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Efficacy of *Moringa oleifera*, lactoferrin and Syner-tox in counteracting aflatoxin effects in broiler chickens

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ABSTRACT

The main strategy in poultry production, is the elimination of mycotoxin adverse impacts. The present study was designed to evaluate the efficiency of *Moringa oleifera* (MO), Lactoferrin (LF), and Syner-tox (ST) in preventing the adverse impact of aflatoxin (AF). 180 eight-day-old broiler chicks divided into nine equal groups (20 each) as follows: Gp1 served as normal non-treated group, Gp2 served as AF non-treated group, Gp3 served as AF + MO treated group, Gp4 served as AF + LF treated group, Gp5 served as AF + ST treated group, Gp6 served as AF + MO + ST treated group, Gp7 served as AF + LF+ ST treated group, Gp8 served as AF + MO + LF treated group, Gp9 served as AF + MO + LF + ST treated group. The used dose of AF was 0.25 mg/kg diet, MO was 3 g/kg diet, LF was 250 mg/kg diet and ST 0.5 ml/liter/5days/week for 4 weeks. A significant reduce in growth performance, many hematological indices, and free amino acid along with modification in antioxidant markers. Conversely, increase in serum nitric oxide, liver and kidney biomarkers and malondialdehyde in liver contents with histopathological changs were observed in AF non-treated. The supplementation of MO, LF and/or ST showed strong antioxidant properties. Furthermore, these treatments enhanced the chickens' immune systems and restored all histopathological alters recorded in AF non-treated Gp. Therefore, the use of MO and LF in the elemination of aflatoxins in poultry feed is suggested as it is cheap and safe.

Introduction

Mycotoxins are poisonous secondary metabolites produced naturally by various fungal species that grow in hot, humid climates and they have a detrimental impact on poultry health and performance (Mgbeahuruike *et al*., 2018). Aflatoxins are mycotoxins that are naturally existing and generated from *Aspergillus flavus* and *Aspergillus parasiticus*, which are normally encountered in a variety range of tropical and subtropical food and feed. It's known to have extremely harmful and carcinogenic influences. Aflatoxin B1 (AFB1) is the most serious of the several harmful aflatoxins (B1, B2, G1, and G2, M1, M2). Additionally, it is regarded as Group I carcinogen. AFB1 supplementation exhibits adverse mutagenic, teratogenic, hepatotoxic, and carcinogenic impacts on humans and different species of livestock (Zhang *et al*., 2016). In poultry, aflatoxicosis results in reduced growth rate, anorexia, poor feed utilization, decreased egg production, and elevated rates of mortality (Ghahri *et al*., 2010). It is also having negative impacts on growth performance, meat characteristics, carcasses, and microflora of intestine (Zaker-Esteghamati *et al*., 2020).

Several phytogenic feed additives are recommended in the animal feed industry as a replacement to antibiotic growth promoters (Awais *et al*., 2013). Plants are high rich source of bioactive components with numerous biological and pharmacological effects (Wallace *et al*., 2010). The application of phytoproducts to check the toxic impacts of aflatoxicosis is a much-accepted concept (Priya *et al*., 2019). *Moringa oleifera* Lam, (Family: *Moringaceae*; common name: drumstick) is a perennial tree, native to India. The leaves are rich sources of minerals, vitamins, and unique phytochemicals of medicinal uses (Umaya *et al*., 2014). It provides multiple advantages, because different tree parts (leaves, immature pods, fruits, and flowers) are edible and included in traditional diets in several countries (Anhwange *et al*., 2004). By supplementing *M. oleifera* leaf powder to feed contaminated with aflatoxin, Umaya *et al*., (2012) found improved hepatic antioxidant status and serum liver enzymes in broilers chickens. The potential anti-AFB1 effects in broilers have been attributed to the hepatoprotective and free radical scavenging characteristics of the phytochemicals kaempferol, quercetin, flavonoids, and β-carotene encountered in *M. oleifera* leaves.

Lactoferrin (LF) is an iron-binding glycoprotein that is linked to transferrin family which plays a critical role in antimicrobial activity functions due to its distinct structure (Cai *et al*., 2018), and it demonstrates immunomodulatory properties (Vega-Bautista *et al*., 2019). Furthermore, LF has previously been shown to enhance poultry performance (Enany *et al*., 2017). Increasing beneficial bacterial populations can help to protect the host against a variety of pathogenic viruses and bacteria (Mohamed and Younis, 2018). LF is derived from various sources, involving secretory fluids such as mucous secretions, exocrine glands (tears or maternal milk), and secondary granules of blood and neutrophils (Geier *et al*., 2011). Several studies have been conducted on LF due to its health benefits and practical applications. LF also is thought to be a potential pre-immune host defense system (Kell *et al*., 2020). Several of LF's effects are believed to be related to its cationic nature. Its cationic nature enables LF to attach to different molecules, whether soluble or adhered to cell surfaces. LF exerts bactericidal impact via destroying the outer membrane of Gram-negative bacteria, as well as immunoregulatory influences via decreasing generate of interleukin-1 (IL-1), IL-2 and tumour necrosis factor- (TNF-α) and enhancing the cytotoxicity of natural killer cells and monocytes (Caccavo *et al*., 2002). Moreover, LF might reduce AFB1-induce cytotoxicity and DNA damage in tissue via reducing oxidative stress mediated by mitogen-activated protein kinase (MAPK) pathways (Zheng *et al*., 2018).

Anti-mycotoxin additives define a group of compounds that when added to animal feed can adsorb, neutralize, or inactivate mycotoxins in the gastrointestinal tract (Oliveira *et al*., 2015). There are several biological compounds applied to control mycotoxins in poultry farms, as well as feed additives (El-Shafei and Saleh, 2016). Syner-Tox is one of the biological controls against mycotoxins. It is a detoxifying commercial compound mixture containing organic acids, soluble enzymes, and dried *Bacillus subtilis* fermentation extract. *Bacillus subtilis* is a type of probiotic

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accepted by human and animals and had a potent detoxifying effect on AFB1 (Gao *et al*., 2011). As a result of climatic changes, the formation of mycotoxins increased with the increase in temperature and relative humidity in the air and surrounding environment. Therefore, the present study planned to investigate the impact of dietary supplementation of *Moringa oleifera*, lactoferrin and Syner-Tox during aflatoxicosis in broiler chickens. The methodology included evaluating growth performance, hematological alterations, oxidative stress markers, alterations in some amino acids, and histopathological changes.

Materials and methods

Ethical approval

The experimental protocol was approved by the Animal Health Research Institute (AHRI) in conformity with the Agriculture Research Center (ARC) and IACUC Committee (ARC, AHRI, IACUC, 66 /24) in Egypt.

Chemicals and treatments

Moringa oleifera (MO)

Moringa oleifera was obtained from the Egyptian Scientific Society of *Moringa*, National Research Center, Giza, Egypt.

Lactoferrin (LF)

Lactoferrin was supplied by Dulex lab nutrition and pharmaceutical company, New Cairo, Egypt, Batch NO:B2117051.

