≪Research Note≫

Effect of the Addition of *Saccharomyces Cerevisiae* Yeast Cell Walls to Diets with Mycotoxins on the Performance and Immune Responses of Broilers

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This study was conducted to evaluate the effect of *Saccharomyces cerevisiae* yeast cell walls (YCWs) in diets with low doses of aflatoxin B1 (AFB1) and ochratoxin A (OTA), alone or in combination, on broiler performance and immune response. A total of 210 male broilers aged 1−21 days were used. Broilers were completely randomized into seven treatments with five replicates of six broilers each, as follows: 1) control diet; 2) control + 350 μg/kg AFB1; 3) Control + 350 μg/kg OTA; 4) Control + 350 μg/kg AFB1 and 350 μg/kg OTA; 5) Control + 350 μg/kg AFB1 and 1.5 kg/ton YCW; 6) control + 350 μg/kg OTA and 1.5 kg/ton YCW; 7) control + 350 μg/kg AFB1, 350 μg/kg OTA, and 1.5 kg/ton YCW. The broilers were housed under environmentally controlled conditions in Petersime battery cages. Weight gain, feed intake, and feed conversion index were measured. The relative weights of the thymus, spleen, and bursa of Fabricius (BF) were evaluated. The local immune response was assessed by quantifying the level of intestinal immunoglobulin A (IgA). The cellular immune response was evaluated using a delayed hypersensitivity test. Hemograms and blood cell counts were also performed. The results showed that mycotoxins decreased performance and reduced the immune response ($p < 0.05$) of broilers. Weight gain and feed conversion improved in the groups receiving YCWs. The YCWs increased ($p < 0.05$) intestinal IgAs and the cellular immune response ($p < 0.05$). The addition of YCWs also affected the relative weight of the thymus, spleen, and BF ($p < 0.05$), and the leukocyte, lymphocyte, and heterophil counts ($p < 0.05$). The addition of YCWs can be an alternative to counterage the negative effect of low doses of AFB1 and OTA in broilers diets.

Key words: mycotoxins, *Saccharomyces cerevisiae*, yeast cell walls

Introduction

Mycotoxins are metabolites produced by fungi, and are present in contaminated feed of productive animals such as poultry. Aflatoxins and ochratoxins are the most common mycotoxins (Gimeno, 2004). Among birds, ducks are the most susceptible to aflatoxin poisoning, followed by turkeys, broilers, laying hens, and quail. Aflatoxin B1 is considered a hepatic carcinogen and affects a wide variety of animals and humans because of its immunosuppressive effect (Dragan and Pitot, 1994). The target organ of ochratoxin alpha (OTA) is the kidney, in which lesions can occur due to acute or chronic exposure (Harwig *et al*., 1983). OTA is approximately three times more toxic than aflatoxin to growing broilers (Huff *et al*., 1992).

The effect of exposure to more than one mycotoxin in animals can be equal to the sum of the expected effects of each mycotoxin individually (an additive effect), lower than the expected effects of each mycotoxin individually (an antagonistic effect), or higher than expected from the sum of the individual effects of each mycotoxin (a synergistic effect) (Tammer *et al*., 2007; Wang *et al*., 2009).

The contamination of animal feed with mycotoxins has led to clays and other aluminosilicates being mixed into the feed in order to absorb the aflatoxin in the animal's digestive tract; however, not all toxins are always adsorbed. Subsequently, the use of the oligosaccharides $\beta1,3-\beta1,6$ glucan and mannan extracted from the cell walls (YCWs) of the yeast *Sac-*

charomyces cerevisiae have been shown to protect poultry from the adverse effects of mycotoxins (Devreese *et al.*, 2013).

