Research Article

Comparative Effectiveness of Silymarin and Choline Chloride (Liver Tonics) in Preventing the Effects of Aflatoxin B1 in Broiler Chicks

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Abstract | The purpose of this study was to compare the effectiveness of two distinct liver tonics (Silymarin and Choline chloride) against aflatoxin B1 (AFB1), as well as the impacts of AFB1 on the growth performance, liver weight, haematological, and serum biochemical responses of broiler chicks. Six nutritional treatment groups with equal numbers of birds each were created. In a randomized complete block design experiment, each group contained 100 birds in five repetitions and was assigned to one of the six feeding regimens. Experimental groups included: (1) Negative control (NC) with the basal diet, (2) NC + 600 mg/kg diet of silymarin, (3) NC + 400 mg/kg diet of choline, (4) Positive control (PC) containing 1 mg AFB1/kg diet, (5) PC + 600 mg/kg diet of silymarin and (6) PC + 400 mg/kg diet of choline. The findings show that the AFB1-treated chicks' growth characteristics dramatically decreased. While total antioxidant capacity (TAC) dropped as a result of AFB1, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, creatinine, and malnodialdhyde (MDA) dramatically rose. After 35 days of feeding, AFB1-treated chicks showed a significant decrease in their total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin concentration (Hb), haematocrit levels (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC), as well as lymphocyte %, neutrophil %, and monocyte %. All the indicators, including feed intake, body weight gain, haematological tests, and serum biochemical tests, were significantly ($p<0.05$) enhanced by both liver tonics. The study concluded that silymarin or choline can be effectively used to decrease the adverse effects of AF in broiler chicken. However, silymarin comparatively more efficiently ameliorate the effects induced by AFB1 than choline chloride.

Keywords | Aflatoxin B1, Liver tonics, Broiler chicks, Blood

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INTRODUCTION

The low molecular weight mycotoxins that fungi create are secondary metabolic products. They hurt economies significantly and are present in about 25% of cultures globally (Magnoli et al., 2017). Mycotoxins (B1, B2, G1, and G2) that can contaminate cereals include aflatoxin, which is a byproduct of the metabolism of the fungus Aspergillus spp (Buszewska-Forajta 2020). Because it has hepatotoxic, carcinogenic, and teratogenic properties, aflatoxin B1 (AFB1), which is produced by the fungus *Aspergillus flavus* and *Aspergillus parasiticus*, is regarded as the most toxic of the four varieties (Anater et al., 2020). (Magnoli et al., 2017). One of the animal species that is most susceptible to mycotoxins is the birds (Magnoli et al., 2017). In addition to affecting the intestinal mucosa, metabolism, and immunity when fed by broilers, AFB1 lowers animal performance (Abdolmaleki et al., 2019).

Mycotoxin toxicity in birds is influenced by a number of variables, including the organism's susceptibility to toxin exposure, exposure duration, health state, and contamination dose (Buszewska-Forajta 2020).

Utilizing liver tonics, which protect the liver from damage, is a successful strategy for combating the effects of mycotoxins. Many flavonolignans, including silybin (50–60%), silychristin (20%), silydianin (10%), and isosilibine (5%), as well as a flavonoid (taxifolin), are present in silymarin (Attia et al., 2019). Silymarin has been shown to have a variety of advantageous impacts on the growth of chickens. In order to promote growth performance, decrease oxidative stress, improve meat quality, increase production of polyunsaturated fatty acids, and stimulate immunological status in broilers, it is thought to be a potential feed addition (Armanini et al., 2021; Bagno et al., 2021). Antioxidant silymarin controls and absorbs intracellular glutathione in its role as an antioxidant. By preventing the mycotoxin from reaching the liver, silymarin also regulates and stabilizes the permeability of the cell's outer membrane. Silymarin increases rRNA synthesis, which accelerates the regeneration of the liver, prevents cirrhosis by converting the liver stellate cells into myofibroblasts, and removes free radicals, eventually protecting the liver (Fraschini et al., 2002).

