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RESEARCH ARTICLE

The Effect of Nano-chitosan in Reducing the Toxicity of Aflatoxin B1 and Fumonisin B1 in Broilers

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ABSTRACT

This study aimed to evaluate the impact of adding nano-chitosan to feed contaminated with aflatoxin and fumonisin on the performance, carcass quality, organ weights, immunoglobulin levels, blood profiles, and histopathology of broilers. A total of 96 Ross-308 male broiler chicks were divided into 8 experimental groups with 3 replicates of 4 chicks each for a period of 42 days. The experimental groups included a control group (no treatment), group receiving 2.81mg/kg of aflatoxin-B1 (AFB1), 332.44mg/kg of fumonisin-B1 (FB1), or a combination of both of AFB1+FB1. In addition, nano-chitosan (NC) was supplemented at the rate of 0.5g/kg to these diets, resulting in the AFB1 + NC, FB1 + NC, and AFB1 +NC groups. Individual body weight (BW) and feed intake (FI) were measured at 0, 7, 21, and 42 days to calculate body weight gain (BWG) and feed conversion ratio (FCR). The carcass traits, internal organ weights, blood components and immunoglobulins (IgA, IgG, and IgM) levels in blood were determined on day 42. Addition of AFB1, FB1, or their combination significantly reduced BW, BWG, FI, FCR, and carcass traits ($P<0.05$). The supplementation of NC partially improved performance values in infected broilers. The cooking loss, drip loss, and freezing loss percentages of meat were significantly higher in the AFB1, FB1, or their combination when compared to the NC groups ($P<0.05$). The hematological and immunoglobulins parameters were significantly reduced in infected broilers while the addition of NC restored these parameters ($P<0.01$). The AFB1, FB1, or their combination caused pathological lesions in the liver of broilers and addition of NC treatment partially restored these anomalies. In conclusion, adding AFB1, FB1, or their combination in the diets depressed performance traits, carcass quality, blood components, and the addition of NC showed improvements in these parameters.

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INTRODUCTION

Mycotoxins are the toxic compounds being produced in food and feed by certain fungi as their secondary metabolites. These fungi can contaminate various feeds and foods in different stages of their production, harvest and storage (Tilley *et al*., 2017). They can develop rapidly in food products when suitable moisture and heat conditions are present in the medium (Ochieng et al., 2021). A study by the FAO (Food and Agriculture Organization of the United Nations) found mycotoxin contamination rates of up to 25% in worldwide crop products (Wang et al., 2023). Presence of mycotoxin can cause about 2% loss in the nutritional and economic values of the feed (Eskola *et al.*, 2020).

Aflatoxin B1 (AF-B1) is a secondary metabolic compound produced by the molds *Aspergillus flavus* and *Aspergillus parasiticus*. It is one of the most prevalent fungal toxins in poultry feeds and causes aflatoxicosis in different production periods (Khatoon *et al.*, 2024).

Interestingly, more than 85% of recorded food and feed poisoning cases are caused by AFB1 (Pitt and Miller, 2021). On the other hand, *Fusarium* species, particularly *Fusarium verticillioides* and *F. proliferatum*, are known to produce fumonisin, as their secondary metabolite (Groff-Urayama *et al.*, 2022). There are about 28 different kinds of fumonisins, but fumonisin B1 (FB1) is considered as the most common toxins in poultry feed. In cases of *Fusarium* fungal contamination, 70–80% of the total food contamination is caused by FB1 (Batikh *et al.*, 2021). The detrimental effects of these toxins have been demonstrated in numerous research works (Bello *et al.,* 2019) and it has been well reported that mycotoxin-induced food poisoning can have serious consequences, including cell death (Awuchi *et al.*, 2022a). Long-term exposure of these mycotoxins in low concentrations may cause immunosuppression and oncogenic disorders (Ahmad *et al.*, 2022; Saha Turna *et al*., 2024). The type and dosage of the mycotoxin, as well as the organs impacted, determine the degree and nature of these health issues (Awuchi *et al.*, 2022b).

Chitosan is a natural polysaccharide synthesized from chitin via deacetylation process that entails the removal of acetyl groups from chitin (Aranaz *et al.*, 2021). Chitosan is considered to have various bioactivities, such as immune-enhancer and antibacterial activities. Also, this polysaccharide has been used as an effective adjuvant for the delivery of biological materials, such as vaccines and drugs. Nano-chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine (Szymczyk *et al.*, 2016).

Chitosan may enhance the digestion and absorption of nutrients by serving as a natural substrate for beneficial microbes (Ayman *et al.*, 2022). Nano-chitosan may also serve as an effective adsorption material, notably in mitigating aflatoxin and fumonisin, hence yielding beneficial effects in diminishing their detrimental effects (Ding *et al.*, 2023). The study was carried out to determine the effects of nano-chitosan addition to AFB1 and FB1 contaminated diets on performance, carcass quality, immunity parameters, and liver histology.

MATERIALS AND METHODS

Experimental design, broilers, and diets: Animal care procedures for the experiment were approved (No: 22/204) by the 'Local Ethics Committee of Animal Experiments' of Erciyes University, Kayseri, Türkiye. A total of 96-one-day old Ross-308 male broiler were individually weighed and divided into 8 experimental groups with 3 replicates of 4 chicks each. The experimental groups were as follows:

Groups	Treatments
Control	C, basal diet, no mycotoxin and nano-chitosan addition
AFB1	Addition of Aflatoxin B1 (2.81mg/kg),
FB1	Addition of Fumonisin B1 (332.44mg/kg),
AFB1+FB1	Addition of AFB1 (2.81mg/kg) +FB1 (332.44mg/kg)
NC	Addition of nano-chitosan, (0.5g/kg)
AFB1+NC	Addition of AFB1(2.81mg/kg) + NC (0.5g/kg)
FB1+NC	Addition of FB1 (332.44mg/kg) + NC (0.5g/kg)
AFB1+FB1+NC	Addition of AFB1 (2.81mg/kg) + FB1(332.44mg/kg) + NC (0.5g/kg)

The chicks were raised in semi-open poultry houses in wire cages with dimensions of 100cm (length) x 100cm (width) x 60cm (height). Feed was provided in feeders for each cage, along with the water system. The lighting program used LED lights with 24hr of daylight for the first 3 days, followed by 23hr of daylight and 1hr of darkness from day 4 to day 42. The poultry house was well ventilated for fresh air. The birds were randomly assigned to the treatments, and their average weight was 41.8±1.01g. The poultry house temperature was adjusted for 35 °C during the first 24hr and then the temperature was reduced to 33 °C until the end of the first week, followed by a decrease of 2 °C every week to meet the birds' thermal needs. Water and feed were provided *ad libitum*.

Procurement of Nano-chitosan: Chitosan nanoparticles (CNPs) with molecular formula $C_{56}H_{103}N_9O_{39}$, having particle size (80-100nm) and a purity of 99.5% was purchased from EPRUI, Biotech Co., Ltd. China.