The major components of YCW are carbohydrates, which belong to a group of oligosaccharides comprising mostly β-glucans and mannan-oligosaccharides (MOSs). YCWs are estimated to contain 85 to 90% oligosaccharides, corresponding to molecules of 1,3-β-glucans (30−50%), 1,6-β-glucans (5−10%), mannoproteins (30−50%), and chitin (1.5−6%) (Klis *et al.*, 2006). The mode of action of the β-D-glucans has not yet been fully determined (Yiannikouris *et al.*, 2006). YCWs are comprised of 10 to 20% protein (Nguyen *et al.*, 1998). The presence of oligosaccharides in YCWs can explain their mode of action via the chemical interaction between β-D-glucans and the cyclic and hydroxyl groups of the mycotoxins, through weak bonds such as hydrogen bonds and Van der Waals interactions. The results of several studies have suggested that the three-dimensional structure of YCWs, which mainly consists of oligosaccharides, is capable of adsorption reactions with specific mycotoxins such as aflatoxins, ochratoxin, and zearalenone (Yiannikouris *et al.*, 2004; Jouany and Diaz, 2005; Ringot *et al.*, 2006). Furthermore, Osborn and Khan (2000) reported that MOSs and glucans play significant roles in the immune system, as well as in the intestine, where they are important and beneficial in maintaining the intestinal microflora health and balance, thereby increasing broiler growth (Czop JR, 1986; Hooge DM. 2004; Kocher *et al.*, 2005; Gómez *et al.*, 2009; Morales *et al*., 2010; Brümmer *et al.*, 2010).

The aim of this study was to evaluate the effect of *S. cerevisiae* YCWs inclusion in sorghum-soya-based broiler diets contaminated with low doses of AFB1 and OTA (alone or in combination) on the performance and immunological parameters of growing broilers.

Materials and Methods

Experiments were conducted using broilers; the bird handling procedures met the requirements set forth by the Institutional Animal Care and Use Committee (Comité Institucional para el cuidado y uso de los animales experimentales - CICUAE FMVZUNAM) based on the Official Mexican Norm (NOM-033-SAG/ZOO-2014).

Concentration of Mycotoxins and Experimental Model

AFB1 and OTA were produced in the Research Unit in Grains and Seeds (Unidad de Investigación en Granos y Semillas - UNIGRAS) of the School of Higher Studies (Facultad de Estudios Superiores - FES), Cuautitlan, National Autonomous University of Mexico (Universidad Nacional Autónoma de México - UNAM).

The Vicam Corp Afla B^{TM} test (Waters Corporation; Milford, MA, USA) was used to determine the AFB1 concentration, and the OchraTest, also from Vicam Corp (Waters Corporation), was used to determine the OTA concentration (Amerongen *et al.* 2005). Once the final concentrations of the mycotoxins had been determined, diets based on sorghum and soya meal were prepared. The sorghum was previously analyzed using the same chromatographic technique with

Table 1. Calculated composition and analysis of the starter basal diet for broilers (1−21 days of age)

Ingredient	Basal diet kg
Sorghum	600.03
Soybean oil meal (48% protein)	317.97
Soya oil	44.74
Monodicalcium phosphate	17.69
Calcium carbonate	11.15
Salt	3.82
DL-Methionine 99%	1.50
L-Lysine HCl	1.00
Vitamin Premix*	1.00
Choline chloride 60%	0.50
Mineral premix**	0.30
Bacitracin 10%	0.15
Antioxidant	0.15
Total	1000

	Calculated analysis
Metabolisable energy (kcal/kg)	3150
Protein	21.00
Dig. lysine (%)	1.10
Dig. met + cys (%)	0.84
Total calcium (%)	0.90
Available phosphorus (%)	0.45

* The vitamin (Vit.) mixture includes the following: 12,000,000 IU Vit. A; 2,500,000 IU Vit. D3; 15,000 IU Vit. E; 2,000 mg/kg Vit. K3; 2,250 mg/kg Vit. B1; 8,000 mg/kg Vit. B2; 45,000 mg/kg Vit. B3; 12,500 mg/kg Vit. B5; 3,500 mg/kg Vit. B6; 20 mg/kg Vit. B12; 1,500 mg/kg folic acid; and 25 mg/kg biotin.

