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Ameliorative effect of a novel enzymatic detoxifier against natural field levels of mycotoxins in the broiler chicken diet

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ABSTRACT

Mycotoxins are considered hidden dangers that threaten the poultry industry globally because they suppress the immunity of birds, reduce their production, and increase their chance of being infected with diseases, which exposes the poultry industry to enormous economic losses. Therefore, this investigation aimed to assess the effectiveness of VemoZyme Detox®, a novel enzymatic detoxifier, in mitigating the detrimental consequences of mycotoxin contamination in broiler chickens. The experiment involved 10,000-day-old, Cobb 500 broiler chicks, which were allotted into two groups of 5000 birds each as follows: T1: received a control basal diet; and T2: birds were provided with a basal diet supplemented with VemoZyme Detox®. The birds underwent comprehensive monitoring, including evaluations of growth performance, blood parameters, mycotoxin levels, hepatic histopathological alterations, and litter bacteriological counts. Broilers receiving dietary VemoZyme Detox® exhibited significant improvements in various aspects, including growth performance, reduced mortality rates, and more favorable feed conversion ratios. Moreover, the enzymatic supplement played a protective role in maintaining hepatic and renal health, as evidenced by reductions in blood aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid, and creatinine. Importantly, although there was no significant difference in mycotoxin levels (zearalenone, fumonisin B1, ochratoxin A, aflatoxin B1) within the feed, VemoZyme Detox® had a significant impact on decreasing mycotoxin levels, particularly those of zearalenone and fumonisin B1. Hepatic histological examinations also revealed healthier conditions in T2, and positive impacts extended to litter samples, as indicated by reduced counts of *Clostridium perfringens* (*C. perfringens*) and *Escherichia coli* (*E. coli*) counts. In conclusion, the use of an enzymatic detoxifier is a promising method for counteracting the negative impacts of mycotoxin contamination in broilers. The results underscore the substantial potential of enzymatic detoxifiers for ensuring the health and productivity of broilers, opening new avenues for safer poultry production.

Introduction

Recent advancements in poultry feeding aim to maximize genetic potential and accommodate shorter growth periods in contemporary broilers (Abd El-Hack *et al*., 2022; El-Saadony *et al*., 2022). Given their immature physiology at the time of marketing, controlling gastrointestinal immaturity is critical for optimal production (Ravindran and Reza Abdollahi, 2021; Kamal *et al*. 2023). Maintaining intestinal integrity in broilers is vital for effective digestion, nutrient uptake, immune strength, and infection prevention (Kamal *et al*., 2019a; Siddiqui *et al*., 2022; Salem *et al*., 2023).

Mycotoxin contamination in poultry feed ingredients poses risks, potentially leading to a compromised gut lining, decreased gut function, and various health issues (Garbaba *et al*., 2018; Akinmusire *et al*., 2019). Over 400 mycotoxins, including aflatoxins, fumonisins, trichothecenes, and ochratoxin A, have been identified in feeds and shown to impact avian health and mortality (Kemboi *et al*., 2020; Nakavuma *et al*., 2020).

Efforts have been made to ensure feed security using eco-friendly additives and technologies (Kamal *et al*., 2019b; Muiruri and Fathima, 2023). Clay-based mycotoxin binders, such as zeolite and montmorillonite, mitigate the adverse effects of mycotoxins, especially aflatoxins, in poultry (Bhatti *et al*., 2018; Mgbeahuruike *et al*., 2021). Similarly, mycotoxin modifiers such as fungi, bacteria, and enzymes can reduce the toxicity of mycotoxins in the digestive system of animals (Ochieng *et al*., 2021).

Recent research has explored the use of bacterial strains and enzymes in poultry feed to mitigate mycotoxin effects, revealing promising results against toxins such as AFB1, zearalenone, and deoxynivalenol (Chang *et al*., 2020; de Souza *et al*., 2020). Enzymes such as fumonisin esterase effectively break down fumonisins into less harmful byproducts, suggesting their safe use in bird diets (Rychen *et al*., 2016; Hamad *et al*., 2023).

Chronic mycotoxicosis caused by low-level mycotoxin consumption increases susceptibility to immune and inflammatory disorders in chickens (Hamad *et al*., 2023). Various studies have explored the use of additives such as immunological boosters, antioxidants, and anti-inflammatory substances to prevent mycotoxicosis (Qu and Liu, 2021). Enzymes such as glucose oxidase exhibit multiple benefits in animal feed, enhancing intestinal function, growth, and immunity and possessing antibacterial and antifungal properties (Wong *et al*., 2008; Wang *et al*., 2018).

