Mycotoxin Res (2017) 33:273–283
DOI 10.1007/s12550-017-0285-y

ORIGINAL ARTICLE

Use of yeast (*Pichia kudriavzevii*) as a novel feed additive to ameliorate the effects of aflatoxin B_1 on broiler chicken performance

A.P. Magnoli[1,2,3] **· M.C. Rodriguez**[1,4] **· M.L. González Pereyra**[1,2] **· V.L. Poloni**[1] **· M.F. Peralta**[3] **· A.J. Nilson**[3] **· R.D. Miazzo**[3] **· G. Bagnis**[5] **· S.M. Chiacchiera**[2,4] **· L.R. Cavaglieri**[1,2]

Received: 6 December 2016 / Revised: 14 June 2017 / Accepted: 22 June 2017 / Published online: 8 July 2017
© Society for Mycotoxin Research and Springer-Verlag GmbH Germany 2017

Abstract The aim of this study was to evaluate the efficacy of autochthonous *Pichia kudriavzevii* as a novel bioadsorbent for aflatoxin B_1 (AFB_1). The selection of this yeast was based on the AFB_1 adsorption capacity previously demonstrated in vitro (Magnoli et al. 2016). One-day-old Cobb broilers (n = 160) were randomly assigned to four dietary treatments (T1: basal diet (B); T2: B + 0.1% yeast; T3: B + AFB_1, 100 μg/kg; T4: B + 0.1% yeast + AFB_1, 100 μg/kg). Performance parameters (average daily weight gain body, average daily consumption, feed conversion ratio, carcass weight, and dead weight), biochemical parameters (albumin, globulin, and albumin/globulin), liver pathological changes, and AFB_1 residual levels in the liver and excreta were evaluated. Significant differences ($P < 0.05$) in performance parameters were observed among treatments and controls: T3 group showed the lowest average daily body weight gain value while in T4 group, the value of this parameter increased significantly ($P < 0.05$). T3 and T4 groups showed the lowest and highest values for average daily feed consumption, respectively. The feed conversion ratio (FC) showed no significant differences among treatments. T3 group showed the lowest dead weight and carcass weight compared with T1 group. The biochemical parameters showed no significant differences among treatments. T3 group showed macroscopic and microscopic liver changes compared to the control. Aflatoxin B_1 levels (μg/g) were detected in broiler livers and showed significant differences among treatments ($P < 0.05$). In conclusion, native *P. kudriavzevii* incorporation (0.1%) in broiler diets containing AFB_1 was shown to be effective in ameliorating the adverse effects of AFB_1 on production.

Keywords Adsorption · Aflatoxin B_1 · Broilers · Liver · Performance · *Pichia kudriavzevii*

✉ L.R. Cavaglieri
lcavaglieri@exa.unrc.edu.ar

[1] Laboratorio de Micología, Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Ruta 36 km.601, (5800), Río Cuarto, Córdoba, Argentina

[2] Member of Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina

[3] Departamento de Producción Animal, Cátedra de Producción Avícola, Facultad de Agronomía y Veterinaria Universidad Nacional de Rio Cuarto, Córdoba, Argentina

[4] Departamento de Química, Facultad de Ciencias Exactas, Físico, Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

[5] Departamento de Patología Animal, Cátedra de Patología Animal, Facultad de Agronomía y Veterinaria Universidad Nacional de Rio Cuarto, Córdoba, Argentina

Introduction

Mycotoxins are toxic secondary metabolites generated by certain species of molds, most notably by *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* genera. The quality of products used in animal feed can be affected as these ingredients are ideal substrates for fungal growth. Under favorable conditions, these can be synthesized and accumulated in the substrate. Mycotoxins can occur during the growth of the crop, during post-harvest storage or during storage of the compound feed (CAST 2003). Contamination of agricultural products and feed with mycotoxins is a problem of global importance. Acute and chronic mycotoxicoses may reduce life expectancy, at least in developing countries, and cause significant economic losses in animal production. Aflatoxins (AFs), which are mainly produced by *Aspergillus flavus* and

Aspergillus parasiticus, are a major concern in poultry production (CAST 2003; Bhat et al. 2010). Aflatoxins cause severe economic losses and health problems in the poultry industry because of their toxicity and frequency of occurrence in feedstuffs (Nemati et al. 2014a). The toxicity of AF in broilers has been widely investigated for their carcinogenic, mutagenic, teratogenic, and growth-inhibitory effects (Oğuz et al. 2000a; Sur and Celik 2003; Magnoli et al. 2011a; Gutleba et al. 2015). Biochemical–hematological, immunological, and other adverse effects of AFs have also been well described (Oğuz et al. 2000a; Manafi et al. 2014; Nemati et al. 2015). Aflatoxins can also cause important gross and microscopic changes in the liver, such as hepatomegaly, paleness, hydropic degeneration, fatty change, bile-duct hyperplasia, and periportal fibrosis (Ortatatli and Oğuz 2001; Bintvihok 2002; Magnoli et al. 2011a; Bovo et al. 2015; Dos Anjos et al. 2015), kidney and spleen lesions (Bilgic and Yesildere 1992; Kumar and Balachandran 2014), impairment of the humoral and cellular immune responses; and increased susceptibility to some environmental and infectious agents (Oğuz et al. 2003; Nemati et al. 2015).

