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Milk Thistle (*Silybum marianum*), Marine Algae (*Spirulina platensis*) and Toxin Binder Powders in the Diets of Broiler Chickens Exposed to Aflatoxin-B1: Growth Performance, Humoral Immune Response and Cecal Microbiota

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Abstract: This research was performed to investigate the effects of milk thistle (MT), toxin binder (TB) and marine algae (*Spirulina platensis*; SP) on the performance, blood indices, humoral immunity and cecal microbiota of broiler chickens exposed to aflatoxin-B_1 (AFB1). A total of 300 one-day-old male chicks were equally divided into five treatments, with six replicates with 10 birds per treatment. Dietary treatments included: (T_1) a control diet (without any feed additive or AFB1); (T_2) control diet + 0.6 mg AFB_1/kg; (T_3) T_2 + 10 g/kg MT; (T_4) T_2 + 1 g/kg TB; and (T_5) T_2 + 10 g/kg SP. BWG and FI were found to be considerably reduced in broilers given AFB1-contaminated diets ($p < 0.05$). The FCR was negatively influenced in birds fed AFB1-contaminated diets ($p < 0.05$). MT, TB, and SP powders also reduced the deleterious effects of AFB1 on the growth of chickens ($p < 0.05$). In comparison with the control birds and the other treatments, broilers given AFB1-contaminated diets had a higher relative weight of abdominal fat ($p < 0.05$). The feeding of AFB1 resulted in a substantial rise in AST and ALT activity ($p < 0.05$). MT, TB, and SP powders significantly decreased blood AST and ALT activity in broilers ($p < 0.05$). The AFB1 and MT groups had the lowest skin thickness ($p < 0.05$) twenty-four hours after injection. The phytohemagglutinin injection results showed that the TB and SP were more efficient than the other additives in removing toxins from the feed sources ($p < 0.05$). The antibody titer against sheep red blood cells (SRBCs) was lower in the AFB1 group compared to the control group at 28 days of age ($p < 0.05$). When comparing AFB1-fed chicks to the control treatment, there was a significant ($p < 0.05$) concentration of cecal *Coliform* bacteria. When MT, TB, and SP powders were added to AFB1-contaminated diet, cecal *Coliforms* were decreased ($p < 0.05$). When fed AFB1-contaminated diets, it can be concluded that MT, TB, and SP are suitable for supporting growth performance, immunological function, and the serum biochemical parameters of broiler chickens.

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1. Introduction

Agriculture represents a vital part of the global economy, with animal production adding considerably to gross domestic product. The contamination of agricultural products with mycotoxins is one of the issues encountered in this sector [1]. Depending on harvesting, storage, and procedures, contamination might occur both before and after harvest. When circumstances are appropriate, the fungus of the genus Aspergillus (A), primarily *Aspergillus flavus* and *Aspergillus parasiticus*, generate aflatoxins (AFs). Broilers are the most susceptible species to AFs [2]. Poor feed conversion ratio (FCR) and growth in broilers, increased mortality rate, anorexia, and movement difficulties are some of the effects, in addition to concern for public health due to the risk of AF residue in broiler meat [3]. For the detoxification of mycotoxin-contaminated feedstuffs, a range of technologies have been explored, including thermal inactivation, physical separation, microbial degradation, irradiation, and other treatments [4]. The use of adsorbent materials in the diet, which can be organic (microbial) or inorganic (primarily clay minerals), is a method for the mycotoxin detoxification. Biological products to reduce mycotoxin availability have received much attention; the most frequent technique for preventing and treating mycotoxicosis in birds is the use mycotoxin adsorbents [5]. Medicinal herbs have recently received a lot of interest as feed additives for reducing the negative effects of mycotoxins.

The medicinal plant milk thistle (MT) (*Silybum marianum*) is commonly used to treat liver disorders. Different flavonolignans, such as silybin, are found in MT extract. The majority of the active compounds in MT are found in seed, which contains about 70–80% silymarin. The incorporation of MT in broiler chickens' diets has led to improved growth performance and health status [6–13]. Silybin shows significant biological activities, including in birds affected by AFB1 [14]. Although there are several commercial adsorbents available in the form of feed additives to mitigate these naturally occurring toxins, there are few data on the prevalence of AFs in commercial broiler diets and on birds' performance.