Syner-Tox® (ST)

Syner-Tox® is manufactured by Agrarian Marketing Corporation Company, USA. Each 1000 ml contains phosphoric acid 65ml, citric acid 80ml, lactic acid 0.2 ml, aspartic acid 0.3ml, sodium potassium tartrate 0.1ml, calcium pantothenate 0.2ml, calcium lactate 0.2ml, papain 0.1ml, propylene glycol 100ml, dried *Bacillus subtilis* fermentation extract 0.3ml, riboflavin 0.3ml, thiamin mononitrate 0.3ml, disodium EDTA 15ml, pyridoxine hydrochloride 2ml, distilled water up to 1000 ml.

Production and estimation of total aflatoxins (Smith, 1997)

Aflatoxigenic *A. flavus* (isolated from poultry feed) were cultivated on Czapex Dox agar media for 5-7 days at 25ºC. 500 ml flasks with 100 g of fine grounded yellow corn were autoclaved for 1 hour at 121ºC. The flask was shaken to prevent cooking yellow corn. It was inoculated with spore suspension of 2 slants of *A. flavus* and incubated for 4 weeks at 25-28ºC. After end of incubation period, the corn was removed from flasks, dried; finely ground and 50 g of each were subjected for estimation of aflatoxins. The estimation of prepared aflatoxins was measured quantitatively by fluorometric method according to the recommended method of AOAC (1990) and Refai and Hassan (2013).

Vaccines and vaccination

All birds were vaccinated against Newcastle disease virus by HitchnerB1 and LaSota on 7 and 21 days of age, respectively, and vaccinated also against infectious bursal disease at 12 days of age. Vaccines are manufactured by KBNP, INC. Company, Chungnam, Korea.

Chickens, feeding and experimental design

One hundred eighty (unsexed) one day old Cobb broiler chicks were obtained from El- Kahera poultry company10th of Ramadan City. They were fed on a balanced commercial ration purchased from Feed Mix Company for 7 days before the experiment. On day 8, the chicks were divided to 9 groups of 20 each at random. The experimental feeding was designed as follows:

Gp1: fed healthy diet and served as normal non-treated group

Gp2: fed diet with 0.25 mg/kg diet aflatoxin (AF) only for 28 days served as AF non-treated group (Ashry *et al*., 2022).

Gp3: fed diet with 0.25 mg/kg diet AF + MO at a dose of 3 g/kg diet (Fouad and El-Rayes2019) for 28 days served as AF + MO treated group.

Gp4: fed diet with 0.25 mg/kg diet AF + LF at a dose of 250 mg/kg diet (Enany *et al*., 2017) for 28 days served as AF + LF treated group.

Gp5: fed diet with 0.25 mg/kg diet AF + ST at a dose of 0.5 ml/liter distilled water/ 5days/ week (Amer *et al*., 2022) for 28 days served as AF + ST treated group.

Gp6: fed diet with 0.25 mg/kg diet AF + MO 3 g/kg diet + ST 0.5 ml/liter distilled water/ 5days/ week for 28 days served as AF + MO + ST treated group.

Gp7: fed diet with 0.25 mg/kg diet AF + LF 250 mg/kg diet + ST 0.5 ml/ liter distilled water/ 5days/ week for 28 days served as AF + LF+ ST treated group.

Gp8: fed diet with 0.25 mg/kg AF diet + MO 3 g/kg diet + LF 250 mg/kg diet for 28 days served as AF + MO + LF treated group.

Gp9: fed diet with 0.25 mg/kg AF + MO 3 g/kg diet + LF 250 mg/kg diet + ST 0.5 ml/liter distilled water/ 5days/ week for 28 days served as AF + MO + LF + ST treated group.

Growth performance

This step included evaluating the weekly body weight (BW), body weight gain (BWG), feed intake, feed conversion rate (FCR) which were indicated throughout the 35-day study period. FCR is calculated according to the equation: FCR= feed intake/weight gain.

Sampling

On day 35 of the experiment, two blood samples were collected by wing vein from each chicken. The first1st sample was collected in a tube containing EDTA as an anticoagulant for hematological profile analysis, meanwhile the second tube without an anticoagulant for obtaining serum. Then, chicks were sacrificed by neck dislocation and liver, and muscle tissue samples were collected. Serum samples were obtained via centrifuging the blood samples at 3000 rpm for 7 minutes. Sera were seperated and kepted at -20°C for biochemical analysis. Concerning tissues, both the liver and muscle were excised and prepared for aflatoxin residue. The liver tissue was divided into three sections, one for antioxidant assessment, the other for aflatoxin residue and the last section in 10% formalin for histopathological examination. The experimental groups and sampling schedule are illustrated in Figure 1.

Biochemical analysis

Manual erythrocyte (RBCs 106 /mm3) and white blood cell count (WBCs, $10^3/mm^3$), Hemoglobin (Hb) concentration (g/dl) and the percentage of packed cell volume (PCV, %) were estimated according toFeldman *et al*. (2000), differential leucocyte count depending on method described by Anderson and Latimer, (1990). Serum total protein and it's electrophoretic pattern were determined by Spectrum kit CAT. NO 310 001 and chemical prepration of polyacrylamide gel electrophoresis using the continuous buffer system of Kaplan and Szalbo (1983) and Davis (1964) respectivly, and the calculated based on SynGene S. No. 17292*14518 sme*mpcs program using Scie Plas TV100 Mini Vertical Gel Unit UK with Power Supply Consort EV714, Belgium. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined by spectrum kit CAT. NO 260 001 and 265 001 respectively. Levels of

creatinine and urea in serum were determined by using spectrum kit CAT. NO 235 004 and 319 005 respectively. Cholesterol, triacylglycerol (TAG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) were estimated by using spectrum kit CAT. NO 230 001, 314 005, 266 003 and 280 001 respectively. Very low density lipoprotein (VLDL) was determined by the formula: VLDL=Triglycerides/5. Additionally, nitric oxide (NO) and total antioxidant capacity (TAC) were estimated by using Bio-diagnostic kit CAT. No. NO 25 33 and TA 2513 respectively. Liver tissue of malonaldehyde (MDA), reduced glutathione (GSH), and catalase (CAT) were estimated by using Bio-diagnostic kit CAT.NO MD 25 29, GR 25 11 and CA 25 17 respectively. Moreover lysine and methionine percentages were estimated by the Amino Acid Analyzer sykam GmbH, Analytischer Messtechnik (Gewerbering 15, D_86922 Erosing, Germany), Genetic Research Center, Giza, Egypt.

