Evaluation of *Saccharomyces cerevisiae* as an antiaflatoxicogenic agent in broiler feedstuffs

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ABSTRACT Aflatoxins (AF) are the most important mycotoxins produced by toxigenic strains of various *Aspergillus* spp. Biological decontamination of mycotoxins using microorganisms is a well-known strategy for the management of mycotoxins in feeds. *Saccharomyces cerevisiae* strains have been reported to bind aflatoxin B_1 (AFB_1). The aim of this study was to evaluate the ability of *S. cerevisiae* CECT 1891 in counteracting the deleterious effects of AFB_1 in broiler chicks. Experimental aflatoxicosis was induced in 6-d-old broilers by feeding them 1.2 mg of AFB_1/kg of feed for 3 wk, and the yeast strain was administrated in feed (10^{10} cells/kg), in the drinking water (5×10^9 cells/L), or a combination of both treatments. A total of 160 chicks were randomly divided into 8 treatments (4 repetitions per treatment). Growth performance was measured weekly from d 7 to 28, and serum biochemical parameters, weights, and histopathological examination of livers were determined at d 28. The AFB_1 significantly decreased the BW gain, feed intake, and impaired feed conversion rate. Moreover, AFB_1 treatment decreased serum protein concentration and increased liver damage. The addition of *S. cerevisiae* strain to drinking water, to diets contaminated with AFB_1, showed a positive protection effect on the relative weight of the liver, histopathology, and biochemical parameters. Furthermore, dietary addition of the yeast strain to drinking water alleviated the negative effects of AFB_1 on growth performance parameters. In conclusion, this study suggests that in feed contaminated with AFB_1, the use of *S. cerevisiae* is an alternative method to reduce the adverse effects of aflatoxicosis. Thus, apart from its excellent nutritional value, yeast can also be used as a mycotoxin adsorbent.

Key words: aflatoxin B_1, *Saccharomyces cerevisiae*, broiler chick, performance, liver histopathology

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INTRODUCTION

Aflatoxins (**AF**) are naturally occurring mycotoxins, produced as secondary metabolites by species of *Aspergillus*, mainly *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus* (Payne, 1998; Ito et al., 2001; Rawal et al., 2010). Among the various dangerous AF and their metabolites, aflatoxin B_1 (**AFB_1**) is the most widespread and potent naturally occurring hepatocarcinogen in animals and humans (Ellis et al., 1991; Massey et al., 1995; Pitt et al., 2000). On the other hand, the economic impact of AF contamination occurs at all levels of agricultural commodities and animal production, at various commercial stages, including both products and by-products (Kubena et al., 1990; CAST, 2003). Losses caused by AF contamination are associated with the frequency of contamination of agricultural products and with the time of exposure of animals to chronic levels of these compounds through the intake of contaminated rations (CAST, 2003). Due to the ubiquitous nature of *Aspergillus* species in the environment, some level of mycotoxin contamination of feed grain is unavoidable (Wilkinson et al., 2003; Matur et al., 2010).

Poultry are exposed to AF by eating feeds prepared with contaminated raw materials, and the produced mycotoxicosis, aflatoxicosis, apart from affecting poultry health, reduces production and affects the availability of certain products and their marketing (Osuna, 1996; Oğuz, 2011). The natural incidence of potential aflatoxigenic strains and AF in Argentinean poultry feed were previously reported (Dalcero et al., 1997, 1998; Magnoli et al., 2002). Aflatoxicosis in poultry causes listlessness, anorexia with low growth rate, poor food utilization, weight gain decrease, egg production

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decrease, increased susceptibility to environmental and microbial stresses, and increased mortality (Bailey et al., 1998; Oğuz and Kurtoglu, 2000; Oliveira et al., 2007). Anemia, mutagenicity, carcinogenicity, and teratogenicity were also reported (CAST, 2003; Rawal et al., 2010). With increasing knowledge and awareness of AF as a potent source of health hazards to both human and farm animals, a great deal of effort has been made to completely eliminate the mycotoxin or reduce its content in foods and feedstuffs to significantly lower levels (Rustom, 1997).

