC+SIL+SP	2.20^{b}	0.230^{a}	0.260^{a}	0.155^{b}	0.673^{c}	6.00^{ab}	330.0^{ab}	52^{b}	0.80^{a}	65.0^{b}
SEM	0.865	0.016	0.008	0.003	0.050	0.180	3.451	1.62	0.052	4.87
p-value	0.033	0.001	0.028	0.001	0.002	0.001	0.004	0.0001	0.0001	0.0001

$^{a–c}$Means with different superscripts in each column are significantly different ($p \leq 0.05$).
NC: Negative control; PC: Positive control; SL: Silymarin; SP: *Spirulina platensis*; SEM standard error of the mean; BW: Body weight; LW: liver weight; GMET: geometric mean titer; NDV: Newcastle disease virus; IBD: infectious bursal disease.

Coliforms, Salmonella sp., and E. coli in cecum: Intestinal microbial populations in broiler chickens exposed to AF are presented in Table 6. Coliforms, *E. coli*, and *Salmonella* sp. in the cecal of chicks fed an AF - contaminated diet increased ($p \leq 0.03$) against to the positive control group. The negative effect of AF was reverted by SIL and/or SP, reducing the concentration of coliforms, *E. coli,* and *Salmonella sp.* in the broiler's cecum.

Table 6. Presence of coliforms, *Salmonella sp.,* and *E. coli* in the cecum of broilers exposed, or not, to aflatoxin and treated with silymarin and/or *Spirulina platensis* (n= 6 samples per treatment)

Treatments	Bacteria concentration (log10CFU/g)		
	Coliforms	*Salmonella*	*E. coli*
NC	1.70^{b}	1.05^{b}	1.80^{c}
PC	3.00^{a}	1.86^{a}	3.12^{a}
PC+SIL	2.00^{b}	1.16^{b}	2.00^{b}
PC+SP	1.85^{b}	1.20^{b}	2.00^{b}
PC+SIL+SP	1.90^{b}	1.12^{c}	1.90^{bc}
SEM	0.22	0.27	0.28
p-value	0.001	0.025	0.001

$^{a–c}$Means with different superscripts in each column are significantly different ($p \leq 0.05$).
Negative control; PC: Positive control; NC: SL: Silymarin; SP: *Spirulina platensis*; SEM standard error of the mean.

Meat nutritive value and AF residues in liver tissue and meat: Against the negative control group, meat from broilers fed diets containing AF showed lower protein levels ($p \leq 0.05$) and high levels of fat, cholesterol, and triacylglycerols ($p \leq 0.04$). AF residue in the liver and meat of broilers from positive control was higher ($p \leq 0.0001$), since in negative control there was no AF. Content of protein, total fat, cholesterol, and triacylglycerols were improved, as well as AF residues in the liver were reduced ($p \leq 0.05$) due to the addition of SIL and/or SP in the diets. The inclusion of the SIL and/or SP resulted in non-detectable AF residues in the broiler's meat (Table 7).

Table 7. Breast meat nutritive value and aflatoxin (AF) deposits in liver and meat of broilers exposed, or not, to aflatoxin B1 and treated with silymarin and/or *Spirulina platensis* (n= 6 samples per treatment)

Treatments	Breast meat nutritive value				AF residue (ng/g)	
	Protein (%)	Total fat (%)	Cholesterol (mg/100g)	Triacylglycerols (mg/100g)	Liver	Meat
NC	21.80[a]	0.60[c]	79.2[c]	57.0[c]	nd	nd
PC	18.10[b]	1.12[a]	107.2[a]	65.00[a]	2.86[a]	1.08
PC+SIL	21.00[a]	0.70[c]	87.2[b]	59.00[bc]	0.132[b]	nd
PC+SP	21.10[a]	0.86[b]	86.0[b]	60.0[bc]	0.140[b]	nd
PC+SIL+SP	21.60[a]	0.67[c]	80.0[c]	58.00[c]	nd	nd
SEM	0.48	0.0k83	2.47	1.25	0.55	0.05
p-value	0.042	0.001	0.002	0.036	0.0001	0.0001

[a–c]Means with different superscripts in each column are significantly different ($p \leq 0.05$).
NC: Negative control; PC: Positive control; SL: Silymarin; SP: *Spirulinaplatensis*; SEM standard error of the mean; nd = non-detectable levels (detection limit: 0.025 ng/g).

