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Original Research Article

A *Saccharomyces cerevisiae* RC016-based feed additive reduces liver toxicity, residual aflatoxin B1 levels and positively influences intestinal morphology in broiler chickens fed chronic aflatoxin B1-contaminated diets

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ABSTRACT

The present study was conducted to investigate the ability of *Saccharomyces cerevisiae* RC016 (Sc)-based feed additive to reduce liver toxicity, residual aflatoxin B_1 (AFB_1) levels and influence intestinal structure in broiler chickens fed chronic aflatoxin B_1-contaminated diets. A total of 100 one-day-old male commercial line (Ross) broiler chickens were divided into 4 treatments, with 5 pens per treatment and 5 broiler chickens per pen. Birds were randomly assigned to 4 treatments, which were namely treatment 1 (T_1), control diet (CD); T_2, CD + Sc at 1 g/kg; T_3, CD + AFB_1 at 100 μg/kg; T_4, CD + Sc at 1 g/kg + AFB_1 at 100 μg/kg. The liver histopathology of broiler chickens fed diets with AFB_1 showed diffused microvacuolar fatty degeneration. The addition of Sc showed normal hepatocytes similar to the control. The small intestine villi from AFB_1 group showed atrophy, hyperplasia of goblet cells, prominent inflammatory infiltrate and oedema. In contrast, the small intestine villi from birds that received the yeast plus AFB_1 showed an absence of inflammatory infiltrate, and atrophy; moreover, a lower number of goblet cells compared to the groups with AFB_1 was observed. The morphometric intestine studies showed that a significant decrease ($P < 0.05$) in the crypt depth values when Sc was applied to AFB_1-contaminated diets. Although the intestinal villus height and apparent adsorption area did not show significant differences ($P > 0.05$), there was a tendency to improve these parameters. The residual levels of AFB_1 in livers were significantly reduced ($P < 0.05$) in the presence of the yeast. The present work demonstrated that the addition of Sc alone or in combination with AFB_1 in the broiler chicken diets had a beneficial effect in counteracting the toxic effects of AFB_1 in livers besides improving the histomorphometric parameters and modulating the toxic effect of AFB_1 in the intestine.

1. Introduction

Mycotoxins are secondary metabolites produced by *Aspergillus*, *Penicillium* and *Fusarium* fungal species. Frequently, mycotoxins are found in very low concentrations in plants, which is impossible to control by chemical or biological methods and tend to accumulate causing a negative impact on animals (Speijers and Speijers, 2004). Aflatoxin B_1 (AFB_1) is one of the most toxic mycotoxins classified by the International Agency for Research on Cancer as group 1 carcinogens (IARC, 2012). The effect of AFB_1 in broiler chickens mainly

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depends on the time and dose of exposure (Oguz et al., 2003). Moreover, it has been widely known as hepatotoxic and hepatocarcinogenic agent, causing immunosuppression during chronic intoxications due to its ability to bind DNA, affecting protein synthesis. Aflatoxin B_1 has been shown to produce morphological alterations of the intestinal epithelium by increasing the depth of the crypts and decreasing the height of the villi, mainly at the level of the small intestine (duodenum and jejunum). This alteration results in a modification of the functionality of the small intestine, affecting the absorption of the nutrients and consequently the productive parameters (Yunus et al., 2010).

Adsorbent agents incorporated in diets sequester the toxins in the gastrointestinal tract forming insoluble complexes that are eliminated in the faeces (Yiannikouris et al., 2006). Thus, reducing the bioavailability of mycotoxins decreases their toxic effects. Several zeolites, bentonites and clinoptilolite, which are natural adsorbents, have been evaluated *in vitro* and *in vivo* for their ability to adsorb aflatoxins (Magnoli et al., 2011; Nemati et al., 2014). Although these products are widely available as commercial feed additives, they have negative effects reducing nutritional value of feeds or producing undesirable side effects (Zain, 2011). Organic compounds such as *Pichia* sp. yeast based product and yeast cell wall-based products have also been suggested to reduce *in vivo* the negative effects produced by mycotoxins (Roto et al., 2015; Magnoli et al., 2017). While *in vitro* studies are extensive, few *in vivo* studies have demonstrated the effectiveness of biological adsorbents by evaluating the effects on intestinal integrity and liver toxicity with chronic experimental levels of AFB_1. Thus, the aim of this study was to investigated the ability of *Saccharomyces cerevisiae* RC016 (Sc)-based feed additive to reduce liver toxicity, residual AFB_1 levels and to influence on intestinal morphology in broiler chickens fed chronic AFB_1-contaminated diets.

2. Materials and methods

The working protocol and the used techniques comply with the regulations of the Subcommittee on Animal Bioethics under the Ethics Committee of Scientific Research, as established in Resolution 253/10 of the Superior Council of the National University of Rio Cuarto.