Techniques for the elimination or inactivation of AF include physical and chemical methods. However, no universally applicable, effective, and practical methods are currently available. Thus, novel approaches have focused on preventing AFB_1 absorption in the gastrointestinal (**GI**) tract of humans and animals (Phillips, 1999; El-Nezami et al., 2000; Gratz et al., 2007). Biological decontamination of mycotoxins using microorganisms is a well-known strategy for the management of mycotoxins in foods and feeds. Therefore, a promising alternative is the use of microorganisms as AFB_1-sequestering agents. Inclusion of such microbes in the diet may reduce the toxic effects of mycotoxins on farm animals because an AFB_1-microorganism complex may decrease availability of the mycotoxin and consequently its absorption in the GI tract (Gratz et al., 2007; Hernandez-Mendoza et al., 2009; Pizzolitto et al., 2012a). In the poultry industry, *S. cerevisiae* has been used as a general performance promoter in poultry feeds and has recently been shown to have beneficial effects against AFB_1 exposure (Madrigal-Santillán et al., 2006; Shetty and Jespersen, 2006; Firmin et al., 2011). Animal feeding experiments with whole yeast and yeast cell wall have shown that the addition of *S. cerevisiae* in the diet resulted in reduced mycotoxin toxicities, indicating possible stability of the yeast-mycotoxin complex through the GI tract (Çelýk et al., 2003; Santin et al., 2003). Although much literature has been devoted to the protective effects of yeasts against AFB_1 in poultry by their inclusion in animal diets, there is no information available analyzing the efficacy of yeasts to reduce bioavailability and diminish the toxic effects of AF in broiler chicks through their inclusion in drinking water. On the other hand, in previous studies we demonstrated that *S. cerevisiae* CECT 1891 showed high efficiency in AFB_1 removal from liquid media (Bueno et al., 2007; Pizzolitto et al., 2011). The objective of this investigation was to evaluate the efficacy of *S. cerevisiae* CECT 1891 strain to protect against aflatoxicosis in broiler chicks by its administration in feed, in the drinking water, or a combination of both treatments. In the present study, the measured parameters were serum biochemistry (concentration of proteins, albumin, and globulins), productive parameters [feed consumption (**FC**), BW gain, and feed conversion ratio (**FCR**)] and relative liver weights and histopathologic changes.

Table 1. Composition (g/kg of diet) and proximate analyses (g/kg of diet) of the basal diet

Item	Grower diet
Ingredient	
Yellow corn	697.5
Soybean oil meal	220.0
Meat and bone meal	73.0
Vitamin and mineral mix[1]	1.5
NaCl	2.5
Oyster shell	3.5
DL-Methionine	2.0
Total	1,000.0
Proximate composition of the test diets	
CP	214.0
Crude fat	50.0
Crude fiber	25.0
Calcium	9.5
Methionine	5.0
Tryptophan	2.3
ME (kcal/kg)	3,100.0

[1]Vitamin and mineral mix provided the following (mg/kg of feed): vitamin A, 10×10^6 IU; vitamin D_3, 3×10^6 IU; vitamin K, 33 g, vitamin B_1, 1 mg; vitamin B_2, 2.5 mg; vitamin B_6, 2.5 mg; vitamin B_{12}, 0.0125 mg; folic acid, 0.25 mg; nicotinic acid, 25 mg; calcium pantothenate, 10 mg; biotin, 0.01 mg; choline chloride, 240 mg; manganese, 87.5 mg; iron, 60 mg; copper, 7.5 mg; zinc, 68.75 mg; I, 1.0 mg; Se, 0.2 mg; and butylated hydroxytoluene, 0.312 mg.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

In this experiment, 1-d-old Ross male broiler chicks were obtained from a commercial hatchery. The birds were kept under continuous incandescent electric lamps with basal diet and water available for ad libitum consumption until they were 6 d old. A standard starter corn soybean meal diet, which meets NRC requirements, was fed from d 1 until the end of the experiment (NRC, 1994). The composition and proximate analyses of experimental diets are presented in Table 1.

On d 6, a total of 160 chicks were randomly selected by similar BW and divided into 8 treatments (**T1** to **T8**) with 4 replicate groups per treatment and 5 chicks per replicate group distributed in 32 pens measuring 90 cm × 100 cm × 39 cm (length × width × height). All pens had a 1 cm^2 wire mesh bottom with a removal tray placed underneath to allow cleaning. The pens were also equipped with a feeding trough placed outside and one water cup placed inside. From d 7 until d 28, chicks received the corresponding experimental diets. The different treatments resulted from the combination of the presence or absence of *S. cerevisiae* CECT 1891 in drinking water (5×10^9 cells/L) with the presence or absence of AFB_1 (1.2 mg/kg) and *S. cerevisiae* CECT 1891 (10^{10} cells/kg) supplementation in feed (Table 2). During the treatments (lasting 3 wk), birds were monitored daily for signs of morbidity and mortality and also weighed weekly.

Table 2. Treatment groups[1]

	Saccharomyces cerevisiae CECT 1891		Aflatoxin B_1
Treatment	Feed (cells/kg)	Water (cells/L)	Feed (mg/kg)
T1	0	0	0
T2	10^{10}	0	0
T3	0	5×10^9	0
T4	10^{10}	5×10^9	0
T5	0	0	1.2
T6	10^{10}	0	1.2
T7	0	5×10^9	1.2
T8	10^{10}	5×10^9	1.2

[1]Each treatment group consisted of 4 replicates containing 5 birds each (20 birds per treatment).