Toxofix Arka (Noavaran Arka Tejarat Kabudan. Co., Urmia, Iran) is a novel multicomponent toxin-binder with a strong affinity for polar and nonpolar toxins, such as AF, *Fusarium*, and *Zearalenone*. It has uses three key effective strategies of adsorption, biotransformation and bioprotection to remove the vast variety of mycotoxins. *Spirulina platensis* (SP) is important due to it medicinal properties, and its high nutritional value has initiated new perspectives of research on different feed additives to the diets of animals. These algae may operate as immune stimulants or performance enhancers, hence increasing the feed quality for specific animal species. Aćimović [15] and Tufarelli et al. [16] discovered that nutritional supplementation with algae improved the hepatic and intestinal antioxidant capacity of old laying hens and helped to detoxify aflatoxin-contaminated diets [17]. It was reported by Suwarno et al. [18] that carotenoid compounds including beta-carotene and beta-cryptoxanthin significantly reduced aflatoxin production by *Aspergillus flavus* in an in vitro study. Another in vitro study found that commercially obtained carotenoid inhibited aflatoxin biosynthesis by >70% for 38 *Aspergillus* genotypes isolated from maize. Algae, and in particular *Spirulina platensis*, naturally contain high amounts of carotenoids.

Because of the importance of AF detoxification in poultry diets, the current study investigated growth performance, carcass characteristics, antibody titers, serum blood indices, and cecal microbial population in broiler chickens fed with an aflatoxin-contaminated diet.

2. Materials and Methods

2.1. Birds, Diets and Experimental Design

In a completely randomized design, 300 one-day-old male Ross 308 broiler chickens (initial BW of 43 $\pm$ 1 g) were assigned to five dietary treatments. We included six replicates of ten chickens for each treatment type. Dietary treatments included: (T1) a control diet

(without any feed additive or AFB1); (T2) control diet + 0.6 mg AFB1/kg; (T3) T2 + 10 g/kg MT; (T4) T2 + 1 g/kg TB; and (T5) T2 + 10 g/kg SP. The corn and soybean meal used for formulating the experimental diets were analyzed for DM and CP by near-infrared spectroscopy (NIRS) at the Athar Daneh Azerbijan Laboratory. Diets were formulated (Table 1) based on Ross recommendations of starter (1–10 d), grower (11–24 d), and finisher (25–42 d) periods.

Table 1. Ingredients and chemical composition of the basal diets.

Ingredients, %	Starter (1–10 d)	Grower (11–24 d)	Finisher (25–42 d)
Maize	49.03	59.6	65.99
Soybean meal, 44% CP	26.86	16.05	10.12
Corn gluten meal, 60% CP	10.00	11.48	11.50
Wheat	5.58	5.00	5.00
Soybean oil	3.50	3.34	3.09
Dicalcium phosphate	1.95	1.80	1.83
Limestone	1.45	1.23	1.00
DL–methionine	0.52	0.58	0.57
Vitamin–mineral premix *	0.50	0.50	0.50
Sodium chloride	0.36	0.36	0.36
Lysine–HCL	0.25	0.06	0.04
Calculated chemical analysis			
ME, kcal/kg	3000	3100	3200
CP, %	23.00	20.00	18.00
Calcium, %	1.00	0.90	0.89
Available Phosphorus, %	0.48	0.45	0.43
Chloride, %	0.22	0.22	0.22
Sodium, %	0.17	0.18	0.18
Methionine, %	0.58	0.53	0.46
Lysine, %	1.42	1.17	1.07
Methionine + Cysteine, %	1.09	0.81	0.78

* Supplied per kilogram of diet: vitamin A—12,500 IU; vitamin E—10 mg; vitamin D—2200 IU; niacin—35.0 mg; d-pantothenic acid—12 mg; riboflavin—3.63 mg; pyridoxine—3.5 mg; thiamine—2.4 mg; folic acid—1.4 mg; biotin—0.15 mg; vitamin B—0.03 mg; Mn—60 mg; Zn—40 mg; Fe—1280 mg; Cu—8 mg; I—0.3 mg; Se—0.2 mg.

