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Hydroalcoholic Extract of Saffron Petals, Yeast Cell Wall and Bentonite Reduce Contamination Effects With *Aflatoxin B_1* and *Ochratoxin A* in Exposed Broilers

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Keywords: *aflatoxin B_1* | *ochratoxin A* | saffron petal extract | toxin binder | yeast cell wall

ABSTRACT

Background: Contamination is not surprising in light of the ubiquitous nature of the fungi that produce aflatoxin and ochratoxin A. The presence of these toxins in the broiler diet leads to increased losses, an increased feed conversion ratio, and decreased productivity.

Objectives: This study aimed to investigate the effects of the use of hydroalcoholic extracts of saffron petals, yeast cell walls and bentonite in the diets of broiler chickens contaminated with *aflatoxin B_1* and *ochratoxin A*.

Methods: In a completely randomised design, 350 one-day-old Ross 308 broilers were allocated into seven treatment groups and five replications: a negative control diet (without toxins or additives), a positive control diet (2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A (mg/kg) and diets containing toxins with a commercial toxin binder or yeast cell wall, processed bentonite or saffron petal extract alone or together.

Results: Compared with those in the positive control treatment, the relative weights of the carcasses, breasts and drumsticks improved with the addition of toxic adsorbent compounds ($p < 0.05$). Compared with the control treatment, the inclusion of a toxin binder had a significant effect on the concentration of glucose ($p < 0.05$). The concentrations of alanine aminotransferase and gamma-glutamyltransferase enzymes in the yeast cell wall + processed bentonite + saffron petal extract treatment were lower than those in the other treatments ($p < 0.05$). Toxin adsorbent compounds significantly improved the morphology of the small intestine in chickens fed contaminated diets ($p < 0.05$).

Conclusion: The inclusion of toxic adsorbent compounds can reduce the negative effects caused by the presence of *Aflatoxin B_1* and *Ocratoxin A*. Saffron petal extract can potentially be used to modulate diets contaminated with *Aflatoxin B_1* and *Ocratoxin A*, which is best achieved with 750 mg/kg saffron petal extract along with 0.1% yeast cell wall extract and 1% processed bentonite.

1 | Introduction

Globally, food and feed are severely contaminated with mycotoxins. Among the hundreds of known fungal toxins, aflatoxins, ochratoxins, trichothecenes, zearalenone and citrinins are known. Aflatoxins are among the most dangerous mycotoxins and are responsible for causing 4.6%–28.2% of all liver cancers (Mesgar et al. 2022). The main producers of aflatoxin

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are *Aspergillus* species, especially *Aspergillus flavus*, *Aspergillus parasitics*, *Aspergillus prize* and so forth (Mesgar et al. 2022). Ochratoxin is a mycotoxin produced by fungi of the genera *Aspergillus* and *Penicillium* and is currently present in many agricultural products worldwide. Ochratoxins are more toxic than aflatoxins are (Bryła et al. 2023). Ochratoxin has less intestinal absorption ability than aflatoxin does, and the intestine is more damaged than other organs because it is more exposed to this toxin (Grenier and Todd 2013). The consumption of feeds contaminated with mycotoxins leads to reduced absorption of nutrients (Qu et al. 2017), poor performance, suppression of the immune system, residues in animal products, increased susceptibility to infectious and parasitic diseases, and, as a result, hurts human health. Contamination of feed with these toxins causes great economic losses to the industry by reducing poultry performance (Wade, Sapcota, and Verma 2017). Many attempts have been made to prevent the absorption of aflatoxins in the gastrointestinal tract by using sorbents or compounds that change or detoxify these mycotoxins and their metabolites, which include the use of sorbents, minerals, organic adsorbents, microorganisms, microbial metabolites and plant compounds or generally a combination of them to inhibit these toxins and improve liver and immune system function (Ma et al. 2015). Some of minerals as zinc have advantage of boosting the antioxidant system and improving the functioning of the immune system (Niknia et al. 2022).One of the best adsorbents for aflatoxin degradation is chemically neutral bentonite clays. These materials have outstanding physical and chemical properties, including surface area, enlargement, absorption, cation exchange, low cost, high safety and rheological and colloidal properties, and are often used in the food industry (Assaf et al. 2019). Bentonites bind only to aflatoxins and leave other toxins unchanged in the digestive tract (Assaf et al. 2019). The yeast cell wall is also an aflatoxin adsorber with peptidoglycans and polysaccharides in the cell wall and has a high ability to adsorb fungal toxins. It has been suggested that these oligosaccharides, which are isolated from the cell wall of Saccharomyces yeast and block the binding sites of pathogenic bacteria in the small intestine mucosa, reduce damage to the intestinal wall and thus reduce the rate of replacement of intestinal cells and improve the ability to use nutrients. The oligosaccharides in the cell wall of yeast can stimulate appetite and, as a result, increase feed consumption in broilers (Azizpour and Moghadam 2015).

Saffron has been traditionally used as an herbal medicine. More than 150 different compounds, including carbohydrates, polypeptides, lipids, minerals and vitamins, are found in saffron (Khorasany and Hosseinzadeh 2016). Saffron contains picrocrocin, safranal, crostin, alpha-crocin, lycopene, zeaxanthin and alpha and beta-carotene. Elements such as zinc, iron, copper, selenium, magnesium, phosphorus, calcium and manganese and several vitamins, such as vitamins A and C, folic acid, riboflavin and niacin, are rich (Qadir, Bashirand, and John 2020). Compared with the control diet, the aflatoxin B_1 diet caused lower feed consumption and body weight gain (BWG), and the inclusion of 350 and 700 mg/kg saffron extract in the quail diet partially compensated for the loss of body weight and feed consumption caused by aflatoxin. The feed conversion ratio (FCR) also increased significantly in the groups contaminated with aflatoxin. The inclusion of 350 mg/kg saffron hydroalcoholic extract in the quail diet is recommended (Hosseini Vashan and Pirai 2018). On the basis of these findings, saffron petal extract can be used to reduce the harmful effects of aflatoxins.

In previous experiments, animal responses were evaluated according to the experimental doses used. On the basis of recent mycotoxin surveys (Liu, Wang, Jia et al. 2018; Liu, Wang, Liu, et al. 2018; Jahanian et al. 2019), these doses were placed in three different categories: realistic (< 0.3, < 0.3), occasional (> 0.3, > 0.3) and unrealistic (> 2, > 2) doses. The EU limits aflatoxin and ochratoxin A in finished feed (0.02 and 0.1 for poultry) according to the European Commission Recommendation 2006/576/EC and the European Commission Directive 2003/100/EC; three USA limits in finished feed (0.1 and no advisory or guidance levels established for poultry) according to the Food and Drug Administration Regulatory Guidance for Toxins and Contaminants (Grenier and Todd 2013(.