AF Production and Diet Preparation

Aflatoxins were produced via fermentation of rice by *Aspergillus parasiticus* NRRL 2999 (USDA, Agricultural Research Service, Peoria, IL). The sterile substrate, placed in Erlenmeyer flasks, was inoculated with 2 mL of the mold aqueous suspension containing 10^6 spores/mL. Cultures were allowed to grow for 7 d at 25°C in darkness. On the d 7, Erlenmeyer flasks were autoclaved and culture material was dried for 48 h at 40°C in a forced-air oven and ground to a fine powder. The AFB_1 levels in rice powder were measured by thin-layer chromatography and HPLC (Trucksess et al., 1994; AOAC International, 1995). The milled substrate was added to the basal diet to provide a level of 1.2 mg of AFB_1/kg of feed. The concentrations of AFB_1 in each diet were confirmed by HPLC. The natural level of AFB_1 in the basal diet was 10 μg/kg of AFB_1 and in the contaminated diet was 1.2 mg of AFB_1/kg of AFB_1. The dose selected for AFB_1 is a dose proven to reduce the productive performance also affecting the serum biochemical parameters and liver weight in broilers (Raju and Devegowda, 2000; Dersjant-Li et al., 2003; Tedesco et al., 2004; Devegowda and Murthy, 2005; Denli et al., 2009).

Saccharomyces cerevisiae Culture

Saccharomyces cerevisiae CECT 1891 (Spanish Type Culture Collection, University of Valencia, Spain) was grown on yeast extract peptone dextrose (**YPD**) broth (0.5% yeast extract and 0.5% peptone, 4% glucose) in an orbital incubator at 150 rpm for 24 h at 25°C. The inoculum of the yeast strain was prepared from an overnight culture in YPD broth at 25°C. Then, 250-mL Erlenmeyer flasks containing 100 mL of YPD were inoculated with 1 mL of the respective inoculum. Then, the solid phase was separated by centrifugation at 3,000 $\times$ *g* for 30 min at room temperature and lyophilized. The sample was processed in a Labconco Freeze Dryer (Freezone 6), with a temperature of −50°C and a negative pressure of 50 mBar. Lyophilized cells of *S. cerevisiae* CECT 1891 were incorporated in feed (10^{10} cells/kg), in the drinking water (5×10^9 cells/L), or both.

Variables Measured

Production Parameters. The production parameters were measured weekly during treatment period (7 to 28 d of age). Feed consumption, BW gain, and feed conversion ratio were determined.

Biochemical Parameters. At the end of the experiment, blood samples (2 mL per bird) were collected from broiler chicks for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2,500 $\times$ *g* for 15 min at room temperature) and stored at −20°C until further analysis. The concentration of total protein was measured by following the Biuret method and albumin by following the bromocresol green method (Oğuz et al., 2000; Rosa et al., 2001).

Necropsy, Gross Pathology, and Histopathology. At 28 d of age, necropsy was performed on 2 birds from each cage (8 birds per treatment). The livers were collected and were carefully examined macroscopically, and gross lesions were recorded first. Then the organs were weighed after examination and the organ to BW ratios were calculated (weight of organ/100 g of live BW). After being weighed, the organs were fixed in 4% paraformaldehyde and processed for paraffin embedding by standard methods. The paraffin-embedded sections of 5-μm thickness were cut, stained with hematoxylin and eosin, and examined under light microscopy. Liver sections of all birds were microscopically examined.

Statistical Analysis

Data were evaluated with ANOVA for a complete randomized design, using the GLM procedure of SAS software (SAS Institute, 1985). When the ANOVA showed significance, Tukey's significant-difference test was applied.

RESULTS

In the present study, the effects of different diets on growth performance (BW gain, FC, and feed conversion ratio) were determined and are shown in Tables 3 and 4. On d 7, there were no significant treatment effects. On d 14, AFB_1 decreased FC, BW, and increased feed:gain