Enzymes such as lactonohydralases, carboxypeptidases, and proteases aid in detoxifying mycotoxins, while multienzyme compounds improve broiler utilization of nutrients and reduce enteric pathogens (Boudergue *et al*., 2009; Yang *et al*., 2010). Supplements such as Detoxizyme® have shown positive effects on broiler growth, enzyme activity, and toxin reduction (Ademola *et al*., 2015; Saleh *et al*., 2023). Prevention and control of several mycotoxins, especially trichothecenes, ochratoxin, zearalenone, and fumonisin, which are poorly adsorbed by mycotoxin binders, requires novel strategies; thus, our hypothesis evaluated the combination of safe antimycotoxicosis-specific biologically active microbial enzymes and nontoxic environmentally friendly feed grade toxin binders against mycotoxins to fight poultry feed mycotoxins.

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Materials and methods

Ethical approval

The experimental protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Cairo University. Approval number: Vet CU 25122023849.

Experimental birds

A total of 10,000-day-old (Cobb 500) chicks were kept on built-up litter and then randomly classified into 2 groups with 20 replicates (250 birds/replicate) in each group to match the average body weight (BW) in each group. The birds were kept under daily observation until the end of the experiment at 28 days of age. The experimental groups were classified as T1: the control birds that were supplied with a basal corn soya-based diet; T2: the 2nd group was fed a basal diet supplemented with 300 g/MT of a mixture of specific natural antimycotoxin enzymes and toxin binders (VemoZymeDetox®, VEMO99, Bulgaria), which was obtained from 3A Pharma, Tanta, Egypt, Reg. No. 1/4, Code No. M20211013-2865N. VemoZyme Detox® contains total protease activity from Bacillus licheniformis 30 million U/kg (alkaline proteases (peptidase) 10 million U/kg, neutral protease 12.5 million U/kg, acid protease 7.5 million U/kg), glucose oxidase from Aspergillus niger 10000 U/kg, bentonite (90% montmorillonite) 980 g/kg, and activated charcoal 3 g/kg.

Diet and housing

The birds were fed a diet based on the Cobb 500 (cobb-vantress, 2022) requirements for the as-hatched chicken catalog, as shown in Table 1, with a 3-stage feeding strategy (starter, 0–10 days; grower, 11–21 days; and finisher, 22–28 days). The diets used were ad libitum, the starter diet was crumbled, and the grower and finisher diets were pellets. To maintain the path, a resident with open windows and a 23-hour light-and-dark cycle was employed. The diet contains natural field levels of mycotoxin contamination, and no additional experimental levels of mycotoxins have been applied. Every day, the temperature was maintained at 24-26°C, and the relative humidity was 60-70%. At 5 days of age, all chicks received the Hitchner-IB vaccine via eye drops. At 9 days, inactivated H5N1 was administered via subcutaneous injection; at 14 days, Gumboro Interme-

Table 1. Composition of the experimental starter, grower, and finisher diets.

Ingredient, g/kg	Starter (1–10 days)	Grower (11–25 days)	Finisher (26–28 days)
Yellow corn	507	548	578
Soybean meal, 46%	370	317	280
Corn gluten meal, 60%	38	50	50
Soya oil	17	21	31
Calcium carbonate	14	13.8	12.6
Dicalcium phosphate	20	17.5	16
Salt	2.3	2.4	2.3
Sodium sulfate	1.8	1.6	1.6
Dl Methionine, 99%	2.7	2	1.9
l-Lysine HCl, 98%	2.5	2.3	2.2
l-Threonine	1.1	0.7	0.6
Choline chloride, 60%	0.8	0.8	0.8
Wheat bran	19.25	19.35	19.45
Premix*	3	3	3
Anti-coccidia	0.15	0.15	0.15
Anti-clostridia	0.1	0.1	0.1
Antimycotoxin Vemozyme Detox®	0.3	0.3	0.3
Chemical Analysis on DM basis			
AME kcal	3000	3040	3140
Crude protein, %	23	21	19
Fat, %	6.3	4.5	6.9
Digestible LYS, %	1.28	1.24	1.15
Digestible M and C, %	0.95	0.92	0.87
Digestible THR, %	0.86	0.83	0.77
Digestible ARG, %	1.37	1.33	1.25
Digestible ILE, %	0.9	0.87	0.85
Digestible LEU, %	1.87	1.83	1.84
Digestible VAL, %	0.96	0.93	0.91
Calcium, %	0.96	0.96	0.87
Available P, %	0.48	0.48	0.44
Sodium, %	0.16	0.16	0.16
Chloride, %	0.23	0.23	0.23

*Composition (per 3 kg): Vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 10,000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30,000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10,000 mg, manganese 60,000 mg, zinc 50,000 mg, iron 30,000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

diate Plus was administered via eye drops; and at 21 days, Lasota was administered via eye drops.

Growth performance parameters

The BW was reported for each bird each week. The average live body weight gain (BWG) and feed intake (FI) were recorded to calculate the FCR for each replicate (Abdelfatah *et al*., 2023). After calculating the liveability percentage (100 – mortality), a performance index such as the European Production Efficiency Factor (EPEF) was used to evaluate the growth performance index of the broilers, as suggested by Aviagen (2018). The EPEF was calculated according to the following formula: European Production Efficiency Factors (EPEF) = (Livability%*BW(kg)/Age(d)*FCR)*100.