DISCUSSION

Effect of Aflatoxin (AF): Mycotoxins are secondary metabolites produced by pathogenic fungi under suitable environmental conditions (Saleemi *et al.,* 2023). The use of AF-contaminated diets resulted in poor productive performance: in fact, FBW, BWG, and FI were reduced by 20, 20.3, and 4.5%, respectively, compared to chickens fed diets without AF (Table 2). These findings suggest that aflatoxin may impair the effective absorption and utilization of essential nutrients, negatively affecting their growth rate and the amount of food consumed. This phenomenon can be attributed to the toxic effects of aflatoxin on the metabolism and digestive function of poultry. These results agree with by the discoveries of Cravens *et al.* (2015), Armanini *et al.* (2021), and Lin *et al.* (2022) in broilers and by Chen *et al.* (2014) in Pekin ducklings. Dietary AFB 1 has a detrimental influence on broiler growth performance due to decreased of utilization proteinaceous and energetic substances, which could be brought about by a decline in the broilers' metabolic and digestive performance (Rajput *et al.,* 2017). Indeed, it is necessary to evaluate the impact of the dose and duration of exposure to aflatoxin on animal mortality.

The FDA's regulatory criteria for aflatoxin in feed indicate that the utmost amount of aflatoxin that can be present in poultry is 100 µg/kg (Xie *et al.,* 2022). AFB1 is called as silent killer because it is unavoidable contaminant and its prolonged contamination leads to adverse toxicopathological effects (Imram *et al.,* 2020). AFB1 intoxication leads to a decrease in body weights, feed intake in a dose-related manner (Ashraf *et al.,* 2022). Notably, it has been observed that broiler production performance is negatively impacted by AFB 1 at doses extending between 40 to 1500 µg/kg (Fouad *et al.,* 2019). The mortality rate was greater in the chicks given the AF-diet, in line with the findings of Hedayati *et al.* (2014), Zafar *et al.* (2017), and (Saleemi *et al.,* 2020).

Chronic AF ingestion may induce damage in the liver (Hua *et al.,* 2021; Sang *et al.,* 2023) and kidneys (Li *et al.,* 2018; Wang *et al.,* 2022a), causing a failure in these organs, which could result in higher mortality rates. It is well known that the liver is the main organ involved in the metabolism of toxins, however during the metabolization process reactive metabolites can be generated which damage liver cells and lead to inflammation and necrosis, compromising liver function (Sang *et al.,* 2023). The kidneys, on the other hand, can be damaged by aflatoxin through various mechanisms, including the direct accumulation of toxic metabolites or the induction of oxidative stress (Li *et al.,* 2018). The nephrotoxic effects of aflatoxin can therefore cause damage to kidney cells, compromise filtration function and lead to disturbances of water and electrolyte balance. AF analogous findings were seen by Amiridumari *et al.* (2013) in a study with broilers. The authors showed that 500 ppb AF caused a reduction in serum levels of calcium and HDL and heightened the serum concentrations of creatine, AST, and ALT. Armanini *et al.* (2021) also indicated a rise in ALT levels in broilers fed diets with AF and fumonisin, relative to the control

group. Recently, Feshanghchi *et al.* (2022) and Galli *et al.* (2022) noted an increase in ALT and AST due to the inclusion of 0.6 mg/kg and 300 µg/kg AF in the broiler's diet.

The elevated concentrations of TC and LDL and reduced concentrations of HDL may indicate a disruption of lipid metabolism, consistent with hepatocellular deterioration due to the chronic ingestion of AF. The liver is the organ most impacted during aflatoxicosis because it is responsible of the AF biotransformation into secondary toxic intermediates that can attach the cellular biomolecules such as RNA, DNA, and proteins. In addition, protein synthesis inhibition in the liver could induce biochemical changes during aflatoxicosis (Amiridumari *et al.,* 2013; Ahmed *et al.,* 2022).