** The mineral mixture includes the following: 200 mg/kg Selenium; 200 mg/kg Cobalt; 300 mg/kg Iodine; 12,000 mg/kg Copper; 50,000 mg/kg Zinc; 50,000 mg/kg Iron; 110,000 mg/kg Manganese.

immunoaffinity columns to avoid any mycotoxin contamination. When mixing the ingredients to obtain a nutritionally adequate sorghum-soya-based diet (Table 1), according to the recommendations for the Ross 308 broiler strain, YCWs (Safmannan, Phileo, Lesaffre Animal Care), AFB1, and OTA were added homogeneously. The eight experimental diet treatments were as follows:

- Treatment 1: control diet
- Treatment 2: T1 + 350 μg/kg AFB1
- Treatment 3: T1 + 350 μg/kg OTA
- Treatment 4: T1 + 350 μg/kg AFB1 and 350 μg/kg OTA
- Treatment 5: T1 + 350 μg/kg AFB1 and 1.5 kg/ton YCW
- Treatment 6: T1 + 350 μg/kg OTA and 1.5 kg/ton YCW
- Treatment 7: T1 + 350 μg/kg AFB1, 350 μg/kg OTA and 1.5 kg/ton YCW

A completely randomized design with seven experimental treatments was used. Each treatment included five repetitions of six chickens each. Once prepared, the diets were analyzed to confirm that each mycotoxin was present at the correct amount.

The experiment was conducted with 210 Ross 308 male broilers aged 1 to 21 days from a commercial hatchery. Broilers were individually weighed and randomly allocated to the different treatments. Each treatment consisted of five

replicates of six broilers each. The birds were housed throughout the test in Petersime battery cages[TM] (Incubators & Brooders, Zulte, Belgium) under a controlled environment. The experiment was conducted at the Centre for Education, Research, and Extension in Poultry Production (Centro de Enseñanza, Investigación y Extensión en Producción Avícola - CEIEPAv), Faculty of Veterinary Medicine and Animal Science, National Autonomous University of Mexico, located in Tláhuac Delegation, Federal District, Mexico.

Water and feed were administered ad libitum. During the experiment, the broilers and feed were weighed weekly to determine the weight gain, feed intake, and feed conversion index.

Systemic Humoral Immune Response

To evaluate the systemic response to YCWs at 9 days of age, the broilers simultaneously received a live-virus vaccine against Newcastle disease via the ocular route, and a killed-virus vaccine against Newcastle disease subcutaneously. Ten blood samples without anticoagulants were taken per replication prior to vaccination and 13 days after vaccination. The sera were frozen at $-20℃$ for subsequent determination of the specific serum antibody titers against the Newcastle disease virus using a hemagglutination inhibition test.

Local Humoral Immune Response

A 10-cm sample of duodenum was taken from 12 broilers per treatment. The samples were washed with 10 ml of sterile ice-cold phosphate-buffered saline (PBS) solution by passing the PBS through the fraction of the intestinal tract three times. The contents were collected and centrifuged at 1200 rpm for 10 minutes. The supernatant was collected and frozen at $-20℃$ for subsequent evaluation using an ELISA.

IgA Assessment

The concentrations of immunoglobulin A (IgA) in the intestinal washings were evaluated using the Chicken IgA ELISA Quantitation Set (Bethyl Laboratories, Inc., Montgomery, TX, USA) following the manufacturer's specifications. Briefly, chicken IgA in a carbonate buffer solution (0.05 M, pH 9.6) was placed in 96-well flat-bottomed plates. The plates were incubated at 4℃ overnight and then washed three times with 0.05% PBS-Tween 20. A blocking solution (0.5% skimmed milk and 0.2% sucrose in PBS) was added, and the plates were incubated for 30 minutes and then washed. The intestinal washings were incubated for 1 hour at 37℃ and then washed five times with 0.05% PBS-Tween-20. An HRP conjugate (goat anti-chicken IgA-HRP[1]) was added and the plates were incubated at 37℃ for 1 hour and washed again. ABTS substrate was added, the plates were incubated for 20 minutes, and the reaction was stopped with stop solution (H_2SO_4 2 M). The density was read at 405 nm.

Cellular Immune Response
Hematology

Blood samples were collected with an ethylenediaminetetraacetic acid (EDTA) S-Monovette (Sarstedt AG & Co.KG, Sarstedtstraße 1, 51588 Nümbrecht, Alemania) from the radial veins of 12, 21-day-old chickens per treatment group. A differential leukocyte count was performed using blood smears stained with Wright's stain. Total counts were indirectly determined by calculating the percentage of leukocytes in defined areas on the blood smears (Campbell, 1995).