These two mycotoxins can be found as simultaneous contaminants of feed ingredients and finished feed. The contamination is not surprising in light of the ubiquitous nature of the fungi that produce aflatoxin and ochratoxin A. This association and the extreme toxicity elicited by both mycotoxins prompted an investigation to determine the synergistic toxicity and describe the major effects of these mycotoxins when they are administered simultaneously (Huff and Doerr 1981).

Therefore, the aim of this study was to explore the detrimental effects or consequences of aflatoxin B_1 and ocratoxin A contamination at 2.5 and 2 mg/kg (0.25% and 0.2%, respectively) in the broiler diet via hydroalcoholic extracts of saffron petals, yeast cell walls and bentonite.

2 | Materials and Methods

2.1 | Preparation of Aflatoxin B_1 and Ochratoxin A

To implement this project, first, in the laboratory, *A. flavus* was purchased from the Mushroom and Bacteria Collection Center, Iran Scientific and Industrial Research Organization and potato dextrose agar culture medium from the method of (Shotwell et al. 1966) was propagated; then, by fementation on rice, aflatoxin B_1 was produced (Al Anas et al. 2022). Standard *Aspergillus* extracellular vials cultured on wheat were used to produce ochratoxin (Trenk, Butz, and Chu 1971). By using fungal isolates of aflatoxin and ochratoxin in the laboratory environment, the reproduction of the fungus and the production of toxins of these two fungi were carried out, and the fungi were then placed on corn under storage conditions for 7–10 days at a temperature of 28–30°C. After drying and grinding, the two poisons were separated in the laboratory, and aflatoxin was measured via thin layer chromatography (AOAC 2000). The toxin concentrations of ochratoxin A were measured via high-performance liquid chromatography at the Mabna Veterinary Laboratory (Karaj, Iran).

Notably, owing to the pollution and danger of waste containing mycotoxins and the possibility of the spread of contamination related to this type of fungal toxin in the surrounding

COMPOSITION	PRIMARY FERMENTATION
Proteins	max. 25 %
Mannans	26 % [+/- 2 %]
ß-Glucans	27 % [+/- 2 %]
Ash	max. 6 %
Fibers	max. 3 %
Solubility	max. 8 %

FIGURE 1 | Composition of the yeast cell wall.

environment, the laboratory wastes resulting from working with mycotoxins and fungal toxins are first disinfected with distilled water and finally set on fire in areas far from human and animal habitats.

2.2 | Yeast Cell Wall Preparation

The yeast cell wall was purchased from the Research and Development Department of Razavi Yeast Company, which is located in Fariman City, Iran. This product is made from the autolysis of special strains of saccharose yeast that produce maximum amounts of mannan and beta-glucan. The yeast culture environment, especially the temperature, was monitored closely and in a timely manner. The number of particles per milligram was measured via a hemocytometer (Hamza et al. 2019). The composition of the yeast cell wall was as follows (Figure 1).

2.3 | Saffron Petal Extract Preparation

To prepare saffron petal extract, the soaking method was used. Saffron petals were gathered from the Torbat-Heydarieh District in the Khorasan Razavi Province, which is located in northeastern Iran. Postharvest, the petals were shade-dried and subsequently pulverised. The method employed for the extraction of saffron petals was elucidated in detail in our previous publication (Vakili, Toroghian, and Torshizi 2022). In brief, the pulverised petals were subjected to shaking with 50% aqueous ethanol at a 1:10 ratio for 2 h. This mixture was then filtered and concentrated via a rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany). This was followed by spray-drying, sieving and packaging processes. The yield of the ethanolic saffron petal extract was 42%. The extract, in its powdered form, was stored in dark containers at 4°C until further use. The resulting powder was subsequently combined with equal proportions of calcium carbonate powder via a rapid mill composed of a ceramic jar with a lid and round ceramic balls at 350 rpm. Once the supplement was prepared, it was first mixed with a 10 kg diet in a small mixer before being remixed horizontally. The prepared extract was stored in a refrigerator for various investigations in the next steps (Ashrafi Yourghanloo and Gheibi 2019).

Major bioactive constituents (secondary metabolites) of Iranian saffron petal extract.

Constituent	Content
Total phenolic compounds (mg)	3.42 ± 0.11
Total flavonoids (mg/g)	2.75 ± 0.07
Kaempferol (% w/w)	12.6 ± 0.12
Crocin (% w/w)	0.6 ± 0.03
Anthocyanin (mg/l extract)	1712 ± 0.24

Source Vakili, Toroghian, and Torshizi (2022).

2.4 | Experimental Design and Experimental Diets

This experiment was carried out after approval by the Animal Ethics Committee of the Agricultural Research Institute of Iran. All procedures involving animals were approved by the Saffron Institute, University of Torbat Heydarieh, Iran (code: 169869; date of approval: 16 October 2023). This research was financially supported by the Saffron Institute, University of Torbat Heydarieh. The grant number was P/169869. A total of 350 one-day-old male broiler chickens (Ross 308) were obtained from Fariman Broiler Breeder Company (Mashhad, Iran). This experiment was carried out in the poultry breeding research hall of the Research and Education Center for Agriculture and Natural Resources in Razavi Khorasan, located in southern Mashhad. This hall is equipped with environmental control systems. Using 350 one-day-old Ross 308 broilers in 7 treatments, 5 replicates and 10 broiler chickens per replicated pen were used in a completely randomised design. The chickens were reared in the pen system under hygienic conditions in a ventilated and temperature-controlled area. Chickens were reared in experimental pens with dimensions of 1 × 1 m with free access to water and feed, and the management factors of the breeding strain guide management (Ross 2022) were applied from 0 to 42 days to provide light (23 h of light and 1 h of darkness). In accordance with the veterinary instructions, the vaccination program for bronchitis, Newcastle, influenza and Gamboro diseases was carried out at 4, 14 and 21 days. Furthermore, they were randomly allocated into seven treatments with five replicates (10 chickens in each replicate). The experimental rations were set in three ration phases, 0–10, 11–24 and 25–42 days, on the basis of the recommended requirements of the Ross strain and UFFDA software, and all the requirements were calculated according to the Ross recommendation-2022- and then prepared in the form of mash and consumed by chickens. The commercial toxin binder used was purchased from the biomin company. The experimental treatments included the following: Treatment 1, no toxin or additive; Treatment 2, a diet containing 2.5 mg/kg aflatoxin + 2.5 mg/kg ochratoxin A; Treatment 3, a diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + commercial toxin binder; Treatment 4, a diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + 0.1% yeast cell wall; Treatment 5, a diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + 1% processed bentonite; Treatment 6, a diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + 750 mg/kg saffron petal extract and Treatment 7, a diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + 0.1% yeast cell wall + 1% processed bentonite + 750 mg/kg saffron petal extract. The compositions of the initial, growth and final experimental diets are reported in Table 1. The analyzed mycotoxin levels in the diets

TABLE 1 | Composition of the diets and calculated nutrients in different periods of the experiment (%).