Higher acid uric and creatinine levels in serum due to AF may occur in birds with renal failure since metabolites from AF may be transported, generated, or decomposed in the kidney (Li *et al.,* 2018). In addition, the liver and kidney are considered organs for AF accumulation (Wang *et al.,* 2022b). The liver injury caused by AF, with a rise in expansion of bile ducts and hyperplasia, the accumulation of fat in hepatocytes (fatty infiltration), the presence of empty spaces within liver cells (vacuolation), and the expansion of liver cells (hepatocyte hypertrophy) (Yunus *et al.,* 2011; Attia *et al.,* 2013; Yavus *et al.,* 2017), might explain the greater liver weight.

Lower levels of serum calcium due to the presence of AF in diets may be probably due to the inhibition, by AF, of the hydroxylation reactions in the liver and kidney responsible for activating vitamin D_3, leading to a state of vitamin D_3 deficiency (Nassar *et al.,* 1985). Glahn *et al.* (1991) showed that aflatoxin-treated broilers presented lower plasmatic levels of calcium, 25-hydroxy-vitamin D, and 1,25-dihydroxy-vitamin D. In addition, Costanzo *et al.* (2015) stated that AF downregulated the vitamin D receptor expression in cultured human cells.

The liver and spleen grew larger, while the bursa and thymus shrank (Table 5). This alteration in organ size correlated with a weakened immune response of infectious bursal disease (Juranova *et al.,* 2001). In fact, IBD is associated with a significant compromise of both humoral and cellular immunity (Sharma *et al.,* 1989).

The hepatic injury caused by AF, with proliferation and hyperplasia in the bile ducts, vacuolation, fatty infiltration and enlargement of hepatic cells (Yunus *et al.,* 2011; Attia *et al.,* 2013; Yavus *et al.,* 2017), could explain the greater liver weight.

The spleen serves as the largest lymphoid organ within the body, harboring a significant quantity of both T and B lymphocytes. AF is known for its immunotoxicity in lymphoid organs, causing oxidative stress, inflammation (Zhao *et al.,* 2019), congestion of red pulp, and necrosis, which impair spleen function (Peng *et al.,* 2014). The lower weight of the bursa and thymus may be due to the necrosis of the parenchyma and atrophy, causing lymphoid tissue depletion in these organs, as stated by Peng *et al.* (2015) and Yavus *et al.* (2017). Conversely, a decrease in the proportional weight of the bursa of Fabricius may be due to its necrosis or cell depletion. Studies have shown that during aflatoxicosis, the size of bursal follicles diminishes as a result of a reduction in both cortical and medullary lymphocytes, along with cell cycle arrest in broilers' bursal cells (Hu *et al.,* 2018). This phenomenon could also explain the increase in the relative weight of the spleen, serving as a compensatory mechanism for the damage inflicted on the bursa of Fabricius (Teleb *et al.,* 2004). The enlarged spleen may indicate compromised immune function. This effect was further confirmed by the spleen/bursa ratio, which provides insights into the development and growth of these lymphoid organs.

According to Solis-Cru *et al.* (2019), the AFB1 group's larger spleen/bursa ratio may be a sign of bursa atrophy as well as increased migration and proliferation of lymphocyte subpopulations to the spleen, which can be utilized as a field indicator of immunological state. Comparable findings were discovered by Hedayati *et al.* (2014), Peng *et al.* (2015), Yavus *et al.* (2017), and Liu *et al.* (2018). Furthermore, the degradation of the three immune organs may be linked with its hepato-toxicity. It's conceivable that 8,9-epoxide and AFB1-DNA adducts generated in the liver could accumulate and consequently affect splenocytes, thymocytes, and bursal cells (Peng *et al.,* 2015). Greater liver weight was also noted by Feshanghchi *et al.* (2022) noticed an elevation in liver weight of broilers fed diets containing 0.6 mg AF. However, the weight of the bursa, thymus, and spleen remained unaffected by the mycotoxin. Fawaz *et al.* (2022), however, did not observe any effect of 40 µg/kg AF on the weight of the liver, spleen, and bursa. It has been shown that AFB1 can stimulate the generation of reactive oxygen species (ROS) and oxidative stress, leading to cellular and DNA damage (Choi *et al.,* 2010). An organism's antioxidant system can neutralize the damaging effects of ROS, and significant endogenous antioxidant enzymes such GST, T-SOD, CAT, and GSH-Px are essential for scavenging ROS and preserving the intracellular oxidative stability (Rajput *et al.,* 2017).