Sample preparation and serum analysis

The same three birds from each replicate were used for weekly collection of blood samples from their wing vein. Blood samples were collected in heparinized test tubes, and the plasma was separated immediately by centrifugation (3000 rpm for 20 min at 5°C). The plasma was stored at -20°C until further analysis. A commercial colorimetric kit was used to quantify the amounts of alanine transaminase (ALT) and aspartate transaminase (AST) (Egyptian Company for Biotechnology). The absorbance was measured using a spectrophotometer (Unico UV-2000; Spectra Lab Scientific Inc., USA) calibrated at a wavelength of 545 nm (Saleh *et al*., 2023). Using commercially available kits, the producer's instructions were followed to establish the amounts of creatinine and uric acid calorimetrically (Diamond Diagnostics, Egypt) (Saleh *et al*., 2023).

Mycotoxin analysis

The detection of mycotoxins in biological materials such as feed and excrement via liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is popular. Previously described chromatographic techniques and chromatographic and mass spectrometric factors were utilized in this investigation (Malachová *et al*., 2014).

During the last 3 days of the trial, three birds from each replicate, all of the same weight, were moved to different cages to determine the ration and droppings mycotoxins. The experimental "finisher" pelleted feed was fed to the birds, and samples were taken for analysis of mycotoxins. Samples were collected after mixing numerous samples from different locations at each collection place. Next, the samples were shipped in polystyrene cartons with cooling gel that had been previously frozen to -20°C to the Animal Health Research Institute (AHRI) for extraction and quantitative measurements via LC–MS/MS-based analysis for multimycotoxin contamination. All the samples were maintained in a chilling room at -20°C until analysis. Following the completion of the analysis and data review, each sample was properly disposed of. During the last 3 days of the trial, droppings from each replicate were collected and weighed to determine the mycotoxin content. The birds' weight and feed consumption were recorded daily for 3 days, and the droppings they passed were collected, weighed, and stored in a freezer. In a drying oven, all the samples were dried for a full day at 60°C. The total dried samples were homogenized and then finely powdered for testing in accordance with previous methods (Lim *et al*., 2018).

Histomorphological evaluation

To obtain liver tissue samples, 3 birds were randomly selected from each replicate on day 28, after which the hepatic tissue samples were submerged in 10% formalin in saline. After fixation, the tissue samples were washed in xylene, dehydrated in ethyl alcohol at escalating concentrations (from 70% to 100% alcohol), and then trained for histological examination. Then, sections 4-5 µm in thickness were stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008).

Microbiological analysis of the litter samples

Fresh litter samples were collected weekly from each replicate in an aseptic manner by placing a hand into an inverted Ziploc bag, securing the sample and sealing the bag after reverting it to its right side. Colony counting of *E. coli* and *C. perfringens*: One gram of each sample was serially diluted 10 times after being diluted 1 to 9 times (w/v) in sterile PBS with slight adjustment, and colony counting was carried out following previous methods (Quinn, 1994). To summarize, the samples were diluted and incubated anaerobically in Reinforced Clostridia Agar Medium (Oxoid) for one day at 37°C for *C. perfringens*. Additionally, the dilutions were inoculated in EMB media for *E. coli* colony counting and kept aerobically for one day at 37°C.

Data analysis

The Statistical Package for Social Sciences, version 25.0 (SPSS, Inc., Chicago, IL), was utilized for the data analysis. First, every piece of information was coded into variables. The Kolmogorov–Smirnov test was applied to determine whether the data were normal. The results are presented using a combination of inferential and descriptive statistics, including the Friedman and Kruskal–Wallis H tests. The collected results were subjected to a post hoc Dunn's test. A P - value of less than 0.05 was used for each test.

Table 2. Effect of antitoxin enzyme supplementation on growth performance in Cobb 500 broilers.

Item		Age (days)			
		7	14	21	28
Body weight (g)	T1	160.8±2.6^{b}	421.7±2.95^{b}	831.7±5.1^{b}	1381.7±5.1^{b}
	T2	172.8±2.03^{a}	481.5±6.4^{a}	931.2±11.5^{a}	1521.2±12.1^{a}
Body weight gain (g)	T1	118.2±2.6^{a}	260.8±1.01^{b}	410±2.4^{b}	550±1.9^{b}
	T2	130.4±2.02^{a}	308.7±4.5^{a}	449.7±5.4^{a}	590±3.5^{a}
Feed intake (g)	T1	140.8±0.48^{a}	457.5±1.9^{a}	1073.3±2.6^{a}	2194.3±13.55^{a}
	T2	137.8±0.31^{b}	451.7±0.95^{b}	1069.2±2.27^{b}	2044.2±13.56^{b}
FCR	T1	0.88±0.01^{b}	1.09±0.004^{a}	1.29±0.005^{a}	1.59±0.005^{a}
	T2	0.79±0.008^{a}	0.94±0.011^{b}	1.15±0.012^{b}	1.34±0.004^{b}
Mortality (%)	T1	0.83±0.08^{a}	1±0.14^{a}	1.75±0.09^{a}	2.38±0.11^{a}
	T2	0.42±0.08^{b}	0.71±0.1^{b}	1.17±0.08^{b}	1.58±0.05^{b}
EPEF, index	T1	260.4±7.3^{b}	274.8±2.5^{b}	301.5±2.7^{b}	303.3±0.69^{b}
	T2	308.4±6.5^{a}	364.2±8.6^{a}	381.8±8.3^{a}	397.9±3.7^{a}

In each row, mean values followed with different superscript letters^{a,b,c} are significantly different ($P \leq 0.05$). The values are expressed as the means±SEs.