2.1. Yeast biomass production

S. cerevisiae RC016 isolated from animal ecosystem was identified by molecular techniques through DNA extraction and 18S rRNA and 28S rRNA amplification and analysis, comparing sequences with the Basic Local Alignment Search Tool (BLAST) within the National Centre for Biotechnology Information (NCBI) database (Armando et al., 2012). The strain is currently deposited in the culture collection of the Universidad Nacional de Río Cuarto collection centre, located in Río Cuarto, Córdoba, Argentina.

S. cerevisiae RC016 biomass was obtained from 24-h culture in Yeast-Peptone-Dextrose broth added 1 g of PO_4H_2K per litre in a BioFlo 2000 fermentor (New Brunswick Scientific Co., Inc., Enfield, CT, USA) operated at 28 °C, at 3.6223 g and aeration 1.5 vessel volume per minute during 8 h. The pH value was adjusted to 5 with 6 mol/L sodium hydroxide (NaOH). The working volume was 2 L. The fermenter was first inoculated with 4.5×10^6 cells/mL and samples were taken every hour during 10 h. The optical density at 640 nm was measured and the number of viable cells was counted in a haemocytometer by the trypan blue exclusion assay. After biomass was produced, cells were harvested and concentrated by centrifugation (20 min, $698.75 \times g$) at room temperature, and the pellets were lyophilized and homogenized to be incorporated into the control diet in order to provide a concentration of 1 g yeast/kg feed (0.1%). The levels of inclusion of Sc were selected according to bibliographic references (Seidavi et al., 2017).

2.2. Aflatoxin B_1 production

Enough AFB_1 concentration to contaminate feed for the experiment was produced. Seven-day culture plugs from a reference strain *Aspergillus parasiticus* NRRL2999 were inoculated in 250-mL Erlenmeyer flasks containing 25 g autoclaved rice and 10-mL distilled water. Cultures were incubated in the dark at 28 °C for 7 d, manually stirring the flasks vigorously for 1 min once a day during the first 5 d to enhance the dissemination of conidia in the rice. After incubation, the cultures were autoclaved. The content of flasks was placed in a metallic tray, covered with paper, let dry at 60 °C in a forced air oven and ground with a laboratory mill. Aflatoxin B_1 content of the resulting powder was quantified by high-performance liquid chromatography (HPLC) according to the methodology described by Trucksess et al. (1994). The ratio of AFB_1 to AFG_1 concentration in the culture was 2:1. Aflatoxin B_1 is the most abundant in food and contaminated feed, toxic and carcinogenic to human beings and animals. Therefore, the AFB_1 effect was only tested. The analyses were performed in triplicate. The milled contaminated substrate (60.0 ± 1.1 μg/g) was added to the control diet pre-premixing to provide a concentration of about 100 μg of AFB_1/kg of feed.

2.3. Aflatoxin B_1 determination in feed

Feed sampling for AFB_1 analysis was carried out following the recommendations of the European Union (Regulation 401/2006 and its modification by Regulation 178/2010). Food samples (1 kg) were homogenized and quartered to obtain a laboratory sample. Twenty-five grams of ground feed were extracted with 125 mL of methanol/water (60:40, vol:vol), 80 mL hexane, and 2 g NaCl and shaken 30 min in an orbital shaker. The mixture was filtered using Whatman No 4 filter paper (Whatman, Inc., Clifton, NJ, USA) and 25 mL of the methanol/water phase of the filtrate was extracted twice with 25 and 15 mL of chloroform, respectively. The chloroform phase was vacuum-dried using a rotatory evaporator and the extract was redissolved in 200 μL of mobile phase. The concentration of AFB_1 in each diet was estimated by HPLC-fluorescence according to Trucksess et al. (1994). The mobile phase was methanol–acetonitrile–water (1:1:4, vol:vol:vol), pumped at a flow rate of 1.5 mL/min. For derivatization, aliquots (200 μL) were mixed with 700 μL of acetic acid–trifluoroacetic acid–water (20:10:70) solution and allowed to stand for 9 min at 65 °C in the dark (AOAC, 1994). An HPLC Waters Alliance 2695 system coupled to a fluorescence detector (Waters 2487) was used. Excitation and emission wavelengths were set on of 395 and 470 nm, respectively. Separation was carried out in a C18 Luna Phenomenex column (150 mm × 4.6 mm, 5 μm). Standards for the calibration curve were prepared by dilution of a stock solution of AFB_1 2.06 μg/mL. The concentrations of chromatographic standards were 0.005, 0.010, and 0.015 μg/mL of AFB_1. Standard solutions for the calibration curves were prepared daily. Although the levels of other mycotoxins were not determined, the animals did not present symptomatology associated with deoxynivalenol, nivalenol, zearalenone, ochratoxin A, fumonisin $B_1 + B_2$ and T-2 and HT-2 mycotoxins. The levels of AFB_1 in the broilers control diet were 3 ± 1 μg/kg (natural contamination) and 100 ± 6 μg/kg for the contaminated diets of AFB_1. The analysis was developed in triplicates.