A number of biological processes in chicken depend on choline, a newly discovered vitamin B4 that is primarily found in the form of phospholipids. It is necessary for the development and maintenance of cell membranes and organelles, such as mitochondria and microsomes, as well as for the healthy maturation of the bone's cartilage matrix (Arele et al., 2015). Additionally, it is a crucial part of acetylcholine, the most prevalent neurotransmitter in the nervous system that facilitates the passage of nerve impulses across synapses (Wauben and Wainwright, 1999). Choline's physiologically active methyl groups are its most noticeable structural property, and they enable it to play a crucial role as a labile methyl donor in the production of methionine from homocysteine after being oxidised to betaine (Zhang et al., 2013). Additionally, choline is thought to be a lipotropic agent that inhibits aberrant lipid accumulation and the growth of fatty liver (Halver, 2002). Insufficient choline causes perosis and growth retardation in young fowl. In chicks and turkey poults, perosis is the main clinical symptom of a choline deficit, whereas quail develop swollen hocks and bowed legs (NRC, 1994).

The vitamin levels in the liver were altered by aflatoxicosis in three different ways: (i) nonsignificant reductions in niacin, pantothenate, and thiamine; (ii) significant reductions in vitamin B6, choline, folate, and riboflavin that were associated with aflatoxin dosage; and (iii) a significant change in biotin that was unrelated to aflatoxin dosage (Michael et al., 1980). As a result of a shortage of phosphatidylcholine, which the liver utilises to convert too much triglyceride into lipoproteins, choline deficit in humans results in hepatosteatosis (Fatty liver) (Buchman et al., 1995). Additionally, choline shortage damages the liver by raising blood aminotransferases, a sensitive sign of liver issues (Rahmani et al., 2012). Thus, AFB1-induced liver damage might be treated with choline chloride. Taking into account all of these details, our study's initial objective was to examine the effects of silymarin and choline chloride on broiler chicken growth performance, haematological, and serum biochemistry in order to reduce the impact of AFB1 in liver cells.

MATERIALS AND METHODS

Production of aflatoxin B1

By cultivating *Aspergillus parasiticus* (NRRL 2999) (source: National Institute of animal Health, Dokki, Cairo, Egypt) on rice using a modified version of the method developed by Kubena et al. (1990) and modified by West et al. (1973) aflatoxin B1 (AFB1) was produced from the *Aspergillus parasiticus*. The fermented rice was autoclaved, ground into a powder, and the amount of AF was determined using spectrophotometric analysis (Wiseman et al., 1967) with modifications of Nabney and Nesbitt 1965). To achieve the necessary amount of 1.0 ppm of AFB1/kg of diet, rice powder was added to the basic diet. The levels of AF that were identified in the control diet were below the detection thresholds.

Ration

A commercial-style diet of corn and soybean meal served as the foundation. Up until 21 days of age, chickens were given a starter-grower commercial ration; from days 22 to 35, they were moved to a finisher diet (Table 1). The ration did not include any antibiotics, anticoccidial, or antifungal medications. The ration was created to meet or surpass the NRC (1994) recommendations for chicken nutrition. There was allowed access to feed and water for the birds. The basal food was examined for any probable residual mycotoxins, such as aflatoxins, ochratoxins, zeralenone, and fuminsin, before experimental birds were fed it (Rottinghaus et al., 1982).

Experimental chickens

The 600 mixed-sex, day-old broiler chicks (Ross 308) utilized in this investigation were bought from a business hatchery. The bird pens were meticulously cleaned and sterilised. Over the course of the trial, birds were kept on a 24-hour continuous light schedule (35 days). During the period of brooding, the temperature was maintained at the desired level. At 7 days old, chickens were given intra-ocular route of the Hitchner B1+H120 vaccine to protect them from Newcastle Disease (ND) and infectious bronchietis,

Table 1: Composition and calculated analysis of Starter and Finisher diets.

Ingredients	Starter-grower (1-21d)	Finisher (22-35d)
Yellow corn	54.40	62.00
Soybean meal, 44%	27.00	24.05
Corn Gluten meal, 60%	10.00	6.19
Soy bean oil	4.55	4.00
Limestone	1.10	1.00
Di-calcium phosphate	2.20	2.05
Vit & min. premix*	0.30	0.30
DL-Methionine	0.05	0.01
L-lysine (HCl)	0.15	0.15
Na Cl	0.25	0.25
Total	100	100
Calculated analysis: **		
CP, %	23.03	20.02
ME (Kcal/kg)	3204	3201
Calcium, %	1.05	0.97
Available phosphorus, %	0.45	0.42
Lysine, %	1.14	1.03
Methionine, %	0.52	0.41
TSAA, %	0.90	0.73

*Each 3kg contain: VitA 12000000IU, Vit D3 2000 000 IU, Vit E 10g, Vit K3 2g, Vit B1 1g, Vit B2 5g, Vit B6 1.5g, Vit B12 10mg, Nicotinic acid 30g, Pantothenic acid 10g, Folic acid 1g, Biotin 50mg, Choline chloride 250g, Iron 30g, Copper 10g, Zinc 50g, Manganese 60g, Iodine 1g, Selenium 0.1g, Cobalt 0.1gand carrier (CaCo3) to 3 kg. **According to tables of NRC (1994).

and at 9 days old, they were given intramuscular injections of the inactivated H5N2 vaccine to protect them from avian influenza. Using the 228 E strain and La Sota vaccines, respectively, immunization against infectious Bursal illness and ND was performed at the age of 15 days.