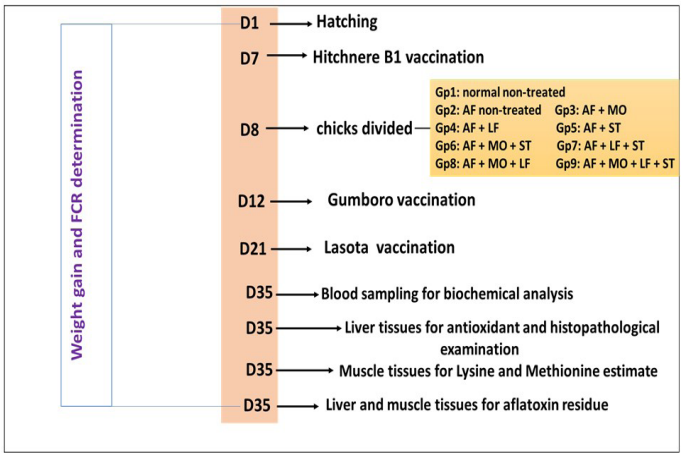

Fig. 1. A timeline illustrating the protocol of study, involving the day (D) number, vaccination, groups, and sampling techniques.

Measurement of total aflatoxin residues

Liver and muscle tissues were subjected to direct examination using a fluorometric assay to determine total aflatoxin residues, according to Hussain *et al.* (2010)

Histopathological examinations

Liver specimens from chickens of different groups were collected and immediately fixed in 10% buffered neutral formalin solution for 24 hours, dehydrated in gradual ascending ethanol, cleared in xylene, and embedded in paraffin wax. 5 µm thickness paraffin sections were sliced using a microtome (Leica RM 2155, England). The sections were prepared and then routinely stained with hematoxylin and eosin stains and investegated microscopically for any histopathological changs (Suvarna *et al*., 2018).

Statistical analysis

The results were statistically analyzed by the One-Way Analysis of Variance (ANOVA) test. Data were given as mean ± standard error (SE) using SPSS 14.0 (2006), and then the Duncan test was performed. Statistical significance was set at $p<0.05$.

Results

The results of performance features (Table 1) displayed a remarkable decrease ($P<0.05$) in BW, BWG and feed intake with a noticeable increase of FCR in aflatoxin non-treated group (Gp2) compared with normal chickens (Gp1). However, chickens that treated with *Moringa*, lactoferrin and/or Syner-Tox groups (Gp3 – Gp9) showed a remarkable increase ($P<0.05$) in BW, BWG and feed intake with a noticeable decrease of FCR when compared with aflatoxin non-treated group (Gp2).

The observed data in Table 2 demonstrated that, a remarkable decrease ($P<0.05$) in Hb, RBCs, PCV, MCHC, WBCs and absolute lymphocyte in Gp2 when compared with Gp1. In strict contrast, each treated groups (Gp3-Gp9) displayed a statistically significant increase ($P<0.05$) in all the prior measurements when compared with aflatoxin non-treated group (Gp2). However, a noticeable increase in MCV, MCH and absolute eosinophil associated with non-significant increase in absolute heterophil, and monocyte were observed in Gp2 compared with Gp1. However, each treated groups (Gp3-Gp9) displayed an improvement in each of these measurements when compared with aflatoxin non-treated group Gp2.

Moreover, our data created in Table 3 displayed that, serum AST, ALT, creatinine, urea, cholesterol, triacylglycerol (TAG), LDL and VLDL remarkably increased ($P<0.05$) in aflatoxin non-treated birds as compared with Gp1. However, each treated groups suggested a remarkable decrease in all the prior measurements when compared with aflatoxin non-treated group. Conversely, a remarkable decrease in serum HDL was note in the aflatoxin non-treated birds as compared with Gp1. Each treated group revealed a remarkable increase in HDL compared with the aflatoxin non-treated group.

As shown in Table 4, the aflatoxin non-treated group (Gp2) displayed a remarkable decrease in serum total protein, prealbumin, albumin, beta 1,2 and gamma 1,2, when compared with normal control group (Gp1). However, treatment of aflatoxicosis chickens with *Moringa*, lactoferrin and/or Syner-Tox groups (Gp3 – Gp9) displayed an improvement in each of these measurements when compared with aflatoxin non-treated group Gp2.

Additionally, our data created in Table 5 displayed that, serum NO and MDA concentrations in the liver were remarkably increased ($P<0.05$) in aflatoxin non-treated birds as compared with Gp1. However, each treated group showed a remarkable decrease in NO and MDA when compared with aflatoxin non-treated group. Conversely, a remarkable decrease in serum TAC, levels of CAT, GSH in liver tissue and muscle tissue lysine and methionine ration were observed in the aflatoxin non-treated

Table 1. Effect of *Moringa oleifera*, lactoferrin and/or Syner-Tox on growth performance during aflatoxicosis in broiler chickens.

Groups	Initial weight (g)	Final weight (g)	Body Weight gain (g)	Feed intake (g)	Feed conversion rate (FCR)
Gp1 normal non-treated	44.20±0.58^{a}	1814.00±8.28^{d}	1769.80±8.17^{d}	2505.6±128.69^{a}	1.50±0.11^{b}
Gp2 AF non-treated	43.80±0.97^{a}	1138.00±24.78^{e}	1094.20±24.74^{e}	2174.0±108.98^{b}	2.00±0.15^{a}
Gp3 AF + MO	43.60±1.08^{a}	1956.00±25.81^{c}	1912.40±26.31^{c}	2594.6±99.66^{a}	1.50±0.15^{b}
Gp4 AF + LF	43±1.00^{a}	2050±24.70^{ab}	2007.00±24.68^{ab}	2483.2±106.08^{a}	1.36±0.17^{b}
Gp5 AF + ST	43±0.55^{a}	2019.60±9.80^{ab}	1976.60±10.03^{ab}	2606.8±113.53^{a}	1.45±0.17^{b}
Gp6 AF + MO + ST	43.40±0.93^{a}	2002.00±22.89^{bc}	1958.60±22.52^{bc}	2561.8±100.49^{a}	1.41±0.14^{b}
Gp7 AF + LF+ ST	43.20±1.07^{a}	2058.40±26.18^{ab}	2015.20±25.79^{ab}	2551.2±97.02^{a}	1.39±0.17^{b}
Gp8 AF + MO + LF	43.00±1.30^{a}	2077.60±5.98^{a}	2034.60±6.80^{a}	2551.8±93.40^{a}	1.40±0.18^{b}
Gp9 AF+MO + LF + ST	42.80±1.16^{a}	2068.00±21.54^{a}	2025.20±22.01^{a}	2575.2±103.22^{a}	1.39±0.16^{b}

The results are displayed as Mean±SE. n=5 chickens; Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table 2. Effect of *Moringa oleifera*, lactoferrin and/or Syner-Tox on blood profile during aflatoxicosis in broiler chickens.