Table 3. Effects of *Saccharomyces cerevisiae* CECT 1891 on growth performance[1] of broiler chicks fed diets containing 1.2 mg of aflatoxin B_1/kg of diet at d 7 and 14 of treatment

	d 7			d 14		
Treatment	FC (g)	BW gain (g)	Feed:gain ratio (g:g)	FC (g)	BW gain (g)	Feed:gain ratio (g:g)
T1	1,550 ± 132[a]	980 ± 20[a]	1.58 ± 0.14[a]	4,423 ± 98[a]	2,600 ± 53[a]	1.70 ± 0.03[a]
T2	1,657 ± 131[a]	982 ± 20[a]	1.69 ± 0.16[a]	4,450 ± 130[a]	2,593 ± 105[a]	1.71 ± 0.02[a]
T3	1,582 ± 88[a]	980 ± 50[a]	1.61 ± 0.10[a]	4,428 ± 138[a]	2,595 ± 18[a]	1.71 ± 0.04[a]
T4	1,607 ± 90[a]	973 ± 25[a]	1.67 ± 0.23[a]	4,472 ± 93[a]	2,492 ± 71[a]	1.80 ± 0.03[b]
T5	1,427 ± 89[a]	905 ± 75[a]	1.59 ± 0.18[a]	4,150 ± 132[b]	2,260 ± 48[b]	1.84 ± 0.03[bc]
T6	1,497 ± 118[a]	922 ± 40[a]	1.63 ± 0.17[a]	3,983 ± 186[b]	2,113 ± 103[b]	1.89 ± 0.03[c]
T7	1,545 ± 38[a]	978 ± 20[a]	1.58 ± 0.06[a]	4,493 ± 107[a]	2,617 ± 71[a]	1.72 ± 0.01[a]
T8	1,450 ± 108[a]	922 ± 36[a]	1.57 ± 0.13[a]	4,053 ± 125[b]	2,267 ± 85[b]	1.80 ± 0.01[b]

[a–c]Values within columns with no common superscripts are significantly different ($P \leq 0.05$).

[1]Results are reported as the mean ± SD for 4 lots (number of birds per treatment: 20). FC = feed consumption.

(T5); yeast added to the feed (T6) did not alter the effects of AFB_1; yeast administered in the drinking water (T7) appeared to spare the effect of AF and was not significantly different from the controls ($P < 0.05$); but when yeast was administered in both the drinking water and the feed (T8), there was not a significant protective effect. On d 21, a significant decrease in BW gain and FC was observed in birds fed AFB_1 alone ($P < 0.05$), and the feed conversion ratio (g of feed/g of gain) was significantly ($P < 0.05$) increased by AFB_1 intake (T5). Also on d 21, the presence of *S. cerevisiae* CECT 1891 in the diet without AFB_1 (T2 to T4) showed significant differences among treatments; when yeast was administered in the drinking water (T7), the inhibitory effects of AFB_1 were diminished and the impaired growth performance recovered in relation to those obtained for the control treatments ($P < 0.05$); when yeast was added to both the drinking water and the feed (T8), there was not a protective effect of the AF on FC and BW, but there was a small effect on the AFB_1 on feed:gain, with values not significantly different from control values (T1). Additionally, the changes in the biochemical parameters for different treatments were examined, and results are shown in Table 5. Compared with the control, a decrease in the serum levels of the total protein, albumin, and globulins was observed in chicks exposed to diets containing AFB_1 and *S. cerevisiae* CECT 1891 after 21 d of treatment. It was interesting to observe that the diet of birds treated simultaneously with AFB_1 plus *S. cerevisiae* CECT 1891 added to the drinking water (T7) showed a reduction in the negative effects of aflatoxicosis on biochemical parameters. No treatment difference was found between chicks fed the control diet and chicks fed the diets containing *S. cerevisiae* CECT 1891 alone.

Figure 1 shows the macroscopic appearances of livers from chicks that had been fed the control diet, *S. cerevisiae* CECT 1891, AFB_1, and a combination of AFB_1 and yeast cells in feed, drinking water, or both at d 21 of treatment. Livers of broilers whose fed diets contained AFB_1 (T5), AFB_1 plus *S. cerevisiae* CECT 1891 in feed (T6), and AFB_1 supplemented with *S. cerevisiae* CECT 1891 in feed and in drinking water (T8) were friable and pale in appearance (Figure 1B, C, and E), whereas livers of broilers that consumed a diet with AFB_1 supplemented with *S. cerevisiae* CECT 1891 in drinking water (T7) were normal in appearance (Figure 1D).

The effect of AFB_1 on the liver tissue of the birds that were fed the control diet (T1) and the diet amended with AFB_1 (T5) and AFB_1 supplemented with *S. cerevisiae* CECT 1891 in drinking water (T7) is shown in Figure 2. Compared with the control (Figure 2A), histopathologies of livers from birds fed T5 showed multifocal and varied cytoplasmic vacuolation with perilobular location when stained with hematoxylin

Table 4. Effects of *Saccharomyces cerevisiae* CECT 1891 on growth performance[1] of broiler chicks fed diets containing 1.2 mg of aflatoxin (AF)/kg of diet at d 21 of treatment