2.4. Experimental design

A total of 100 one-day-old male broiler chickens vaccinated against Mareck disease were divided into 4 treatments with 25

broiler chickens each. The broiler chickens were fed *ad libitum* with each of the experimental diets from 1 to 22 d of age. On d 1, birds were weighed individually (BW ± SD) and were allocated randomly into treatments. The birds were provided continuous fluorescent lighting with feed and water available *ad libitum* until they were 22 d old. During the experimental period, broiler chickens received the diet corresponding to each treatment. A standard corn-soybean meal starter diet commercial (basal diet) that met of Ross 308 Guidelines (Aviagen. Ross 308 Broiler, 2014) requirements was used to formulate the different experimental diets (Table 1).

The experimental diets for each treatment were as follows: treatment 1 (T_1), control diet (CD); T_2, CD + Sc at 1 g/kg; T_3, CD + AFB_1 at 100 μg/kg; T_4, CD + Sc at 1 g/kg + AFB_1 at 100 μg/kg.

Broiler chickens were monitored daily for signs of morbidity and mortality. At the end of the feeding trial, residual levels of AFB_1, macroscopic (colour and size) and microscopic changes in the liver, histopathological changes and morphometric parameters (villus length and width, and crypt depth) in the intestines of the broiler chickens were evaluated.

When broiler chickens reached 22 d old, the feeding trial was terminated, and 5 broiler chickens from each treatment were selected randomly and sacrificed by cervical dislocation. The livers and duodenal loops were removed and fixed in 10% neutral buffered formalin. The fixed tissues were trimmed, embedded in paraffin, and stained with hematoxylin–eosin for histopathological examination by optical microscopy studies. Part of the liver was conserved a −20 °C for residual AFB_1 levels analysis.

2.5. Liver and intestinal histopathology

Portions of approximately 6 mm^2 of liver and duodenal tissue sampleswere fixed in 4% (vol/vol) buffered-saline formaldehyde pH 7.2 to 7.4 at 4 °C, dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) and xylene solutions, embedded in paraffin and cut in ±4 μm histological serial-sections. The histological sections were stained with hematoxylin–eosin for microscopic analysis. Liver slides were examined for characteristic intoxication signs and hepatocellular degeneration of livers was evaluated according to Magnoli et al. (2011). Intestine were examined for damage and inflammation using a standard histopathological grading system described by Del Carmen et al. (2013), histological findings identical to normal mice (grade 0); mild mucosal and/or submucosal inflammatory infiltrate (admixture of neutrophils) and oedema, punctate mucosal erosions often associated with capillary proliferation, muscularis mucosae intact (grade 1); grade 1 changes involving 50% of the specimen (grade 2); prominent inflammatory infiltrate and oedema (neutrophils usually predominating) frequently with deeper areas of ulceration extending through the muscularis mucosae into the submucosa; rare inflammatory cells invading the muscularis propria but without muscle necrosis (grade 3); grade 3 changes involving 50% of the specimen (grade 4); extensive ulceration with coagulative necrosis bordered inferiorly by numerous neutrophils and lesser numbers of mononuclear cells; necrosis extends deeply into the muscularis propria (grade 5); grade 5 changes involving 50% of the specimen (grade 6). High histological scores indicate increased damage in the intestines. Digital images were captured with an Axiophot microscope (Carl Zeiss, Thornwood, NY) fitted with high-resolution Power shot G6 7.1 megapixels digital camera (Canon INC, Japan). Digital image analysis and morphometric measurements were performed with Axiovision AxioVs40 V4.6.3.0 software (Carl Zeiss, Göttingen, Germany).

Table 1
Ingredients of the experimental diet (g/kg, as fed basis).

Item	Content
Macro ingredients	
Milled corn CP 8.0%	641.7
Soybean meal	289.0
Meat meal 40%	53.3
Sodium chloride	2.6
Calcite 38%	4.3
Micro ingredients	
Premix of vitamin and mineral[1]	4.0
Baking soda	1.4
DL-methionine	1.1
L-lysine	2.6
Total (Macro + Micro)	1,000

[1] Premix contains the following per 2.5 kilogram powder: calcium 27.0%, starch 0.04%, crude fibre 0.03%, vitamin A 4,000,000 IU, vitamin D_3 800,000 IU, vitamin E 12,000 IU, vitamin B_1 800 mg, vitamin B_2 2,000 mg, vitamin B_6 1600 mg, vitamin B_{12} 8,000 μg, vitamin K_3 800 mg, pantothenic acid 4,000 mg, niacin 16,000 mg, biotin 60,000 μg, folic acid 400 mg, choline chloride 60,000 mg, iron 16,000 mg, iodine 400 mg, copper 4,000 mg, manganese 32,000 mg, zinc 24,000 mg, selenium 60 mg.