Groups	Hb (g/dl)	RBCs ($x10^6$/cmm)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($x10^3$/cmm)	Lymphocyte ($x10^3$/cmm)	Heterophil ($x10^3$/cmm)	Monocyte ($x10^3$/cmm)	Eosinophil ($x10^3$/cmm)
Gp1 normal non-treated	12.84±0.07^{b}	3.95±0.06^{b}	34.94±0.48^{b}	88.55±0.29^{b}	32.55±0.30^{b}	36.76±0.31^{ab}	18.90 ±0.46^{b}	11.76±0.31^{a}	6.27±0.19^{b}	0.64±0.05^{ab}	0.23±0.04^{c}
Gp2 AF non-treated	9.13±0.08^{e}	2.56±0.03^{c}	27.80±0.24^{c}	108.82 ±1.55^{a}	35.72±0.38^{a}	32.84 ±0.30^{d}	13.06±0.32^{c}	5.30±0.17^{b}	6.50±0.15^{b}	0.76±0.05^{a}	0.50±0.03^{a}
Gp3 AF+ MO	12.47±0.1^{c}	3.92±0.05^{b}	33.86±0.54^{b}	86.37±0.55^{b}	31.82±0.29^{bc}	36.86±0.49^{ab}	19.09±0.52^{b}	11.57±0.40^{a}	6.52±0.17^{b}	0.69±0.06^{ab}	0.31±0.05^{bc}
Gp4 AF + LF	12.80±0.1^{b}	3.93±0.07^{b}	33.94±0.99^{b}	86.36±1.56^{b}	32.62±0.42^{b}	37.84 ±1.04^{a}	19.68±0.58^{ab}	12±0.36^{a}	6.62±0.30 ^{b}	0.67±0.06^{ab}	0.39±0.06^{abc}
Gp5 AF + ST	11.93±0.07^{d}	3.85±0.07^{b}	33.26±0.74^{b}	86.48±1.00^{b}	31.05±0.43cd	35.93±0.66^{bc}	21.06±0.67^{a}	12.39±0.47^{a}	7.52 ±0.15^{a}	0.72±0.07^{ab}	0.43±0.08^{ab}
Gp6 AF + MO + ST	12.66±0.11^{bc}	3.92±0.06^{b}	34.30±0.87^{b}	87.45±1.17^{b}	32.30±0.19^{b}	36.97±0.68^{ab}	19.36±0.40^{ab}	11.55±0.43^{a}	6.85±0.09^{ab}	0.62±0.07^{ab}	0.34±0.07^{abc}
Gp7 AF + LF+ ST	13.26±0.09^{a}	4.41±0.10^{a}	37.87±0.37^{a}	85.91±1.30^{b}	30.09±0.51^{d}	35.02±0.16^{c}	19.86±0.58^{ab}	12.35±0.37^{a}	6.75±0.26 ^{b}	0.52±0.09^{a}	0.24±0.04^{c}
Gp8 AF + MO + LF	12.14±0.08^{d}	4.08±0.10^{b}	35.07±0.49^{b}	86.15±1.02^{b}	29.83±0.55^{d}	34.62±0.28^{c}	20.26±0.70^{ab}	12.47±0.47^{a}	6.97 ±0.36 ^{ab}	0.53±0.05^{a}	0.29±0.05^{bc}
Gp9 AF + MO + LF + ST	11.88±0.14^{d}	3.91±0.08^{b}	33.35±0.69^{b}	85.30±0.09^{b}	30.42±0.32^{d}	35.66±0.36^{bc}	19.75±0.64^{ab}	11.93±0.52^{a}	6.87±0.28^{ab}	0.59±0.06^{ab}	0.36±0.05^{abc}

The results are displayed as Mean±SE. n=5 chickens; Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table 3. Effect of *Moringa oleifera*, lactoferrin and/or Syner-Tox on liver, kidney and lipid profile biomarkers during aflatoxicosis in broiler chickens.

Groups	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Cholesterol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Gp1 normal non-treated	67.6±1.21^{d}	14.4±1.50^{e}	0.4±0.01^{cde}	7.68±0.42^{d}	138.6±1.03^{f}	175.8±1.85^{c}	53.6±1.08^{c}	49.84±1.52^{d}	35.16±0.37^{c}
Gp2 AF non-treated	98.4±1.36^{a}	37.2±0.86^{a}	0.89±0.02^{a}	22.92±0.33^{a}	195.8±1.59^{a}	202.4±1.78^{a}	40±0.45^{e}	115.32±1.62^{a}	40.48±0.36^{a}
Gp3 AF + MO	73.4±1.03^{c}	19.8±0.86^{d}	0.43±0.01^{b}	19.24±0.37^{c}	153±1.63^{d}	179.4±0.51^{c}	52±0.95^{c}	65.12±1.96^{c}	35.88±0.10^{c}
Gp4 AF + LF	67±1.14^{de}	21.2±1.07^{cd}	0.43±0.01^{b}	21.08±0.49^{b}	177.6±1.72^{b}	184.8±1.71^{b}	52.6±0.51^{c}	88.04±1.11^{b}	36.96±0.34^{b}
Gp5 AF + ST	76.8±1.39^{b}	23.6±1.03^{bc}	0.41±0.01^{bcd}	21.18±0.35^{b}	171.6±0.81^{c}	179.6±1.29^{c}	47.6±0.75^{d}	88.08±1.12^{b}	35.92±0.26^{c}
Gp6 AF + MO + ST	65±0.71^{de}	23.2±0.86^{bc}	0.39±0.00^{cde}	20.54±0.42^{bc}	167.4±1.29^{e}	153.2±1.59^{e}	52.2±0.66^{c}	84.56±1.19^{b}	30.64±0.32^{e}
Gp7 AF + LF+ ST	66.4±0.93^{de}	23.8±0.66^{bc}	0.42±0.01^{bc}	20.52±0.63^{bc}	142.8±1.85^{ef}	163.2±1.53^{d}	59.4±0.51^{b}	50.76±1.92^{d}	32.64±0.31^{d}
Gp8 AF + MO + LF	63.6±1.08^{e}	23.2±1.07^{bc}	0.38±0.00^{de}	19.28±0.40^{c}	145.8±1.93^{e}	175±1.92^{c}	62.8±0.37^{a}	48±1.53^{d}	35±0.38^{c}
Gp9 AF+MO + LF + ST	67.4±1.03^{d}	25.6±1.02^{b}	0.37±0.00^{e}	20.28±0.40^{bc}	128.8±1.36^{g}	144.4±1.47^{f}	57.8±0.66^{b}	42.12±1.52^{e}	28.88±0.29^{f}

The results are displayed as Mean±SE. n=5 chickens; Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table 4. Effect of *Moringa oleifera*, lactoferrin and/or Syner-Tox on serum T. protein and its sub-fractions (g/dl) during aflatoxicosis in broiler chickens.