	Starter, 1–10 days							Grower, 11–24 days							Finisher, 25–42 days						
Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Corn	54.43	54.43	54.43	54.43	54.43	54.43	54.43	42.5	42.5	42.5	42.5	42.5	42.5	42.5	45.99	45.99	45.99	45.99	45.99	45.99	45.99
Soybean meal (44%)	35	35	35	35	35	35	35	30.29	30.29	30.29	30.29	30.29	30.29	30.29	25.56	25.56	25.56	25.56	25.56	25.56	25.56
Fish meal	3.07	3.07	3.07	3.07	3.07	3.07	3.07	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oil	3.29	3.29	3.29	3.29	3.29	3.29	3.29	3.57	3.57	3.57	3.57	3.57	3.57	3.57	3.76	3.76	3.76	3.76	3.76	3.76	3.76
Dicalcium phosphate	1.73	1.73	1.73	1.73	1.73	1.73	1.73	1.47	1.47	1.47	1.47	1.47	1.47	1.47	1.49	1.49	1.49	1.49	1.49	1.49	1.49
Oyster shell	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Vitamin supplement[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral supplement	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.24	0.24	0.24	0.24	0.24	0.24	0.24
L-Lysine	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Commercial toxin binder	—	—	0.1	—	—	—	—	—	—	0.1	—	—	—	—	—	—	0.1	—	—	—	—
Bentonite	—	—	—	—	1	—	1	—	—	—	—	1	—	1	—	—	—	—	1	—	1
Saffron petal extract (mg)	—	—	—	—	—	750	750	—	—	—	—	—	750	750	—	—	—	—	—	750	750
Yeast cell wall	—	—	—	0.1	—	—	0.1	—	—	—	0.1	—	—	0.1	—	—	—	0.1	—	—	0.1
Aflatoxin B_1	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Ochratoxin	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Nutrients composition																					
Energy (kcal/kg)	2980	2980	2980	2980	2980	2980	2980	3050	3050	3050	3050	3050	3050	3050	3100	3100	3100	3100	3100	3100	3100
CP (%)	22	22	22	22	22	22	22	20	20	20	20	20	20	20	18	18	18	18	18	18	18
Lysine (%)	1.43	1.43	1.43	1.43	1.43	1.43	1.43	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Methionine + cysteine (%)	1.07	1.07	1.07	1.07	1.07	1.07	1.07	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Tryptophan (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.26	0.26
Ca (%)	1.05	1.05	1.05	1.05	1.05	1.05	1.05	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.85	0.85	0.85	0.85	0.85	0.85	0.85
P (%)	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.42	0.42	0.42

Note: Aflatoxin B1 was detected at concentrations lower than 8 μg/kg, and ochratoxin A was detected at concentrations lower than 10 μg/kg.

[a]Mineral supplement per kilogram of diet containing 0.62 mg of manganese sulfate, 0.05 mg of iron sulfate, 0.528 mg of zinc oxide, 0.063 mg of copper sulfate, 0.063 mg of calcium iodate, 0.13 mg/choline mg 56/1 of selenium, and 0.006 mg of antioxidant. Vitamin supplement per kilogram of diet containing vitamin AIU 25/56 international units, vitamin IU3D 5/12 international units, vitamin EIU 113/0 international units, vitamin Kmg 0/0, vitamin mg1B 011/0, vitamin mg2B 041/vitamin mg12B 0001/pantothenic acid g 061/0, folic acid mg 600/niacin mg 185/pyridoxine mg 018/biotin mg 0001/0.

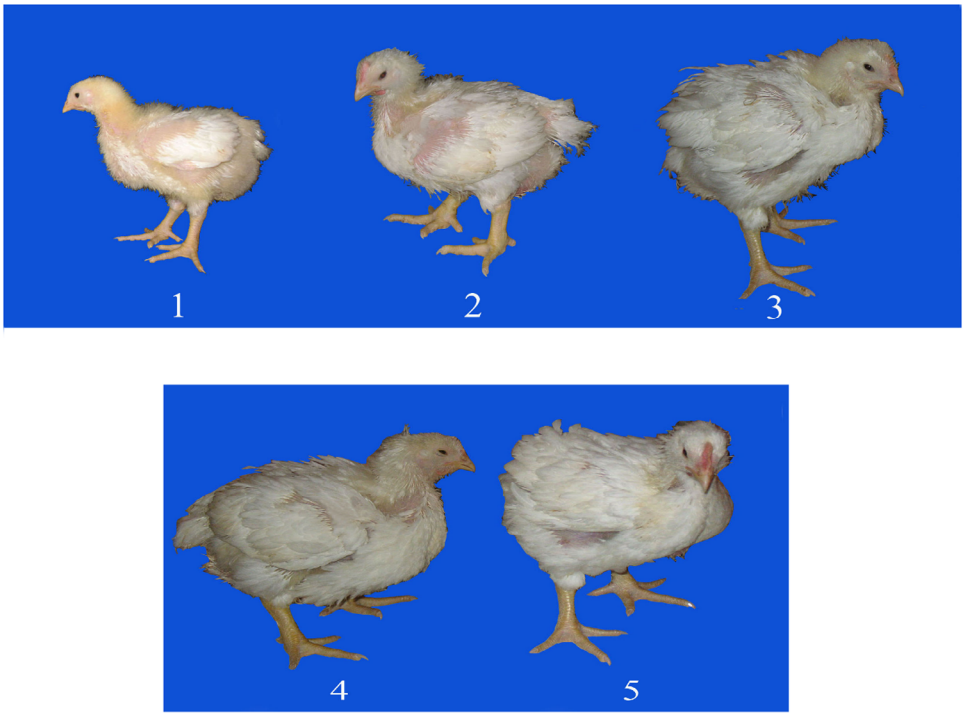

FIGURE 2 | Gradual change in the appearance of poultry receiving aflatoxin-B1 and ochratoxin during the weeks of the experiment.

measured by high-performance liquid chromatography are given in Table 1.