Results

Growth performance

Table 2 provides an overview of the effects of dietary VemoZyme Detox® treatment on various parameters in Cobb 500 broilers over 28 days. In the control group T1, there were significant reductions in BW, BWG, FI, and EPEF, with values of 1381.7±5.1, 550±1.9, 2194.3±13.55, and 303.3±0.69, respectively. This group also experienced a higher mortality rate of 2.38±0.11 and an elevated FCR of 1.59±0.005. Conversely, in group T2, there were significant increases in BW, BWG, FI, and EPEF, with values of 1521.2±12.1, 590±3.5, 2044.2±13.56, and 397.9±3.7, respectively. Additionally, this group exhibited an improved FCR of 1.34±0.004 and a decreased mortality rate of 1.58±0.05 throughout the experimental period.

Blood parameter analysis

The impact of dietary VemoZyme Detox® on blood parameters is presented in Table 4. At 28 days of age, the chickens in the T2 group exhibited significant reductions (P ≤ 0.05) in ALT (13.33±0.57), AST (72.28±7.74), uric acid (2.87±0.12), and creatinine (0.54±0.02) compared with those in the T1 group (16.38±0.47, 117.3±5.63, 3.56±0.15, 0.68±0.03, respectively).

Mycotoxin analysis

Fig. 2 illustrates the impact of dietary Vemozyme Detox® on the levels of four mycotoxins in broiler feed and droppings. The mycotoxin levels did not significantly differ (P ≤ 0.05) between the feed samples in the control group (T1) and the treated group (T2). However, in T2, the droppings' mycotoxin concentrations were significantly (P ≤ 0.05) lower (ZEN (0.00±0.02), FUM (0.00±1.02), OTA (0.02±0.14), and AFB1 (0.39±0.44)) than those in the T1 group (ZEN (0.24±0.01), FUM (0.29±0.15), OTA (1.65±0.02), and AFB1 (6.92±0.0).

Postmortem lesions

As shown in Fig. 3 and 4, the postmortem lesions (PMs) of the freshly dead T1 chicken at 28 days of age revealed muscular hemorrhages on the thigh and breast muscles; the liver was enlarged and friable in texture with subcapsular hemorrhages, accompanied by fatty degeneration; the kidney was enlarged with nephritis and/or nephrosis with focal hemorrhagic areas with distended ureters with urates; and the intestine revealed enteritis with an unabsorbed yolk sac. Birds in the T2 group had no significant PM lesions with normal PM findings.

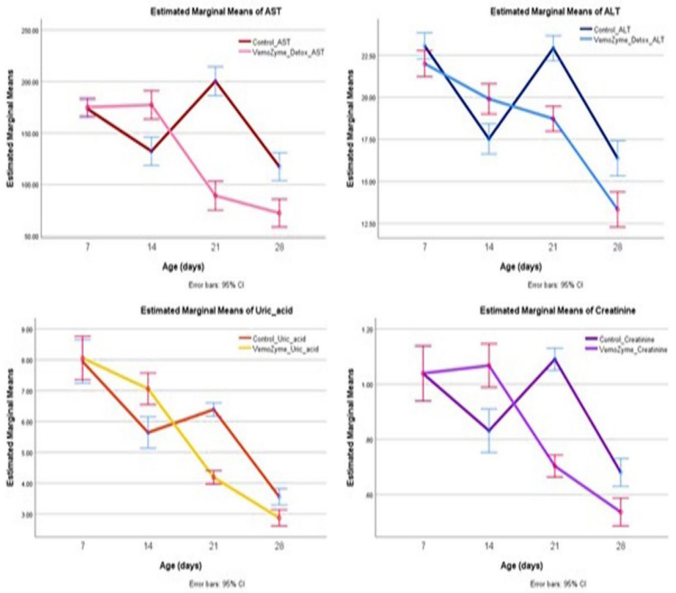

Fig. 1. Effect of antitoxin enzymes supplementation on liver and kidney function blood parameters in Cobb 500 broilers.

Liver histopathology

As presented in Fig. 5, histopathological analysis of the hepatic tissue

Table 3. Effect of antitoxin enzyme supplementation on liver and kidney function blood parameters in Cobb 500 broilers.