Production of Aflatoxin B1: AFB1 was prepared by growing *Aspergillus flavus* 3357 on rice as per the method of Shotwell *et al.* (1966), and the rice grains containing the growing mold were sterilized in an autoclave at 121°C for 10min, then dried and ground to a fine powder.

Production of Fumonisin B1: Fumonisin B1 was prepared by growing the mold *Fusarium moniliforme* on sterilized yellow corn as per the method of (Shephard *et al.*, 1999), and the corn was then dried at 45°C until the next day and ground into a fine powder.

Analysis of AFB1 and FB1: Extraction was doe following the protocol described by Shenzhen Lvshiyuan Biotechnology Co., Ltd., China. Crushed samples (1.0 ± 0.05g) were mixed with 5ml of extraction solution (methanol: deionized water, 7:3), shaken for 5min, and centrifuged at 4000rpm for 10min. The supernatant (100µl) was diluted with redissolving solution (1:19) and shaken. A 50µl aliquot was analyzed using an ELISA spectrometer (450nm) with a Bio-TEK instrument ING. AFB1 and FB1 were estimated using a commercial kit (Shenzhen Lvshiyuan Biotechnology Co., China).

Histological examination: At the end of the study periods, slaughtering was done, and liver samples were collected from 6 birds in each treatment and fixed in 10% phosphate-buffered formaldehyde (pH 7.4), for histopathological examination. Sections of 4mm thickness were obtained in a rotary microtome, deparaffinized, rehydrated, and stained with hematoxylin-eosin (Ijaz *et al.*, 2023). Observation of histopathology of liver were carried out via an Olympus-CX41 RF light microscope (Olympus Corporation, Tokyo, Japan).

Determination of blood profiles: At the time of slaughtering, blood samples were collected from the birds (6 samples from each treatment, 2 samples from each replicate). The blood was collected in tubes containing the anticoagulant (K-EDTA). Whole blood was used to conduct hematological analysis, which included the red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), the packed cell volume (PCV) percentage, heterophil percentage, lymphocytes percentage and the

ratio of heterophil cells to lymphocytes (L/H) percentage. The concentration of IgA, IgG, and IgM was measured using different commercial kits (Life Diagnostics, USA).

Determination of meat cooking loss: To determine meat cooking loss the chest and hip meat samples were cut into cubes with a diameter of 1cm x 1cm and placed in sealed heat-resistant polyethylene bags. Then, the bags were placed into water bath at 75 °C for 1hr and re-weighed according to Honikel (1998). Cooking loss (CL) was calculated with this formula, CL= [(weight before cooking - weight after cooking): weight before cooking] × 100.

Determination of meat-dropping loss: To determine drip loss, 1cm x 1cm cubed meat samples taken from the breast and hip were weighed and the samples were hung in an inflatable bag with a string to prevent them from touching the bag and collapsing in the bag. After a 24hr storage period at 4 °C temperature, the samples were removed, the drip was gently removed with blotting paper and weighed. Drip loss was calculated as a percentage of the initial weight.

Determination of meat freezing loss: To determine meat freezing loss the samples were taken from the chest and hip (1cm x 1cm cubed meat samples) and placed in polyethylene bags. After that, samples were frozen at a temperature of -20 °C until well frozen for 24h, then taken out of the freezer, and left at room temperature until they were completely thawed. The exuding liquid was removed and re-weighed; the freezing loss was calculated by the difference between the weights.

Statistical analysis: The data were analyzed using IBM SPSS Statistics 26. To conduct an analysis of variance, one-way ANOVA was used and the Duncan test with $P<0.05$ was employed to detect significant differences between the means.

RESULTS

The effects of AFB1, FB1, and NC on body weight (g) and average daily body gain of broilers are shown in Table 1. Body weight and body weight gain was significantly ($P<0.05$) higher in NC group compared to AFB1+FB1, FB1 and AFB1 groups on day 7, 21 and 42. Supplementation of NC to the infected groups partially restored the body weight and body weight gain in broilers. The effects of AFB1, FB1, and NC on the average daily feed intake (FI) and feed conversion ratio (FCR) of broilers are shown in Table 2. Broilers in the control and NC groups had significantly higher feed intake in all periods compared to aflatoxin-treated birds individually or in combination. The addition of NC to toxin-contaminated diet improved feed intake but not to the extent of control level. Additionally, FCR was significantly poorer in toxin-exposed diets, with the highest value recorded in AFB1+FB1 group ($P<0.01$). NC supplementation restored FCR bringing it closer to the control. No significant difference was observed in FCR during the 35-42 days.

Table 3 presents the effects of aflatoxin B1, fumonisin B1, and nano-chitosan on the internal organs of broilers. Broilers in the aflatoxin-treated groups had significantly ($P<0.05$) lower carcass weight and carcass yield compared to the control and NC groups. The lowest value was observed in the AFB1 + FB1 group. NC supplementation improved these parameters in the toxin-exposed group but did not fully restore them to the control level. Liver, heart, and spleen percentages were significantly ($P<0.05$) higher than AFB1, FB1, and their combination groups compared to the control. The NC addition reduced these organs weight in toxin-exposed birds. The bursa of Fabricius percentage was significantly ($P<0.01$) lower in toxin-exposed groups compared to the control, but NC supplementation partially improved it.

Table 4 provides the effects of aflatoxin B1, fumonisin B1, and nano-chitosan on carcass yield. Aflatoxin B1 and fumonisin B1 exposure significantly ($P<0.01$) increased hip percentage while reducing wing and neck percentages compared to the control group. The lowest neck percentage was observed in the AFB1 group. Nano-chitosan supplementation mitigated these effects, improving carcass traits in toxin-exposed groups. However, chest percentage remained statistically unchanged across all groups.

Table 5 shows the effects of AFB1, FB1, and NC on the cooking loss percentage. Aflatoxin B1 and fumonisin B1 significantly ($P<0.01$) increased cooking loss, and freezing loss in both chest and hip meat compared to the control. The highest values were observed in the Aflatoxin B1 and fumonisin B1 group, indicating a compounded negative effect. Nano-chitosan supplementation mitigated these losses, with values approaching the control group, particularly, in the Aflatoxin B1 and NC and FB1 +NC groups. However, freezing loss remained relatively higher in the FB1 + NC and AFB1 +FB1 + NC groups, suggesting incomplete protection.

Table 6 presents the results of aflatoxin B1, fumonisin B1, and nano-chitosan on blood profile analysis of broilers. Aflatoxin treatment significantly ($P<0.01$) reduced RBC count, Hb count, and PCV while increasing WBC count and heterophil percentage, indicating immunosuppression and hematological stress. The combined effect of aflatoxin showed the most severe effects. Nano-chitosan supplementation mitigated these alterations, with RBC, Hb, and PCV values in the AFB1 +NC and FB1 + NC groups approaching control levels. Additionally, WBC counts and heterophil percentages decreased in NC-supplemented groups, lowering H/L ratio compared to individual toxin groups. However, the AFB1 + FB1+ NC group showed partial recovery, suggesting incomplete protection.