The presence of potential AF-producing strains and AFB_1 in commercial poultry feeds has been extensively reported in previous studies (Oliveira et al. 2006; Fraga et al. 2007; Monge et al. 2013). The AF content of most poultry feedstuffs in Argentina is higher than the maximum allowed level of 20 μg/kg of feed (Monge et al. 2013). The US Food and Drug Administration (FDA) set regulatory levels of 20 μg/kg aflatoxin for poultry feeds (Bhat et al. 2010). In countries of the American continent, poultry production grew extensively in recent years. In Argentina, the poultry industry is one of the most important animal production systems and has acquired great economic significance, mainly in the meat production sector (Lamelas et al. 2010). The aim of producers, researchers, and governments is to develop effective prevention management and decontamination technologies to minimize the toxic effects of AF in animal production. Several zeolites, bentonites and clinoptilolite, which are natural adsorbents and members of the heulandite–stilbite group, have been evaluated in vitro (Chiacchiera et al. 2000) and in vivo for their ability to adsorb AF (Miazzo et al. 2000, 2005; Oğuz et al. 2000a, 2000b; Rosa et al. 2001; Daković et al. 2008; Magnoli et al. 2011a, 2011b; Nemati et al. 2014b). Although these products are widely available as commercial feed additives (sold as "mycotoxin binders"), they need to be incorporated at high levels and have negative effects since they can reduce nutritional value of feeds or produce undesirable side effects (Zain 2011). Other organic compounds such as dairy strains of lactic acid bacteria-based products (El-Nezami et al. 1998, 2000), yeast, and yeast cell wall-based products have also been suggested to reduce negative effects on animal health produced by mycotoxins, especially zearalenone, T-2 toxin, and aflatoxin in poultry (Freimund et al. 2003; Santin et al. 2003; Onwurah et al. 2013; Roto et al. 2015). Yeast cell walls have been studied for their ability to complex with several mycotoxins without reducing the bioavailability of certain nutrients and causing no environmental impact. Other studies have indicated that *Saccharomyces cerevisiae* cell walls can be added to contaminated feeds, to selectively bind mycotoxins (Yiannikouris et al. 2003, 2004). These authors claim that the yeast-toxin complex passes through the digestive tract without any negative effect on animals or any carry-over to edible animal products such as milk, eggs, or meat (Yiannikouris et al. 2003, 2004).

Dried yeast and yeast cell walls added to AFB_1-contaminated rat feed was found to result in a significant reduction in the feed's toxicity (Baptista et al. 2004; González Pereyra et al. 2014). Many authors have studied the use of yeast and yeast cell walls (*S. cerevisiae)* from different origins (brewery industry, bakery, among others). However, very few have used autochthonous yeasts to control mycotoxicoses.

The aim of this study was to evaluate the efficacy of autochthonous *Pichia kudriavzevii* strain isolated from broiler feedstuff, to prevent the toxic effects of aflatoxin in broilers fed diets containing natural contamination levels of AFB_1. Therefore, performance and biochemical parameters, macroscopic and microscopic changes, and residual levels of AFB_1 in liver and excreta were determined.

Materials and methods

Chemicals

Aflatoxin B_1 (> 99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and its concentration was confirmed by HPLC-fluorescence according to an Association of Official Agricultural Chemists (AOAC) (1995) method. Demineralized water (HPLC grade) was obtained with Labconco equipment (model 90901-01, Labconco, Kansas City, MO). Organic solvents as acetonitrile and methanol were purchased from Sintorgan (Buenos Aires, Argentina). Trifluoroacetic acid was purchased from Sigma Chemical Co. (St Louis, MO, USA) and hexane was purchased from Cicarelli (Santa Fe, Argentina).