Delayed Hypersensitivity Test

The broilers were evaluated at 20 days of age using the delayed hypersensitivity test (Edelman et al., 1986; Corrier and DeLoach, 1990), which measures the response of broilers to intradermal inoculation. Broilers were inoculated with phytohemagglutinin (PHA-A) at 0.1 mg/0.1 ml concentration, which was injected into the interdigital webbing of phalanges 3 and 4 of the left leg of three broilers per replicate (12 broilers per treatment). The same procedure was performed in the interdigital webbing of the right leg using sterile saline (0.1 ml) as a control. The reading was taken 24 hours after inoculation (broiler was 21 days of age). The thickness of the interdigital webbing was determined with a digital Vernier caliper before inoculation and 24-hours later. The following formula was used to calculate the increase in the thickness of the broilers' interdigital webbing: cutaneous hypersensitivity response = the thickness of the interdigital webbing of the left leg 24 hours after inoculation – the thickness of the interdigital webbing of the left leg before inoculation (Corrier and DeLoach, 1990).

Organ Histopathology

The broilers were sacrificed by cervical dislocation at 21 days of age to allow organ sampling for the histopathological analysis of the thymus, spleen, bursa of Fabricius (BF), liver, and kidney. Thymus, spleen, BF, liver, and kidney samples were acquired from 12 broilers per treatment. The thymus and the BF were weighed, and the relative weights were obtained (Perozo-Marín et al., 2004). All samples were preserved and fixed in 10% buffered formalin for processing and for the generation of histological sections for microscopic observation.

Statistical Analysis

Data corresponding to each variable studied were analyzed in a completely randomized design with seven experimental treatments. Because a difference ($p < 0.05$) existed between treatments, the data were compared using Tukey's test with the statistical package SPSS version 20. Antibody titer data from the hemagglutination inhibition test were transformed into logarithm base 2 for statistical analyses.

Results

Animal Performance

The results of the production variables are shown in Table 1. The body weight ($p < 0.05$) was affected in the treatments contaminated with AFB1, OTA, and AFB + OTA compared with treatments without mycotoxins. Additionally, body weight improved ($p < 0.05$) with the addition of YCWs to the diets. Differences ($p < 0.05$) in feed intake were detected among the treatments, with decreased intake observed with the OTA and AFB + OTA treatments compared with treatments without mycotoxins and AFB1. An improvement in the feed conversion index ($p < 0.05$) was observed with the addition of YCWs.

Relative Weights of Lymphoid Organs

The relative weights of the thymus, spleen, and BF are

Table 2.   **Effect of the addition of *Saccharomyces cerevisiae* yeast cell walls on performance and relative weight of the thymus, spleen, and bursa of Fabricius in broilers fed diets contaminated with low doses mycotoxins**

	Weight at 21 days (g)	Feed conversion (g*g)	Feed intake (g)	Thymus weight (% LW)	Spleen weight (% LW)	Bursa of Fabricius weight (% LW)
Control diet	834a	1.260a	1025a	0.42a	0.11a	0.26a
Control + 350 μg/kg AFB1	792b	1.277a	1019ab	0.36b	0.09b	0.22b
Control + 350 μg/kg OTA	797b	1.250a	1006b	0.36b	0.09b	0.22b
Control + 350 μg/kg AFB1 and 350 μg/kg OTA	786b	1.248a	993b	0.36b	0.09b	0.21b
Control + 350 μg/kg AFB1 and 1.5 kg/ton YCW	845a	1.237b	1044a	0.38ab	0.10a	0.27a
Control + 350 μg/kg OTA and 1.5 kg/ton YCW	835a	1.250a	1042a	0.38ab	0.10a	0.28a
Control + 350 μg/kg AFB1, 350 μg/kg OTA and 1.5 kg/ton YCW	812ab	1.240b	1009b	0.38ab	0.10a	0.28a
MEE	4.4	4.2	0.01	0.01	0.001	0.001

a, b Values within a column not sharing a common superscript are statistically different ($p<0.05$).