2.6. Intestinal morphology

Morphometric measurements of intestinal variables were carried out on 2 slides per animal's intestine, 2 sections per slide and 5 fields per section. The morphometric measurements taken from the intestinal histological sections included villus length and width, intestinal crypt depth and quantification of goblet cells. Digital images were captured with an Axiophot microscope (Carl Zeiss, Thornwood, NY) fitted with high resolution Power shot G6 7.1 megapixels digital camera (Canon INC, Japan). Digital image analysis and morphometric measurements were performed with Axio vision AxioVs40 V4.6.3.0. software (Carl Zeiss, Göttingen, Germany). Later, apparent absorptive surface area was calculated using the following formula according to Iji et al. (2001).

Apparent absorptive surface area = (3.1 × Villi width + 3.2 × Villi height) × 1 − (2 × Villi height).

2.7. Residual levels of aflatoxin B_1 in livers

A total of 20 livers ($n = 20$) were selected, i.e. 5 livers from each treatment. Aflatoxin B_1 in the liver tissue was extracted according to AOAC (1995) as described by Tavčar-Kalcher et al. (2007) with some modifications. Briefly, the ground liver sample (50 g) wasmixed thoroughly with 5 mL of a 20% aqueous citric acid solution and diatomaceous earth (10 g). The toxin was extracted with 100 mL dichloromethane by stirring for 30 min at room temperature. The organic phase was filtered through Whatman No 4 filter paper (Whatman International Ltd., Maidstone, UK). The water was removed by the addition of 5 g of anhydrous sodium sulphate and the extract was filtered a second time. Twenty milliliters of the filtrate was evaporated to dryness at 60 °C. The residue was redissolved in 20 mL of acetonitrile–H_2O (75:25, vol:vol) and extracted with 10 mL of hexane for the removal of fat. The mixture was thoroughly mixed, centrifuged and 10 mL of the aqueous phase were evaporated to dryness. For cleaning, the dry extract was redissolved in 10 mL of methanol–H_2O (80:20, vol:vol), 90 mL of distilled water was added and passed through a preconditioned OASIS, HLB, 6 mL (200 mg) SPE cartridges (Waters Corporation, Milford, MA, USA) according to the methodology described bySørensen and Elbæk (2005). Solid phase extractions were performed on a Vac Elut 20 position Manifold SPE (Agilent Technologies Inc., Santa Clara, CA, USA). The toxin was eluted with 7 mL of methanol, evaporated to dryness and stored at −20 °C until analysis. The extracts were redissolved in 500 mL of methanol–H_2O (20:80, vol:vol) and AFB_1 was quantified by HPLC according to Magnoli et al. (2016).

2.8. Statistical analysis

Data were analysed by general linear and mixed model (GLMM) using InfoStat (version 2.03 for Windows 2012; University of Cordoba, Argentina) software 2008 (Di Rienzo et al., 2008). The parameters data were analysed by analysis of variance (ANOVA). Means and standard error (SEM) were compared using the Fisher's protected least significant test ($P < 0.05$ and $P < 0.0001$).

3. Results

3.1. Liver and intestine histopathology

During the experimental period no signs of morbidity or mortality were observed.

Macroscopic changes in the colour, size, weight, consistency, and shape the livers from broiler chickens fed the different diets were not observed (Fig. 1).

Fig. 2 shows the photomicrographs of haematoxylin and eosin-stained liver sections of chickens in different dietary treatments. Livers from control and *S. cerevisiae* groups did not show histopathological alterations (Fig. 2A and B). In contrast, histological analysis results revealed significant damage ($P < 0.05$) in the liver tissue of broiler chickens that consumed 100 μg/g AFB_1 alone (Fig. 2C) showing diffuse microvacuolar fatty degeneration throughout the organ. These effects were prevented in livers from broiler chickens fed diets with AFB_1 plus addition of Sc, showing normal hepatocytes similar to the control (Fig. 2D). Liver tissues had moderate hydropic and an unmarked peripheral degeneration. Also, there was no proliferation of bile ducts. Hepatocytes from 3 hepatic lobules showed generalized vacuolar type cytoplasm. Moreover, they showed a marked decrease in the fat microvacuoles being similar in appearance to the livers of broiler chickens fed with yeast alone (Fig. 2B) and the livers of broiler chickens fed control diet (Fig. 2A).

3.2. Intestinal morphology

Fig. 3 shows the representative microstructure of intestinal villi of broilers at 22 d of age from each treatment. Non histopathological alterations were observed in the small intestine of control broiler chickens (grade 0) (Figs. 3A and 4A). The villi in the small intestine from the yeast group showed long villi with slight atrophy, absence of hyperplasia of the goblet cells, hyperemia and inflammatory infiltrate (grade 1) (Figs. 3B and 4B). The small intestine villi from the AFB_1 group showed an important atrophy, hyperplasia of goblet cells, prominent inflammatory infiltrate and oedema (neutrophils usually predominating) (grade 3) (Figs. 3C and 4C). In the group with yeast plus AFB_1, the absence of hyperemia, normal villi, lower number of goblet cells and atrophy compared to the group with AFB_1 was observed (grade 1) (Figs. 3D and 4D).