Item		Age (days)			
		7	14	21	28
ALT (U/I)	T1	23.06±0.52^{a}	17.53±0.42^{b}	22.92±0.24^{a}	16.38±0.47^{a}
	T2	22±0.18^{a}	19.9±0.48^{a}	18.72±0.47^{b}	13.33±0.57^{b}
AST (U/I)	T1	173.4±5.96^{a}	132.3±8.04^{b}	200.3±3.19^{a}	117.3±5.63^{a}
	T2	175.1±1.62^{a}	177.3±5.5^{a}	89.17±9.42^{b}	72.28±7.74^{b}
Uric acid (mg/dl)	T1	7.94±0.48^{a}	5.64±0.26^{b}	6.39±0.12^{a}	3.56±0.15^{a}
	T2	8.06±0.15^{a}	7.06±0.26^{a}	4.19±0.09^{b}	2.87±0.12^{b}
Creatinine (mg/dl)	T1	1.04±0.07^{a}	0.83±0.04^{b}	1.09±0.02^{a}	0.68±0.03^{a}
	T2	1.04±0.02^{a}	1.07±0.04^{a}	0.7±0.02^{b}	0.54±0.02^{b}

In each row, mean values followed with different superscript letters^{a,b,c} are significantly different (P ≤ 0.05). The values are expressed as the means±SEs.

Table 4. Effect of antitoxin enzyme supplementation on litter clostridial and E. coli counts in Cobb 500 broilers.

Item			Age (days)			
			7	14	21	28
Clostridia (cfu/g)	T1		1.53x10^{6} ±0.06^{a}	5.98x10^{6} ±0.15^{a}	37.4x10^{6} ±2.24^{a}	60.6x10^{6} ±2.76^{a}
	T2		1.40x10^{6} ±0.12^{a}	1.73x10^{6} ±0.1^{b}	2.47x10^{6} ±0.09^{b}	5.28x10^{6} ±0.49^{b}
		Sig.	0.33	<0.001	<0.001	<0.001
E coli (cfu/g)	T1		1.42x10^{6} ±0.29^{a}	12.3x10^{6} ±0.54^{a}	29x10^{6} ±2.29^{a}	51.7x10^{6} ±3.22^{a}
	T2		1.28x10^{6} ±0.31^{a}	5.13x10^{6} ±0.73^{b}	10.1x10^{6} ±0.55^{b}	15.5x10^{6} ±0.96^{b}
		Sig.	0.76	<0.001	<0.001	<0.001

In each row, mean values followed with different superscript letters^{a,b,c} are significantly different (P ≤ 0.05). The values are expressed as the means±SEs.

revealed a normal histological picture of T2, while portal inflammatory cell infiltration associated with necrotic hepatocytes and aggregation of bacterial clusters were detected in examined sections from birds in T1.

Microbiological analysis of the litter samples

Table 4 shows the data concerning the influence of dietary VemoZyme Detox® on bacteriological counts in litter samples throughout 4 weeks in broilers. Dietary VemoZyme Detox® in T2 presented significantly lower (P ≤ 0.05) *C. perfringens* and *E. coli* counts in litter samples from 14 to 28 days of age than in those from T1 at 28 days of age.

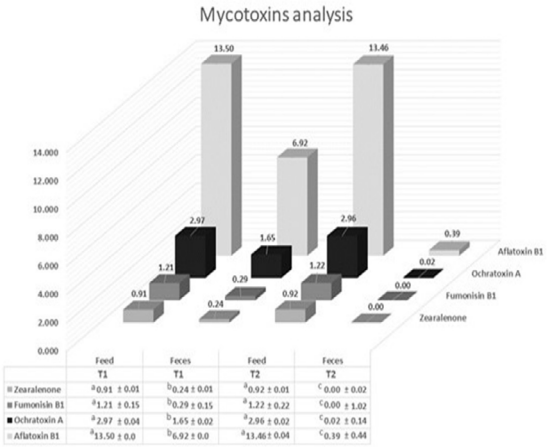

Fig. 2. Effect of antitoxin enzymes supplementation on feed and dropping mycotoxin levels in Cobb 500 broilers.
[a,b,c]The mean values placed at the rows by different superscript letters are significantly different (P ≤ 0.05). Values are expressed as the mean±SE.

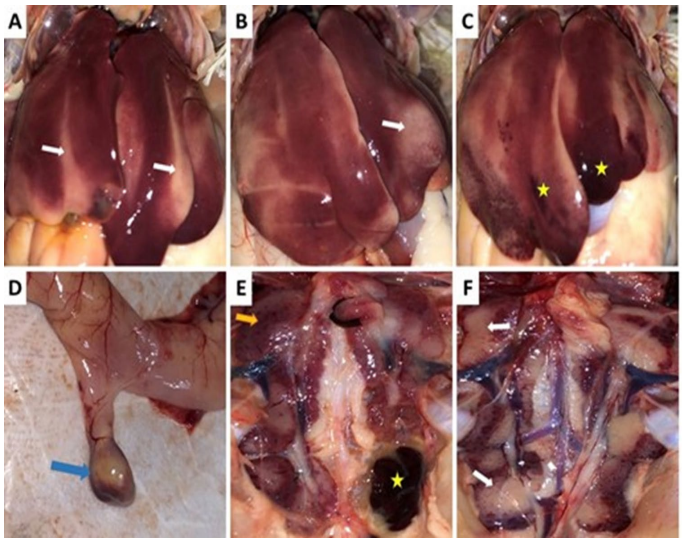

Fig. 3. PM of birds in T1 at the end of the experiment, A, B & C: liver enlarged with friable texture and subcapsular hemorrhage (yellow star) with streaks of fatty degeneration (white arrow); D: intestine showed enteritis with unabsorbed yolk sac (blue arrow). E & F: kidney showed enlargement with severe nephritis (yellow arrow) and severe focal hemorrhages (yellow star), focal nephrosis (white arrow).