2.5 | Measured Parameters

2.5.1 | Growth Performance

For each feeding stage, the BWG, feed intake (FI) and FCR were recorded. The body weights of the chicks were recorded individually at 0, 10, 24 and 42 days with a digital scale with an accuracy of 0.01 g. The daily feed consumption was calculated in grams per day (day/bird/g), and to correct losses during the test period, the information about the bird was lost (experimental unit, date and weight), and the amount of feed consumption was recorded. The FCR at the end of each period was calculated by dividing the feed consumption of each experimental unit (g) by the gram weight and BWG. The conversion factor of feed consumption in each week and each growth period as well as in the whole period was calculated by dividing the average daily feed consumption of chicks in each specified period by the average daily weight gain of that period. The FCR is defined as the feed consumed (g) per BWG (g) according to the following formula: FCR = FI/BWG (Alharthi et al. 2022) (Figure 2).

2.6 | Blood Biochemical Parameters and Serum Enzymatic Activity

At the age of 42 days, one bird was randomly selected from each experimental unit. To check the serum indicators, 2.55 cc of blood was taken from the wing vein via a 5 cc syringe. The blood samples were centrifuged for 20 min at 5000 rpm, and the obtained plasma was stored in a freezer at −20°C until analysis. The isolated sera were transferred to the laboratory. In the laboratory, the concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase enzymes (γ-GT), as well as the blood parameters of total serum protein, albumin, bilirubin, glucose, uric acid, calcium and phosphorus, were measured through biochemical tests according to the instructions of the Biosystem S.A., Barcelona (Spain).

2.7 | Carcass Weight and Organs

To check the effects of the experimental treatments, at the end of 42 days, one bird from each experimental unit was selected, killed, and cleaned with lukewarm water and a filling machine. After the contents of the digestive tract were emptied, the weights of the liver, heart, digestive tract and bursa of Fabricius were measured via a digital scale with an accuracy of 0.01 g, and their relative weights were calculated by dividing the obtained weights by the live body weight of the bird (Figures 3–6).

The weights of the muscles of the breast, drumsticks and wings were measured and calculated relative to the live weight of the bird. In addition, parts of the jejunum of the small intestine were separated for morphological tests.

2.8 | Intestinal Morphology

A 5-cm sample was taken from the small intestine and kept in a 10% formalin solution until it was transferred to the laboratory. After fixation, the ileum tissue was treated with paraffin, cut with

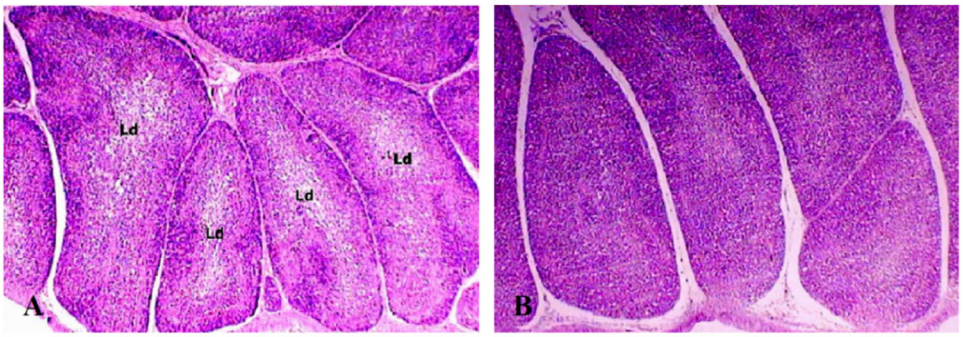

FIGURE 3 | (A) Depletion of the lymph tissue of the centre of the bursa of Fabricius follicle in the treatment group receiving aflatoxin and ochratoxin to (B) normal cells of the lymph tissue of the bursa of Fabricius as a result of the consumption of aflatoxin and ochratoxin.

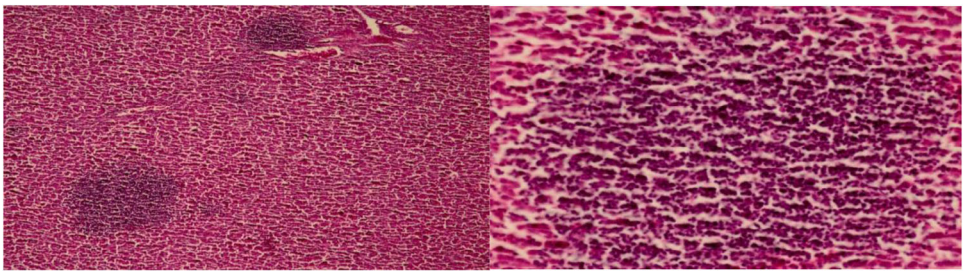

FIGURE 4 | Infiltration of lymphocyte cells of different sizes in liver tissue.

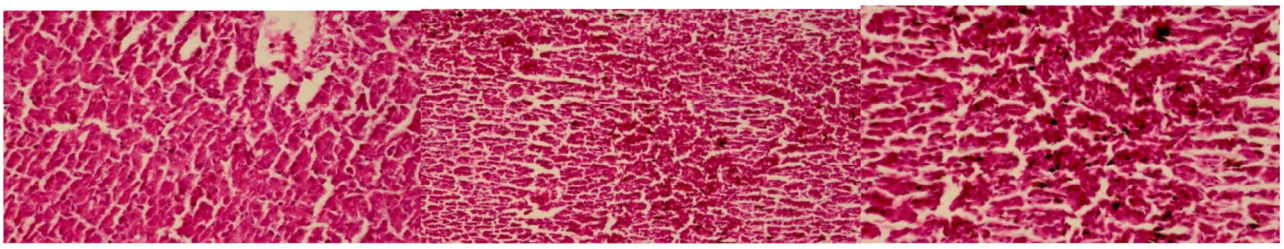

FIGURE 5 | Infiltration of lymphocyte cells of different sizes in liver tissue.

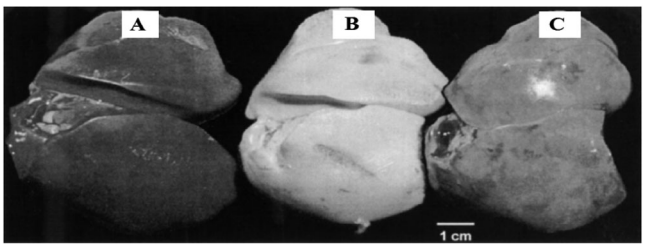

FIGURE 6 | (A) Control, (B) poultry liver receiving 1% bentonite with aflatoxin and ochratoxin, (C) poultry liver receiving 0.1% yeast cell wall with aflatoxin and ochratoxin.

a microtome, and subjected to haematoxylin and eosin staining to examine the relevant tissues (Viveros et al. 2011).

The morphostructural characteristics of the ileum, such as the villus height, crypt depth and goblet cell count, were assessed on ileum tissues via standard histopathological protocols (Poloni et al. 2020).