Throughout trial, all birds had free access to feed and water. During the study, mash for the diet was provided. Birds were maintained with a heater following the temperature program based on instructions for Ross 308 broilers [19]. Air humidity was kept at 60–70% in the 1st week of age and at 50–60% in the 2nd–6th weeks of age by spraying water on the floor. Throughout the experiment, 23L:1D lighting software was used. Toxofix Arka was used as commercial TB (Noavaran Arka Tejarat Kabudan Company, Science and Technology Park, Urmia, Iran) containing high-quality bentonite as a mineral component and activated charcoal with a high-binding capacity for aflatoxin and other toxins. These had a suitable combination of biological components (specific mycotoxin-decomposing bacteria such as Lactobacillus sp., Bacillus sp., and bifidobacteria). Moreover, they included a scientifically selected blend of plant extracts (*Allium sativum* and *Curcuma longa*) and antioxidants (quercetin and punicalagin) to support the liver and immune system. The chemical composition of MT seed is reported in Table 2.

The marine algae used in this study was *Spirulina platensis* (SP) as pure SP powder (~100%; Nour Darou Company, Gonbad Kavous, Golestan, Iran). The chemical composition of the SP powder is presented in Table 3.

Table 2. Nutrient and bioactive content of milk thistle seeds.

Dry matter (%)	94.14
Crude protein (%)	16.13
Crude fat (%)	15.56
Crude fiber (%)	11.90
Ash (%)	15.34
Calcium (%)	0.72
Phosphorus (%)	0.39
Gross energy (kcal/kg)	5012
Beta-carotene (mg/kg)	8.93
Silychristin (mg/kg)	2851
Silybin B (mg/kg)	8864
Silymarin (mg/kg)	100

All the values were analyzed at the Viromed Central Analytical Laboratory, Pardis Technology Park, Tehran, Iran.

Table 3. Nutrient content of *Spirulina platensis*.

Chemical Composition (% on DM Basis)	
Moisture	7.2
Crude protein	57.6
Crude fat	5.3
Crude fiber	3.8
Ash	13.8
Mineral concentrations (%)	
Calcium	2.20
Phosphorus	0.40
Magnesium	0.85
Iron	1.23
Manganese	0.80
Zinc	1.87
Vitamins and carotenoids (mg/g)	
Total carotenoids	6.8
Chlorophyll	7.4
Chlorophyll b	4.2
Vitamin A	1.34
Vitamin E	1.05
Ascorbic acid	8.24

Data obtained from the manufacturer (The Nour Darou Company, Gonbad Kavous, Golestan, Iran).

2.2. Aflatoxin-B1 Production

The AFB1 was created by the fermentation of rice grains from an *A. parasiticus* PTCC-5286 culture, and its AFB1 concentration was evaluated. About 30 kg rice (mesh size 2.00 mm) was placed in 100 L containers; then, we added 10 L distilled water to each and autoclaved the mixture [20]. The media in the container was inoculated with 500 mL A. flavus (1×10^8 spores/mL) and incubated at 28 °C for 7 days. The AFB1 concentration in the moldy rice was measured as 10 mg/kg and modulated to 0.6 mg AFB1/kg in the feed.

2.3. Birds' Performance, Serum Biochemistry, and Organ Weight

Cumulative body weight gain (BWG) and feed intake (FI) were recorded at the end of 42 d of age to calculate the feed conversion ratio (FCR). Three broilers from each replicate were randomly selected and slaughtered at the end of the experiment (42 days). The weights of the carcass, liver, abdominal fat, spleen, bursa of Fabricius, and thymus of the broiler chickens were measured, and their relative weights were calculated. Three broilers with similar BW to the mean BW of each replication were chosen at the end of the experiment, and blood samples were taken from the wing vein. Then, serum total protein, glucose, total cholesterol, triglyceride, alanine aminotransferase (ALT), aspartate amino-

transferase (AST), uric acid, and lactate dehydrogenase (LDH) enzymes were assessed with a spectrophotometer using commercial kits (Alcyon 300, USA, and Pars Azmon Kits, Iran).