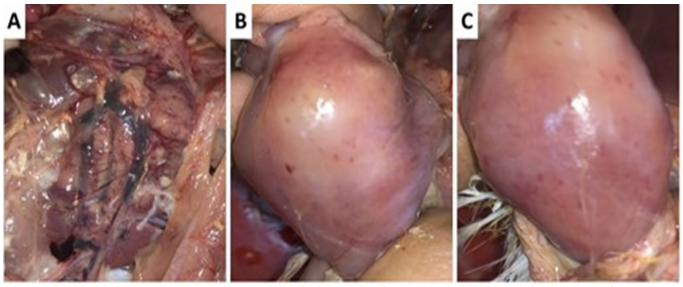

Fig. 4. PM of birds at T1 at the end of the experiment. A: Kidney showing enlargement with severe nephrosis and ureters distended with urates (yellow arrow); B & C: petechial hemorrhages on thigh muscles.

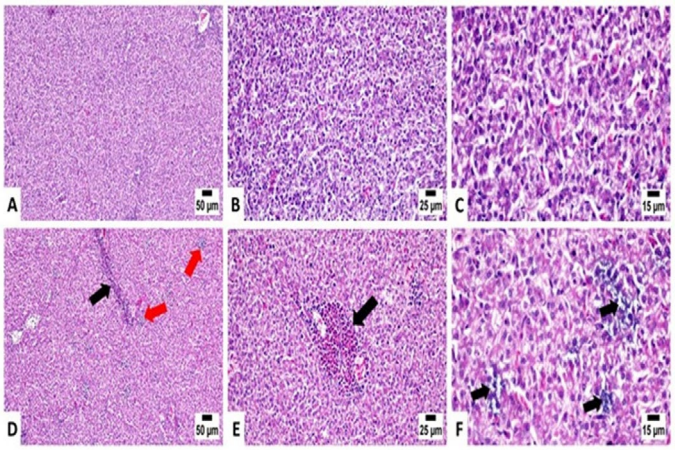

Fig. 5. Effect of antitoxin enzymes supplementation on liver histopathology in Cobb 500 broilers. (A, B, C); Photomicrograph of the liver, T2 group at 28 days showing a normal histological structure of hepatic parenchyma and apparently normal hepatocytes arranged in hepatic plates (H&E). Photomicrograph of the liver, T1 group at 28 days showing portal inflammatory cells infiltration (black arrow) associated with necrotic hepatocytes and aggregation of bacterial clusters (red arrow) (H&E) (D), numerous portal heterophilic infiltration (H&E) (E), and multifocal area of bacterial clusters admixed with necrotic hepatic plates (arrows) (H&E) (F).

Discussion

Contaminated poultry feed containing mycotoxins is a substantial concern within the poultry industry, and mycotoxins have been recognized for their detrimental impact on broiler chicken health and productivity, resulting in significant economic losses (Saleh *et al*., 2023). Moreover, prolonged exposure to small amounts of mycotoxins in avian products raises safety concerns for consumers of these products (Ochieng *et al*., 2021). To combat poultry feed mycotoxins, the authors propose using a combination of environmentally friendly, nontoxic feed-grade toxin binders and safe, biologically active microbial enzymes that are specific to mycotoxicosis. The authors also plan to thoroughly assess the practical and financial effectiveness of this combination before implementing it in commercial settings.

Anti-mycotoxin multienzymes assume a crucial role in degrading and detoxifying mycotoxins present in broiler feed, a function that significantly impacts productivity (Llamas-Moya *et al*., 2020). The current findings provide compelling evidence that dietary supplementation of broiler feed with 300 g of Vemozyme Detox® per ton led to significant improvements in various growth parameters. These improvements encompassed BW, BWG, FI, EPEF, and FCR throughout the study for 28 days (Table 2). These findings align with those of Schatzmayr *et al*. (2006), who demonstrated that the growth performance of broilers could be positively affected by feed supplemented with bacterial enzymes capable of detoxifying mycotoxins in the gut before absorption. Conversely, our findings diverge from those of Ademola *et al*. (2015), who reported no substantial variations in broiler growth performance when fed a ration with Detoxizyme®. The disparities in outcomes can be attributed, in part, to the initial high contamination of maize mycotoxins before the commencement of their study, which may have dampened the efficacy of the enzymes. In contrast, Bedford *et al*. (2022) have underscored the role of exogenous enzyme supplementation in animal feed, emphasizing its capacity to enhance nutrient breakdown, thereby increasing the nutritional value of feed, which leads to improved growth efficiency and enhanced feed utilization. Feed intakes remain consistent, which is crucial for broiler production, where maintaining optimal feed consumption is essential. Furthermore, the reduced FCR among birds receiving VemoZyme Detox® demonstrated its potential to improve nutrient utilization. This finding is in line with other research showing that enzymatic feed additives can enhance the FCR (Diarra *et al*., 2022), which is a pivotal factor for efficient broiler production.