Experimental design

Six hundred Ross 308 d-old broiler chicks were divided, based on a completely randomized design, into 6 experimental groups and five replicates (20 chicks per replicate) for 35 days. During the experiment, all the environmental conditions were the same for the treatments, receiving food and water, *ad libitum*, from hatch to d 35. Experimental groups included: (1) Negative control (NC) with the basal diet, (2) NC + 600 mg/kg diet of silymarin, (3) NC + 400 mg/kg diet of choline, (4) Positive control (PC) containing 1 mg AFB1/kg diet, (5) PC + 600 mg/kg diet of silymarin and (6) PC + 400 mg/kg diet of choline. From the day of hatch to the age of five weeks, all groups were kept under strict observation (end of the study). The Institutional Animal Ethical Committee and National Regulations on Animal Welfare were followed in conducting the experiment (IAEC).

Growth performance determination

Data on live body weight and feed intake (FI) were collected weekly during the experimental time and data were used to estimate body weight gain (BWG), feed intake, and feed conversion ratio (FCR). Mortality was logged upon incidence.

Blood Hematology and Biochemistry

At 35 days into the experiment, blood samples were taken from five birds per group. The birds were manually restrained, and blood was drawn from the ulnar vein using an insulin syringe. This material was divided into two tubes, one containing ethylenediamine tetraacetic acid (EDTA) to get whole blood for haematological examination and another tube containing serum but not containing an anticoagulant. The serum was then extracted from the blood and stored at -20 °C for biochemical analysis after being centrifuged at 3500 rpm for 10 minutes. Complete blood count (CBC) in haematology is a test that measures haemoglobin level (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The haematological features were assessed using an Abbott CELL-DYN 3700 haematology analyzer (Chicago, IL, USA). As a stress indicator, the heterophil to lymphocyte ratio was assessed. Aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured

Table 2: Growth performance in broiler chicks fed on 1 mg/kg aflatoxin B1 contaminated feed with two different liver tonics.

Treatments	Growth parameters					
	IBW, (g)	FBW, (g)	BWG, (g)	FI, (gm/bird)	FCR (g feed:g gain)	Mortality %
Negative control (NC)	40.00	2180[a]	2140.0[a]	3685[a]	1.721[c]	0.00
NC+ Silymarin	40.05	2220[a]	2179.95[a]	3710[a]	1.701[c]	0.00
NC+ Choline chloride	40.00	2190[a]	2150.0[a]	3690[a]	1.716[c]	0.00
Positive control (AFB1)	40.10	1720[d]	1679.9[d]	3250[c]	1.934[a]	17.00a
AFB1+ Silymarin	40.08	2150[b]	2109.9[b]	3700[a]	1.753[bc]	3.00b
AFB1+ Choline chloride	40.05	2100[c]	2059.9[c]	3645[b]	1.769[b]	4.00b
SEM	1.255	15.225	13.224	10.555	0.528	0.002
p-value	0.488	0.0001	0.0001	0.002	0.024	0.0001

[a,b,c,d]Means within column with different superscripts differ significantly P < 0.05; SEM: Standard Error of the mean; IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

Table 3: liver function, kidney function and oxidative status in broiler chicks fed on 1 mg/kg aflatoxin B1 contaminated feed with two different liver tonics.

Treatments	Liver weight, %	Liver function		Kidney function		Oxidative status	
		AST U/l	ALT, U/l	Creat. mg/dl	Uric acid, mg/dl	MDA, nmol/ml	TAC, nmol/ml
Negative control (NC)	2.52[c]	102.55[c]	178.22[c]	0.965[d]	3.90[b]	0.88[d]	3.58[a]
NC+ Silymarin	2.50[c]	99.52[c]	169.58[c]	0.955[d]	3.88[b]	0.80[d]	3.66[a]
NC+ Choline chloride	2.56[c]	100.51[c]	180.11[c]	0.977[cd]	3.89[b]	0.82[d]	3.61[a]
Positive control (AFB1)	3.96[a]	156.38[a]	248.22[a]	1.22[a]	6.22[a]	2.58[a]	1.59[d]
AFB1+ Silymarin	2.82[bc]	118.27[bc]	182.55[c]	0.980[c]	4.05[ab]	1.04[c]	3.22[b]
AFB1+ Choline chloride	3.04[b]	122.52[b]	200.25[b]	0.998[b]	5.11[b]	1.56[b]	3.00[c]
SEM	0.228	0.361	0.412	0.055	0.158	0.528	0.144
p-value	0.0001	0.0001	0.0001	0.025	0.0001	0.025	0.001