Table 7 presents the effects of aflatoxin B1, fumonisin B1, and nano-chitosan on immunoglobulins of broilers. Treatment of Aflatoxin B1 and fumonisin B1 significantly ($P<0.01$) reduced IgA, and IgM levels, with the lowest values observed in the AFB1 + FB1 group, indicating immunosuppression. Nano-chitosan supplementation improved immunoglobulin levels, with the NC-group showing the highest level. The AFB1+NC, FB1+NC, and AFB1+FB1+NC groups exhibited partial restoration, suggesting that nano-chitosan mitigated, but did not fully counteract the immunosuppressive effects of mycotoxin.

The liver histological examination is shown in Fig. 1. The liver tissues were analyzed by microscopy and clearly showed structural differences between groups. With the control group, normal liver architecture was

Table 1: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on body weight weekly and average daily body gain of broilers, g.

Groups	Body weight, g/bird				Daily body weight gain, g/bird		
	0 d	7 d	21 d	42 d	0-21 d	21-42 d	0-42 d
Control	42.60+0.88	166.28±2.09^{ab}	863.08±15.30^{b}	2963.67±57.69^{b}	39.10±0.73^{b}	100.03±2.02^{b}	69.56±1.37^{b}
AFB1	41.40+1.08	154.06±2.23^{d}	639.75±13.59^{f}	2084.00±45.65^{f}	28.46±0.65^{f}	68.77±1.61^{f}	48.62±1.09^{f}
FB1	42.00+0.84	154.99±1.65^{cd}	683.08±14.02^{e}	2264.08±37.92^{e}	30.53±0.67^{e}	75.29±1.14^{e}	52.91±0.90^{e}
AFB1+FB1	41.40+1.08	147.07±1.29^{e}	602.75±10.38^{g}	1982.17±39.13^{f}	26.70±0.50^{g}	65.69±1.37^{f}	46.19±0.93^{f}
NC	42.80+1.00	169.12±2.33^{a}	925.00±4.13^{a}	3197.42±15.61^{a}	42.05±0.20^{a}	108.21±0.55^{a}	75.13±0.37^{a}
AFB1+ NC	41.60+0.93	157.78±2.33^{cd}	784.08±12.40^{c}	2678.25±29.60^{c}	35.34±0.59^{c}	90.20±0.82^{c}	62.77±0.71^{c}
FB1+NC	41.80+1.01	160.85±1.64^{bc}	787.92±4.62^{c}	2679.00±16.62^{c}	35.52±0.22^{c}	90.05±0.57^{c}	62.79±0.40^{c}
AFB1+FB1+NC	41.00+0.91	156.58±1.81^{cd}	750.58±2.97^{d}	2535.08±11.74^{d}	33.74±0.14^{d}	84.98±0.42^{d}	59.36±0.28^{d}
P	0.610	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, P: probability, **:P<0.01.

Table 2: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on average daily feed intake and feed conversion ratio (FCR) of broilers.

Groups	Daily feed intake, g/bird/day			Feed conversion ratio, g feed/g gain		
	0-21 d	21-42 d	0-42 d	0-21 d	21-42 d	0-42 d
Control	50.69±0.31^{b}	164.15±1.20^{b}	107.42±0.76^{b}	1.30±0.03^{e}	1.64±0.04^{c}	1.55±0.03^{c}
AFB1	43.14±0.33^{d}	140.48±5.63^{de}	91.81±2.98^{e}	1.52±0.04^{b}	2.04±0.06^{a}	1.89±0.05^{a}
FB1	44.91±0.86^{d}	140.94±5.37^{de}	92.92±3.09^{de}	1.47±0.04^{bc}	1.87±0.07^{b}	1.76±0.06^{b}
AFB1+FB1	43.75±0.73^{d}	135.03±3.69^{e}	89.39±2.21^{e}	1.64±0.04^{a}	2.06±0.07^{a}	1.94±0.06^{a}
NC	54.34±0.45^{a}	179.47±1.49^{a}	116.90±0.96^{a}	1.29±0.01^{e}	1.66±0.02^{c}	1.56±0.02^{c}
AFB1+ NC	48.67±0.69^{c}	156.43±1.34^{bc}	102.55±1.01^{bc}	1.38±0.04^{ade}	1.74±0.03^{bc}	1.63±0.03^{bc}
FB1+NC	47.73±0.09^{c}	151.82±0.38^{cd}	99.78±0.21^{c}	1.35±0.01^{de}	1.69±0.01^{c}	1.59±0.01^{c}
AFB1+FB1+NC	47.21±1.07^{c}	150.57±4.48^{cd}	98.89±2.77^{cd}	1.40±0.03^{cd}	1.77±0.05^{bc}	1.67±0.04^{bc}
P	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, P: probability, **:P<0.01.

Table 3: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on internal organs.

Groups	Hot carcass weight, g	Carcass yield, %	Liver, %	Heart, %	Spleen, %	Bursa fabricius, %
Control	2242.67±29.29^{b}	75.41±0.34b	2.52±0.03^{c}	0.56±0.02^{c}	0.23±0.01^{c}	0.40±0.03^{a}
AFB1	1496.50±25.58^{f}	72.40±0.36cd	3.66±0.05^{a}	0.83±0.05^{a}	0.41±0.01^{a}	0.23±0.01^{c}
FB1	1609.17±37.56^{e}	72.52±0.36de	3.51±0.06^{a}	0.81±0.05^{a}	0.40±0.01^{a}	0.23±0.01^{c}
AFB1+FB1	1437.33±24.33^{f}	72.07±0.37e	3.66±0.10^{a}	0.82±0.05^{a}	0.40±0.01^{a}	0.24±0.02^{c}
NC	2483.33±22.84^{a}	76.68±0.64a	2.51±0.03^{c}	0.63±0.04^{bc}	0.24±0.00^{c}	0.40±0.02^{a}
AFB1+ NC	2014.67±26.81^{c}	73.92±0.29c	3.06±0.11^{b}	0.65±0.0^{bc}	0.33±0.01^{b}	0.32±0.02^{b}
FB1+NC	2014.83±21.09^{c}	73.78±0.21c	2.99±0.10^{b}	0.66±0.04^{bc}	0.32±0.01^{b}	0.31±0.02^{b}
AFB1+FB1+NC	1838.67±14.23^{d}	72.85±0.24cde	3.21±0.09^{b}	0.71±0.04^{ab}	0.33±0.01^{b}	0.33±0.02^{b}
P	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, P: probability, **:P<0.01.

Table 4: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on carcass yield in 42 days old of broilers.