2.4. Humoral Immune Response

Three broilers from each experimental unit were chosen at random and assigned a color at the age of 35 days. Before injection, the thickness of the third digit on the right foot was measured, and 0.1 mL of phytohemagglutinin solution was injected subcutaneously. The thickness of the injection site was evaluated 24 and 48 h after injection, and the difference was used as the measure of the proliferation of immune cells (Barati et al., 2018). At 21 and 28 days of age, 5 mL of 5% SRBC solution was injected into pectoral muscle to assess humoral immunity. After that, 3 mL of blood from the same birds was taken 7 days after the first injection of SRBC solution (28 and 35 days). The serum samples were used to determine the overall response titer (SRBC) through the microtiter hemagglutination technique and the IgG titer. The antibody sensitive to mercaptoethanol (IgM) was obtained after measuring the quantity of antibodies resistant to mercaptoethanol (IgG) and subtracting IgG from SRBC [21].

2.5. Cecal Microbial Population

Three chickens from each pen that had not been injected with SRBC were slaughtered by neck cut on day 42 for cecal content sampling. The gut was then removed and 1 g of cecal content was taken and transferred into a sterile container using sterile forceps. The samples were transferred to tubes containing phosphate buffer (PBS) with a pH of 7.2 and thoroughly mixed. The *E. coli* were grown on eosin methylene blue agar, salmonella was grown on salmonella–shigella agar (SS) and *Coliforms* were grown on McConkey agar (Darmstadt, Germany). Colony-forming units (CFU) were defined as distinct colonies measuring at least 1 mm in diameter [22].

2.6. Statistical Analysis

A completely randomized design was used, and the general linear model (GLM) procedure was used to analyze the data by SAS Software 2004. Tukey's range test was used to separate the means when treatment means were significant at $p < 0.05$.

3. Results

The effects of the MT, TB and SP powders on the BWG, FI and FCR of broiler chickens exposed to AFB1 are shown in Table 4. Broilers fed AFB1-contaminated diets had substantially decreased BWG and FI ($p < 0.05$). Birds fed an AFB1-contminated diet exhibited poor FCR ($p < 0.05$). Furthermore, MT, TB, and SP powders reduced the deleterious effects of AFB1 on BWG, FI, and FCR in the chickens ($p < 0.05$).

Table 4. Effect of dietary treatments on the growth performance of broiler chickens at 42 days of age exposed to aflatoxin B_1.

Treatment	BWG [6]	FI [7]	FCR [8]
Control [1]	2517.9 [a]	4010.1 [a]	1.59 [b]
AFB1 [2]	2033.3 [c]	3615.7 [b]	1.77 [a]
MT [3]	2263.6 [b]	3976.2 [a]	1.75 [a]
TB [4]	2385.1 [ab]	3920.1 [a]	1.64 [ab]
SP [5]	2248.1 [b]	3723.6 [a]	1.65 [ab]
Pooled SEM	47.83	96.43	0.04
p-value	0.001	0.030	0.005

[a–c] Means with different superscripts in each column are significantly different ($p < 0.05$).[1] Control diet; [2] control diet + 0.6 mg AFB1/kg; [3] control diet +10 g/kg milk thistle; [4] control diet + 1 g/kg toxin binder; [5] control diet + 10 g/kg *Spirulina platensis*; [6] body weight gain; [7] feed intake; [8] feed conversion ratio.

Table 5 summarizes the relative weights of carcass traits. In comparison to the control birds and other treatments, broilers given an AFB1-contaminated diet had a higher relative weight of abdominal fat ($p < 0.05$).