[a,b,c,d]Means within column with different superscripts differ significantly P < 0.05; SEM : Standard Error of the mean; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Creat: creatinine; MDA: malnodialdhyde; TAC: total antioxidant capacity.

Table 4: Hematological parameters in broiler chicks fed on 1 mg/kg aflatoxin B1 contaminated Feed with two different liver tonics.

Treatments	Hematological parameters						
	RBCs x 10^6/ µl	WBCs x 10^3/ µl	Hb gm%	PCV %	MCV %	MCH Pg	MCHC %
Negative control (NC)	3.14[a]	23.00[a]	10.96[a]	31.58[a]	101.56[a]	35.38[a]	33.78[a]
NC+ Silymarin	3.25[a]	23.93[a]	11.02[a]	30.85[a]	98.55[a]	35.75[a]	33.85[a]
NC+ Choline chloride	3.19[a]	23.25[a]	10.85[a]	30.22[a]	99.45[a]	35.22[a]	33.49[a]
Positive control (AFB1)	2.68[c]	17.62[c]	8.68[c]	22.55[c]	88.65[c]	29.85[c]	30.48[c]
AFB1+ Silymarin	3.10[a]	22.29[b]	10.58[a]	28.25[b]	95.94[ab]	32.84[ab]	33.00[ab]
AFB1+ Choline chloride	2.96[b]	21.67[b]	9.86[b]	30.00[a]	90.55[b]	30.33[b]	32.05[b]
SEM	0.125	0.452	0.228	0.365	1.528	0.524	0.358
p-value	0.0001	0.0001	0.0001	0.0001	0.025	0.011	0.035

[a,b,c,d]Means within column with different superscripts differ significantly P < 0.05; SEM : Standard Error of the mean; RBC: red blood cells; WBC: white blood cells; Hb: haemoglobin; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC; mean corpuscular haemoglobin concentration

Table 5: Differential leukocyte count in broiler chicks fed on 1 mg/kg aflatoxin B1 contaminated Feed with two different liver tonics.

Treatments	Differential leukocyte count				
	Heterophil (H) ($\times10^3$/µl)	Lymphocyte (L) ($\times10^3$/µl)	Eosinophil ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)	H/L ratio
Negative control (NC)	6.88[a]	15.35[a]	0.55	0.22	0.448[b]
NC+ Silymarin	7.08[a]	16.08[a]	0.56	0.21	0.440[b]
NC+ Choline chloride	6.93[a]	15.55[a]	0.55	0.22	0.445[b]
Positive control (AFB1)	5.66[b]	11.28[c]	0.48	0.20	0.501[a]
AFB1+ Silymarin	7.00[a]	14.96[ab]	0.52	0.21	0.480[b]
AFB1+ Choline chloride	6.75[a]	14.00[b]	0.50	0.20	0.474[b]
SEM	0.224	1.258	0.055	0.0245	0.005
p-value	0.025	0.001	0.554	0.851	0.002

[a,b,c,d]Means within column with different superscripts differ significantly P < 0.05.SEM : Standard Error of the mean.

in the serum for the liver function test, and uric acid and creatinine were measured in the blood for the renal function test using a commercial kit in accordance with the manufacturer's instructions using a chemistry analyzer (URIT-800). The total antioxidant capacity (TAC) and malnodialdhyde (MDA) of the broiler chick plasma were measured according to Erel (2004).

Statistical analysis

The data of the current study were statistically analyzed (SAS, 2002) using one-way analysis of variance (ANOVA). The means, exhibiting significant differences in ANOVA, were compared using the Duncan multiple range test at p<0.05.