Groups	Chest percentage	Hip percentage	Wing percentage	Neck percentage
Control	37.08±1.67	37.99±0.61^{ab}	12.77±0.37^{ab}	3.89±0.22^{a}
AFB1	35.79±1.35	40.38±0.98^{a}	11.01±0.50^{c}	2.91±0.09^{d}
FB1	35.24±1.06	39.77±0.57^{a}	11.63±0.28^{bc}	3.00±0.15^{cd}
AFB1+FB1	35.46±2.39	40.14±1.70^{a}	11.41±0.50^{bc}	3.12±0.10^{cd}
NC	38.63±1.12	37.94±0.42^{ab}	13.24±0.38^{a}	3.60±0.15^{ab}
AFB1+NC	38.34±1.27	36.19±0.97^{b}	11.70±0.41^{bc}	3.04±0.19^{cd}
FB1+ NC	39.14±1.98	37.94±0.50^{ab}	12.04±0.49^{abc}	3.24±0.11^{bcd}
AFB1+FB1+NC	36.47±2.14	36.71±0.75^{b}	12.24±0.43^{abc}	3.47±0.15^{abc}
P	0.581	0.012**	0. 014**	0.000**

a, b, c: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, P: probability, **:P<0.01.

Table 5: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on cooking %, drop %, and freezing % loss of broiler meat

Groups	Cooking loss percentage		Drip loss percentage		Freezing loss percentage	
	Chest	Hip	Chest	Hip	Chest	Hip
Control	16.84±0.89^{b}	21.65±0.80^{c}	2.50±0.29^{c}	2.36±0.35^{b}	2.45±0.18^{d}	2.15±0.25^{b}
AFB1	21.31±0.85^{a}	25.17±1.16^{ab}	5.26±0.41^{a}	4.49±0.84^{a}	6.99±0.90^{a}	4.29±0.39^{a}
FB1	20.23±0.89^{a}	25.20±0.45^{ab}	4.72±0.35^{ab}	4.62±0.55^{a}	7.19±0.70^{a}	4.52±0.80^{a}
AFB1+FB1	19.01±0.59^{ab}	27.15±0.96^{a}	5.36±0.33^{a}	5.03±0.60^{a}	7.08±0.56^{a}	4.33±0.78^{a}
NC	16.57±0.95^{b}	21.14±1.06^{c}	2.54±0.27^{c}	2.03±0.32^{b}	3.06±0.22^{d}	2.07±0.21^{b}
AFB1+ NC	18.31±1.58^{ab}	22.78±1.52^{bc}	3.78±0.64^{bc}	3.48±0.69^{ab}	5.73±0.65^{ab}	3.03±0.42^{ab}
FB1 +NC	16.85±1.29^{b}	24.90±0.35^{ab}	4.11±0.52^{ab}	3.35±0.43^{ab}	6.69±0.31^{a}	3.45±0.52^{ab}
AFB1+FB1+NC	18.28±0.93^{ab}	23.99±0.80^{bc}	3.95±0.60^{ab}	3.67±0.79^{ab}	4.65±0.63^{bc}	4.08±0.16^{a}
P	0.021*	0.001**	0.000**	0.009**	0.000**	0.002**

$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: cano-chitosan, P: probability, **:P<0.01.

observed with its well-structured hepatocytes having spherical nuclei and normal sinusoids with just a couple of Kupffer cells distributed. On the other hand, the AFB1 group had lymphocytic nodules, increased Kupffer cell infiltration, and disrupted sinusoids. Atypical hepatic morphology was observed for the FB1 group with

Table 6: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on blood hematocrit profile analysis of broilers

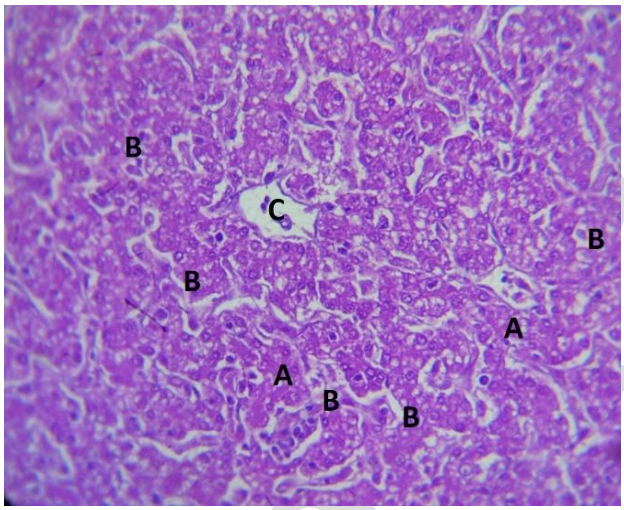
Groups	RBC (x 10^6 /mm^3)	WBC (x 10^6 /mm^3)	Hbg/dl	PCV %	Heterophil %	Lymphocytes %	H/L
Control	2.67±0.03^{a}	23.46±0.63^{a}	8.72±0.33^{a}	40.00±0.82^{a}	24.33±0.61^{c}	61.33±1.12^{a}	0.40±0.01^{e}
AFB1	2.39±0.07^{b}	31.29±1.64^{d}	4.54±0.41^{d}	32.83±1.05^{c}	27.50±0.56^{ab}	56.83±1.01^{b}	0.49±0.01^{abc}
FB1	2.43±0.12^{ab}	28.61±1.42^{cd}	5.14±0.45^{cd}	33.00±1.13^{c}	28.00±0.68^{a}	57.33±0.92^{b}	0.49±0.02^{ab}
AFB1+FB1	2.38±0.07^{b}	30.51±1.44^{d}	4.40±0.37^{d}	32.00±1.51^{c}	28.33±0.56^{a}	57.17±1.49^{b}	0.50±0.02^{a}
NC	2.67±0.01^{a}	23.41±1.02^{a}	8.94±0.38^{a}	39.33±0.92^{ab}	25.00±0.58^{c}	61.17±1.19^{a}	0.41±0.02^{de}
AFB1+ NC	2.61±0.03^{ab}	26.07±0.79^{b}	6.92±0.60^{b}	37.33±2.30^{ab}	26.00±0.58^{bc}	58.00±1.21^{ab}	0.45±0.01^{bcd}
FB1 +NC	2.63±0.04^{a}	26.21±1.04^{b}	7.08±0.63^{b}	38.17±1.25^{ab}	25.67±0.56^{bc}	58.00±1.03^{ab}	0.44±0.01^{cde}
AFB1+FB1+NC	2.59±0.14^{ab}	27.37±1.27^{bc}	6.50±0.67^{bc}	35.50±1.59^{bc}	25.83±0.60^{bc}	58.17±1.30^{ab}	0.44±0.01^{cde}
P	0.022*	0.000**	0.000**	0.000**	0.000**	0. 050*	0.000**

$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, RBC: red blood cell, WBC: white blood cell, Hb: hemoglobin, PCV: packed cell volume, H/L: heterophil/lymphocytes, P: probability, **:P<0.01.