Table 2 shows the results obtained from the morphometric measurements, villus height, villus width, crypt depth, the goblet cells number and apparent adsorption area for the different treatments assayed. The morphometric intestinal studies showed that AFB_1 had a significant toxic effect on crypt depth, adsorption area and villus height compared to the control ($P < 0.05$). In broiler chickens fed diets with AFB_1 the villus height and the apparent absorption area showed the lowest values in relation to the control. The crypt depth was inversely affected in relation to the other parameters mentioned above. On other hand, when yeast was applied in the diets with the toxin, a significant decrease ($P < 0.05$) in the values of the crypt depth, were observed while height intestinal villus and apparent adsorption area did not show significant differences, however a tendency to improve these parameters was observed.

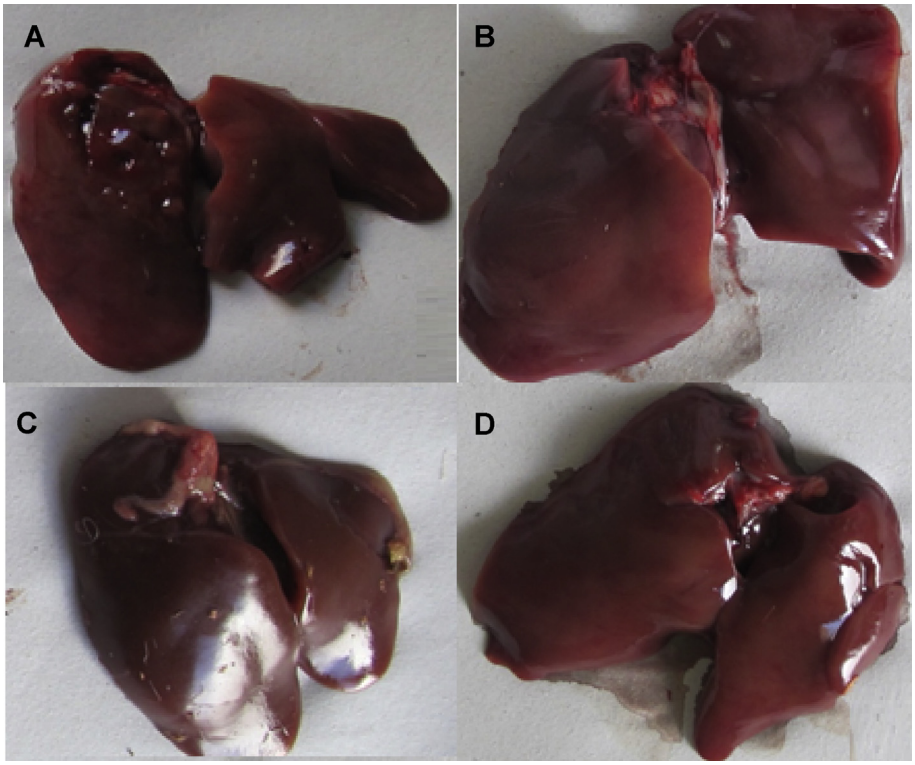

Fig. 1. Representative livers from broilers (22 d old) in different treatments. (A) Treatment 1 (T_1): control diet (CD); (B) T_2: CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; (C) T_3: CD + aflatoxin B1 (AFB1) at 100 μg/kg; (D) T_4: CD + Sc at 1 g/kg + AFB1 at 100 μg/kg.