RESULTS

Growth performance

The results from Table 2 demonstrate the impact of silymarin supplementation, choline chloride without or with aflatoxin B1 contaminated meals on broiler growth performance from 0 to 35 days of the experiment. When compared to the control group, the findings of the experiment showed that feed containing AFB1 at a dose of 1 mg/kg feed resulted in significantly (P≤0.05) decreased body weight gain, feed intake, while feed efficiency and mortality rate were increased. When compared to the control group, the growth performance of the birds fed silymarin or choline chloride alone dramatically improved. The adverse effects of AFB1 on body weight gain, feed intake, feed efficiency and mortality rate were mitigated by the addition of silymarin and choline chloride to the AFB1-containing diets, but the group treated with silymarin showed better progress than the group treated with choline chloride.

Liver weight and Blood constituents

The effects of dietary interventions on liver weights and several blood parameters are demonstrated by the data in Table 3. In chicks given AFB1, the weights of the livers dramatically increased. When silymarin or choline chloride was added to meals containing AFB1, the toxin's effects on liver weight were lessened. The addition of silymarin or choline chloride to the control diet, however, had no impact on the weight of the liver. Two enzymes, Alanine aminotransferase (AST) and Aspartate aminotransferase (ALT), were elevated in the liver function test by feeding feed contaminated with aflatoxin, although liver tonics improved AST and ALT activates significantly (P≤0.05). As may be seen in Table 3, Silymarin performed better than choline chloride. In the renal function test, uric acid and creatinine were the two variables that were examined. Following AFB1 feeding, uric and creatinine levels both rose. Silymarin demonstrated more significant outcomes than the choline chloride seen in Table 3. Each of TAC and MDA was influenced significantly by supplementing diets with silymarin or choline (Table 3). Regarding the effect of contaminated diet with AFB1 showed significantly (P≤0.05) the highest MDA but recorded significantly (P≤0.05) the lowest TAC values compared other groups. Silymarin and Choline chloride significantly improved the adverse effects of aflatoxin on oxidative status.

Hematological parameters

When compared to the control group, the birds fed contaminated diets without additives displayed significant decreases in the total erythrocytic count, haemoglobin concentration, packed cell volume, and mean corpuscular volume, which were linked to leukopaenia, heteropaenia, and lymphopaenia. Eosinophil and monocyte counts underwent negligible changes. The CBC at day 35 following treatment was significantly (p<0.05) enhanced by silymarin or choline chloride (Table 4 and 5).

DISCUSSION

Growth performance

In the current study, dietary aflatoxin contamination decreased feed intake, final body weight, body weight gain and impaired FCR values. The lowering of feed intake caused by aflatoxin intoxication appears to be caused by a number of potential processes. First, a defensive mechanism during aflatoxicosis is a decrease in appetite (Rauber et al., 2007). Second, aflatoxins postpone gastric emptying (Rotter et al., 1996). Third, aflatoxins appear to trigger interleukin-6 and tumour necrosis factor-a, leading to anorexia and subsequent weight loss (Schobitz et al., 1994). Aflatoxin challenge-induced body weight suppression may be relevant to a number of different routes. The first way that aflatoxicosis inhibits protein synthesis is by causing problems with the enzymes and substrates needed for the initiation, transcription, and translation stages (Eaton and Gallagher, 1994). Second, aflatoxins lower the perceived absorptive surface area of villi, which in turn decreases nutrient absorption (Verma et al., 2002; Awad et al., 2008). Third, aflatoxins cause a decrease in feed consumption, which lowers body weight. According to Oguz et al. (2000b), either the birds' immune systems were significantly compromised or disease resistance decreased during the entire experimentation period of this study, which resulted in an increase in the mortality rate owing to aflatoxicosis (Pasha et al., 2007). While the outcomes differed from those of Oguz et al. (2000b) and Tedesco et al. (2004). This discrepancy may be brought on by differences in immune response, dose, sex, exposure time, and age. Broilers that fed on diets naturally polluted with AFB1 and AFB2 showed decreased body weight, average daily increase, and feed intake, according to Liu et al. (2020). Aflatoxin exposure also decreased food intake by 11%, feed conversion by 6%, and weight increase by 11%, according to Andretta et al. (2011).