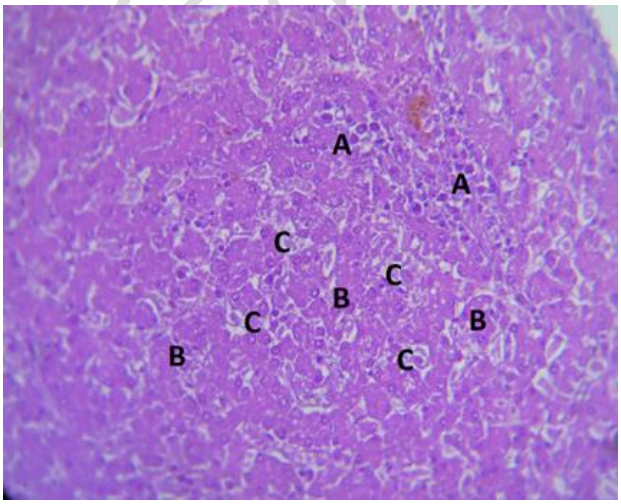
Table 7: Effects of aflatoxin B1, fumonisin B1, and nano-chitosan on immunoglobulins of broilers

Groups	IgA (mg/dl)	IgG (mg/dl)	IgM (mg/dl)
Control	5.96±0.48^{ab}	5.01±0.24^{ab}	6.35±0.32^{ab}
AFB1	3.37±0.36^{de}	2.47±0.23^{c}	3.57±0.52^{d}
FB1	3.32±0.31^{de}	2.88±0.30^{c}	3.60±0.30^{d}
AFB1+FB1	3.13±0.24^{e}	3.04±0.17^{c}	3.31±0.33^{d}
NC	6.63±0.39^{a}	5.29±0.47^{a}	7.19±0.45^{a}
AFB1+ NC	4.48±0.45^{cd}	4.20±0.28^{b}	4.70±0.19^{cd}
FB1 +NC	5.14±0.51^{bc}	4.21±0.30^{b}	5.37±0.75^{bc}
AFB1+FB1+NC	4.55±0.56^{cd}	4.56±0.26^{ab}	5.03±0.61^{bc}
P	0.000**	0.000**	0.000**

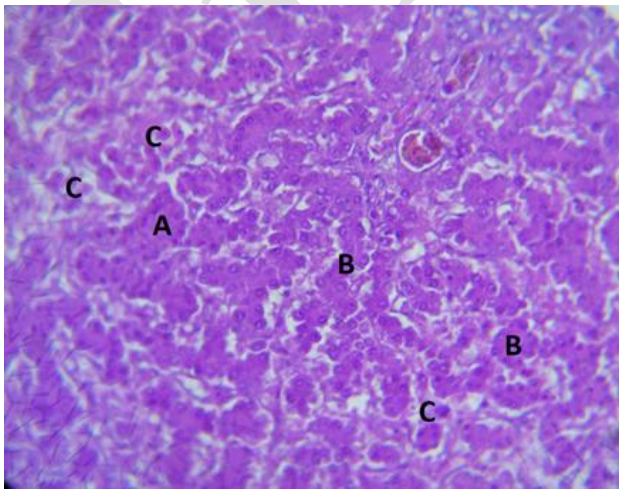
$^{a, b, c}$: Differences between the averages are significant in the same column with a different letter. AFB1: aflatoxin B1, FB1: fumonisin B1, NC: nano-chitosan, IgA: immunoglobuline A, IgG: immunoglobuline G, IgM: immunoglobulin M, P: probability, **:P<0.01.



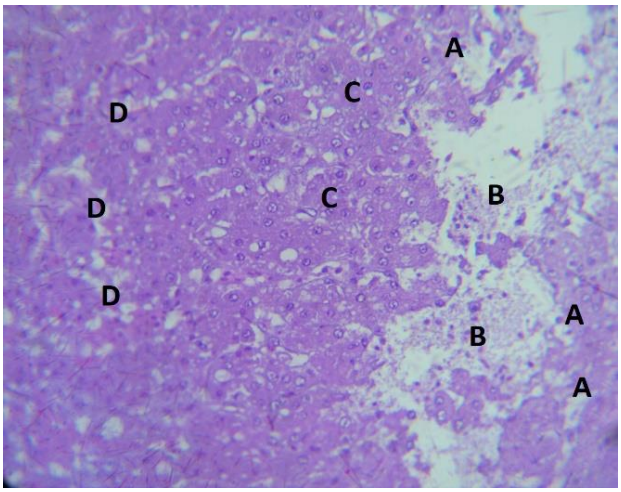
Control: A liver cell groups, **B** blood sinusoid with Kupffer cell, **C** central vein, (H2EX40).



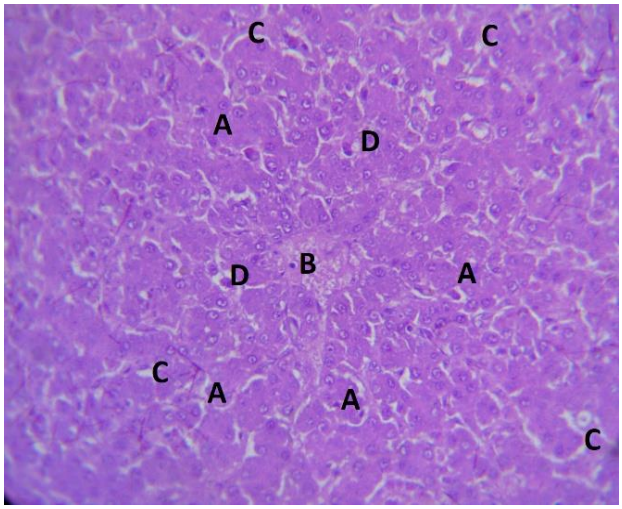
AFB1 A modular aggregation of WBCs, **B** groups of liver cells, **C** channels of blood sinusoids with Kupffer cells, (H2EX40).



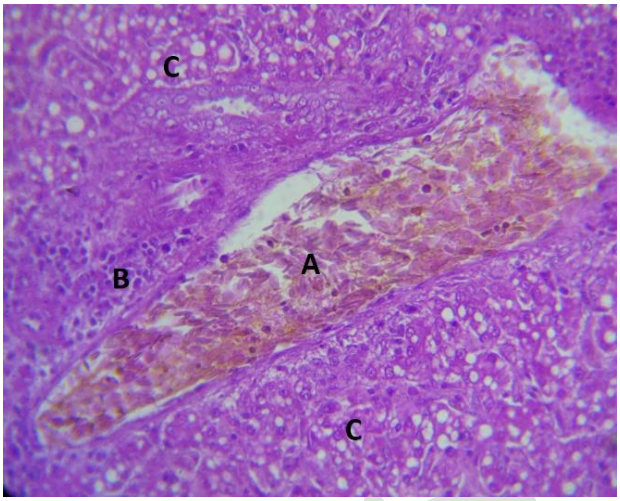
FB1: A hypertrophic liver cells, **B** the normal architecture of liver cells, **C** blood sinusoids with Kupffer cells and cellular debris, (H2EX40).