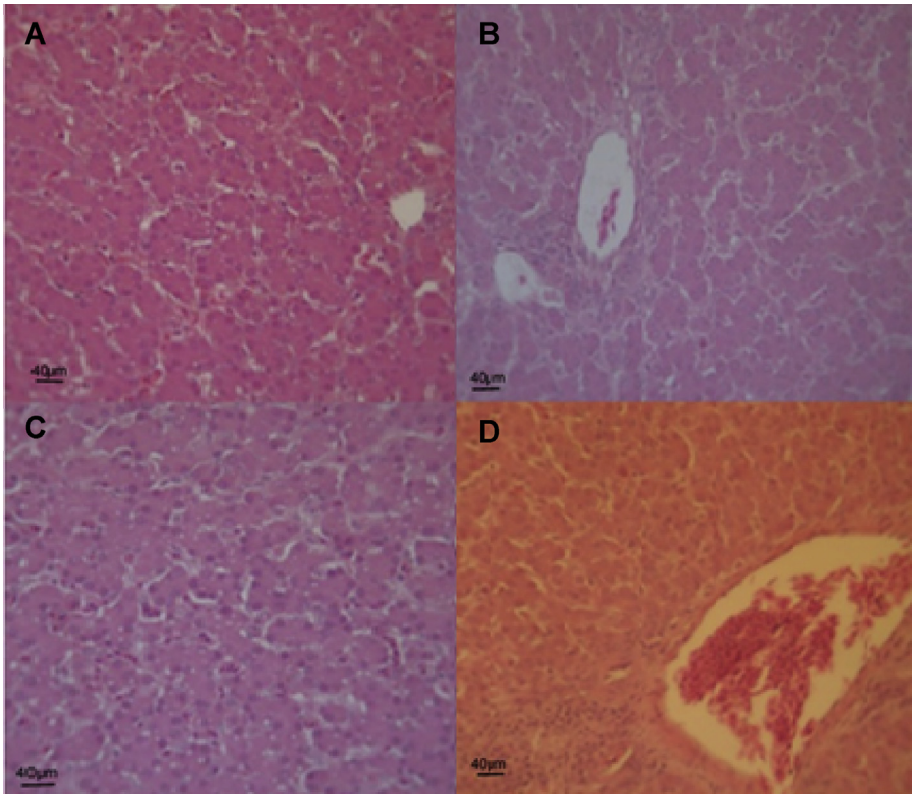

Fig. 2. Photomicrographs (optical microscopy) of haematoxylin and eosin-stained broiler liver sections in 4 treatments, 40 × magnification. (A) Treatment 1 (T_1): control diet (CD); (B) T_2: CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; (C) T_3: CD + aflatoxin B1 (AFB1) at 100 μg/kg; (D) T_4: CD + Sc at 1 g/kg + AFB1 at 100 μg/kg, Scale bar = 40 μm.

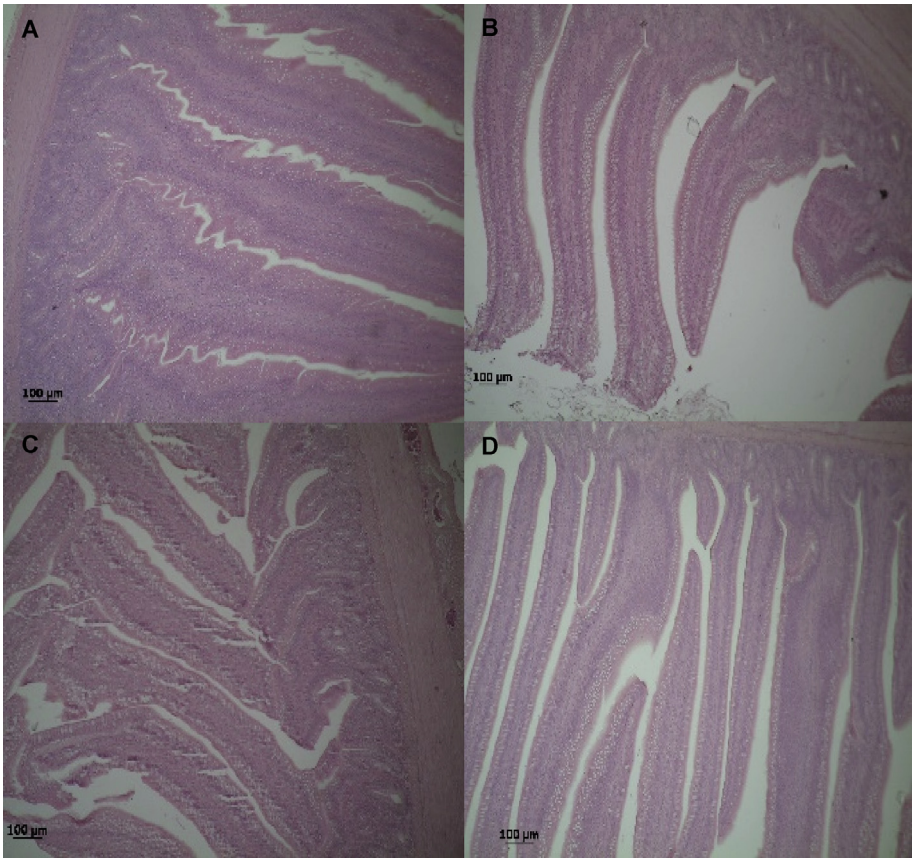

Fig. 3. Photomicrographs (optical microscopy) of representative microstructure of intestinal villi of broilers at 22 d of age in 4 treatments, 10 × magnification. (A) Treatment 1 (T_1): control diet (CD); (B) T_2: CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; (C) T_3: CD + aflatoxin B_1 (AFB_1) at 100 μg/kg; (D) T_4: CD + Sc at 1 g/kg + AFB_1 at 100 μg/kg. Scale bar = 100 μm.