Table 5. Effect of dietary treatments on the carcass traits of broiler chickens at 42 days of age exposed to aflatoxin B_1.

Treatments	Carcass [6]	Thigh	Breast	Liver	Abdominal Fat	Bursa of Fabricius	Thymus	Spleen
Control [1]	60.09	23.16	37.70	2.10 [b]	0.96 [b]	0.18	0.15	0.12
AFB1 [2]	57.52	24.33	39.53	2.86 [a]	1.68 [a]	0.21	0.19	0.16
MT [3]	59.23	23.90	39.84	2.08 [b]	1.18 [b]	0.17	0.17	0.13
TB [4]	59.85	23.91	38.89	2.04 [b]	0.99 [b]	0.13	0.15	0.12
SP [5]	60.22	23.73	40.29	2.23 [b]	1.05 [b]	0.16	0.16	0.12
Pooled SEM	1.40	0.95	0.91	0.18	0.13	0.02	0.02	0.01
p-value	0.650	0.931	0.332	0.001	0.003	0.153	0.611	0.285

[a,b] Means with different superscripts in each column are significantly different ($p < 0.05$). [1] Control diet; [2] control diet + 0.6 mg AFB1/kg; [3] control diet +10 g/kg milk thistle; [4] control diet + 1 g/kg toxin binder; [5] control diet +10 g/kg *Spirulina platensis*. [6] Carcass, liver, abdominal fat, bursa of Fabricius, thymus and spleen are presented as percentage of live body weight, and breast and thigh are presented as percentage of carcass weight.

Table 6 shows the blood serum biochemical characteristics of broiler chicks exposed to AFB1. Dietary supplements had no effect on blood serum glucose, total protein, triglyceride, cholesterol, or uric acid ($p > 0.05$). When compared to the control, AFB1 diets resulted in a substantial increase in AST and ALT activity ($p < 0.05$). MT, TB, and SP powders significantly lowered serum AST and ALT activity in chickens ($p < 0.05$).

Table 6. Effect of dietary treatments on the blood parameters of broiler chickens at 42 days of age exposed to aflatoxin B_1.

Treatments	Glucose (mg/dL)	Total Protein (g/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Uric Acid (mg/dL)	AST [6] (U/L)	ALT [7] (U/L)
Control [1]	158.83	4.21	108.61	147.33	6.48	276.83 [b]	5.16 [b]
AFB1 [2]	166.16	3.24	90.61	138.33	8.47	380.00 [a]	9.00 [a]
MT [3]	165.16	3.66	100.96	142.16	7.31	297.00 [b]	7.00 [ab]
TB [4]	162.66	3.82	103.70	141.66	7.08	286.00 [b]	6.66 [ab]
SP [5]	163.00	4.01	103.96	141.66	7.13	291.67 [b]	6.83 [ab]
Pooled SEM	4.05	0.32	6.20	6.36	0.54	20.08	0.80
p-value	0.744	0.301	0.342	0.902	0.155	0.008	0.042

[a,b] The means with different superscripts in each column are significantly different ($p < 0.05$). [1] Control diet; [2] control diet + 0.6 mg AFB_1/kg; [3] control diet +10 g/kg milk thistle; [4] control diet +1 g/kg toxin binder; [5] control diet + 10 g/kg *Spirulina platensis*; [6] aspartate aminotransferase; [7] alanine transaminase.

Table 7 shows the outcomes of experimental treatments in broiler chickens exposed to AFB1 in response to the phytohemagglutinin skin challenge. The AFB and MT groups had the lowest skin thickness 24 h after injection ($p < 0.05$). According to the findings of the phytohemagglutinin injection, TB and SP appear to be more effective than other additives at removing toxins from feed ($p < 0.05$). The results of the skin challenge with phytohemagglutinin 48 h after injection revealed no significant changes between treatments ($p > 0.05$).

Table 7. Effect of dietary treatments in response to skin challenge with phytohemagglutinin (mm) in broiler chickens exposed to aflatoxin B_1.