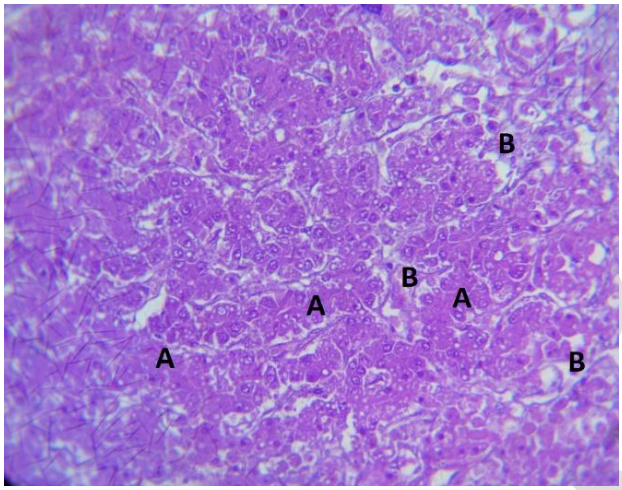
AFB1+FB1: A extensive degeneration of liver cells, **B** cavitation of liver tissue with cellular debris and WBCs, **C** liquefication of liver cells, **D** Kupffer cells in the blood sinusoids, (H2EX40).



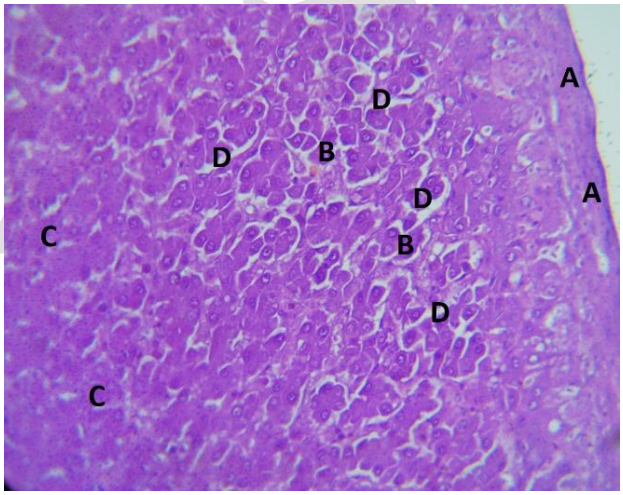
NC: A groups liver cells present like a honey-comb, **B** central vein with blood, **C** blood sinusoids, **D** Kupffer cells, (H2EX40).



AFB1+NC: A the great size of the portal vein with hemolysed blood masses, **B** WBCs aggregation, **C** vacuolations of the cytoplasm of liver cells, (H2EX40).



FB1+NC: A hypertrophy of liver cells, **B** blood channels with Kupffer cells, (H2EX40).



AFB1+FB1+NC: A collagen bundle of the capsule, **B** hypertrophic of liver cells, **C** hypertrophic cells, **D** blood sinusoids, (H2EX40).

Fig. 1: The liver histological examination

hypertrophic hepatocytes, degenerated remnants, and more abundance of Kupffer cells. The AFB1+FB1 group demonstrated heavy degeneration, vacuolated cytoplasm, and high infiltration of inflammatory cells. The NC group showed a honeycomb-oriented arrangement of hepatocytes and sinusoids were structurally normal. On the other hand, the AFB1+NC group showed the dilatation of the portal vein with hemolyzed blood, infiltration of leukocytic cells, and vacuolated hepatocytes. Hypertrophic hepatocytes were relatively balanced with Kupffer cells in the FB1+NC group sinusoids. Finally, there was a collagen capsule enclosing hypertrophy cells and a complicated sinusoidal network with sparse Kupffer cells in the AFB1+FB1+NC group.

DISCUSSION

The supplementation of broiler diets with AFB1, FB1, and AFB1+FB1 negatively influenced BW, BWG, FI, and FCR values. Other compounds such as aflatoxin B1 and fumonisin B1 are believed to sap protein metabolism. This happens because these toxins interrupt protein synthesis, thereby inducing a rise in amino acid catabolism, and a decreased metabolic efficiency. Simultaneously, the production of digestive enzymes responsible for breaking down starches, proteins and nucleic acids decrease more than normal secretion, in addition to the malabsorption of fat and reduced absorption of fat-soluble vitamins (Min et al., 2021). We obtained similar results as previous studies (Li *et al.*, 2022; Qing *et al.*, 2022), showing that AFB1 and FB1 addition to the rations inhibited BW, BWG, FI, and FCR values in broilers. A significant improvement in performance traits was revealed in groups fed with the addition of NC. Chitosan is composed of glycoproteins with anions of a negatively charged sialic acid, whose charge in an acid environment of a stomach turns positive. Chitosan promotes a positive charge interaction, providing an opportunity for a complexation of a negatively charged molecule of AFB1 and FB1, and effectively reduces them in terms of bioavailability (Hernández-Martínez *et al.*, 2023). Greater reactivity and a larger nano chitosan surface can contribute even more to adsorption of toxins, preventing them from absorption in the gastrointestinal

tract. As a result, concentrations of mycotoxins in an organism decrease, and unabsorbed toxins will be eliminated through an excretion (Delgado-Cedeño et al., 2022). NC can decelerate a transition of food through the gastrointestinal tract, providing a larger opportunity for absorption and digesting processes of the nutrients (Dawood et al., 2020).

A considerable increase in comparative heart and liver weights could be noticed in groups that received AFB1, FB1, and a mixture of both when compared with experimental groups. As noticed in agreement with Atamaleki *et al.* (2020), continuous mycotoxin build-up in liver tissue induces widespread tissue impairment, and in the long-term compromising its important role in detoxification and blood filtering processes. Besides, increased cardiac output and blood circulation in response to toxicity can result in increased weight of the liver. Tulayakul *et al.* (2018) suggested that mycotoxins generate increased demand for oxygen, and in consequence, cardiac workload and heart enlargement follow. Besides, a reduction in cardiomyocytes' blood supply combined with toxicity-related impairment in red blood cells and reduced absorption of nutrition accentuate such physiologic derangement (Lakshmeesha *et al.*, 2019). AFB1 and FB1 have deleterious effects on immune function through inhibition of development and growth of the bursa of Fabricius, and suppression of both antibody-dependent and cellular immune functions. Besides, these toxins synergistically act and, in combination, produce a considerable change in immune organ weight, with a considerable impact in spleen (Kolawole et al., 2020).