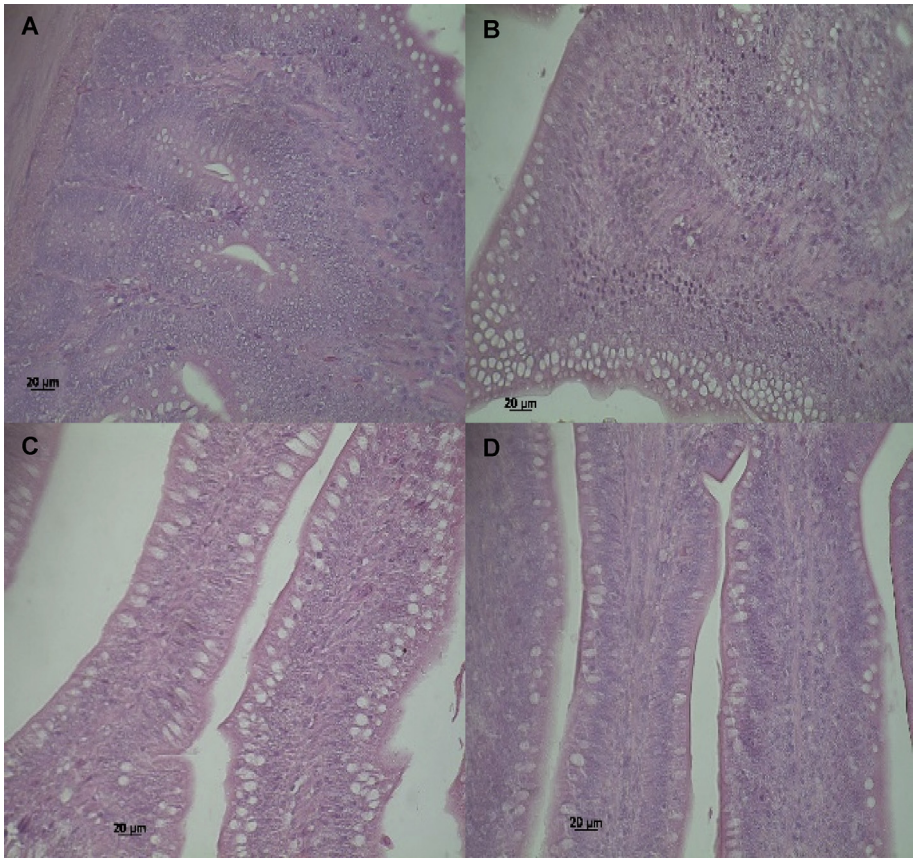

Fig. 4. Photomicrographs (optical microscopy), 40 × magnification. The representative microstructure of intestinal villi at 22 d of age from each treatment. (A) Treatment 1 (T_1): control diet (CD); (B) T_2: CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; (C) T_3: CD + aflatoxin B_1 (AFB_1) at 100 µg/kg; (D) T_4: CD + Sc at 1 g/kg + AFB_1 at 100 µg/kg. Haematoxylin and eosin stain. Scale bar = 20 µm.

Table 2
Length of intestinal villi, depth of crypts, adsorption area, villus width and goblet cells determinations of broilers in different treatments.

Item[1]	Intestinal villus height, µm	Villus width, µm	Crypt depth, µm	Apparent adsorption area, μm^2	Goblet cells, number of cells per villus
T_1	3,012.59 ± 535.98^{b}	2,123.71 ± 1055.88^{c}	250.22 ± 190.94^{a}	6,519.9 ± 1206.6^{b}	205.8 ± 71.33
T_2	1,891.53 ± 521.38^{a}	630.46 ± 454.53^{bc}	291.59 ± 7.31^{b}	6,231.5 ± 411.63^{b}	196.4 ± 47.90
T_3	1,733.67 ± 72.23^{a}	1,317.73 ± 257.42^{ab}	283.92 ± 17.94^{b}	2,557.1 ± 912.63^{a}	203.1 ± 76.20
T_4	1,840.39 ± 42.76^{a}	390.62 ± 114.72^{a}	254.29 ± 28.42^{a}	2,590.8 ± 392.55^{a}	208.9 ± 87.43

$^{a, b, c}$ With in a column, means without common superscripts are significantly different ($P < 0.05$) according to the Fisher's protected least significant test.
[1] Treatment 1 (T_1), control diet (CD); T_2, CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; T_3, CD + aflatoxin B_1 (AFB_1) at 100 µg/kg; T_4, CD + Sc at 1 g/kg + AFB_1 at 100 µg/kg.

There was no significant difference in the number of goblet cells among the assayed treatments.

3.3. Residual levels of aflatoxin B_1 in livers

Table 3 shows the residual levels of AFB_1 in livers of broiler chickens in different treatments. The livers of treatment control (T_1) and treatment with yeast (T_2) did not show detectable residual levels of AFB_1. Livers from animals fed diets with 100 µg/kg of AFB_1 (T_3) showed the presence of AFB_1 in livers (1.26 µg/g). The AFB_1 residual levels in broiler chickens livers fed diets with Sc plus AFB_1 (T_4) were significantly lower (1.01 µg/g) than those receiving AFB_1 alone ($P < 0.05$).