Groups	T. protein	prealbumin	albumin	alpha1	alpha2	beta1	beta2	gamma1	gamma2
Gp1 normal non-treated	4.68±0.15^{a}	0.23±0.01^{a}	0.90±0.04^{b}	0.82±0.04^{a}	0.48±0.01^{a}	0.46±0.03^{a}	0.36±0.02^{a}	1.08±0.03^{a}	0.36±0.01^{a}
Gp2 AF non-treated	2.82±0.03^{d}	0.13±0.01^{c}	0.68±0.02^{c}	0.36±0.00^{d}	0.27±0.02^{c}	0.35±0.02^{b}	0.24±.01^{b}	0.67±0.06^{b}	0.12±0.01^{d}
Gp3 AF + MO	3.35±0.16^{c}	0.14±0.01^{c}	0.82±0.04^{b}	0.60±0.04^{b}	0.37±0.02^{b}	0.35±0.03^{b}	0.32±0.01^{a}	0.62±0.09^{b}	0.14±0.01^{d}
Gp4 AF + LF	3.53±0.05^{c}	0.16±0.01^{bc}	0.95±0.03^{b}	0.54±0.03^{bc}	0.39±0.00^{b}	0.35±0.03^{b}	0.35±0.03^{a}	0.65±0.02^{b}	0.14±0.00^{d}
Gp5 AF + ST	3.37±0.04^{c}	0.15±0.01^{bc}	0.92±0.00^{b}	0.48±0.01^{c}	0.43±0.00^{ab}	0.40±0.02^{ab}	0.25±0.01^{b}	0.60±0.02^{b}	0.14±0.00^{d}
Gp6 AF + MO + ST	3.49±0.08^{c}	0.13±0.01^{c}	0.91±0.08^{b}	0.55±0.01^{bc}	0.38±0.03^{b}	0.38±0.02^{ab}	0.32±0.01^{a}	0.67±0.03^{b}	0.15±0.02^{d}
Gp7 AF + LF+ ST	4.22±0.14^{b}	0.21±0.01^{a}	1.15±0.03^{a}	0.54 ±0.06^{bc}	0.39±0.04^{b}	0.39±0.02^{ab}	0.37±0.01^{a}	0.95±0.10^{a}	0.20±0.00^{c}
Gp8 AF + MO + LF	4.16±0.06^{b}	0.17±0.02^{b}	1.08±0.02^{a}	0.50±0.04^{c}	0.37±0.03^{b}	0.37±0.03^{b}	0.37±0.01^{a}	1.08±0.02^{a}	0.23 ±0.00^{bc}
Gp9 AF+MO + LF + ST	4.00±0.06^{b}	0.21±0.00^{a}	1.14±0.07^{a}	0.48±0.01^{c}	0.41±0.03^{ab}	0.41±0.02^{ab}	0.36±0.04^{a}	0.76±0.08^{b}	0.24±0.00^{b}

The results are displayed as Mean±SE. n=5 chickens; Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table 5. Effect of *Moringa oleifera*, lactoferrin and/or Syner-Tox on oxidative stress biomarkers and amino acids during aflatoxicosis in broiler chickens.

Groups	Serum		Liver			Muscle	
	NO (nmol/L)	TAC (mU/L)	MDA (nmol/g)	CAT (U/g.tissue)	GSH (nmol/g)	Lysine (%)	Methionine (%)
Gp1 normal non-treated	15.91±0.65^{b}	1.42±0.05^{c}	4.14±0.16^{f}	105.2±2.63^{abcd}	23.38±0.41^{c}	4.55±0.01^{a}	1.30±0.03^{b}
Gp2 AF non-treated	24.06±0.31^{a}	0.70±0.01^{e}	7.2±0.11^{a}	83.80±1.84^{e}	16.18±0.43^{d}	1.82±0.01^{f}	0.94±0.00^{f}
Gp3 AF + MO	12.72±0.61^{c}	1.63±0.03^{b}	5.12±0.14^{cd}	100.80±2.82^{cd}	22.39±0.63^{c}	3.68±0.01^{b}	1.05±0.01^{e}
Gp4 AF + LF	13.36±0.78^{c}	1.05±0.04^{d}	5.38±0.11^{c}	108.06±2.15^{ab}	22.96±0.67^{c}	2.86±0.05^{c}	1.36±0.01^{a}
Gp5 AF + ST	13.29±0.54^{c}	1.03±0.02^{d}	5.74±0.09^{b}	101.42±1.28^{bcd}	23.4±0.49^{c}	2.63±0.01^{e}	1.25±0.01^{c}
Gp6 AF + MO + ST	12.48±0.49^{c}	1.05±0.04^{d}	4.76±0.16^{de}	109.51±2.17^{a}	25.64±0.20^{b}	2.70±0.01^{d}	1.08±0.01^{e}
Gp7 AF + LF+ ST	16.32±0.81^{b}	1.89±0.04^{a}	5.18±0.11^{c}	105.71±1.48^{abc}	27.26±0.34^{a}	2.81±0.01^{c}	1.18±0.01^{d}
Gp8 AF + MO + LF	13.90±0.42^{c}	1.95±0.01^{a}	4.7±0.09^{e}	98.89±2.11^{d}	27.30±0.47^{a}	2.86±0.01^{c}	1.16±0.01^{d}
Gp9 AF+MO + LF + ST	14.05±0.45^{c}	1.05±0.02^{d}	5.06±0.09^{cde}	102.24±1.98^{bcd}	26.62±0.69^{ab}	2.67±0.01^{de}	1.24±0.01^{c}

The results are displayed as Mean±SE. n=5 chickens; Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

birds as compared with Gp1. Each treated group suggested a remarkable increase in all the prior measurements when compared with aflatoxin non-treated group.

Figure 2 illustrates aflatoxin in non-treated groups that received aflatoxin only and had noticeable levels of aflatoxin residues in the liver and muscle tissues. However, the third group treated *Moringa* with aflatoxin exhibited improved aflatoxin elimination in the liver and muscle tissues. The administration of *Moringa*, lactoferrin and/or Syner-Tox groups (Gp3 – Gp9) with aflatoxin displayed decrease levels of detoxification.

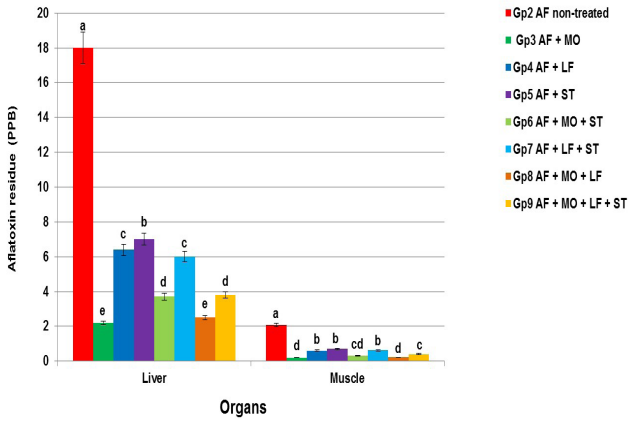

Fig. 2. Effect of *Moringa oleifera*, lactoferrin and/or syner-tox on total aflatoxin residue in liver and muscle during aflatoxicosis in broiler chickens.