The pathological effects of AFB1 and FB1 in blood profiles can be attributed to interfering with amino acid availability. This is through damaging gut cells, interfering with absorption and depriving the organism of important factors for significant processes, such as for erythropoiesis (Saleemi et al., 2020). All these observations agree with Li *et al.* (2022), in supporting NC's viability in overcoming mycotoxin toxicity's pathologic effect. Mycotoxins cause anemia by inhibiting the absorption of iron from small intestines, and decrease serum iron levels and iron-binding capacity, and therefore, cause a reduction in the amount of available iron for the generation of hemoglobin in the bone marrow (Ding *et al.*, 2023). It was reported by Ochieng *et al.* (2021) that the WBC count and ratio increased, and the lymphocyte ratio decreased, and the H:L ratio was an indicator of tension in birds. Fang *et al.* (2018) reported a notable decrease in red blood cell count and percentage, alongside an increase in heterophil fraction, in broilers administered 2.5–3.5mg/kg of AFB1. Schrenk *et al.* (2020) reported a notable increase in the number of heterophils and a decrease in the proportion of lymphocytes in birds administered 2.45mg/kg of AFB1, being consistent with our findings.

With disrupted formation of red blood cells, AFB1 and FB1 compromise erythropoiesis (Armanini *et al.*, 2021). A fall in WBC accompanied by an increase in H/L corresponds to AFB1 (Sousa *et al.*, 2020). Likewise, with a notable increase in WBC count and H/L, a reduction in RBC count and in PCV is accompanied (300mg/kg of FB1, Deepthi *et al.*, 2017). NC promotes broiler wellness via increased count of RBC, rise in PCV, and a healthy H/L ratio. NC strengthens immune function via its interaction with receptor proteins, an interaction that in turn triggers immune stimulation (AbedAllaw, 2016). NC encloses both AFB1 and FB1, a mechanism in which absorption is hindered, and toxicity is averted in cells. Fang *et al.* (2018) validated NC's protective function in countering mycotoxin toxicity, a fact corroborated and supported by regulating agencies.

Both AFB1 and FB1 suppressed the broilers' production of immunoglobulins (IgA, IgG, and IgM) in the present study. As per Hou *et al.* (2022), constant administration of AFB1 produced a less profound impact on the immune system but a profound impact on performance in birds. Long-term intake of mycotoxin-contaminated diets with low mycotoxin concentrations, such as AFB1 and FB1, is, nevertheless, a key cause of disease in birds, though. Infections and compromised efficacy of vaccines through mycotoxin toxicity (Qing *et al.*, 2022) are such key disease risks in birds. AFB1 and FB1 have been confirmed to impair immune function through a considerable drop in IgA, IgG, and IgM, with high concentrations of mycotoxins in diets having a deeper impact. This agrees with Marin *et al.* (2011), whose experiments revealed lower concentrations of antibody production and reduced immunoglobulin when animals consumed these toxins. High mycotoxin consumption over a long duration depresses the immune system, destroys nervous tissue, makes animals susceptible to infection, and brings about financial loss in chicken production (Bezerra da Rocha *et al.*, 2014).

The most affected organ was found to be the liver, whose detoxification function renders it most vulnerable to such toxins. Several abnormalities, including enlargement, pallor, fat-like texture, necrosis, and tissue degradation, were observed macroscopically, agree with Murugesan *et al.* (2015). Histopathological examinations showed that AFB1 and FB1 pathologically caused extensive hemorrhages in and around blood vessels and cell infiltration. These toxic metabolites compete with proteins and nucleic acids, leading to increased lipid accumulation in cells, hepatic enlargement, and shedding of the epithelial lining of bile ducts. Short-term exposure to high concentrations of these toxins potentiated these effects, whereas sustained exposure induced dose- and time-dependent processes such as hepatic fat accumulation and hepatic enlargement associated with cholestasis (Kövesi *et al.*, 2021).

Nano-chitosan supplementation showed protective effects on the liver against mycotoxin-induced toxicity through its antioxidant and free radical scavenging activity. It increased cell membrane integrity, suppressed lipid peroxidation and contributed to the regulation of immune function. It also prevented inflammatory infiltration of cells and restored structure and function in chickens exposed to mycotoxins through its mitigative mechanism (Shahzad *et al.*, 2023).

Conclusions: Addition of nano-chitosan to aflatoxin B1 and fumonisin B1 containing diets provided significant improvements in live weight, feed conversion ratio, organ health, blood parameters, and immunoglobulin levels in broilers compared to the non-added groups. Histological analysis revealed notable tissue recovery, suggesting that nano-chitosan may serve as a protective dietary additive to mitigate mycotoxin-induced damage.

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REFERENCES

AbedAllaw, 2016. The Effect of Adding Different Levels of Chitosan to The Diet In The Some Physiological Characters on Broiler Chickens. Tikrit Univ J Agric Sci 20:1813–1646.

Ahmad S, Anwar S and Pratap PD, 2022. The Characteristic, Occurrence of Aflatoxin and Associated Risk with Human Health. Microbiol Res J Int 32:39–50.

Aranaz I, Alcántara AR, Civera MC, *et al.,* 2021. Chitosan: An overview of its properties and applications. Polymers, 13(19), 3256.

Armanini EH, Boiago MM, de Oliveira PV, *et al.*, 2021. Inclusion of a phytogenic bend in broiler diet as a performance enhancer and anti-aflatoxin agent: Impacts on health, performance, and meat quality. Res Vet Sci 137:186–193.

Atamaleki A, Sadani M, Raoofi A, *et al.*, 2020. The concentration of potentially toxic elements (PTEs) in eggs: A global systematic review, meta-analysis and probabilistic health risk assessment. Trends Food Sci Technol 95:1–9.

Awuchi CG, Ondari EN, Nwozo S, et al., 2022. Mycotoxins' toxicological mechanisms involving humans, livestock and their associated health concerns: A review. Toxins, 14(3), 167.

Awuchi CG, Ondari EN, Nwozo S, *et al.*, 2022b. Livestock and Their Associated Health Concerns : A Review. Toxins (Basel) 14:1–33.

Ayman U, Akter L, Islam R, *et al.*, 2022. Dietary chitosan oligosaccharides improves health status in broilers for safe poultry meat production. Ann Agric Sci 67:90–98.

Batikh MM, El-nabarawy AM, Shakal MAS, *et al.*, 2021. The The effect of mycotoxins in naturally contaminated diet on the pathogenicity of Escherichia coli in broiler chickens. World's Vet J 11:745–757.

Bello A, Dersjant-Li Y and Korver DR, 2019. The efficacy of 2 phytases on inositol phosphate degradation in different segments of the gastrointestinal tract, calcium and phosphorus digestibility, and bone quality of broilers. Poult Sci 98:5789–5800.

Bezerra da Rocha M, Freire F da CO, Erlan Feitosa Maia F, *et al.*, 2014. Mycotoxins and their effects on human and animal health. Food Control 36:159–165.