Malondialdehyde (MDA) is one of the primary aldehydes formed during the secondary oxidation of polyunsaturated fatty acids its measurement serves as a key indicator of lipid peroxidation occurring inside the cell (Addeo *et al.,* 2021).

The actual level of glutathione (GSH) in cells is regulated by a more complex mechanism. GSH depletion may occur due to a reduction in feed intake, which leads to a lower intake of methionine or cysteine, the amino acids that are essential for the synthesis of GSH. Another factor that can contribute to GSH depletion is the

reduction of glutathione disulfide (GSSG), which can occur under conditions of oxidative damage.

High MDA levels, MDA/GSH ratios, and lower GSH concentrations were observed in the hepatic tissue of the broilers examined in this research, indicating oxidative stress in the liver as a result of AF contamination. AF can cause tissue damage through its metabolite, aflatoxin-exo-8,9-epoxide (AFO). Glutathione-S-transferase (GST) in the liver conjugates AFO with reduced GSH to neutralize the metabolite, and this process may contribute to the depletion of GSH stores (Ahmed *et al.,* 2022). Another potential reason for the lower GSH concentration may be related to the hepatic synthesis of GSH, since GSH is mainly produced in the liver (Lin *et al.,* 2022).

As the primary indicator of lipid peroxidation, MDA can be increased by inhibition of protein synthesis and lowering the levels of transferrin and ceruloplasmin in the liver, with consequent augment on concentrations of unbound copper and iron in the liver. Another reason for the high MDA and MDA/GSH ratio may be a depression in vitamins concentration, particularly vitamins E and A, which are essential for lowering lipid peroxidation (Shabani *et al.,* 2016). Finally, a reduction in the function of the antioxidant enzymes glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase increases the MDA levels (Shashi and Thakur, 2022).

The findings align with Wang *et al.* (2022a), who documented that the administration of 750 µg/kg AF per body weight led to heightened levels of MDA and H2O2 in mice kidneys, alongside diminished total antioxidant capacity and reduced activity of catalase, superoxide dismutase, and GSH enzymes. Similar result was verified by Nabi *et al.* (2022) in broilers.

When broilers in the second group (AF) were fed a diet tainted with aflatoxin, there was a notable decrease in the levels of ND and IBD antibodies. Aflatoxin-induced protein synthesis inhibition may be the cause of this, since it leads to the destruction of tissues and cells with high protein turnover, like the immune system, the liver, and the gut epithelium, which are the most vulnerable to the harmful effects of AF. It has been shown that exposure to AF in poultry inhibits their immune system in this way (Hossein and Gurbuz, 2016). Additionally, atrial fibrillation may suppress thymus gland development or affect the relative weight of the bursa of Fabricius, leading to severe deficiencies in both cellular and humoral immune response in chickens (Liu *et al.,* 2018). Inhibition of macrophage functions, T lymphocyte activity, or cytokine expression by atrial fibrillation can result in vaccine failure or pathogen persistence, as evidenced in numerous studies by reduced immunoglobulin production (Yunus *et al.,* 2011).

A strong correlation has been observed by recent epidemiological data to link broiler ration AF contamination to Newcastle disease outbreaks (Yunus *et al.,* 2011). Furthermore, the reduction in antibody titers against Newcastle disease could suggest significant immunosuppression caused by FA contamination, with negative implications on the overall health and performance of farm animals. Indeed, lower antibody titers against NDV and IBD were seen in broilers fed diets with AF (Yunus *et al.,* 2011).