Additives that absorb mycotoxin can have a variety of effects. There are two primary methods: one is called "adsorption," in which the additive adheres to the mycotoxin surface; the other is called "biotransformation," in which the additives break down or change the metabolic forms of the mycotoxin into less harmful molecules (Vila-Donat et al., 2018). To reduce the toxic consequences of mycotoxins, such as lipid peroxidation and liver failure, natural additives that are hepatopotectors have been sought after (Sakamoto et al., 2018). Our findings demonstrate that the treatment of silymarin and choline chloride reversed the detrimental effects of AFB1 on growth performance. The findings of the current study showed that silymarin played a significant role in growth due to the improvement in nutrient availability and absorption along with silymarin's positive effects as an antioxidant element that protects nutrients from oxidation. As a result, the majority of feed in commercial diets, particularly the essential ingredients, became available to the birds to benefit from all of it. These findings in the current study concurred with those published by Kalorey et al. (2005) and Surai (2015) who claimed that silymarin increased body weight when mycotoxins were present in feed. According to a recent study by Tsiouris et al. (2021), adding a detoxifying agent containing modified zeolite, Bacillus (B.) subtilis, Bacillus licheniformis, Saccharomyces cerevisiae cell walls, and silymarin to broiler diets helped to reduce the negative effects of aflatoxin and ochratoxin. Asghar and Masood (2008) proved that silymarin may be used as a dietary natural antioxidant supplement, similar to the majority of vitamins, particularly vitamin E, in avoiding free radical-related disorders (Abdulwahid, 2015). It has been hypothesized that silymarin can penetrate the nucleus and affect RNA polymerase enzymes and rRNA transcription, increasing the synthesis of ribosomes. This in turn accelerates the synthesis of proteins and DNA, which improves the cytoplasm's biosynthetic machinery and speeds up the production of both structural and functional proteins (Sonnenbichler, 1986). The results of this study suggested that silymarin may lessen the toxic effects of mycotoxins in the intestine. Additionally, silymarin can function as a chelating agent, which means it may help prevent the growth of bacteria and toxins while enhancing nutrient absorption (Radhika et al., 2016). Also, broiler chicks' growth performance was increased by adding choline chloride to their diets (Igwe et al., 2015).

Liver weight and Blood constituents

Because most aflatoxins bioactivate in the liver to the reactive 8, 9-epoxide form, which is known to bind DNA and proteins and cause damage to the liver's structures and increase liver weight, the liver is thought to be aflatoxin B1's primary target organ (Bailey et al., 2006). Increased lipid deposits in the liver as a result of poor fat metabolism could be the cause of the rise in liver weight (Pasha et al., 2007). Inhibition of the formation of phospholipids and cholesterol are the main mediators of hepatic lipidosis. The movement of lipid from the liver is consequently impacted by this (Manegar et al., 2010). Due to the build-up of fat in the cytoplasm of the hepatocytes, the livers of these chicks also had a friable and light yellow appearance. However, Magnoli et al. (2011) found that for at least 46 days throughout the broiler production cycle, relative liver weight remained unaffected when toxin levels were relatively low (50 g/kg of dietary AFB1). In the present study AFB1 at 1mg/kg level of inclusion caused significant increase in liver weight. The presence of silymarin or choline reduced the magnitude of these increase, thus indicating a direct protective effect of silymarin or choline on the liver. Choline chloride has a beneficial effect in diminishing the fat from the liver affected with AFB1. Choline is effective

in fatty liver diseases caused by a shortage of highly unsaturated phospholipids. Choline is also the cofactor vital for the formation of such phospholipids (Humphreys, 1988). Both liver tonics effectively cured the chicks but silymarin was more efficient.

To evaluate damage to liver cells, the liver's capacity to synthesis proteins, and the excretory processes of the liver, liver function tests are performed, including measuring serum levels of liver enzymes (Giannini et al., 2005). Results from elevated serum enzyme tests typically reveal liver damage before those from other measures of liver function. The two important enzymes, ALT and AST, are found in liver cells. Similar parallel increases in ALT and AST have been observed in this study, which are common in liver injury cases. In the current study, birds taking 1mg of AFB1 had significantly higher blood AST and ALT activity (Table 3). The increase in AST and ALT levels may be caused by necrosis, which causes hepatic cell disintegration, or by increased membrane permeability (Ozer et al., 2008). Normal serum biochemistry levels for the functions of the liver and kidney were obtained by adding silymarin or choline chloride to the diet. Similar findings on blood biochemistry were made by Tedesco et al. (2004) in broilers fed milk thistle diets exclusively. Additionally, in our study, the aflatoxin-treated group given silymarin or choline chloride did not vary from the untreated control group in terms of AST, ALT, creatinine, and uric acid activity in the blood plasma. It has been shown that the activation of AFB1 in the livers of humans and rats is a complicated process regulated by a number of cytochrome P450 enzymes (Yunus et al., 2011). The cytochrome P450 system can be inhibited by silymarin, which prevents AFB1 activation (Tedesco et al., 2004).