Table 3.   **Effect of the addition of *Saccharomyces cerevisiae* yeast cell walls on the local humoral, cellular, and blood cell immune responses in broilers fed diets contaminated with low doses of mycotoxins**

	IgA (ng/ml)	Delayed hypersensitivity (mm)	Lymphocytes (10^9/L)	Leukocytes (10^9/L)	Heterophils (10^9/L)
Control diet	216b	0.47a	3.2a	7.8b	3.3ab
Control + 350 μg/kg AFB1	198b	0.39b	3.1ab	7.2ab	3.1b
Control + 350 μg/kg OTA	206b	0.37b	3.1ab	6.8b	2.6b
Control + 350 μg/kg AFB1 and 350 μg/kg OTA	193b	0.30b	3.0b	6.7b	2.6b
Control + 350 μg/kg AFB1 and 1.5 kg/ton YCW	234a	0.50a	3.3a	8.2a	3.7a
Control + 350 μg/kg OTA and 1.5 kg/ton YCW	231a	0.50a	3.2a	8.2a	3.6a
Control + 350 μg/kg AFB1, 350 μg/kg OTA and 1.5 kg/ton YCW	212ab	0.47a	3.1a	7.3ab	3.0b
MEE	2.9	0.02	0.06	0.08	0.09

a, b Values within a column not sharing a common superscript are statistically different ($p<0.05$).

shown in Table 3. A detrimental effect of mycotoxins was observed in these organs, as well as improved weight, when the YCWs were added to the diet ($p<0.05$). Moreover, differences ($p<0.05$) between the treatments contaminated with mycotoxins and treatments without mycotoxins were observed.

Humoral and Cell-mediated Immune Responses

Including YCWs in the diet of broilers improved the local humoral immune response ($p<0.05$), increasing the concentration of intestinal IgA. A similar effect was observed for the cellular immune response ($p<0.05$), and delayed basophil hypersensitivity was increased with the addition of YCWs (Table 2). However, for the systemic humoral immune response, no significant differences ($p>0.05$) in the antibody titers against Newcastle disease were observed among the treatments.

The blood profile showed significant differences among treatments ($p<0.05$) in leukocytes, lymphocytes, and heterophils. The mycotoxins decreased the blood concentration of these cells. An increase in the concentration of lymphocytes and heterophils was observed with the addition of YCWs (Table 2).