4. Discussion

Aflatoxins contamination is a constant hazard to the poultry industry that results in substantial economic losses to producers due to sub-lethal but toxic effects of AFB_1. In the present study, the liver histopathology of broiler chickens fed diets with Sc did not show the typical pattern of subclinical aflatoxicosis demonstrated with AFB_1; the macroscopic and microscopic alterations of the tissue were not observed, highlighting its beneficial effect. The effects of AFB_1 in of broiler chickens are well known; other researchers reported microscopic lesions of livers as target organs in broiler chickens fed dietary whit levels 50 and 100 µg/kg AFB_1 (Magnoli et al., 2011). Azizpour and Moghadam (2015) reported that the addition of yeast cell wall (0.05% and 0.1%) mitigated the negative effects of AFB_1 on the liver histopathology in broiler chickens. Also, Magnoli et al. (2017) using *Pichia kudriavsevii* (0.1%) demonstrated the effectiveness to prevent the toxic effects of AFB_1 in the liver macroscopy and histopathology of broiler chickens fed diets with 100 µg/kg of AFB_1 at 22 d of age.

Morphological parameters such the length of the villi, depth of the crypt, villus to crypts ratio and surface area of the villi are usually used to investigate the effects of microorganisms on

Table 3
Residual levels of aflatoxin B_1 (AFB_1) in livers of broilers fed different diets.

Item[1]	AFB_1, μg/g
T_1	nd
T_2	nd
T_3	1.26 ± 0.04[a]
T_4	1.01 ± 0.03[b]

nd = not detected.
[a, b] With in a column, means without common superscripts are significantly different ($P < 0.05$) according to the Fisher's protected least significant test.
[1] Treatment 1 (T_1), control diet (CD); T_2, CD + *Saccharomyces cerevisiae* RC016 (Sc) at 1 g/kg; T_3, CD + aflatoxin B_1 (AFB_1) at 100 μg/kg; T_4, CD + Sc at 1 g/kg + AFB_1 at 100 μg/kg.

intestinal morphology and cell proliferation. These parameters but especially the area was positively related with the absorptive efficiency of the small intestine in broiler chickens considered indicators of intestinal functions (Matur and Eraslan, 2012). The present study showed long villi only with slight atrophy when Sc was added demonstrating a preventive effect on the histomorphological damage in the intestine caused by AFB_1 that decreased height of the intestinal villi, number of goblet cells small intestine's surface area for absorption. These results were similar to those demonstrated by Zhang et al. (2005) who observed that supplementation of diets with yeast cell wall showed longer and intact villi in relation to controls without cell wall. Liew et al. (2018) and Wang et al. (2018) evaluated the damage of the small intestine induced by AFB_1 in broiler chickens and found similar AFB_1 lesions than those in the present study.

The microorganisms used as probiotics affect the functions and counts of the goblet cells in the intestinal mucosa. The mucus secreted by these cells is one of the factors that make up the intestinal barrier preventing the invasion of pathogens in the digestive tract (Matur and Eraslan, 2012). In the present study, the number of goblet cells in the small intestine of the broiler chickens was similar among all treatments. However, when histological evaluation was performed goblet cells hyperplasia and inflammatory infiltrate was observed in broiler chickens fed diets with AFB_1, similar to results reported by Liew et al. (2018) who demonstrated an accumulation of lymphocytes in intestine indicating the occurrence of inflammation in rats feed with AFB_1. However, the presence of Sc showed normal and reduced atrophy compared to the AFB_1 treatment.

The liver is the target organ where AFB_1 is metabolized, detoxified and/or conjugated with nucleic acids and proteins (Liew et al., 2018). There is a percentage of **a**flatoxin that can accumulate without changes in the liver, muscle and other edible animal tissues (Magnoli et al., 2011). In the present study, no detectable residual AFB_1 levels were found both in control livers and those from animals receiving dietary yeasts. Chicks fed diets with AFB_1 (100 μg/kg) showed the presence of residual levels in livers, whereas Sc addition (1 g/kg) significantly reduced them in livers. Similar results were reported by Magnoli et al. (2017) who observed a decrease in AFB_1 residual levels in broiler chickens liver fed diets with AFB_1 (100 μg/kg) and the yeast *Pichia kudriavzevii* (0.1%).

5. Conclusion

In conclusion, the probiotic Sc administration was effective in counteracting the toxic effects caused by low levels of AFB_1 in broiler chicken livers and gut histomorphometry. Moreover, residual levels of AFB_1 were prevented in livers. These results are promising for the development of future feed additives that provide benefits for both food safety and consumer health. More experiments are needed to optimize the way to incorporate these additives in feed and to evaluate their viability over time.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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