	S. cerevisiae CECT 1891		AFB_1			
Treatment	Feed (cells/kg)	Water (cells/L)	Feed (mg/kg)	FC (g)	BW gain (g)	Feed:gain ratio (g:g)
T1	0	0	0	8,138 ± 76.5[a]	4,192 ± 127.7[a]	1.95 ± 0.05[ab]
T2	10^{10}	0	0	8,348 ± 162.9[a]	4,202 ± 189.2[a]	1.99 ± 0.03[ab]
T3	0	5×10^9	0	8,273 ± 108.3[a]	4,297 ± 172.5[a]	1.93 ± 0.05[a]
T4	10^{10}	5×10^9	0	8,299 ± 80.5[a]	4,057 ± 141.8[a]	2.05 ± 0.05[b]
T5	0	0	1.2	7,773 ± 56.4[b]	3,598 ± 130.0[b]	2.15 ± 0.03[c]
T6	10^{10}	0	1.2	7,600 ± 269.1[b]	3,562 ± 93.9[b]	2.13 ± 0.04[cb]
T7	0	5×10^9	1.2	8,076 ± 136.3[a]	4,100 ± 77.0[a]	1.97 ± 0.01[ab]
T8	10^{10}	5×10^9	1.2	7,800 ± 91.7[b]	3,760 ± 75.5[b]	2.08 ± 0.02[bc]

[a–c]Values within columns with no common superscripts are significantly different ($P \leq 0.05$).

[1]Results are reported as the mean ± SD for 4 lots (number of birds per treatment: 20). FC = feed consumption.

Table 5. Effects of *Saccharomyces cerevisiae* CECT 1891 on biochemical indicators[1] of broiler chicks fed diets containing 1.2 mg of aflatoxin (AF)/kg of diet

	(g/100 mL)			
Treatment	Serum total protein	Serum albumin (ALB)	Serum globulins (GLOB)	Ratio of serum ALB/GLOB
T1	2.39 ± 0.09[a]	1.50 ± 0.10[a]	0.89 ± 0.05[a]	1.69
T2	2.37 ± 0.06[a]	1.49 ± 0.03[a]	0.88 ± 0.03[a]	1.69
T3	2.44 ± 0.11[a]	1.51 ± 0.08[a]	0.93 ± 0.07[a]	1.62
T4	2.49 ± 0.08[a]	1.58 ± 0.13[a]	0.91 ± 0.05[a]	1.74
T5	1.72 ± 0.01[b]	0.95 ± 0.09[b]	0.77 ± 0.06[b]	1.23
T6	1.87 ± 0.19[b]	1.10 ± 0.13[b]	0.77 ± 0.05[b]	1.43
T7	2.36 ± 0.10[a]	1.46 ± 0.10[a]	0.90 ± 0.08[a]	1.62
T8	1.69 ± 0.02[b]	1.02 ± 0.07[b]	0.67 ± 0.05[b]	1.52

[a,b]Values within columns with no common superscripts are significantly different ($P \leq 0.05$).

[1]Results are reported as the mean ± SD for 20 animals per treatment, at d 21 of treatment.

and eosin (Figure 2B). Addition of *S. cerevisiae* CECT 1891 in drinking water to the diet containing 1.2 mg of AFB_1/kg caused liver changes that included moderate and diffuse cellular swelling as depicted in Figure 2C.

Data presented in Figure 3 show the effects of dietary treatment on relative liver weights (g/100 g of BW). Relative liver weight was increased with AFB_1 contamination (T5). In addition, birds fed the combination (AFB_1 plus *S. cerevisiae* CECT 1891) T6 and T8 had increased relative weights of livers, and even birds fed T8 had heavier livers compared with birds fed AFB_1 alone (T5). However, addition of *S. cerevisiae* CECT 1891 to the drinking water to the diet containing AFB_1 (T7) diminished the toxic effect of AFB_1 on relative weights of livers.

DISCUSSION

Aflatoxins are important to the poultry industry because of their toxicity (Huff et al., 1992; CAST, 2003) and occurrence in feedstuffs (Dalcero et al., 1997, 1998; Astoreca et al., 2011). Moreover, mycotoxin contamination is very costly for the animal industry and is a feed safety concern because of potential mycotoxin residue in meat and eggs (Zhao et al., 2010). The hypothesis that foods and feeds can always be potentially contaminated with AF should be considered. Thus, mycotoxin sequestration in the GI tract by adsorbing agents, such as yeasts, could be a promising strategy to protect against the toxic effect of these feed contaminants. Inclusion of such microbes in the diet may reduce the toxic effects of mycotoxins on animals because AFB_1-microorganism complex may decrease availability of the mycotoxin and consequently its absorption in the GI tract (Gratz et al., 2007).