MDA is frequently measured as a lipid peroxidation indicator, and rising lipid peroxidation levels are linked to oxidative stress (Ozen et al., 2009), which can result in pathological states and disorders (Niki, 2009). As previously noted by Naaz et al. (2007), an increase in the level of MDA may be due to the suppression of enzymatic antioxidants (such as GSH-PX activity) and depletion of non-enzymatic antioxidants (such as GSH) in the liver in the AFB1-treated group compared to the control group chickens. In this study, birds who fed AFB1 showed signs of oxidative stress, a metabolic disease marked by an unbalanced oxidant/antioxidant system. Antioxidant enzymes are therefore in charge of removing the surplus of free radicals produced in the body, hence minimizing cellular and tissue damage (Sakamoto et al., 2018). The liver is the principal organ engaged in the response to poisoning and is the organ that AFB1 targets. As a result, the liver undergoes a number of alterations in the metabolism of proteins, carbohydrates, and lipids (Liu et al., 2020). AFB1 in the liver causes oxidative disorders as seen in this study, i.e., elevated levels of MDA. Mughal et al. (2017) also found that AFB1 resulted in oxidative stress due to excessive ROS generation; in addition to playing a crucial role in hepatocyte apoptosis. Numerous researchers have shown that the activation of AFB1 in human and rat livers is a multistep process regulated by various cytochrome P450 enzymes (Gallagher et al., 1996). Silymarin has been shown to be able to inhibit the cytochrome P450 system, which in turn inhibits the activation of AFB1, according to BaerDubowska et al. (1998). According to Mira et al. (1994), silymarin is a powerful antioxidant that can affect the enzyme systems involved in glutathione and superoxide dismutase as well as operate as a scavenger of free radicals (Valenzuela et al., 1989).

Regarding choline chloride, the polyherbal formulation (PHF) incorporates A. nilotica and C. longa, which are rich sources of polyphenols and curcuminoids, respectively, and can mimic the hepatoprotective effect of choline. Narayanan K et al. (2013) demonstrated the protective effect of A. nilotica on acetaminophen-induced hepatotoxicity, wherein pre-treatment with A. nilotica (250 mg/kg) orally in rats attenuated the liver damage and enhanced serum activities of ALT, AST, alkaline phosphatase, liver weight, and total bilirubin levels caused by administration of acetaminophen. Similar to that, Ali (2016) showed that there was a significant reduction in total cholesterol and triglycerides at 500 mg/kg of A. nilotica in both male and female rats. Additionally, Tranchida et al (2015) found that the supplementation of C. longa extracts caused an effect on transmethylation pathway and/or osmotic regulation by elevating the liver betaine content, which plays a role in liver lipid metabolism. These results strongly suggest that improvement in performance parameters (BW and FCR) and alleviation of AST activity, liver histopathology and lipid content (abdominal fat and breast muscle) of the PHF supplemented group to normal could be related to its hepatoprotective and lipotropic activity. These results demonstrate that PHF has choline-like properties.

The elevated levels of uric acid and creatinine may signify renal tissue damage from aflatoxin. This significant change in renal parameters was observed in birds fed aflatoxin treatments, and it was consistent with data reported from Bintvihok and Kositcharoenkul (2006). In comparison to choline chloride, silymarin was more effective in reducing the adverse effects of AFB1 on blood enzymes, creatinine, uric acid, and MDA while also increasing TAC value. This may be due to its anti-inflammatory, antioxidant, anti-fibrotic, anti-carcinogenic, anti-lipid peroxidative, membrane stabilizing properties and liver regeneration processes (Radko and Cybulski, 2007; Ghonaim et al., 2022).

Hematological parameters

The WBC count, RBC count, PCV%, and Hb concentration of the group fed the contaminated diet were significantly (P≤0.05) lower than those of the other groups, according to data in Table (4 and 5). These results corroborated those made by Elaroussi et al. (2006) and Pande et al. (2006) who studied the effects of mycotoxins on chicks fed on contaminated feed. However, mycotoxins' effects on RBC and WBC numbers result in anaemia. There have been reports of anaemia with a considerable decline in PCV and Hb concentration levels, which has been linked to an iron shortage or an issue with the aemopoietic system (Elaroussi et al., 2006).The decline in a number of leucocytes was reported to be a reflection of a decrease primarily of lymphocytes and to a lesser extent monocyte (Chang et al., 1979) or heterophils (Mohiuddin et al., 1993). Such a lymphocytopenia may be a sensitive and helpful sign of mycotoxins that possibly may occur due to a direct an impact on germinal centers of lymphoid tissues and implies change of the immune function. The effects of mycotoxins on blood cells resulted in cell damage, which decreases blood volume and lowers haemoglobin concentration. These findings corroborated those of Mohiuddin et al. (1986), who noted that the effects of AFB1 cause narcotization and haemorrhage in the digestive tract, which decreases blood volume and lowers haemoglobin concentration. According to Abdel-Wahhab et al. (2002), aflatoxins alone can cause normocytic normochromic anaemia due to a drop in haemoglobin concentration and total red blood cell counts. Numerous causes, including the reduction of protein synthesis as shown by reduced serum albumin (Kaneko, 1989), a decrease in total iron binding capacity (Harvey et al., 1991), and hemopoietic cellular abnormalities of AF, may be to blame for this decline in the haematological parameters (van Vleet and Ferrans, 1992).