Multienzymes play vital roles in fortifying digestive health by degrading macromolecules, modifying the physiology of the broiler's gut, and reshaping the composition of its bacterial community. These actions cumulatively bolster nutrient digestibility (Madigan-Stretton *et al*., 2020). In response to this issue, glucose oxidase has been incorporated into poultry feed production, primarily with the goal of enhancing growth rates and bolstering antioxidant activity. Our data were in accordance with those of Wu *et al*. (2019), who noted that the addition of glucose oxidase improved growth performance, as indicated by increased average daily gain and improved FCR in 21-day-old broilers. This augmentation in growth performance may be due to the amelioration of mycotoxicosis effects, the modification of the intestinal microbiota composition, and the improvement in digestibility and nutrient absorption due to the pres-

ence of glucose oxidase. Furthermore, our positive results with the addition of Vemozyme Detox® in terms of performance can be linked to the contents of acid, neutral, and alkaline proteases, as reported by Freitas *et al*. (2011), as their study emphasized the impact of proteases on the improvement of nutrient digestion and bioavailability. Similarly, Aghili *et al*. (2019) showed that supplementation with a high dose of enzymes significantly improved the villus height and crypt depth of the jejunum ($P \leq 0.05$). Importantly, this supplementation also led to a significant decrease in mortality ($P \leq 0.05$; Table 2). High mortality rates can adversely affect the economics of broiler production. The decrease in mortality suggested that VemoZyme Detox® contributes to enhanced overall health and reduced stress in broilers.

The evaluation of mycotoxin levels revealed that while feed samples did not significantly differ among the control and treated birds, the levels of mycotoxin in the droppings of the treated group, particularly for zearalenone and fumonisin B1, were significantly ($P \leq 0.05$) lower (Fig. 2). Mycotoxins are known to impair broiler health and productivity (Zain, 2011). The decrease in droppings' mycotoxin levels suggested that VemoZyme Detox® can aid in vivo detoxification and transformation of mycotoxins, mitigating mycotoxin absorption and its negative effects. In concurrence with this, Madigan-Stretton *et al*. (2020) noticed improvements in villus height, width, and crypt depth in the duodenum with the superdosing of multienzymes. Additionally, there was an increase in villus width and the number of goblet cells in the jejunum. These results indicate that the extension of intestinal villi, facilitated by enzyme supply and mycotoxin detoxification, enhances the availability of nutrients for absorption, thereby favoring an anabolic response and muscular growth (Attia *et al*., 2012).

As demonstrated in Table 3, throughout the 28-day study, hepatic function enzymes (ALT and AST), as well as renal function metabolic indicators (uric acid and creatinine), exhibited a significant ($P \leq 0.05$) reduction in T2, in contrast to T1, mainly after 21 days of age (Fig. 1). It appears that the Vemozyme Detox® supplement effectively ameliorates the adverse effects of mycotoxins on hepatic and renal tissues, possibly through the presence of glucose oxidase and mycotoxin detoxification enzymes, as discussed by Qu and Liu (2021), as their research suggested that the glucose oxidase supply reduces the inflammatory reaction induced by mycotoxin exposure by decreasing liver function enzyme levels. Additionally, Alharthi *et al*. (2022) reported that the addition of bentonite has a similar ameliorative effect on aflatoxin-exposed livers. These results align with those of Amer *et al*. (2018), who reported that bentonite supplementation overcomes the negative effects of aflatoxins, improving growth performance and decreasing the relative weights of hepatic and renal tissues, which are typically elevated by aflatoxin exposure.

Lower uric acid and creatinine levels in T2 birds suggest reduced renal stress. This is a promising indicator of overall bird health, as high uric acid and creatinine levels can indicate renal dysfunction (Mueller *et al*., 2022). Previous research conducted by Ademola *et al*. (2015) indicated similar effects on ALT and AST levels and a reduction in uric acid levels due to the combined action of multienzymes in Detoxizyme®. On the other hand, Attia *et al*. (2020) reported significant reductions in ALT and AST levels in multienzyme-supplemented birds compared with those in control birds. Additionally, studies conducted by Yang *et al*. (2010) revealed that supplementation with a multienzyme compound comprising carbohydrases and proteases resulted in enhanced energy utilization and higher levels of protein, phosphorus, and calcium in broilers, which mirrored the improved hepatic functions.