The results obtained in this study showed that dietary AFB_1 severely affected productive performance. The adverse effects of AFB_1 on growth performance have been related with a decrease in protein and energy utilization, probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds (Denli et al., 2009). The failure of AFB_1 to affect feed conversion could be attributed to feed rejection by birds probably as a protective mechanism (Zhao et al., 2010). No significant differences in productivity parameters were found between chicks fed the control diet and chicks fed the diets containing *S. cerevisiae* CECT 1891 alone, indicating that the microorganism was inert and nontoxic. Similar results were obtained by Yalçinkaya et al. (2008), who evaluated the effects of feed supplemented with different percentages of mannan oligosaccharides from *S. cerevisiae* (0.05, 0.1, and 0.15%). The present study showed that only the presence of *S. cerevisiae* CECT 1891 in the drinking water (T7) completely returned the growth performance parameters to normal values, showing a protective effect of the yeast strain against aflatoxicosis. The inclusion of yeast freeze-dried to the feed (T6 and T8) did not protect and in addition avoided the beneficial effect of yeast in the drinking water (T8). Contrary to our results, the addition of yeasts to diet contaminated with AFB_1 have been shown to improve broiler chicken productivity (Stanley et al., 1993; Parlat et al., 2001; Çelýk et al., 2003). The differences observed in response to protection against aflatoxicosis are probably due to differences in product formulations. The authors mentioned above supplemented feed with live yeast; however, we added lyophilized yeast, thus this difference may be responsible for antagonistic results. The yeasts are rehydrated in the drinking water, and in consequence they are ingested in living form by birds, and thus the results were comparable with those of the other authors. However, we have no explanation for why the combined yeast treatments (T8) did not work as well as when yeast is only administered in the drinking water (T7).

Regarding the biochemical evidence, no statistical differences were observed in biochemical parameters of the animals from the different treatment groups fed without AFB_1. On the other hand, birds fed AFB_1-contaminated feed had decreased serum proteins, characteristic of aflatoxicosis. The decrease in these biochemical variables has been previously attributed to the inactivation of biosynthetic enzymes and impairment of protein synthesis by AF (Madheswaran et al.,

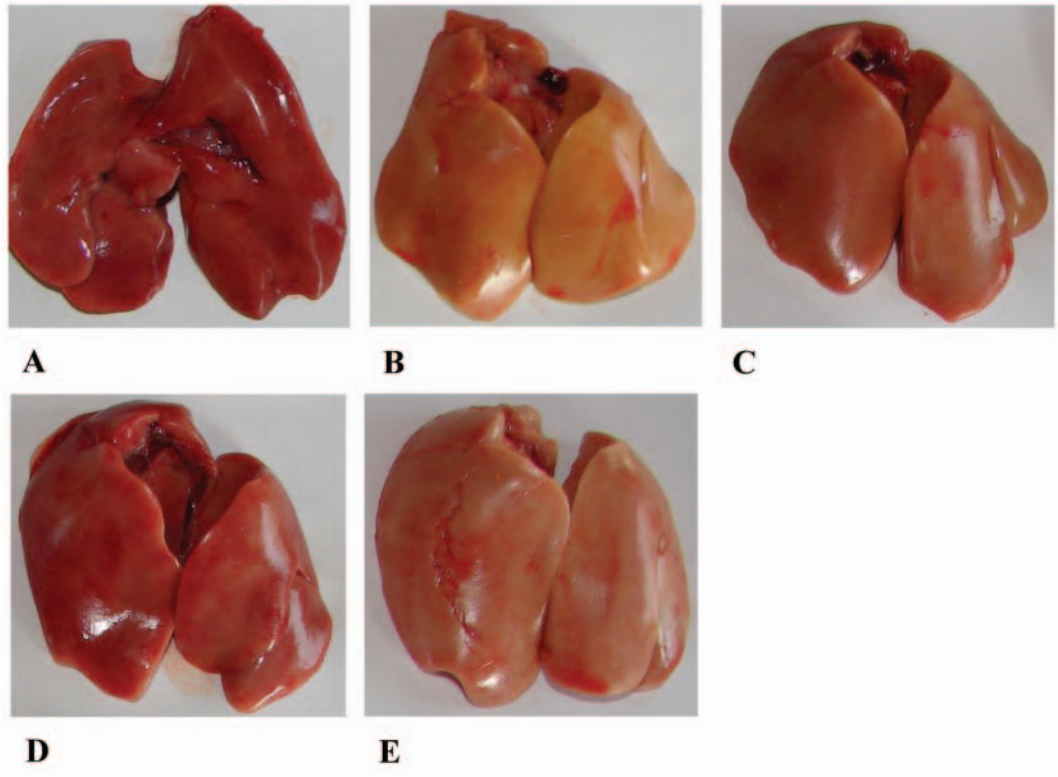

Figure 1. Representative livers from broiler chicks fed T1: the control diet (A), T5: 1.2 mg of aflatoxin B_1 (AFB_1)/kg of feed (B), T6: 1.2 mg of AFB_1/kg of feed plus 10^{10} cells of *Saccharomyces cerevisiae* CECT 1891 (SC)/kg of feed (C), T7: 1.2 mg of AFB_1/kg of feed plus 5×10^9 cells of SC/L of water (D), T8: 1.2 mg of AFB_1/kg of feed plus 10^{10} cells of SC/kg of feed and 5×10^9 cells of SC/L of water (E).