Silymarin supplementation, however, has an impact on the overall Hb concentration. These findings were consistent with those made by Ajay et al. (2009), who discovered that silymarin can enhance iron absorption. Additionally, Sultan et al. (2018) demonstrated that the presence of silymarin improved haematological markers. According to Talebi et al. (2015), silymarin considerably contributes to maintaining serum biochemical and haematological parameters within the normal range. The toxic and suppressive effects of mycotoxicosis can be effectively reduced with its use.

The increase in endogenous antioxidants maintaining the integrity of the plasma membrane and the reduction in lipid peroxidation may be the potential mechanisms by which Silymarin exerts its protective effects. This would prevent some target enzymes from leaking out and harming the cells (Upadhyay et al., 2010). The four flavonoids silybin, isosilybin, silydianin, and silychristin make up silymarin, which is well known (Pradhan and Girish, 2006). Silybin is one of them and is thought to have very powerful biological effects, such as high hepatoprotective and nephroprotective effects against a variety of exo- or endo-toxicants (Shaker et al., 2010). According to some research, Silybum marianum could be added to feed to shield chicks from the harmful effects of mycotoxins such aflatoxins, fumonisins, or ochratoxin A. (Muhammad et al., 2012). According to this study, Silymarin may be an effective "addition to" mycotoxin binders for reducing the negative effects of mycotoxin-contaminated feed in chick farms.

The mechanism of choline for liver protaction, choline is crucial to the process of fat metabolism. It promotes the transportation of fat as lecithin or increases the use of fatty acids in the liver itself to prevent aberrant fat accumulation (fatty livers) (Xue et al., 2010). Due to its ability to influence fat metabolism by accelerating fat clearance or reducing fat deposition in the liver, choline is referred to as a "lipotropic" element. In broiler liver, fat content was decreased by adding choline at 760 mg per kg of feed for birds fed various energy sources (Rao et al., 2001). In diets containing 0.30% methionine and 0.43% cystine in the starter phase and 0.25% and 0.42% methionine and cystine, respectively, in the finisher phase, Spires et al. (1982) discovered that supplemental choline could replace up to two-thirds of the supplemental methionine required in broiler diets from 0 to 47 days. Choline deficiency in young fowl causes growth retardation and perosis. In chicks and turkey poults, perosis is the predominant clinical symptom of a choline shortage, whereas in quail, swollen hocks and bowed legs are signs of the same condition (NRC, 1994).

The risk of AF to human health may come from direct consumption of mycotoxins-contaminated grains that have been infected by fungi, as well as through secondary contamination through products for animals and poultry that have residual mycotoxins or their metabolites in them. Our findings generally imply that silymarin or choline chloride may protect broiler chicks from the harmful effects of AFB1 and may indirectly prevent the onset of liver and kidney disease in humans.

CONCLUSION

The current study has clarified how AFB1 negatively affects broiler chick development, haematological, and serum biochemical markers connected to the liver and kidney. Silymarin, one of the two liver tonics, had more ameliorating effects than choline chloride, but both considerably rescued the broiler chicks from AFB1 damage. However, additional research is required to examine the AFB1 effect in greater detail and to examine the protective effectiveness of liver tonics in chicks. This is only preliminary informa-

tion on aflatoxin treatment; further research is necessary to fully comprehend the pharmacodynamics of silymarin and choline chloride as well as the distribution of each in different tissues.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

NOVELTY STATEMENT

This research work was able to determine that the effect of liver tonics (Silymarin and Choline chloride) to minimize the adverse effects of AFB1 in liver cells on growth performance, hematology and serum biochemistry in broilers.

AUTHORS CONTRIBUTION

Authors contributed equally and have read and approved the final manuscript.

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