Histological Findings

In the histological sections, no significant changes in the liver, kidney, spleen, thymus, and BF were observed (data not shown). The histological findings in the livers of the broilers fed diets with AFB included vacuolar degeneration, bile duct hyperplasia, and moderate multifocal fibrosis with mild focal necrosis. Qualitatively, moderate lymphoid depletion was observed in the thymus, spleen, and BF. Moreover, moderate epithelial hyperplasia with the presence of epithelial cysts, as well as mild villous edema, were detected in the BF (Fig. 1). In the OTA treatment group, no microscopic morphological alterations were observed at the structural level in the kidney, although OTA is nephrotoxic. The same morphological changes described for aflatoxin were observed in the thymus and spleen (Fig. 2). No apparent pathological changes were observed in broilers fed diets with YCWs and without mycotoxins. The AFB + OTA interaction (Fig. 3) in the liver caused a moderate diffuse vacuolar degeneration in the hepatocytes, bile duct hyperplasia, and moderate multifocal fibrosis, as well as moderate focal necrosis. No apparent pathological changes were observed in the kidney. Moderate lymphoid depletion and mild multifocal necrosis were observed in the thymus. Moderate
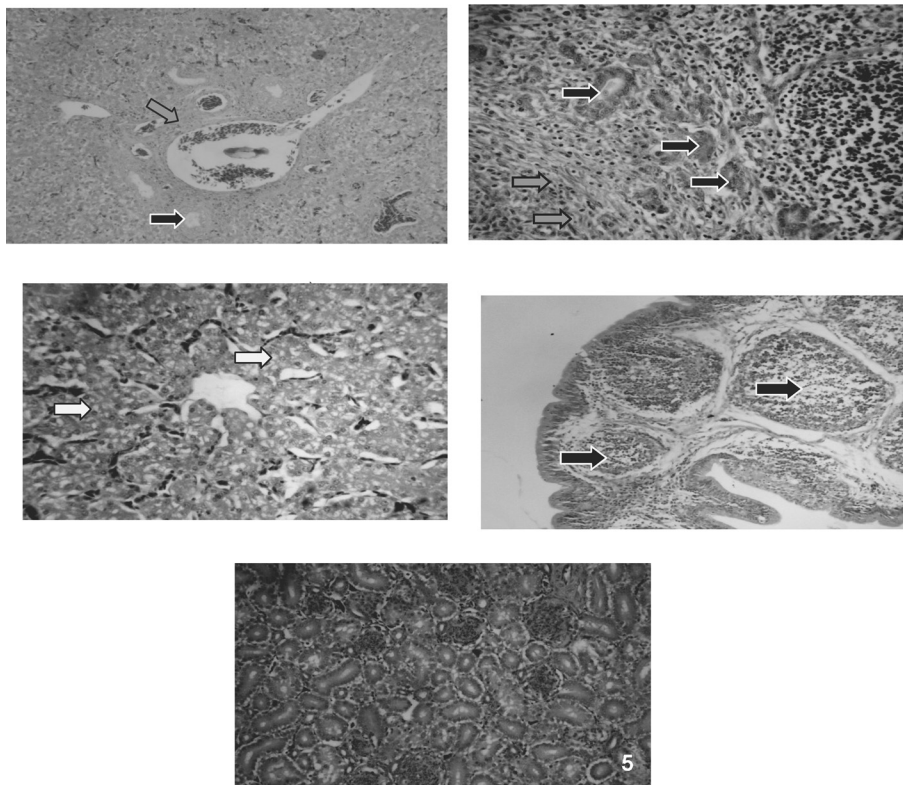
Fig. 1. Histological sections from the aflatoxin B1 (AFB) treatment. 1) Liver with fibrosis (gray arrow), 2) Liver bile duct hyperplasia (black arrows), and 3) Liver fatty degeneration (white arrows) and 4) the bursa of Fabricius with lymphoid depletion (black arrows) are shown. 5) Kidney no microscopic morphological alterations.
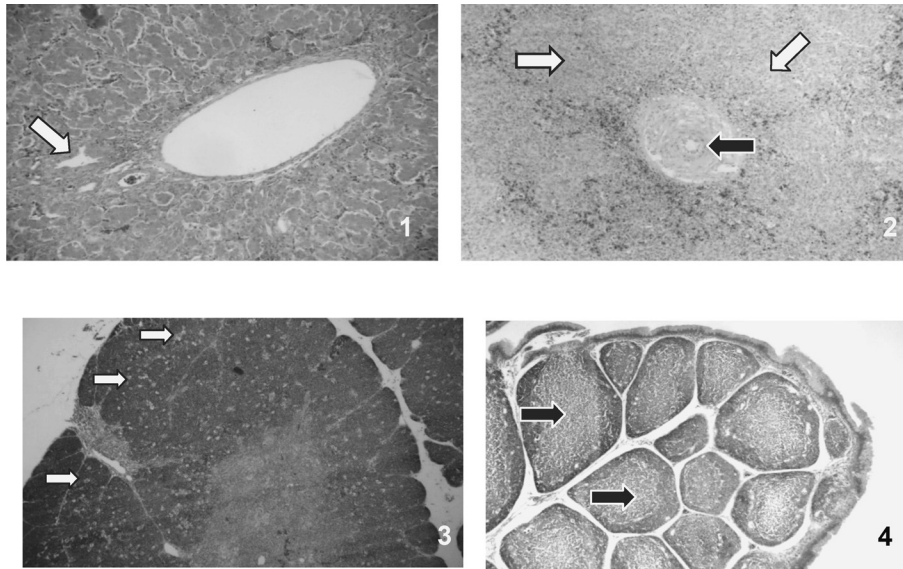
Fig. 2. **Histological sections from the ochratoxin A (OTA) treatment.** 1) Liver (Non Significants Changes), Bile duct without apparent pathological changes (white arrows), 2) spleen with mild lymphoid depletion (white arrows), central arteriola (black arrow), 3) thymus with apoptosis (white arrows), and 4) bursa of Fabricius with lymphoid depletion (black arrows) are shown.
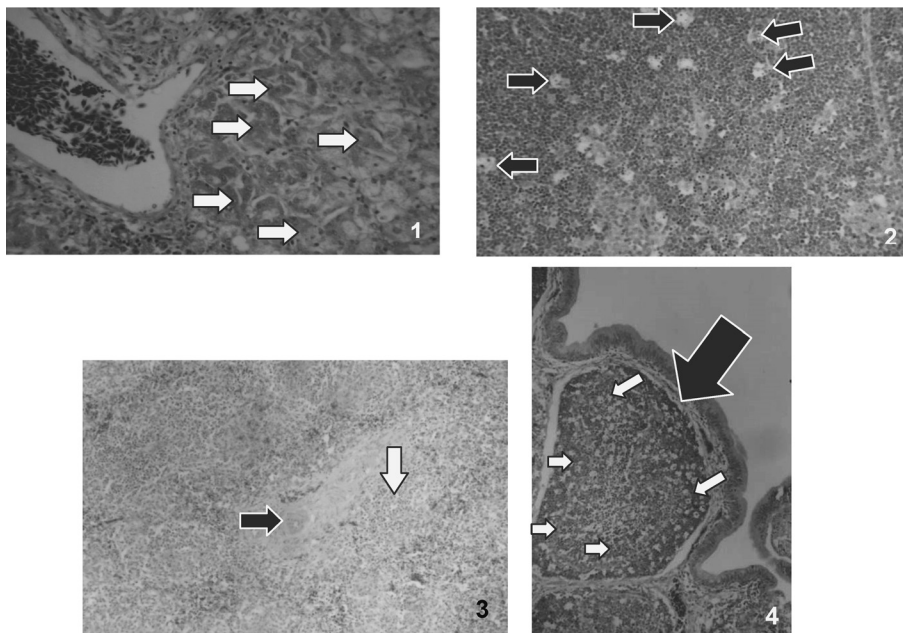
Fig. 3. **Histological sections from the aflatoxin B + ochratoxin A (AFB + OTA) treatment.** 1) Liver with bile duct hyperplasia (white arrows), 2) thymus with apoptosis (black arrows), 3) spleen with lymphoid depletion (white arrow), central arteriola (black arrow), and 4) bursa of Fabricius with lymphoid depletion (black arrow) and apoptosis (white arrows) are shown.