2004). Results obtained in the present report show that serum biochemical changes could be ameliorated by *S. cerevisiae* CECT 1891 administration to the diet at doses of 5×10^9 cells/L of drinking water in broiler chicks given 1.2 mg of AFB_1/kg of diet (T7). On the other hand, the biochemical parameters for broilers fed diets containing AFB_1 plus *S. cerevisiae* CECT 1891 in feed (T6) or in feed and drinking water (T8) did not completely return to normal values, showing an inhibition of the protein synthesis.

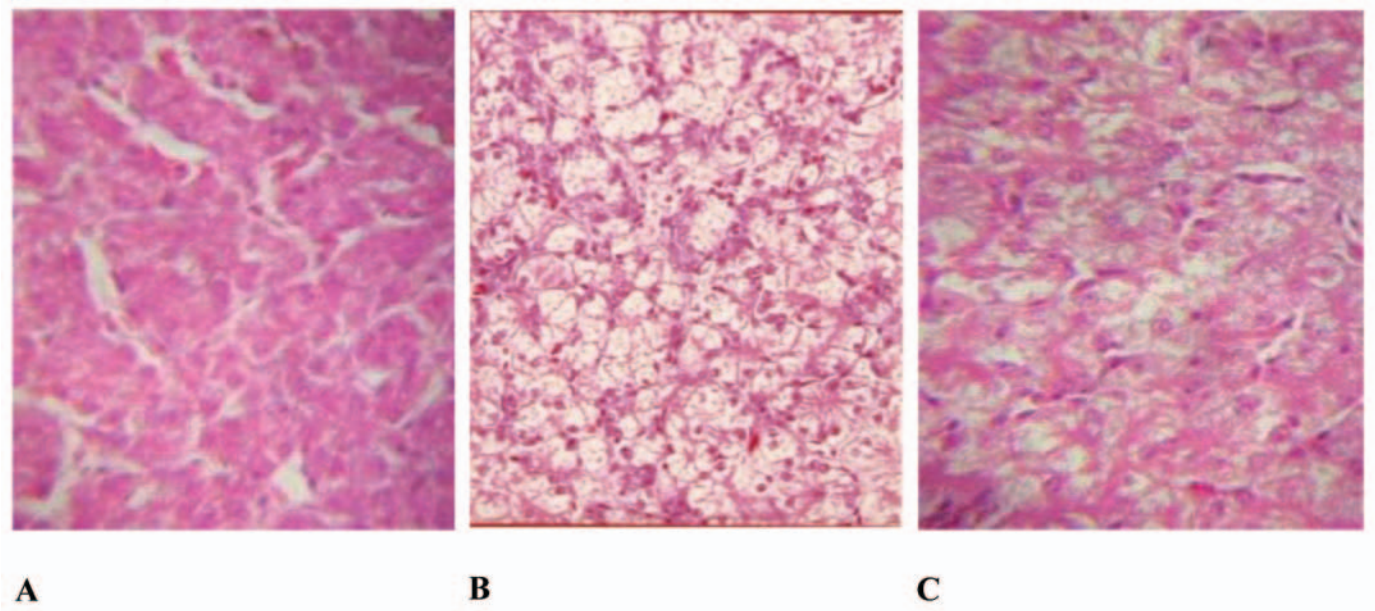

Figure 2. Photomicrographs of hematoxylin- and eosin-stained liver sections of chicks fed the following diets: T1: the control diet (A), T5: 1.2 mg of aflatoxin B_1 (AFB_1)/kg of feed (B), T7: 1.2 mg of AFB_1/kg of feed plus 5×10^9 cells of *Saccharomyces cerevisiae* CECT 1891/L of water (C).