2.9 | Statistical Analysis

All the data were analysed via ANOVA via the GLM procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC). Duncan's multiple range test was used to identify significant differences among the treatments. Statistical significance was considered at $p < 0.05$, and the results are reported as the means and SEMs.

3 | Results

3.1 | The Performance

3.1.1 | Feed Consumption, Weight Gain and FCR

The effects of the use of different types of toxin binders and saffron petal extract on the performance of broiler chickens are reported in Table 2. The results revealed that the use of bentonite, yeast shell, or a type of commercial toxin binder had no significant effect on the feed consumption, BWG, or FCR of

TABLE 2 | Effects of experimental treatments on the performance of broilers (g).

	1–10 days			10–24 days			24–42 days		
Treatments	**FI**	**BWG**	**FCR**	**FI**	**BWG**	**FCR**	**FI**	**BWG**	**FCR**
T1	275.15	219.70	1.25	1244.4	831.2	1.50	2006.80	1155.80	1.74
T2	272.20	215.10	1.27	1240.4	802.6	1.55	2002.60	1139.40	1.76
T3	278.10	220.20	1.26	1246.4	816.5	1.53	2012.60	1155.40	1.74
T4	278.30	223.20	1.25	1248.7	806.7	1.55	2016.50	1180.00	1.71
T5	275.10	221.06	1.24	1248.9	824.8	1.51	2020.00	1173.90	1.72
T6	275.70	219.80	1.25	1245.3	803.1	1.55	2017.80	1173.00	1.72
T7	277.80	224.10	1.24	1247.3	805.8	1.55	2022.40	1185.3	1.71
SEM	3.25	3.72	0.02	10.43	9.23	0.05	14.32	17.45	0.06
p value	0.42	0.082	0.091	0.125	0.221	0.211	0.223	0.152	0.111

Note: Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxerin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract

TABLE 3 | Effects of experimental treatments on the carcass yield and weight ratio of broiler carcass components (% of body weight).

Treatments	**Carcass**	**W + B + N + T**	**Drumsticks**	**Breast**
T1	63.93[a]	19.99	20.63[a]	23.31[a]
T2	58.57[c]	19.60	19.84[b]	19.83[c]
T3	60.55[b]	19.88	20.32ab	20.35[b]
T4	60.78[b]	19.85	20.70ab	20.23[b]
T5	60.62[b]	19.84	20.60[a]	20.18[b]
T6	61.14[b]	19.87	20.40ab	20.87[b]
T7	63.54[a]	19.98	20.87[a]	22.69[a]
SEM	2.71	1.20	0.40	0.60
p value	0.05	0.121	0.023	0.032

Note: Lowercase letters indicate significant differences between treatments ($p < 0.05$). Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxerin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract.

broilers during the breeding period ($p > 0.05$). On Days 24–42, the greatest increase in weight and feed consumption was related to Treatments 4 and 7, respectively, but this increase was not significant ($p > 0.05$).

3.1.2 | Carcass Traits and Organs

In Tables 3 and 4, the effects of the use of toxin binders on the components of the carcasses of broiler chickens are reported. The use of toxin binder had a significant effect on the relative weights of the carcass, drumsticks and breast meat ($p < 0.05$). The relative weights of the carcasses, breast meat and drumstick meat increased significantly in Treatments 1 and 7 compared with those in the other treatments ($p < 0.05$). The relative weights of the intestine, spleen, pancreas and abdominal fat increased significantly in treatment 4, and the relative weights of the liver, kidneys and bursa increased significantly in Treatment 2 ($p < 0.05$). The results revealed that the experimental treatments had no significant effect on the relative weights of the gizzard and heart ($p > 0.05$). In Treatment 2, the relative weight of the kidneys also significantly ($p < 0.05$) increased when both aflatoxin and ochratoxin A were present.

3.2 | Blood Biochemical Parameters and Serum Enzymatic Activity

The results reported in Tables 5 and 6 were used to investigate the effects of several types of toxin binders and saffron petal extracts on blood parameters and liver enzymes. The concentration of

TABLE 4 | Effects of experimental treatments on the relative weights of internal organs (g/100 g of live weight).

Treatments	Liver	Heart	Full intestine	Spleen	Abdominal fat	Pancreas	Gizzard	Kidneys	Bursa fabricious
T1	2.153^{b}	0.14	0.23^{b}	0.53^{c}	0.22^{a}	0.42^{b}	0.47	0.42^{a}	1.93^{c}
T2	3.977^{a}	0.16	0.27^{b}	0.44^{d}	0.25^{a}	0.44^{b}	0.50	0.86^{c}	2.35^{a}
T3	2.146^{b}	0.13	0.23^{b}	0.43^{d}	0.24^{a}	0.41^{b}	0.48	0.47^{a}	2.15ab
T4	2.825^{b}	0.21	0.32^{a}	0.82^{a}	0.15^{b}	0.56^{a}	0.53	0.53^{b}	0.95^{e}
T5	2.247^{b}	0.16	0.23^{b}	0.52^{c}	0.24^{a}	0.43^{b}	0.48	0.51^{b}	1.72^{cd}
T6	2.241^{b}	0.14	0.26^{b}	0.53^{c}	0.21^{a}	0.45^{b}	0.50	0.50^{b}	1.44^{d}
T7	2.271^{b}	0.17	0.27	0.62^{b}	0.20 ^{a}	0.48	0.50	0.49^{b}	1.37^{d}
SEM	0.05	0.11	0.05	0.05	0.05	0.05	0.19	0.07	0.03
p value	0.013	0.068	0.033	0.049	0.011	0.020	0.294	0.026	0.035

Note: Lowercase letters indicate significant differences between treatments ($p < 0.05$). Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract.

TABLE 5 | Effects of experimental treatments on the blood parameters of broilers at the end of the experimental period.