The proliferation and differentiation of immune cells is a continuous process, and the immunosuppression caused by mycotoxins (Jolly *et al.,* 2008; Yunus *et al.,* 2011) is due to the inhibition of DNA, RNA, and protein synthesis, such as IgG and IgM, beyond the lower cytokine production, such as interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17, and others in broilers and pigs. AF also inhibits the development of various cell types, such as lymphocytes (Taranu *et al.,* 2005; Jolly *et al.,* 2008; Liu *et al.,* 2018). Antibodies boost adaptive immunity and engage with the complement system to protect the body Ingestion of contaminated diets resulted in higher concentrations of coliforms, *Salmonella* sp., and *E. coli* in the broiler's gut (Table 6). The gastrointestinal tract is the primary organ exposed to the mycotoxin and is anticipated to be impacted by AF. Aflatoxin may cause intestinal dysbiosis, with a reduction in *Lactobacillus spp.* as lactic acid bacteria strains possess the capability to detoxify toxins like AF (Yasmeen *et al.,* 2021) and prevent the proliferation of harmful bacteria (Subramanian *et al.,* 2015). Analogous findings were found by Jahanian *et al.* (2021) who demonstrated that the inclusion of 0.5 and 2 ppm in diets for broilers, increased the concentration of *E. coli* and *Salmonella spp.* at 28 and 42 days of age, and increased the concentration of total negative bacteria at 4 weeks of age. Galarza-Seeber *et al.* (2016), in a study with chickens, also reported that AF (1.5 and 2 ppm) increased the quantity of Gram-negative bacteria in the cecum. Feshanghchi *et al.* (2022) showed that coliform concentration was reduced due to 0.6 mg/kg AF, but the presence of *E. coli* and *Salmonella spp.* was not affected.

Meat's nutritive value reflects the AF-induced impaired protein synthesis and lipid metabolism (Abdel-Daim, *et al.,* 2021; Ahmed, *et al.,* 2022). Broiler meat showed reduced nutritive value due to the low protein concentration and higher content of total fat, cholesterol, and triacylglycerols. There are scarce results within the literature regarding the impact of AF on meat nutritive value (Table 7).

Effects of the supplementation with silymarin (SIL) and/or Spirulina platensis (SP): *S. platensis,* commonly known as spirulina, a filamentous cyanobacterium rich in numerous phytocontents, including pigments, amino acids, fatty acids, minerals, and carotenoids (Saleemi *et al.,* 2023) has considerable nutritional value and potential bioactive properties. Its rich and diversified composition

includes a wide range of micronutrients such as essential amino acids, polyunsaturated fatty acids, vitamins and minerals, β-D-glucans, and other polysaccharides with the ability to absorb mycotoxins (Utama *et al.,* 2021; Yadavalli *et al.,* 2023). Glucans may absorb about 50% of toxin molecules, such as aflatoxins (Yiannikouris *et al.,* 2006), which reduces its absorption in the digestive tract and decreases the harmful effects of AF. As shown by Oguz *et al.* (2022), in an *in vitro* study, glucomannans can bind mycotoxins via hydrogen, ionic, or hydrophobic interactions and showed great binding capacity (85.2% in medium with pH 3.0 and 96.3% in medium with pH 6.8, similar to the pHs of stomach and intestines). In addition, the high content of amino acids, fatty acids, vitamins, and minerals in SP, is important to the metabolic pathways in the liver and to the regulation of hepatic enzymes (Mahmoud *et al.,* 2018), improving liver function.

The inclusion of SIL or SP in the AF contaminated diets counteracts the negative effects of AF, improving the productivity level, biochemical profile, oxidative stress level, weight of lymphoid organs, nutrient composition of the meat, reducing the concentration of coliforms and *Salmonella* in the cecum, and level of AF residuals in the liver in relation to the positive control group; nevertheless the results were comparable to the negative control group when both substances were included in the diets, showing a synergic effect resulting in decreasing toxicity of AF by consecutive mechanisms.