The results of our study, particularly the examination of PM lesions in the muscles, kidney, intestine, and liver (Figs. 3 and 4) and liver histopathology (Fig. 5), revealed signs of inflammation in the control group (T1), while T2, the hepatic parenchyma, retained a normal histological structure, indicative of healthy liver tissue. In contrast, T1 hepatic photomicrograph exhibited portal inflammatory cell infiltration and necrotic hepatocytes. Bakeer *et al*. (2013) reported similar PM lesions due to mycotoxicosis in chickens. These findings align with the observations of Gao *et al*. (2022), who highlighted the significant contribution of glucose oxidase to improving liver functions in broilers by reducing key indicators such as AST, ALT, and liver lesion scores. Similarly, our results are consistent with those of Amer *et al*. (2018), who reported that the addition of bentonite effectively decreased histopathological alterations in hepatic, renal, and intestinal tissues caused by aflatoxin-contaminated diets. Mgbeahuruike *et al*. (2018) also demonstrated the potency of bentonite in preventing histopathological effects associated with aflatoxicosis. Furthermore, Miazzo *et al*. (2005) noted that the addition of bentonite to broiler diets contaminated with aflatoxin and fumonisin resulted in a reduction in multifocal and varied cytoplasmic vacuolization by mitigating the severity of hepatic histopathological alterations linked to aflatoxicosis. In line with our findings revealing fatty liver changes, Attia *et al*. (2020) observed that broiler diets supplemented with antitoxin multienzymes led to reduced plasma cholesterol and low-density lipoprotein levels while elevating high-density lipoprotein and albumin levels. However, in contrast to these results, El-Katcha *et al*. (2014) did not observe significant alterations in lipid profiles in chickens that received the same feed without enzyme supplementation.

The influence of multienzymes on avian immunity was revealed by the presence of an unabsorbed yolk sac in the PM image of the T1 group (Fig. 3). Research has indicated that aflatoxicosis can lead to decreased immunological responses that induce opportunistic bacteria to be pathogenic and develop lesions such as unabsorbed yolk sacs (El-Moneim *et al*., 2020). Our data parallel those of Liu *et al*. (2017), who proved that the use of multiple antitoxin enzymes improved immunity, reduced *C. perfringens* numbers, and decreased antibody levels compared with aflatoxins in broilers. Furthermore, when antitoxin multienzymes were mixed with pig feed and subjected to fumonisin, these enzymes lessened the negative impacts of mycotoxins on hepatic tissues, lungs, and jejunum while improving immunity (Grenier and Applegate, 2013). The improved immunological function, attributed to increased nutritional availability resulting from multiple enzyme supplements, was further corroborated by previous research (Yang *et al*., 2010; Saleh and Alzawqari, 2021). The observed tissue lesions in the muscles, liver, kidney, and intestines are likely due to the disruptive effects of mycotoxins. Mycotoxins can interfere with hematopoiesis; metabolize proteins, fats, and carbohydrates; dilate arterioles; cause tissue congestion; disrupt calcium absorption and coagulation factors; and reduce cellular metabolic activity. These effects lead to weakened blood vessels and result in hemorrhages in different organs (Zabiulla *et al*., 2021).

The microbiological analysis of the litter samples suggested the potential antimicrobial effects of VemoZyme Detox® enzymes. Notably, there was a significant decrease in clostridia and *E. coli* bacterial counts in the T2 group compared to those in the T1 group (Table 4). This reduction in the litter sample density can be attributed to the ameliorating effect of glucose oxidase. Glucose oxidase catalyzes the transformation of glucose into gluconic acid and hydrogen peroxide, a process that significantly consumes intestinal oxygen. The end product, gluconic acid, contributes to a decrease in gastrointestinal pH, creating an acidic environment favorable for the development of lactic acid bacteria. Additionally, the hydrogen peroxide generated during this reaction sterilizes and directly inhibits the growth and proliferation of bacteria such as *E. coli*, *Salmonella* spp., *Pasteurella multocida*, and *Staphylococcus* spp., as previously reported (Fang *et al*., 2015). Furthermore, Borda-Molina *et al*. (2019) noted that the addition of protease enzymes decreases clostridia counts. Notably, contrasting findings were reported in studies conducted by Madigan-Stretton *et al*. (2020), who reported no significant differences in the prevalence of linked bacteria or microbial diversity within multienzyme-treated groups.

Conclusion

This study revealed that the anti-mycotoxin multienzyme VemoZyme Detox® has multifaceted benefits for broiler production. It has the potential to enhance growth performance, maintain essential blood parameters, reduce mycotoxin absorption, protect liver health, and mitigate bacterial proliferation. These findings hold significant promise for the poultry industry, as supplementation with VemoZyme Detox®, which is used at a dose of 300 g/ton in a broiler ration, can lead to healthier and more productive broilers. Nevertheless, further work is necessary to explore the underlying mechanisms and refine the application strategies for this enzymatic detoxifier.

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Conflict of interest

The authors confirm that they have no competing interests to declare.

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