Treatments	Glu (mmol/L)	Ca (mmol/L)	P (μmol/L)	Tp (g/L)	Albumin (g/L)	Bil-T (μmol/L)	Bil-D (μmol/L)	Uric acid (mmol/L)
T1	209.7^{c}	7.75^{a}	6.05	23.2	15.7	6.61	2.49	1.95^{bc}
T2	246.5^{a}	11.45^{a}	7.2	36.2	17.5	6.81	2.82	1.30^{c}
T3	218.2^{b}	10.3ab	7.1	33.6	16.2	6.91	2.64	1.61^{c}
T4	220.1^{b}	9.1ab	5.35	30.5	12.3	7.42	2.03	3.30^{a}
T5	219.5^{b}	7.07^{b}	4.85	34.1	13.2	7.24	2.32	2.47ab
T6	220.3^{b}	6.85^{b}	4.47	28.4	10.7	7.45	2.31	3.19^{a}
T7	217.2^{b}	7.67^{a}	4.89	31	12.06	7.37	2.22	2.96ab
SEM	10.14	0.74	0.38	3.2	1.12	0.13	0.08	0.20
p value	0.026	0.008	0.059	0.189	0.065	0.088	0.302	0.011

Note: Lowercase letters indicate significant differences between treatments ($p < 0.05$). Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxerin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract.

glucose increased greatly in the treatment containing aflatoxin and ochratoxin. Compared with the control treatment, the inclusion of toxin binder in the diet of chickens had no significant effect on the concentrations of phosphorus, total protein, albumin, or bilirubin ($p > 0.05$). The calcium concentration in Treatment 2 and the blood uric acid concentration in Treatment 4 were significantly greater than those in the other treatments ($p < 0.05$). AST was not affected by the experimental treatments ($p > 0.05$), but ALT and γ-GT were significantly lower in Treatment 7 than in the other experimental treatments ($p < 0.05$).

3.2.1 | Morphometric Analysis

The results of the morphometric analysis of the intestine are presented in Table 7. Compared with those in the other experimental treatments, the villus height in the jejunum in Treatment 7 was significantly greater ($p < 0.05$). The poison caused by fungal toxins in chickens significantly reduced the villus height and number of goblet cells while increasing the crypt depth ($p < 0.05$). The results revealed that dietary inclusion of toxin absorbents significantly ($p < 0.05$) increased the villus height and number of goblet cells and decreased the crypt depth. In poisoned chickens, the number of goblet cells as a part of the immune system increased significantly ($p < 0.05$) in response to aflatoxins and ochratoxins as exotoxins.

4 | Discussion

4.1 | Feed Consumption, Weight Gain and FCR

There is a consensus among researchers that weight loss, reduced FI and increased FCR occur in broilers fed fungal

TABLE 6 | Effects of treatments on the liver enzymes of broilers at the end of the experiment (U/mL).

Treatments	AST	ALT	γ-GT
T1	182.02	15.75[a]	20.0[a]
T2	202.36	25.42[b]	67.53[b]
T3	195.34	20.0[a]	50.72[b]
T4	213.14	25.18[b]	78.05[b]
T5	212.21	25.40[b]	23.66[a]
T6	210.21	18.59[a]	17.27[a]
T7	184.85	16.2[a]	19.10[b]
SEM	21.37	2.36	3.68
p value	0.063	0.021	0.015

Note: Lowercase letters indicate significant differences between treatments ($p < 0.05$). Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxerin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract.

TABLE 7 | Effects of experimental treatments on changes related to losses (%) in broilers.

Treatments	Villus height, µm	Crypt depth, µm	Goblet cells, *n**
T1	855[b]	155[cd]	7.7[a]
T2	732[d]	189[a]	4.9[c]
T3	799[c]	158[cd]	5.7[bc]
T4	789[c]	166[bc]	5.7[bc]
T5	747[d]	177[b]	5.9[c]
T6	788[c]	171[bc]	5.9[c]
T7	944[a]	149[d]	6.7[b]
SEM	18.35	5.97	0.51
p value	0.04	0.05	0.05

Note: Lowercase letters indicate significant differences between treatments ($p < 0.05$). Treatment 1: no toxin and additives, Treatment 2: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin, Treatment 3: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + commercial binder toxin, Treatment 4: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall, Treatment 5: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 1% processed bentonite, Treatment 6: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxerin + 750 mg/kg saffron petal extract and Treatment 7: diet containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite processed + 750 mg/kg of saffron petal extract.
*In 100 µm of villus height.

toxin-contaminated diets. Compared with individual mycotoxins, cocontamination with aflatoxin and ochratoxin caused a severe reduction in growth performance, and combined feeding with aflatoxin (300 ppb) and ochratoxin (250 ppb) led to synergistic effects on BWG, FI, feed efficiency and immunity in broiler chickens (Sharma, Mandal, and Singh 2016). In the present study, the treatments included the addition of 250 ppb aflatoxin + 200 ppb ochratoxin at realistic levels, which severely reduced growth performance.

Although the use of bentonite, yeast cell wall, a type of commercial binder toxin, and saffron petal extract in the broiler diet did not significantly affect the performance of broiler chickens, weight gain, feed consumption and a better conversion ratio were observed at 24–42 days of age. In a study conducted on broilers, 100, 200, 300 and 400 µg /kg of *ochratoxin A* were added to broiler diets. During the growth period, the BWG significantly decreased at concentrations greater than 100 µg/kg. *Ochratoxin A* contamination does not affect FI but results in a poorer FCR and thus worse feed utilisation efficiency in broiler chickens (Singh, Singh, and Mandal 2017). In one study, the use of 2 mg/kg aflatoxin in the diet of broiler chickens caused a decrease in body weight, feed consumption and the FCR (Rashidi et al. 2020), which is almost consistent with the results of the present study. The adverse effects of aflatoxin on growth performance may be due to the occurrence of anorexia, the inhibition of protein synthesis, and lipogenesis resulting from aflatoxin poisoning. In addition, other researchers have reported that aflatoxin can reduce the activity of pancratolipase, amylase and trypsin and, as a result, reduce the growth rate (Rajput et al. 2017). It has also been determined that the use of yeast cell walls improves the growth of broiler chickens due to the supply of nutrients such as vitamins, enzymes and protein and by preventing the absorption of toxins in the digestive system (Santin et al. 2003). In many studies, the use of yeast cell walls has promoted the adsorption of aflatoxin and ochratoxin, and as a result, the performance of broiler poultry with a diet contaminated with aflatoxin has improved (Zhao et al. 2010). In this study, glucomannan was able to cope with changes in serum biochemical parameters caused by 5.5 mg/kg deoxynivalenol in roosters. However, no positive effects on FI or BWG were observed; however, improved pig performance was observed when glucomannan was included in the diet compared with diets contaminated with 2.5 or 3.8 mg/kg deoxynivalenrol alone (Sun et al. 2014). In a study in which quail chicks aged 7–35 days were tested, FI and body weight decreased in the AFB1-treated group; however, in the probiotic-treated group, nutrition improved. During the test period, the conversion rate increased due to the decrease in feed consumption. The conversion rate was improved in the probiotic-containing group. Treatments containing probiotics can improve growth performance and immunity (Bagherzadeh Kasmani, Quails, and Mehri 2015). There are different results concerning the effects of using saffron in the diet of broiler chickens. In a previous study, the use of saffron petal powder in the diet of broiler chickens increased feed consumption, reduced the conversion rate, and did not affect body weight (Naghous et al. 2015). In contrast, the addition of hydroalcoholic extracts of saffron petals to the diet of quail chicks increased body weight, increased feed consumption, and decreased the FCR (Hosseini-Vashan, Mohammadian, and Afzali 2018b). There are no studies on the effect of saffron in diets containing binder toxin; therefore, the performance of poultry in the present study may differ from that in other experiments because the addition of aflatoxin to the diet reduces the performance of broiler poultry (Wade, Sapcota, and Verma 2017). On the other hand, due to the presence of high levels of substances with antioxidant properties, such as kaempferol,

quercetin, flavonoids and minerals, in saffron petals, possibly by reducing the activity of free radicals and reducing the breakdown of fats, increasing the accessibility of oils and soluble fatty acids and vitamins is possible. Pin fat increases bird growth and reduces the negative effects of aflatoxins (Hosseini and Mollafilabi 2017).