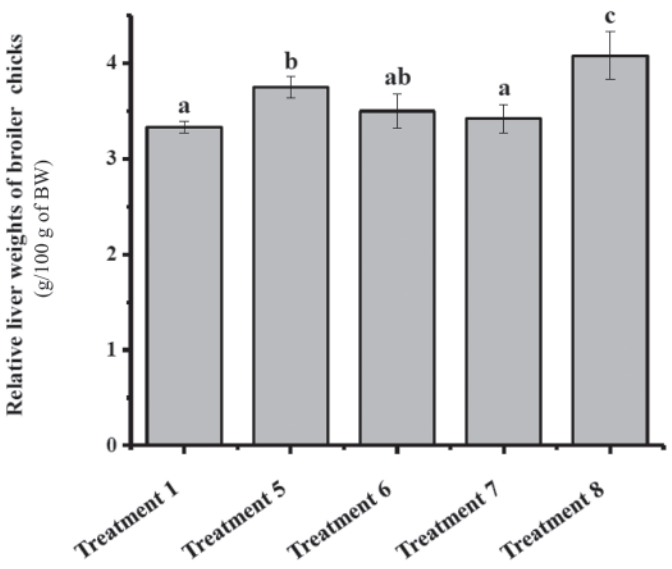

Figure 3. Effect of *Saccharomyces cerevisiae* CECT 1891 on relative organ weights of broiler chicks fed diets containing 1.2 mg of aflatoxin B_1 (AFB_1)/kg of feed. Bars with different letters (a–c) are statistically different ($P < 0.05$). Number of birds per treatment: 8.

The liver is both the main organ for AFB_1 detoxification and the principal target organ (Miazzo et al., 2000, 2005). In our study, a pronounced increase in liver weight was observed in chickens treated with AFB_1. Damaged liver cells can account for most of the changes in serum chemistries because less functional proteins are synthesized and secreted from damaged livers (Zhao et al., 2010). From macroscopic examination of liver, a beneficial effect of *S. cerevisiae* CECT 1891 might be occurring because a slightly darker brownish color can be appreciated in T7, suggesting a protective effect against aflatoxicosis.

Histopathological changes in the livers of chickens exposed to AFB_1 are comparable with those reported in the literature on avian aflatoxicosis (Rosa et al., 2001; Miazzo et al., 2005; Denli et al., 2009). However, the histopathological findings in liver sections of broiler fed diets with AFB_1 plus *S. cerevisiae* CECT 1891 added in feed (T6) or combined in feed and drinking water (T8) indicated a nonprotective effect of the yeast strain. The results showed that some histopathological changes could only be ameliorated by *S. cerevisiae* CECT 1891 administration to the drinking water of broiler chicks fed 1.2 mg of AFB_1/kg of diet. Baptista et al. (2002) reported that thermolyzed yeast did not improve symptoms in liver tissue caused by aflatoxicosis in rats, whereas active yeast had the ability to reduce hepatotoxicity caused by AF.

According to an integrated synthesis of the results reported above, the present work demonstrated that the inclusion of *S. cerevisiae* CECT 1891 in the drinking water of poultry was effective in preventing aflatoxicosis without producing adverse effects. Several hypotheses explain the ability of yeast to alleviate the aflatoxicosis effects. Results of several studies suggest that the binding is the main mechanism of detoxification of AF, consequently limiting their availability to the organism (El-Nezami et al., 1998; Peltonen et al., 2000, 2001; Shetty and Jespersen, 2006; Bueno et al., 2007; Gratz et al., 2007; Armando et al., 2011; Pizzolitto et al., 2011). Thus, we propose that *S. cerevisiae* represses the aflatoxicosis severity via chelating AF. The in vitro assay performed in our laboratory revealed that the process involved in AFB_1 removal by *S. cerevisiae* CECT 1891 is, by nature, reversible, and the kinetics are rapid. Consequently, we propose that the mechanism involved in the removal of AFB_1 by *S. cerevisiae* CECT 1891 is a physical adsorption (physisorption) of the toxin molecule to cell wall components of the microorganisms (Bueno et al., 2007; Pizzolitto et al., 2012b). Our results are consistent with those reported by Raju and Devegowda (2000), who attributed the AF binding by yeast cell walls to mannan oligosaccharides. This finding may indicate that trapping the mutagen in the digestive tract and eliminating it (at least partially) through the same route is a viable mechanism to avoid molecular damage. It will be of interest to evaluate the presence of AFB_1 in the excreta of treated birds to confirm the ability of the yeast strain to bind the mycotoxin.

In conclusion, our study indicated that AFB_1 in the diet at levels of 1.2 mg/kg resulted in reduced growth performance and an alteration of the serum biochemical, liver weight, and histological parameters of broiler chicks. The addition of *S. cerevisiae* CECT 1891 strain to drinking water resulted in significant improvement related to productive and biochemical parameters, hepatotoxicity, and histopathology of livers in broilers. Therefore, the use of *S. cerevisiae* CECT 1891 in AFB_1-contaminated feed is an alternative method to reduce the adverse effects of aflatoxicosis in broilers. To our best knowledge, this is the first study that evaluates the protective effects of a combined diet supplemented with *S. cerevisiae* CECT 1891 both in feed and drinking water.

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