lymphoid depletion occurred in the spleen. The lesions observed in the BF were the result of epithelial hyperplasia, lymphoid depletion, and moderate villous edema, with the presence of epithelial cysts. The combination of AFB + YCW resulted in mild bile duct hyperplasia. No apparent pathological changes were observed in the kidney and thymus. Mild lymphoid depletion occurred in the BF. A similar effect was observed with the combination of OTA + YCW in the liver. No apparent pathological changes were observed in the kidneys or the BF. Mild lymphoid depletion was observed in the thymus and spleen. The combination of AFB + OTA + YCW resulted in bile duct hyperplasia and mild multifocal fibrosis in the liver. No apparent pathological changes were observed in the kidneys. Moderate lymphoid depletion was observed in the BF, spleen, and thymus. Mild focal necrosis was also detected in the thymus (Fig. 3).

Discussion

The addition of low doses of mycotoxin (350 μg/kg AFB1, 350 μg/kg OTA, and AFB1 + OTA) to sorghum + soya-based diets decreased the weight of the broilers at 21 days of age. This effect was greater with the AFB + OTA combination. *S. cerevisiae* YCWs included at 0.15% in the diets of the broilers counteracted the toxic effect, which corroborates the findings of other studies (Aravind *et al*., 2003, Arce *et al*., 2005, 2008; Kocher *et al*., 2005; Gao *et al*., 2009; Gómez-Verduzco *et al*., 2009). In other studies, in which diets were contaminated with lower amounts of AFB1 and OTA (200 μg/kg) (Santin *et al*., 2006; Sawarkar *et al*., 2011), isolated or in combination, differences in body weight were observed due to the low doses of mycotoxin used. Other studies reported a detrimental effect on weight when poultry were fed AFB1 and OTA mixed at levels of 100 ppb or higher (400 and 600 ppb AFB1) (Verma *et al*., 2004; Sakhare *et al*., 2007; Anand *et al*., 2008; Sawarkar *et al*., 2011).

Regarding the relative weight of immune system organs (spleen, thymus, and BF), a negative effect ($p > 0.05$) was found with the diets containing mycotoxins, and this effect occurred in a greater percentage of animals with the AFB + OTA combination. This result is consistent with the findings of several studies (Verma *et al*., 2004; Sakhare *et al*., 2007; Sawarkar *et al*., 2011) in which lower weights were reported in some lymphoid organs when poultry were fed diets contaminated with AFB1, OTA, and a combination of both. Conversely, Santin *et al*. (2006) reported no differences in BF weight. Elaroussi *et al*. (2008) reported that 800 ppb of OTA in broiler diets reduced the BF weight. It is possible that tycotoxins affect the relative weight of the lymphoid organs, leading to a detrimental effect on the immune response. This reduction in the weight of the lymphoid organs may be caused by necrosis or cell depletion (Hoerr *et al*., 1981). The addition of YCWs decreased the organ damage caused by the mycotoxins.