It is well documented that major mycotoxins can adversely affect the growth of animals. The impact on performance varies according to many factors, such as the mycotoxin and species used, the concentration in feed, the use of purified versus naturally contaminated feed, or the ingestion of multitoxin-contaminated feed. Although the effects of low doses are more controversial, reduced performance is among the main characterized effects of mycotoxin intoxication (Grenier and Todd 2013).

4.2 | Carcass traits and organ weight

In this experiment, the relative weights of the carcass, breast and drumsticks were significantly lower in the treatment containing aflatoxin than in the other treatments. Although no difference was observed between the experimental treatments, the relative weights of the remaining head + back + neck + wings were greater in the treatments containing toxin binder and saffron petal extract.

Previous data have shown that toxicity-enhancing synergisms exist between mycotoxins and that symptom patterns are altered during multiple mycotoxicoses. The data also demonstrate that nephropathy is the primary effect of this interaction and, thus, is of diagnostic importance. The interaction effect of both mycotoxins on liver lipid levels was significant ($p < 0.05$), and the combined effect demonstrated that ochratoxin A inhibits lipid accumulation normally induced by aflatoxin. The data show that toxicity-enhancing synergisms exist between mycotoxins and symptom patterns (Huff and Doerr 1981). The percentage of abdominal fat was the highest in the mycotoxins and without toxin binder treatment, and the inclusion of toxin binder and saffron petal extract in the diet containing mycotoxins did not affect the relative weights of the heart or pancreas. In addition to the detoxification of mycotoxins, the liver is the main target organ.

The highest relative weight of the liver was associated with the treatment containing 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A, which was significantly different from the other experimental treatments, and the lowest relative weight of the liver was associated with the treatment without mycotoxins. The liver, the principal detoxification organ, is the anticipated target organ of aflatoxin–ochratoxin A synergism since aflatoxin is a potent hepatotoxin and because ochratoxin A has hepatotoxic properties (Huff and Doerr 1981). Although the relative weight of the liver increased significantly ($p < 0.05$) in response to both aflatoxin and ochratoxin A individually, there was no interaction effect of the two toxins on liver weight. However, synergism was evident in the liver lipid concentrations. Aflatoxin increases liver lipid values, which are important diagnostic characteristics of this mycotoxicosis (Huff and Doerr 1981).

However, when both aflatoxin and ochratoxin A were present, the liver lipid levels did not differ from the control values. Ochratoxin A inhibited or spared this major aflatoxin effect. The positive effects of the addition of bentonite and a cell wall to the diet of broilers contaminated with aflatoxin have been well reported (Zhao et al. 2010). In addition, the treatments containing saffron petal extract had lower relative liver weights than the treatments containing aflatoxin and without toxin binder. Saffron has been shown to moderate the toxicity of aflatoxin (aflatoxin B1) and reduce the degree of liver damage caused by it. In another study conducted on quail chicks, the weights of the spleen, heart and liver increased in diets contaminated with AFB_1. However, the weight of the bursa of Fabricius and testis decreased (Bagherzadeh Kasmani, Quails, and Mehri 2015). In broiler chickens, the relative weights of organs related to the immune system (spleen and bursa of Fabricius) were significantly affected by the experimental treatments. In another study, levels of 200 ppb or higher resulted in increased mortality. OTA contamination at 200 ppb or higher leads to an increase in the relative weights of the liver and kidney and the relative weight of Bursa Fabricius (Singh, Singh, and Mandal 2017). Another experiment conducted on chickens for 5 weeks investigated the effects of dietary contamination (with different levels of 1 and 2 mg/kg) of ochratoxin A along with the addition of a mycotoxin deactivator at 0.2% of the diet. In the treatments containing the mycotoxin deactivator, the relative weights of the liver and kidney were significantly reduced by the OTA-contaminated diets. The results of an experiment revealed the positive effects of adding a mycotoxin deactivation product and that the OTA content in the liver and kidney is a suitable biomarker for the contamination of broilers with this mycotoxin (Joo et al. 2013).

The organ most sensitive to the interactive effect of these mycotoxins was the kidney. Aflatoxin and ochratoxin A fed individually caused significant ($p < 0.05$) and similar increases in kidney relative weight. The kidneys of birds treated with the two toxins simultaneously were pale and swollen, resulting in the distention of kidney lobes beyond the normal skeletal limits. Moreover, the relative weights of the kidneys of the cointoxicated birds were increased twofold over the control values, indicating a highly significant interaction ($p < 0.01$). Because this degree of kidney involvement and liver lipid response are viewed as symptoms atypical of aflatoxicosis, a diagnostician encountering such manifestations would probably discount the possibility of aflatoxin contamination. Furthermore, analysis of ochratoxin alone would not be expected to indicate levels sufficient to produce the symptoms observed. Thus, these data seem to provide some explanation for the occasional appearance of more severe animal nephropathies than the low levels of a particular nephrotoxic element in the feed would suggest. They further suggest an underlying fallacy in assuming that a single toxicant is responsible for the diverse and variable symptoms encountered in field mycotoxicoses. These data clearly indicate that synergistic effects exist between mycotoxins and increase their toxicity. The data also demonstrate that symptom patterns can be altered, confusing preliminary diagnoses during multiple mycotoxicoses. Additional studies on the synergism among mycotoxins should provide valuable diagnostic information and a better understanding of the importance of mycotoxins to the animal industry (Huff and Doerr 1981).

The use of saffron petal powder in the broiler diet did not affect the relative weight of carcass components, including the

bursa of Fabrius and abdominal fat (Naghous et al. 2015). In a previous study, the hydroalcoholic extract of saffron petals caused an increase in the relative weight of the lymphatic organs and a decrease in abdominal fat in quails (Hosseini-Vashan, Mohammadian, and Afzali 2018a).