Treatments	24 h	48 h
Control [1]	1.23 [a]	0.47
AFB1 [2]	0.75 [b]	0.25
MT [3]	0.85 [b]	0.35
TB [4]	1.09 [ab]	0.40
SP [5]	0.98 [ab]	0.38
Pooled SEM	0.08	0.06
p-value	0.006	0.166

[a,b] The means with different superscripts in each column are significantly different ($p < 0.05$). [1] Control diet; [2] control diet + 0.6 mg AFB_1/kg; [3] control diet +10 g/kg milk thistle; [4] control diet + 1 g/kg toxin binder; [5] control diet +10 g/kg *Spirulina platensis*.

Table 8 shows the impact of experimental treatments on SRBC response and immunoglobulin titers in broiler chickens exposed to AFB1. At 28 and 35 days of age, there was no significant change in IgM and IgG titers across treatments ($p > 0.05$). The SRBC titer was considerably lower in the AFB group compared to the control group at 28 days of age ($p < 0.05$). The SRBC titer in treatments with different additives was intermediate between the control and AFB groups ($p < 0.05$). However, by inhibiting AFB1, the TB group showed a closer titer to the control with a higher effectiveness than the other treatments.

Table 8. Effect of dietary treatments in response to SRBC and the immunoglobulin titer in broiler chickens exposed to aflatoxin B_1.

	Anti-SRBC Titer (log_2)					
	28 Days			35 Days		
	IgM	IgG	IgT SRBC	IgM	IgG	IgT SRBC
Treatments						
Control [1]	1.32	4.26	6.29 [a]	1.63	3.33	4.99
AFB1 [2]	0.98	3.49	4.33 [b]	1.04	2.56	3.83
MT [3]	1.09	3.78	4.88 [ab]	1.12	2.84	4.09
TB [4]	1.20	4.05	5.26 [ab]	1.27	3.05	4.30
SP [5]	1.11	3.88	5.11 [ab]	1.18	3.02	4.23
Pooled SEM	0.10	0.18	0.38	0.14	0.28	0.38
p-value	0.222	0.073	0.021	0.071	0.445	0.303

[a,b] The means with different superscripts in each column are significantly different ($p < 0.05$). [1] Control diet; [2] control diet + 0.6 mg AFB_1/kg; [3] control diet +10 bg/kg milk thistle; [4] control diet +1 g/kg toxin binder; [5] control diet +10 g/kg *Spirulina platensis*. Note: SRBCs—sheep red blood cells; IgM—immunoglobulin M; IgG—immunoglobulin G.

Table 9 shows the results of the cecal microbial population of broiler chickens exposed to AFB1. When compared to the control, there was an increase ($p < 0.05$) in cecal *Coliform* population in chicks given an AFB1-contominated diet; moreover, adding MT, TB, and SP powders to the AFB1-contaminated diet significantly reduced cecal *Coliforms* ($p < 0.05$).

Table 9. Effect of dietary treatment on cecal microbial population of broiler chickens exposed to aflatoxin B_1 (Log10 CFU/g).

Treatment	*E. coli*	*Salmonella*	*Coliforms*
Control [1]	1.97	1.00	1.65 [b]
AFB1 [2]	2.67	1.57	2.91 [a]
MT [3]	2.35	1.38	2.13 [b]
TB [4]	2.00	1.18	1.91 [b]
SP [5]	2.10	1.21	2.02 [b]
Pooled SEM	0.27	0.27	0.23
p-value	0.375	0.654	0.011

[a,b] The means with different superscripts in each column are significantly different ($p < 0.05$). [1] Control diet; [2] control diet + 0.6 mg AFB_1/kg; [3] control diet +10 g/kg milk thistle; [4] control diet + 1 g/kg toxin binder; [5] control diet +10 g/kg *Spirulina platensis*.