Improved productive performance was the result of improved FI due to the inclusion of SIL and/or SP, which caused better FBW, BWG, and FCR. The increase in FI must be due to the high crude fiber (11.9%) in SIL (Feshanghchi *et al.,* 2022). SIL has a hepatoprotective effect and reduces the anorexigenic effect of the AF, by inhibiting the cytochrome system in the liver (Guerrini and Tedesco, 2023). In addition, SIL improves nutrient digestibility (Sultan *et al.,* 2018) and the intestinal environment, increasing the concentration of lactic acid bacteria and diminishing the occurrence of harmful bacteria (Bhowmik *et al.,* 2009; Bessam and Mehadadi, 2014). These outcomes corroborate the findings of Chand *et al.* (2011), Abdulwahid and Oleiwi (2021), and Armanini *et al.* (2021).

These results were similar to earlier studies that investigated the positive impacts of algae on the growth efficiency of quail (Amritha, *et al.,* 2016), laying hens (Halle, *et al.,* 2009), and broilers (Kasmani *et al.,* 2023). SP is known for its great protein content with high biological value, which ensures an increase in nitrogen intake (Abdelnour, *et al.,* 2019). Additionally, the polysaccharides of algae were found to enhance gut microbiota by boosting lactic-acid-producing bacteria, which creates an ideal environment for good digestive function and mitigates the adverse impacts of AF (Attia *et al.,* 2013; 2016; Khani, *et al.,* 2017).

Broilers provided diets contaminated with aflatoxin exhibited elevated levels of AST and ALT, which were decreased when SIL and/or SP were provided. In addition, lower antibody titers against NDV or lower immunoglobulins levels due to the presence of AF in broiler's diets were described by Yunus *et al.* (2011), Chen *et al.* (2014), Yasmeen *et al.* (2021), and Lai *et al.* (2022). SIL inhibits lipid peroxidation, stabilizes membrane permeability, protects the liver against injury from toxic compounds (Gillessen and Schimidt, 2020), and reduces the leakage of hepatic enzymes, such as ALT and AST. *S. platensis* exhibits antioxidant and anti-inflammatory characteristics (Mazokopakis *et al.,* 2014), in addition to containing β-D-glucans capable of binding to mycotoxins, thereby decreasing their absorption in the digestive tract (Yadavalli *et al.,* 2023). These beneficial effects help to restore liver function to normal levels.

An increase in ALT activity was reverted by feeding broilers a diet containing 800 mg SIL/kg feed along with 1 mg/kg of aflatoxin (Jamshidi *et al.,* 2007). After ducks consumed zearalenone and deoxynivalenol-containing diets, the incorporation of 0.5% SIL into the contaminated diets mitigated hepatic oxidative stress (Egresi *et al.,* 2020). Subhani *et al.* (2018) reported that the increase in ALT and AST in broilers fed diets contaminated with aflatoxins was reverted using another alga, *Chlorella pyrenoidosa*.

Lipid metabolism was altered in broilers consuming AF contaminated diets; however, the values of total cholesterol, HDL and LDL, were restored to values similar to the negative control group due to the supplementation with SIL and/or SP. SIL increased the excretion of bile acids in rats, depleting the pool of bile acids and increasing bile acid synthesis (Gobalakrishnan *et al.,* 2016). SIL also improve LDL binding to hepatocyte, contributing to lower plasma LDL (Skottová and Krecman, 1998). This may be a reason for the reduction of cholesterol and LDL levels in broilers consuming diets with AF + SIL. SP supplementation may reduce cholesterol and LDL levels and increase HDL levels due to the presence of C-phycocyanin that inhibits lipid peroxidation, of gamma-linolenic acid recognized for its role in controlling cholesterol synthesis, and due to the ability to increase the activity of lipoprotein lipase and hepatic triglyceride lipase (Koite *et al.,* 2022; Rostami *et al.,* 2022).

Compared to a group treated with carbon tetrachloride, silymarin reduce the levels of triglycerides and cholesterol in other bird species, such as Japanese quails (Behboodi *et al.,* 2017). Subhani *et al.* (2018) discovered that marine algae had a protective effect on hepatic enzymes, lessening the harmful impacts of AF. Ascorbic acid, which content in SP is high, may protect birds from the acute toxicity of aflatoxicosis (Alpsoy and Yalvac, 2011). The antioxidant and anti-inflammatory

effects of SIL and/or SP may reverse the renal toxicity caused by AF in broilers and improve the levels of creatinine, uric acid, and calcium in serum. SIL increased creatinine clearance in normal rats (El Menyiu *et al.,* 2018) and reduced uric acid levels in broilers consuming diets containing ochratoxin-A (Eid *et al.,* 2022). A normalization of creatinine and uric acid levels in rats intoxicated with lead or treated with titanium dioxide due to the SP supplementation was demonstrated by Gargouri *et al.* (2020) and Sayed *et al.* (2022), respectively.