4.3 | Blood Biochemical Parameters and Serum Enzymatic Activity

The results of this experiment revealed that the concentrations of phosphorus, total protein, albumin and bilirubin were not affected by the experimental treatments. The amount of uric acid in the treatment containing aflatoxin and without toxin binder was significantly lower than that in the other treatments. Similar to the results of this study, aflatoxin levels decreased plasma protein, and the addition of toxin binder did not affect the experimental treatments (Basmacioglu et al. 2005). Aflatoxins damage the liver, which is the central organ for the metabolism of lipids, proteins and amino acids, causing a decrease in protein content and harming the productivity of birds (Fouad et al. 2019). The glucose concentration decreased in the treatments containing toxins. A decrease in the amount of glucose, cholesterol, triglycerides and calcium in a diet containing aflatoxin has been reported (Eckhardt et al. 2014).

Feeding broilers AFB-contaminated diets significantly increased the detection of liver enzymes in the serum. Increased liver enzyme activity has been reported as an indicator of serological susceptibility to poisoning and kidney disease. Serum ALT, AST and alkaline phosphatase levels are specific for liver damage and are indicative of degenerative changes in liver tissue. They are used as markers for changes in cell viability and cell membrane permeability resulting from liver damage. These enzymes are normally found within hepatocytes, but with damage to hepatocytes and their apoptosis due to toxins, these enzymes are released into the bloodstream, and their levels in circulation increase. When the liver becomes damaged, hepatocytes, liver stellate cells, sinusoidal endothelial cells and Kupffer cells also produce excessive amounts of inflammatory factors. In this study, an evaluation of the serum ALT, AST and GGT levels revealed that their levels were elevated in the serum of birds fed diets contaminated with AFB and OTA (Malekinezhad et al. 2021).

The concentrations of ALT and γ-GT enzymes in the 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin A + 750 mg/kg saffron petal extract + processed bentonite and yeast wall treatment groups were significantly lower. Similar to the results of this study, the amount of liver enzymes in diets containing AFB was greater than that in other treatments (Fouad et al. 2019). Among the liver enzymes, AST was higher in the treatment containing aflatoxin and without binder toxin, but this difference was not significant. Another experiment conducted on chickens revealed that in the context of treatments containing a mycotoxin inactivator, the activities of ALT and aspartate in the blood decreased significantly in response to OTA-contaminated diets (Joo et al. 2013). This is because, during poisoning with aflatoxin B1, which is metabolised mainly in the liver, a secondary metabolite called AFB1-8,9-oxide can bind to proteins, lipids and nucleic acids and then cause cancer and damage liver cells. Following liver damage, the permeability of liver cells increases; as a result, liver enzymes are released from the cell and released into the bloodstream, increasing the activity of serum transaminases (Alharthi et al. 2022). The use of saffron petal extract in mice increased the activity of AST and lactate dehydrogenase enzymes during acetaminophen poisoning, and with the injection of saffron petal extract, their activity decreased to the normal range (Omidi, Rahdari, and Hassanpour Fard 2014).

4.4 | Morphometric Analysis

The histology of the small intestine is an important indicator that reflects the health of the digestive system and the response of the intestine to the components in the feed. In the present study, the experimental treatments significantly affected the intestinal morphology indices. The poison caused by fungal toxins in the mycotoxins significantly reduced the villus height and number of goblet cells while increasing the crypt depth ($p < 0.05$). Shorter and thinner villi in chickens fed aflatoxin are due to impaired protein synthesis and reduced epithelial cell proliferation (Malekinezhad et al. 2021). Nothing is known about the effects of mycotoxins on enterocyte differentiation or migration rates along the length of the villus, but many studies have reported adverse effects of these fungal metabolites on the morphology of the intestinal villi. Villi increase the internal surface area of the intestinal wall, increasing the area available for nutrient absorption. Therefore, whenever the integrity of the intestinal wall is compromised, the effectiveness of nutrient absorption might be affected (Grenier and Todd 2013).

The height of the villi and the number of goblet cells were greater in the treatments containing mycotoxins and without toxin binder. The increase in the height of the villi and their ratio to the depth of the crypts is usually positively correlated with digestion and absorption in the digestive tract of birds, which indicates an increase in the level of absorption (Williams et al. 2008). The height of villi and the number of goblet cells were greater in the 2.5 mg/kg aflatoxin + 2 mg/kg ochratoxin + 0.1% yeast cell wall + 1% bentonite process + 750 mg/kg saffron petal extract treatment than in the other mycotoxin treatments. Saffron petal extract can be used to reduce the harmful effects of aflatoxins (Hosseini Vashan and Pirai 2018). The yeasts *Saccharomyces cerevisiae* and lactic acid bacteria have been used in the poultry feed industry as growth promoters, which play an important role in this effect by increasing the cross-sectional area of absorption in the small intestine (Celyk, Denly, and Savas 2003). The beneficial effects of these compounds have been shown in the absorption of aflatoxin in the digestive system. The use of the cell wall of *S. cerevisiae* yeast and bacteria in aflatoxin-free diets increased the height of villi in the jejunum. Several researchers have confirmed that modified glucomannan, a derivative of the yeast cell wall, can bind several important mycotoxins (Singh, Singh, and Mandal 2017). Research on the effects of aflatoxin and its adsorbents on small intestine histology is very scarce and inconclusive (Firmin et al. 2011).

5 | Conclusion

The use of commercial binder toxin, bentonite, yeast cell wall extract, or saffron petal extract had no significant effect on the

performance indicators of broiler chickens. In addition, toxic adsorbent compounds had a significant effect on the relative weights of carcasses, breast meat and chickens contaminated with toxins. The inclusion of toxin binder in the diet of chickens had a significant effect on the glucose concentration of chickens fed contaminated diets, although the amounts of phosphorus, total protein, albumin and bilirubin were not different from those in the control treatment. The AST enzyme was not affected by the experimental treatments. However, the concentrations of ALT and γ-GT enzymes in Treatment 7 were lower than those in the other treatments. In the treatments containing toxin binder, aflatoxin and ochratoxin, the villi length and number of goblet cells were greater, and the crypt depth was lower.

Author Contributions

Reza Vakili: conceptualiation, writing–original draft, writing–review and editing, validation, methodology, formal analysis, project administration, funding acquisition, supervision. **Ali Zanghaneh**: investigation, resources. **Faezeh Qharari**: software, data curation.**Fathmeh Mortzavi**: visualisation.

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Ethics Statement

All research reported in this research has been conducted in an ethical and responsible manner, and is in full compliance with all relevant codes of experimentation and legislation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data presented in this study are available on request from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70122.

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