Histopathological findings

Normal histomorphological structures of hepatic acini, portal areas, sinusoids and central veins were observed in normal non-treated Gp1 (Fig. 3A). While aflatoxin non-treated Gp2 revealed chronic cholangitis that characterized by hyperplastic biliary epithelium with fibrotic biliary wall (Fig. 3B). Moreover, dilatation of portal vein & circumscribed aggregations of lymphocytes that usually seen at periportal areas. Degenerative changes within most hepatocytes beside focal necrotic areas of hepatocytes with pyknotic nuclei were also seen. Also, hemorrhagic areas & inflammatory cell infiltrates were seen in between hepatic parenchyma (Fig. 3C). AF + MO Gp3 (Fig. 3D) exhibited biliary hyperplasia, and round areas of mononuclear cells infiltrates within some portal areas beside mildly dilated hepatic vasculatures were also seen. AF + LF Gp4 (Fig. 3E) showed still present populations of round cell within portal area beside replaced some necrotic hepatocytes by inflammatory cells that mostly seen at perivascular area. AF + ST Gp5 (Fig. 3F) revealed marked areas of vacuolated hepatocytes and perivascular follicles of lymphocytic aggregations. In the other hand, AF + MO + ST Gp6 (Fig. 3G) showed apparent normal majority of hepatic acini, mild perivascular inflammatory cell infiltrates, and mild inflammatory reaction at portal areas. However, apparently normal hepatic acini and mild inflammatory reaction of some portal triads were detected in AF + LF+ ST Gp7 (Fig. 3H). Apparently normal hepatic cells dilated central veins, and few inflammatory reactions of some portal triads were in AF + MO + LF Gp8 (Fig. 3I). Well improved morphological structures of hepatic acini, sinusoids, portal vein, bile ducts were observed in AF+MO + LF + ST Gp9 (Fig. 3J).

Discussion

Most of research is focused on the decontamination and detoxification of feed from aflatoxin, since their frequent occurrence in feedstuffs cause severe health problems in the poultry industry and large economic losses (Djurdjević *et al*., 2022). The present study exhibited the potential efficiency of *Moringa oleifera*, lactoferrin and Syner-tox in detoxification of feeds contaminated with aflatoxin in broiler chickens. This is constituted by the comprehensive evaluation of liver and kidney biomarkers, oxidative stress, amino acids, and histopathological changes, along with assessment of growth performance, and aflatoxin residue in liver and muscle tissues. In the current study a significant decrease in BW, BWG, FI

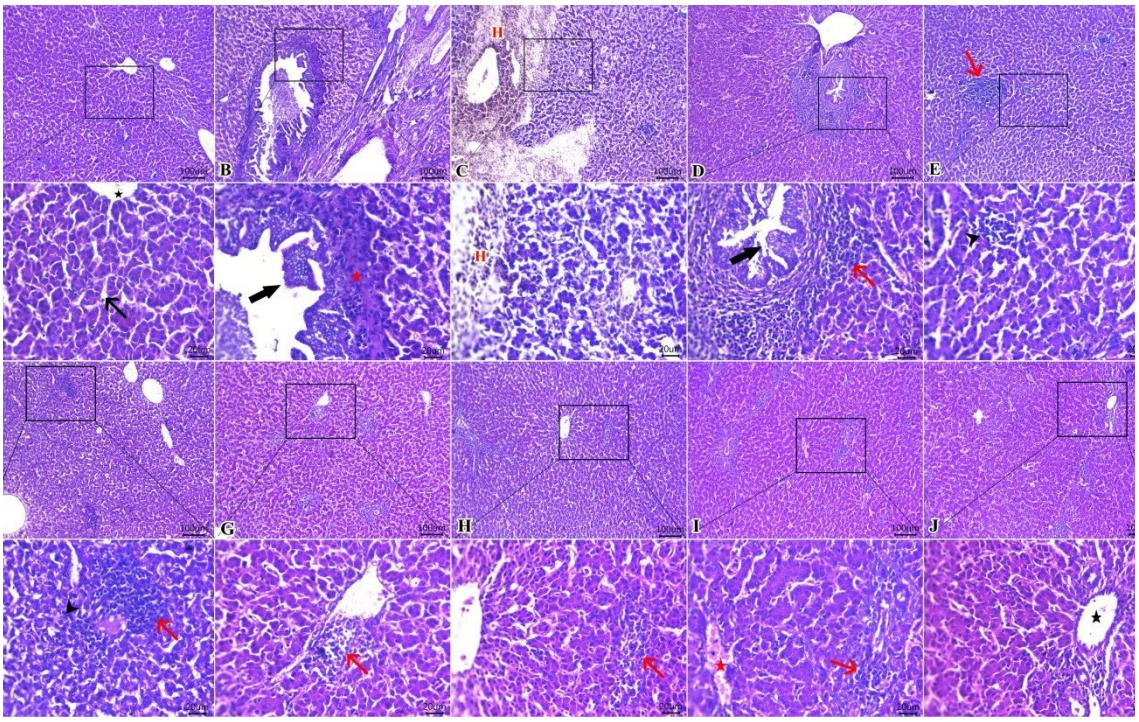

Fig. 3. Photomicrograph of H&E-stained sections of liver showing: A: Normal histomorphological structures of hepatic acini (arrow), portal areas, sinusoids and central vein (star) in normal non-treated (Gp1). B: hyperplastic biliary epithelium (thick arrow) with fibrotic biliary wall (red star), C: hemorrhagic areas (H) and inflammatory cell infiltrates in between hepatic parenchyma in aflatoxin non-treated (Gp2). D: biliary hyperplasia (thick arrow), and areas of mononuclear cells infiltrates (red thin arrow) within some portal areas in AF + MO (Gp3). E: populations of round cells within portal area (red arrow) and replaced some necrotic hepatocytes by inflammatory cells (arrowhead) in AF + LF (Gp4). F: marked areas of vacuolated hepatocytes (arrowhead) and perivascular follicles of lymphocytic aggregations (red arrow) in AF + ST (Gp5). G: normal majority of hepatic acini, mild perivascular inflammatory cell infiltrates (red arrow), and mild inflammatory reaction at portal areas in AF +MO + ST (Gp6). H: apparently normal hepatic acini and mild inflammatory reaction at portal triad (red arrow) in AF + LF+ ST (Gp7). I: apparently normal hepatic cells, dilated central veins (red star) and few inflammatory reaction at portal triad (red arrow) in AF + MO + LF (Gp8). J: Well improved morphological structures of hepatic acini, sinusoids, portal vein (star) ,and bile ducts in AF+MO + LF + ST (Gp9).

and increased FCR occurred in Gp2 (AF non treated group) in comparing with control group. These results concurred with Tawfiek *et al.* (2024) who suggested that the growth suppression and reduced feed efficiency in aflatoxin non-treated groups which may be due to malnutrition and lack of nutrient. The negative effect of dietary AF on broiler growth performance is related to a reduction in protein and energy utilization, which may be caused by a decrease in the digestive and metabolic efficiency which impaired nutrient absorption (Rajput *et al.*, 2017). In contrast, all treated groups with *Moringa oleifera*, lactoferrin and/or Syner-tox showed a significant increase in body performance. Similarly, Fouad and El-Rayes (2019) demonstrated that, BWG and FI in *Moringa* treated chicks were significantly increased compared with control group and there was an amelioration in FCR. This amelioration may be attributed to decreased bacterial load inducing disease in the intestine with mend intestinal lumen health, which enhanced the absorption and utilization of the dietary nutrients. *Moringa* is concentrated in nutrients and in raw form appears to reduce the activity of pathogenic bacteria and improves the digestibility of other foods, helping chickens to express their natural genetic potential (Gaia, 2005). Also, Enany *et al.* (2017) recorded that lactoferrin significantly enhanced growth performance through increase BW and BWG in broiler chickens. Moreover, Shareef and Omar (2012) reported that addition of Syner-tox in the drinking water revealed a marked elevation in FI compared with non-treated group. These findings may be attributed to the high active protease, lipase and amylase enzymes which are secreted by *Bacillus subtilis* as they cause feed decomposition and facilitate the absorption of more nutrients (Li *et al.* 2014).