The antioxidant properties of SIL and SP can reduce free radicals caused by AF, avert peroxidation of the membrane of immune cells (Bendich, 1993), and boost the immune system by increasing antibody output. According to Cook and Samman (1996), the effects on the immune system are due to the antibacterial properties and vitamin C content in SP. In addition, the antioxidant and anti-inflammatory impact of SIL and SP can increase protein synthesis in the liver, spleen, bursa, and thymus. Chand *et al.* (2011) reported higher antibody titers against NDV and IBD due to the SIL supplementation. Dumari *et al.* (2014), however, did not notice a beneficial effect of SIL supplementation on NDV and influenza disease.

Lower levels of free radicals result in a stabilized cell membrane and, low tiers of MDA and MDA/GSH ratio, and high levels of GSH since the antioxidant enzymes will not be extensively used to control the oxidative stress caused by the AF. These results align with Abdalla *et al.* (2018) and Khazaei *et al.* (2022) in studies with SIL in broilers and laboratory animals, respectively, and by Abotaleb *et al.* (2020), Abdel-Moneim *et al.* (2022b) and Alaqil and Abbas (2023) studying the effect of SP in broilers.

Coliforms, *Salmonella spp.,* and *E. coli* presence in the cecum were reduced due to the supplementation with SIL and/or SP. SIL shows a light antimicrobial effect due to its flavonoid content and inhibits bacterial growth by attaching to bacterial membrane proteins with its hydroxyl group, which causes the cells' vital components to seep (Bessam and Mehdadi, 2014). SP can avoid the imbalance of gut microbiota and colonization by pathogens in humans and animals, inhibiting gram-positive and gram-negative bacteria due to its cyclic peptides, phenolics, alkaloids, and lipopolysaccharides compounds (El-Sheekh *et al.,* 2014; Abdel-Moneim *et al.,* 2022a). According to Castillo *et al.* (2014), SP compounds can increase bacterial cell permeability, which leads to cytoplasmic leakage. Jahanian *et al.* (2017) reported lower ileal populations of *E. coli, Salmonella,* and *Klebsiella* due to the supplementation with SIL. Feshanghchi *et al.* (2022) also demonstrated a reduced concentration of coliforms in the cecum of broilers supplemented with SIL and SP.

Both protective agents against AF improve protein synthesis and regulate lipid formation, which can explain the elevated tier of protein and poor levels of cholesterol, total fat, and triacylglycerols in the meat due to the supplementation with SIL and/or SP. Residues in the liver were reduced due to the inclusion of SIL or SP in the diets containing AF, probably due to the reduced AF metabolization in the body and higher excretion in excreta. The improved biochemical constituents could be due to the effects of SIL or SP antioxidant properties and/or their interference in the metabolic pathways of aflatoxin and/or their toxin-binding impacts in the gut.

Conclusion: The present study suggests that dietary supplementation with 0.6 g of SIL/kg and/or 1 g of SP/kg diet is appropriate for maintaining broiler growth performance, immune status, serum constituents, and meat quality in broilers fed AF-contaminated diets at 1 mg/kg diet. It is important to acknowledge that this study was conducted at a specific AF contamination level (1 mg/kg) and may not be generalizable to higher concentrations or different strains of broilers, and further investigations are warranted to elucidate the efficacy of these strategies across a broader range of AF contamination levels and broiler genotype.

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Authors' contribution: conceived and designed the analysis: YAA, RAH. Executed the study, collected data and analyzed the samples: RAH, AEA, WSS. Analyzed the data: KAA, FB. Funding acquisiton: RAH, YAA. Wrote and edited the paper: MCO, YAA, NFA. All authors critically revised the manuscript for important intellectual contents and approved the final version.

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