4. Discussion

The AFs, as the most common mycotoxins, represent a great problem for poultry producers. Mycotoxins have been found to infect over 25% of global crops [23], and it is expected that the AFB1 contamination rate of feed components in many countries will reach 60% in the coming years, posing major public health concerns. The concentration of AFB1 in meals was established at 0.6 mg/kg in this study, which resulted in lower BWG and FI, as well as poor FCR in broiler chickens at 42 days of age, indicating that the birds exhibited subclinical aflatoxicosis. These findings are consistent with those of Barati et al. [21] and Nazarizadeh and Pourreza [5], who found that broilers fed an AFB1-contominated diet had lower feed consumption and reduced FCR. On the other hand, Siloto et al. [24] found no variations in FI in birds fed an AFB1-contaminated diet (1 mg/kg feed). Broilers fed diets including MT, TB, and SP powders may have had fewer negative effects on their overall performance. Silymarin has been shown to improve FI and feed efficiency in broilers when used with MT [14]. In broilers, Fani Makki et al. [8] found that adding silymarin alone enhanced FI and BWG compared to a group fed a diet contaminated with AFB1, but there was no effect on FCR. Supplementing broilers fed an AFB1-contaminated diet with MT enhanced FI and raised BWG. This was in agreement with Chand et al. [6]. By lowering free radicals and enhancing antioxidant enzymes, MT may be able to encourage detoxification. It also binds toxins and restricts their absorption into the hepatocyte by occupying binding sites [14].

The inclusion of TB in AFB1 diets enhanced growth performance and reduced undesirable effects in broilers. The addition of TB (1.0 g/kg of feed) to an AFB1-contaminated diet (0.3 g/kg of feed) decreased the growth inhibitory effects on broiler chicks fed contaminated diets for 21 days, according to a previous study [5]. Cation exchange capabilities, allowing TB to trap molecules inside their pores, were one of the most critical characteristics of the TB employed in this study. The porous nature of TB allows it to attract AFB1 in the gastrointestinal tract, and thus reduces its deleterious effect on broiler chicken performance [20]. Another property of TB is that it contains phenolic compounds from plant extracts which have high antioxidative capabilities. Some botanical extracts have been reported to have antifungal effects [25]. TB, on the other hand, is high in probiotics and some yeasts, such as *Saccharomyces cerevisiae*, which have the ability to reduce the amount of AFB1 in the environment through a mechanism involving the thick peptidoglycan layer of the Gram-positive bacterial cell wall, which interacts with AFB1 and increase its excretion [26]. In the current study, the content of SP was shown to be able to compensate the negative effects of AFB1 in the broilers. Previous research has revealed that the marine alga has a wide range of therapeutic qualities and biological activities, including immunomodulatory, antioxidant, and anti-inflammatory capabilities. The findings of this study correspond with those of Subhani et al. [17], who found that algae might reduce the detrimental effects of AFB1 and have a positive influence on broiler chicken health. Algae's excellent antioxidant properties are one of its most important characteristics. Broilers fed a diet supplemented

with SP had reduced oxidative stress levels, leading to better antioxidant capacity [16]. Data obtained from in vitro and in vivo studies suggest that the vitamins in algae are very effective in preventing mycotoxin-induced damages in cells. For example, different forms of vitamin A inhibited AFB1–DNA adduct [8-hydroxydeoxy-guanosine (8-OHdG)] formation by regulating the metabolism of AFB1 conducted by the cytochrome P450 (CYP450) enzyme system [27]. A previous study found that chicks fed AFB1 at 80 g AFB1/kg feed had considerably greater liver weight than those fed a diet without adsorbents [28]. In the present study, the greater liver weight of birds fed AFB1 was related to increased fat retention, validating the findings of Siloto et al. [24]; moreover, adding MT, TB, and SP to the diet was effective in binding AFB1. When hepatocytes are damaged, transaminases are leaked into the blood, raising serum transaminases activity. Elevated blood AST and ALP concentrations suggest cellular (hepatocyte) damage, such as necrosis or changes in cell membrane permeability, as well as muscle damage caused by AFB1-induced lipid peroxidation [20]. AFB1-contaminated diets increased serum hepatic enzymes including ALT and AST, which were reduced when MT, TB, and SP were supplied. Abou-Shehema et al. [29] reported similar findings, claiming that MT containing silymarin reduced the cytochrome P450 system, limiting AFB1 activation. The TB was composed of complex indigestible carbohydrates (glucomannans and peptidoglycans), phenolic compounds, probiotics, and aluminosilicates such as bentonite, which inhibited mycotoxins from causing damage to the digestive tract. These findings are consistent with those of Subhani et al. [17], who found that marine algae exhibited hepatoprotective properties and lowered liver enzymes, hence reducing the harmful effects of AFB1.