The adverse effects of aflatoxin contaminated diet on hematological parameters were investigated in present study. There were significant reductions in Hb, RBCs, PCV, MCHC, WBCs and absolute lymphocyte in aflatoxin non-treated group compared with normal group. Similarly, Ashry *et al.* (2022) recorded a remarkable reduce in RBCs, HCT, WBCs, and lymphocyte in aflatoxin non-treated chickens. AF can cause damage to the liver, impairing its capability to support erythropoiesis. AF can disrupt hemoglobin Hb synthesis and erythropoiesis, contributing to reduce PCV (Khandros and Weiss, 2010). Aflatoxin administration was reported to cause a noticeable remarkable elevate in heterophil, monocyte, and eosinophil in Gp2 comparing with normal groups. These findings may be attributed to the inflammatory response that is caused by aflatoxin and results in heterophilia (Dönmez *et al.*, 2012). Furthermore, aflatoxin caused immunosuppression as a result of protein deficiency. Thus, chickens are susceptible to infection, particularly, parasitic infection, as noticed by whitish diarrhea in current study. This infection caused eosinophilia and monocytosis (Ashry *et al.*, 2022). On contrast, all treated chickens (Gp3-Gp9) showed amelioration in hematological profile, which is confirmed by Fouad and El-Rayes, (2019) who recorded that *Moringa* modulates the hematological profile (RBCs, Hb, WBCs) of broiler chicks compared with the normal group. This amelioration might be attributed to the antioxidant impact of *Moringa*. Also, Abd El Monsef *et al.* (2024) reported that LF improvement all hematological profile (RBCs, Hb, PCV, WBCs) of broiler chicks when compared with Eimeria tenella non-treated birds. Furthermore, El-Sayed *et al.* (2024) observed that there were noticeable increases in the WBCs, lymphocyte%, RBCs, Hb, and PCV% after dietary administration of *Bacillus subtilis* for 28 days compared with normal groups in broiler chickens. Experimental confirmation indicates that various microbiota-derived components present in the bloodstream participate in steady-state hematopoiesis impact (Belkaid and Harrison, 2017).

The liver and kidney are the target organs for aflatoxin elimination. The findings of our study showed a significant increase in AST, ALT, creatinine, urea, cholesterol, triacylglycerol (TAG), LDL and VLDL associated with a noticeable decrease in HDL in aflatoxin non-treated group when compared with normal group, as recorded by previous studies (Ashry *et al.*, 2022; Gomaa *et al.*, 2022). Hepatotoxic impact of aflatoxin causes hepatic damage through hepatocyte destruction and subsequently leakage of enzyme in the aflatoxicated chickens (Rathod *et al.*, 2017; Tawfiek *et al.*, 2024). Furthermore, the increases in creatinine and urea levels in Gp2 indicated the stressful and deleterious influence on kidney tissue; these results are confirmed by Hamzawy *et al.*, (2013). The cause of increased

urea nitrogen level may be renal failure, gastrointestinal hemorrhage, hypovolemia, elevated catabolism and decrease utilization of amino acids (Denli *et al*., 2009). Moreover, the increases in serum cholesterol and TAG levels are linked with hepatic dysfunction and biliary obstruction (Edrington *et al*., 1995). Additionally, Marcus and Milton (1982) recorded that cholesterol level is related with metabolic functions, which are influenced by the kidney and liver integrity. On contrast, all treated chickens (Gp3-Gp9) showed amelioration in liver, kidney and lipid biomarker. Similarly, Fouad and El-Rayes (2019) suggested that MO modulates the liver and lipid profile of broiler chicks compared with the normal group, who added that MO has properties that can improve liver function. Additionally, it has been used as a natural antioxidant for its antioxidant action and is rich in antioxidant substances (Vongsak *et al*., 2014). Also, MO highly reduced plasma cholesterol, especially LDL, enhanced immune systems in Japanese quail (Moustafa *et al*., 2015). Furthermore, Ling *et al*., (2019) reported that LF provides a beneficial impact on lipid metabolism and decreases total cholesterol and LDL levels through decreases the expression of the protein β-Hydroxy β-methylglutaryl-CoA reductase, which decreases cholesterol synthesis. Additionaly, Ab¬del-Moneim *et al*. (2020) demonstrated that, supplementation of *Bacillus subtilis* extract evoked a noticable reduce in serum creatinine, urea, cholesterol, TAG, LDL and VLDL associated with a noticeable increase in HDL level of treated broilers.

In present study, Table 4 showed a steep decline in total protein, albumin, alpha globulin 1,2 and gamma globulin 1,2 levels among chicks exposed to aflatoxin. Similarly, Ashry *et al*. (2022) reported that, there were significant decreases in total protein and albumin level in aflatoxin non- treated group compared with treated chicks. Decrease total protein may be contributed to suppression the synthesis of protein. Aflatoxin prevents the synthesis of protein, via competitive inhibition of phenylalanine t-RNA synthesis with phenylalanine or because of metabolism disturbances resulting from hepatic degeneration (Rathod *et al*., 2017). In contrast, treatment of aflatoxicosis chickens with MO, LF and/or ST groups (Gp3 – Gp9) displayed a significant increase in total protein and albumin. These results were consistent with Fouad and El-Rayes (2019) who recorded that, *Moringa* supplementation increase serum total protein and albumin in chicks compared with control group. Also, El-Sherbeny and El-Shenawy (2023), suggested that MO, LF and/or ST enhances hepato-renal functions by highly reducing tissue injury, protein loss, and enzyme leakage, which is confirmed via a marked increase of total protein, albumin and globulin levels coupled with a reduced level of AST, ALT, creatinine and urea. In the present study, LF-treated chickens observed elevated levels of gamma globulins, which are immunoglobulins. Subsequently, LF has a liver-preventive and immune-modulatory impact (Assaraj *et al*., 2018). Moreover, the multiple acids which present in ST increase the acidity in the gastrointestinal tract, favouring with the vitamin B complex and aspartic acid in the multiplication of the beneficial probiotic friendly lactic acid bacteria. Papain, which Syner-Tox contains, in its own content, as an anti-inflammatory substance is also help in the protein digestion and hence enhance the immunity as immunomodulator besides protein digestion (Shareef and Omar, 2012).