No differences in the systemic humoral immune response were found among treatments in this study, which is consistent with the findings of Santin *et al*. (2003). In contrast to Gao *et al*. (2009), Raju and Devegowda (2002), and Mehdi and Ghahri (2012), who reported an increase in antibody titers with the addition of YCWs, Kalorey *et al*. (2005), Sakhare *et al*. (2007), Elaroussi *et al*. (2008), and Gómez-Verduzco *et al*. (2009) reported a decrease in antibody tires in broilers intoxicated with AFB1 or OTA (400 and 800 ppb, respectively) and concluded that this effect may be due to synergism between the two mycotoxins.

An effect on the local humoral immune response was observed in this study with the addition of 0.15% YCW, which is in line with the findings of Gao *et al*. (2009) and Gómez-Verduzco *et al*. (2009). This increase in the content of secretory IgA in the intestine due to YCW addition leads to greater protection of the mucosa and intestinal villi against pathogen-induce damage (Gao *et al*., 2009).

An increase in the cellular immune response ($p > 0.05$) was observed with the addition of YCWs in the diet, which is in line with the findings of Gómez-Verduzco *et al*. (2009). This may be based on the chemical structure of the YCWs, which includes mostly sugars, and the likely function of the sugars as lectin-like receptor ligands, which has been described in cell populations of lymphoid origin. In treatments with OTA, greater adverse effects occurred in the cellular response, which corroborates the findings of Devegowda and Murthy (2005).

The blood counts revealed an increased concentration of lymphocytes and heterophils ($p > 0.05$) with the addition of YCW, indicating that YCWs favor an increase in these cell populations, which play an important role in the immune response. A decrease in the percentage of leukocytes, heterophils, and lymphocytes was observed in the broilers fed diets with mycotoxins compared with those fed diets without mycotoxins, according to Kalorey *et al*. (2005), Aravind *et al*. (2003), Sakhare *et al*. (2007), and Basmacioglu *et al*. (2005). This effect may be explained by the results of Celika *et al*. (2000), who demonstrated that aflatoxin-induced immunosuppression resulted in depressed cell functions and a decrease in the number of T lymphocytes in the periphery and in lymphoid tissues (Moura *et al*., 2004; Sawarkar *et al*., 2011).

The histological evaluation of tissues revealed that the highest degree of injury in the liver, spleen, BF, and thymus was observed when broilers were fed diets containing mycotoxins, alone or in combination (AFB1, OTA, AFB1 + OTA), similar to the results of Kalorey *et al*. (2005) and Sakhare *et al*. (2007). However, when 0.15% YCWs was added, a decrease in the severity of the pathological changes was observed, which is in line with the findings of Karaman *et al*. (2005), who reported fatty degeneration and necrosis in the liver, as well as lymphoid depletion in the spleen, thymus, and BF. However, a decrease in lesion severity was found with the addition of glucomannan.

Moreover, in this study, no lesions were found in the kidney following the addition of mycotoxins, particularly with OTA. Santin *et al*. (2003) reported epithelial cell hypertrophy in the proximal tubules in the kidney, vacuolar degeneration and bile duct hyperplasia in the liver, and

lymphoid depletion in the BF of broilers intoxicated with OTA. These differences relating to kidney damage are probably due to the high concentration (2 mg/kg feed) used, whereas a much lower concentration was used in the present study.

One benefit of *S. cerevisiae* YCWs is that they act as immunostimulants, inducing the expression of Toll-like receptors (TLRs) (Roeder *et al*., 2004, Akira *et al*., 2006), which are present on different cell types and recognize pathogen-associated molecular patterns (PAMPs) to initiate the innate immune response. The mannans and zymosan contained in the cell walls of *S. cerevisiae* were found to stimulate TLR4, TLR2, and TLR6, and to increase specific immune responses. In addition, at the doses used, these YCWs function as mycotoxin (AFB1 and, OTA) adsorbents for contaminated feed.

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