Dawood MA, Gewaily MS, Soliman AA, *et al.,* 2020. Marine-derived chitosan nanoparticles improved the intestinal histo-morphometrical features in association with the health and immune response of grey mullet (Liza ramada). Marine drugs, 18(12), 611.

Deepthi BV, Gnanaprakash AP and Sreenivasa MY, 2017. Effect of γ-irradiation on fumonisin producing Fusarium associated with animal and poultry feed mixtures. 3 Biotech 7:1–8.

Delgado-Cedeño A, Hernández-Martínez SP, Ramos-Zayas Y, *et al.*, 2022. Insoluble chitosan complex as a potential adsorbent for aflatoxin B1 in poultry feed. Front Mater 9:1–14.

Ding L, Han M, Wang X, *et al.*, 2023. Ochratoxin A: Overview of Prevention, Removal, and Detoxification Methods. Toxins (Basel) 15:33.

Eskola M, Kos G, Elliott CT, *et al.,* 2020. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate'of 25%. Crit Reviews Food Sci Nut, 60(16), 2773-2789.

Fang J, Zheng Z, Yang Z, *et al.*, 2018. Ameliorative effects of selenium on the excess apoptosis of the jejunum caused by AFB1 through death receptor and endoplasmic reticulum pathways. Toxicol Res (Camb) 7:1108–1119.

Groff-Urayama PM, Padilha-Boaretto JB, Gorges MH, *et al.*, 2022. Use of different adsorbents in broiler diets naturally contaminated by mycotoxins. Acta Sci - Anim Sci 44:1–8.

Hernández-Martínez SP, Delgado-Cedeño A, Ramos-Zayas Y, *et al.,* 2023. Aluminosilicates as a Double-Edged Sword: Adsorption of Aflatoxin B1 and Sequestration of Essential Trace Minerals in an In Vitro Gastrointestinal Poultry Model. Toxins, 15(9), 519.

Honikel KO, 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci 49:447–457.

Hou L, Qiu H, Li A, *et al.,* 2022. Effects of aflatoxin B1 on growth performance, antioxidant status, immune response, and pro-inflammatory cytokine mRNA expression in ISA chicks. Front Vet Sci, 9, 993039.

Ijaz MU, Kalsoom A, Hamza A and Ehsan N, 2023. Sciadopitysin attenuates paraquat induced renal toxicity by modulating Nrf-2/Keap-1 pathway in male albino rats. Asian J Agric Biol 10, 2023110.

Khatoon A, Amin A, Majeed S, *et al.,* 2024. Dietary *Chlorella vulgaris* mitigates aflatoxin B1 toxicity in broiler chicken: Toxicopathological, hematobiochemical and immunological perspectives. Toxicon 251: 108127.

Kolawole O, Graham A, Donaldson C, *et al.,* 2020. Low doses of mycotoxin mixtures below EU regulatory limits can negatively affect the performance of broiler chickens: A longitudinal study. Toxins, 12(7), 433.

Kövesi B, Kulcsár S, Ancsin Z, *et al.*, 2021. Individual and Combined Effects of Aflatoxin B1 and Sterigmatocystin on Lipid Peroxidation and Glutathione Redox System of Common Carp Liver. Toxins (Basel) 13:1–11.

Lakshmeesha TR, Kalagatur NK, Mudili V, *et al.*, 2019. Biofabrication of zinc oxide nanoparticles with Syzygium aromaticum flower buds extract and finding its novel application in controlling the growth and mycotoxins of Fusarium graminearum. Front Microbiol 10:1–13.

Li S, Han M, Zhang Y, *et al.,* 2022. Effect of Curcumin as feed supplement on immune response and pathological changes of broilers exposed to Aflatoxin B1. Biomolecules, 12(9), 1188.

Marin DE, Taranu I, Burlacu R, *et al.*, 2011. Effects of zearalenone and its derivatives on porcine immune response. Toxicol Vitr 25:1981–1988.

Min L, Fink-Gremmels J, Li D, *et al.*, 2021. An overview of aflatoxin B1 biotransformation and aflatoxin M1 secretion in lactating dairy cows. Anim Nutr 7:42–48.

Murugesan GR, Ledoux DR, Naehrer K, *et al.*, 2015. Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci 94:1298–1315.

Ochieng PE, Scippo ML, Kemboi DC, *et al.*, 2021. Mycotoxins in poultry feed and feed ingredients from sub-Saharan Africa and their impact on the production of broiler and layer chickens: A review. Toxins (Basel) 13:633.

Pitt JI and David Miller J, 2021. A Concise History of Mycotoxin Research. J Agric Food Chem 65:7021–7033.

Qing H, Huang S, Zhan K, *et al.*, 2022. Combined Toxicity Evaluation of Ochratoxin A and Aflatoxin B1 on Kidney and Liver Injury, Immune Inflammation, and Gut Microbiota Alteration Through Pair-Feeding Pullet Model. Front Immunol 13:1–16.

Saha Turna N, Comstock SS, Gangur V, *et al.*, 2024. Effects of aflatoxin on the immune system: Evidence from human and mammalian animal research. Crit Rev Food Sci Nutr 64:9955–9973.

Schrenk D, Bignami M, Bodin L, *et al.*, 2020. Risk assessment of aflatoxins in food. EFSA J 18:54–67.

Shahzad U, Mohammed KS, Tiwari S, Nakonieczny J and Nesterowicz R. 2023. Connectedness between geopolitical risk, financial instability

indices and precious metals markets: Novel findings from Russia Ukraine conflict perspective. Res Pol, 80: 103190.

Shephard GS, Sewram V, Nieuwoudt TW, *et al*., 1999. Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the Fusarium section Liseola. J Agric Food Chem 47:5111–5115.

Shotwell OL, Hesseltine CW, Stubblefield RD, *et al*., 1966. Production of aflatoxin on rice. Appl Microbiol 14:425–428.

Sousa MCS, Galli GM, Alba DF, *et al*., 2020. Pathogenetic effects of feed intake containing of fumonisin (*Fusarium verticillioides*) in early broiler chicks and consequences on weight gain. Microb Pathog 147:104247.

Szymczyk P, Filipkowska U, Jóźwiak T, *et al*., 2016. Phosphate removal from aqueous solutions by chitin and chitosan in flakes. Prog Chem Appl Chitin Its Deriv 21:192–202.

Tilley JEN, Grimes JL, Koci MD, *et al*., 2017. Efficacy of feed additives to reduce the effect of naturally occurring mycotoxins fed to Turkey hen poults reared to 6 weeks of age. Poult Sci 96:4236–4244.

Tulayakul P, Mingkhwan R, Hananantachai H, *et al*., 2018. Heavy metal (Cd and pb) and aflatoxin contamination in tissues and eggs from free grazing ducks and their environment in central Thailand. Biol Trace Elem Res 186:514–520.

Wang X, Wang D, Zhang S, *et al*., 2023. and Prevention and Control of Soils. Toxins (Basel) 15:34–48.