The current study displayed decrease levels of lysine and methionine in Gp2. After aflatoxin exposure, the observed early negative impacts involved decreased feed intake, diarrhea, anorexia, and emesis (Pestka and Smolinski, 2005). Aflatoxin significantly reduces amino acids in chickens (Voigt *et al*., 1980). Conversely, a remarkable increase in muscle content lysine and methionine ration were observed in all treated groups (G3-G9 Gps) when compared with aflatoxin non-treated group. The content of amino acids in meat is strictly linked to the meat protein's nutritional value (Wood *et al*., 1996). There are between 16 and 19 amino acids in MO leaves, with 10 of those regarded as essential (Sonkar *et al*., 2019). The level of essential amino acids indicates proteins quality (Yang *et al*., 2020). MO could elevate essential amino acids contents (methionine and lysine) in the breast muscle of chickens, suggesting that adding MO to chickens' diet can mend quality of meat and nutritional value (Jiang *et al*., 2023). Also, *Bacillus subtilis* administration has a significant influence on a broad range of chemical metabolites levels in the chick's intestine, especially those linked to amino acids (Park *et al*., 2020).

Oxidative stress (OS) is one of the primary pathogenic causes of tissue destruction because it alters redox balance in a variety of diseases (Singh and Saso, 2019). Aflatoxin can cause the production of a huge amount of free radicals and increase lipid peroxidation, thereby promoting OS in the liver causing liver damage. The high level of serum NO and liver content of MDA a combined with reduced levels of TAC, CAT, and GSH were recorded in aflatoxin non-treated birds as compared with Gp1. Oxidative stress occurred due to the production of the NO and MDA and reduction of antioxidant enzymes activities. Our results confirmed with the previous studies of showing aflatoxin induced liver damage mediated through OS (Ashry *et al*., 2022; Hassan *et al*., 2022; Al-Habashi *et al*., 2024). Among the several types of natural non-enzymatic antioxidants, GSH is an early biomarker of the OS (Gagliano *et al*., 2006). The decreased GSH level in the current study might be resulting from OS being caused by a catalyzed conjugation reaction. Apoptosis may have resulted from DNA damage and mitochondrial disintegration caused by the imbalance between reactive oxygen species (ROS) and the antioxidant defense system (Sahoo *et al*., 2024). However, each treated group showed a remarkable decrease in NO and MDA, associated with a remarkable increase in serum levels of TAC, CAT, and GSH in liver tissue when compared with aflatoxin non-treated group. Similarly, Fouad and El-Rayes (2019) found that antioxidants markers involving TAC and GSH were high increase in birds fed basal diets administrated with several levels of MO when compared with control group. *Moringa oleifera* has been applied as a natural antioxidant for its antioxidant impacts and is rich in antioxidant substances such as flavonoids, carotenoids, phenols and ascorbic acid (Vongsak *et al*., 2014). MO in both tender and mature leaves have a potent antioxidant property against free radicals, offers highly defense against oxidative stress resulting in prevention of liver and kidney damage (Sreelatha and Padma, 2009). Furthermore, Abd El Monsef *et al*. (2024) stated that, LF treated groups significantly decrease serum NO and MDA levels, while, marked increase in serum TAC; and liver content CAT, and GSH levels were shown comparing with Eimeria tenella non-treated birds, suggesting the antioxidative impacts of LF contributed to its iron-binding features via sequestering iron (Raghuveer *et al*., 2002). Moreover, Amer *et al*. (2022) observed that chickens treated with Syner-Tox® displayed a noticeable reduce in serum NO level at 14th day post-treatment, while GSH and CAT displayed a marked increase at 14th day post-treatment. Also, Chen *et al*. (2019) observed that *Bacillus subtilis* elevated CAT activity which had a beneficial response on antioxidant activity in birds. These results indicated that probiotic bacteria promote antioxidant protection mechanism of chickens. This impact might be related to the probiotic bacteria's efficiency to cause chelate free radicals and capturing ROS (Lin and Yen, 1999).

Residual aflatoxin in the liver and muscle not only impacts the health and performance of broiler chicks but also negatively impacts the health of consumers of broiler chicks' products because of accumulation of aflatoxin in the edible parts of poultry. Consequently, considering public health and safety, it is critical to monitor the quality of chicken products and to analyze aflatoxin residues in different chicken tissues (Salem *et al*., 2018). The finding in the current study of the aflatoxin residue in Gp2 is in line with the results of Ashry *et al*., (2022). In construct, the administration of MO, LF and/or ST groups (Gp3 – Gp9) with aflatoxin displayed decrease residue levels in liver and muscle. Similarly, Fan *et al*. (2013) reported that, *Bacillus subtilis* decreased of aflatoxins residues in the liver by 42–97% in the chicks. In this instance, it is interesting to record here that all amelioration in hematological and biochemical markers in aflatoxicated chicks treated with MO, LF and/or ST were linked with successful elimination of aflatoxins residues in muscle and liver tissues with the best results in Gp3 treated *Moringa* with aflatoxin exhibited significant degradation of aflatoxin from the liver and muscle tissues. The administration of Mo into

poultry feeds has the efficiency to increase the antioxidant impact of liver tissue by amelioration the immune status of the tissue which decreased the deleterious impacts of aflatoxin (Aboelhassan *et al*., 2018).

The liver is a main detoxifying organ in the body, in present study, aflatoxin exposed leads to different liver pathological changes such as hyperplastic biliary epithelium, dilatation of portal vein, aggregations of lymphocytes, necrosis and inflammatory cell infiltrates. These results are nearly similar to those recorded by Denli *et al*. (2009); Hamzawy *et al*. (2013); Hassan *et al*. (2022); Djurdjević *et al*. (2022) and Al-Habashi *et al*. (2024). It is recommended that these negative impacts of aflatoxins are contributed to the release of huge amounts of ROS that damaged cells membrane, DNA, and decay of liver cells (Hassan *et al*., 2021, 2022). In contrast, supplementation of the Mo, LF and/or ST in combination with aflatoxin decreased these pathological and biochemical alters in the liver cells because it's anti-inflammatory and antioxidant impacts. The antioxidants strictly enhance the liver's health and can reduce damage done to the liver and may even hasten liver healing (Mansour *et al*., 2015). Furthermore, Cortes-Alvarez *et al*. (2024) suggested the hepatoprotective effects by the high improvement in the necro-inflammatory score in the rats that received MO compared with non-alcoholic fatty liver non-treated group. These findings indicate that MO has anti-inflammatory and hepatoprotective effects against hepatic damage, contributed to its antioxidant, and anti-inflammatory impacts (Almatrafi *et al*., 2017).

Conclusion

The present study concluded that LF and MO might possess a strong antioxidative and anti-toxicity impact as it decreased the oxidative stress and the aflatoxin residue in liver and muscle tissue. Nevertheless, this should be preceded by future expanding its assessment in the field in the future. Remarkably, the combined applied of LF with MO result in a potent amelioration of antioxidant status of the liver tissues; additionally reduce liver damage. Finally, MO and LF can be recommended to be used for elimination of mycotoxins from animal feed. Furthermore, it could be suggested that MO and LF can be applied as a natural alternative growth stimulator in broiler chicks as it has appositive impacts on growth performance. Further studies are recommended to investigate the primary mechanistic pathways include in anti-mycotoxins agents of MO and LF.

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Conflict of interest

The authors declare that they have no conflict of interest.

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