It has also been suggested that ascorbic acid, which is high in SP, protects animals from the acute toxicity of AFB1 by activating AFB1–epoxide hydroxylase, aldehyde reductase, and CYP3A enzymes located in the enterocytes [27]. Additionally, Simonich et al. [30] reported that chlorophyll and chlorophyll b were potent protective agents against AFB1 carcinogenesis in rat liver and colon, providing supporting evidence that both agents offer protection by inhibiting carcinogen uptake from the gut, thus reducing the availability of AFB1 to the target organ. Increased liver weight is a sign of aflatoxicosis since the main effects of AFs are related to liver damage. Broilers need a strong immune system, especially as birds are frequently exposed to many infections. The immune system is known to be extremely susceptible to AFB1 [5]. Adding AFB1 to broiler diets has been shown to reduce skin reactions to phytohemagglutinin. According to Alhidary et al. [14], silymarin boosted the immune system by quenching free radicals. It has the capacity to protect glutathione supply and to reduce oxidation, as well as having a direct influence on immune cells. Using probiotics, phenolic compounds and yeast may boost immunoglobulin levels and boost the immune system, according to the findings of the TB group. According to a previous study, the beneficial effects of probiotics include lowering gut pH, which prevents bacteria from colonizing through competitive exclusion, the production of organic acids, the production of antibacterial mucin and enzymes, and competition for nutrients in the gut [21].

The SP is a powerful antioxidant that helps to prevent lipid peroxidation. Multiple biological effects of SP polysaccharides have been demonstrated, including anti-inflammatory, antibacterial, antiviral, immunomodulatory, and free radical scavenging. It has the capacity to improve the immunological condition of birds when their immune systems have been compromised by mycotoxin exposure [31]. We detected a considerable increase in *Coliform* populations with the AFB1-contaminated diets, which was reversed by adding MT, TB, and SP into the diets. In chickens, exposure to AFB1 has been demonstrated to impair resistance to many bacterial, viral, and protozoan diseases. The presence of phenolic compounds and silymarin in MT, which have antibacterial and antifungal properties, regulate growth and metabolic factors and lower the amount of harmful bacteria in the gut, have been shown to have a key role in minimizing the negative effects of AFB1 [32].

The use of TB in chicken diets has been pioneered due to its positive effects. The most essential aspect in lowering intestinal bacteria is the inclusion of probiotics, plant chemicals, and mannano-oligosaccharides (MOS) in the composition of TB. The MOS also improves

villus height and the amount of anaerobic and cellulytic bacteria in the gut, which improves lactate consumption and the pH of the stomach. Hydrogen bonds and van der Waals forces bind mycotoxin to yeast wall glucomannan, and this binding is persistent throughout the gut [33]. According to previous studies, probiotics exhibit antibacterial action against *E. coli* and the capacity to adsorb AFB1 [34,35]. It is well known that including algae in the diet can help to reduce the negative effects of mycotoxins. Furthermore, according to some experts, algae play an important function in animal performance and health by regulating the gut's ecological balance [17,23].

5. Conclusions

Based on the obtained data, it was assessed that feeding AFB1-contaminated diets (0.6 mg AFB1/kg) to broilers from 1 to 42 days of age had negative impacts on growth and immunity. Supplementing MT, TB, and SP effectively mitigated the negative effects of AFB1 on the broiler chickens' performance and their blood biochemical characteristics. As a result, using a TB to regulate AFB1 effects on chicks and boost immune function is recommended.

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