Aflatoxin B_1 production

Aflatoxins were produced via fermentation of rice by *A. parasiticus* NRRL 2999 (USDA, Agricultural Research Service, Peoria, IL). The sterile substrate was placed in Erlenmeyer flasks and inoculated with 2 ml of an aqueous suspension containing 10^6 spores/ml. Cultures were allowed to grow for 7 days at 25 °C in the darkness and were shaken daily. On day seven, the Erlenmeyer flasks were autoclaved and the culture material was dried for 48 h at 40 °C in a forced-

air oven and then ground to a fine powder. The powder contained four AFs produced by *A. parasiticus*—AFB_1, aflatoxin G_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2)—being AFB_1 the one produced in higher concentrations. Aflatoxin B_1, AFG1, AFB2, and AFG2 content of the resulting powder was quantified by high performance liquid chromatography (HPLC-fluorescence) detector (Waters 2487), an excitation wavelength of 395 nm and an emission wavelength of 470 nm, 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 (60, 30 μg/g). Levels of AFB_2 and AFG_2 were detectable but not quantifiable. This AF concentrate was used to contaminate feed. The milled contaminated substrate (60 ± 1.1 μg/g) was added to the basal diet pre-premixing to provide a concentration of about 100 μg of AFB_1/kg of feed.

The basal diet was analyzed only for AFB_1: the toxins AFB_2, G_1, G_2, fumonisins, and zearalenona were not quantified. The basal diet was elaborated in an animal feed plant located in the National University of Rio Cuarto. The raw materials for the elaboration of the basal diet were purchased at Agroimperio SRL, Argentina. Ten kilograms of samples of different experimental diets were collected, and subsequently, 25 g subsamples were obtained by the quartet technique according to AOAC (2000) to be analyzed by HPLC-fluorescence.

Yeast production

P. kudriavzevii was obtained from the Collection Centre at the Universidad Nacional de Río Cuarto, Argentina. It was previously isolated from broiler feedstuffs and characterized by sequencing of the 26S D1/D2 rRNA gene domain. Partial 26S-rRNA gene sequences (D1/D2 domains) were amplified using NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers according to Kurtzman and Robnett (2003). The selection of the strain was based on its capacity for binding AFB_1 in vitro (Magnoli et al. 2016). *P. kudriavzevii* was produced in 150 ml of Yeast Peptone Dextrose (YPD) broth in Erlenmeyer flasks inoculated with 1 ml of a pure 24-h culture. Flasks were incubated on an orbital shaker (150 rpm) at 37 °C for 24 h. Then, cells were collected by centrifugation (16,000 g, 5 min) and the pellets were lyophilized and homogenized to be incorporated into the basal diet in order to provide a concentration of 1 g of yeast/kg of feed (0.1%).

Yeast cell viability study

To determine cell viability, *P. kudriavzevii* was grown in YPD broth and incubated with agitation (150 rpm) at 37 °C for 24 h. Decimal dilutions were performed until a 10^7 cell/ml concentration was reached. The cell suspension concentration was determined using a hemocytometer. Viability was confirmed by a standard plate count method using YPD agar, according to Armando et al. (2011).

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). Twenty-five grams of ground feed were extracted with 125 ml of methanol/water (60:40 *v*/*v*), 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, *v*/*v*), 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 × 4.6 mm, 5 μm). The retention time for $AFB_1$was 4.7 min and the limit of detection (LOD) was 0.001 μg/ml. 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. Aflatoxin B_1 levels in feed were 3 ± 1 μg/kg for the basal diet (T1 and T2) (natural contamination) and 100 ± 6 μg/kg of AFB_1 for T3 and T4 diets. The analysis was developed in triplicates.

Experimental design and broilers

One-day-old male chicks (Cobb) vaccinated against Marek's disease were obtained from a commercial hatchery and maintained under continuous fluorescent lighting with feed and water available ad libitum until during all the experiment. On day 1, a total of 160 birds (four replicates/treatment, ten broilers/replicate) were individually weighed and randomly selected. During the experimental period (22 days), broilers received the diet corresponding to each treatment. A standard corn-soybean meal starter diet (basal diet) (Table 1) that met the National Research Council (1994) requirements was used to formulate the different experimental diets. The different diets were prepared by premixing the lyophilized yeast and

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

Item Ingredient	Diet Grower
Yellow corn	697.5
Soybean oil meal	220.0
Meat and bone meal	73
Vitamin and mineral mix[a]	1.5
NaCl	2.5
Oyster shell	3.5
dl-Methionine	2.0
Total	1000.0
Proximate composition of the test diet CP	214.0
Crude fat	50.0
Crude fiber	25.0
Calcium	9.5
Methionine	5.0
Tryptophan	2.3
ME (kcal/kg)	3100.0

[a] Vitamin and mineral mix provided the following (mg/kg of feed): vitamin A, 10×10^6 IU; vitamin D3, 3×10^6 IU; vitamin K, 33 mg, 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. CP crude protein, ME metabolic energy

the AFB_1-contaminated powder and then mixing them with the basal diet in an industrial mixer. The experimental diets for each treatment were formulated as follows: T1: basal diet (B); T2: B + 0.1% yeast; T3: B + AFB_1 (100 μg/kg); and T4: B + 0.1% yeast + AFB_1 (100 μg/kg). Broilers were weighed weekly at the start and end of the study and monitored daily for signs of morbidity and mortality. The feed consumption was determined at the end of the study.

At the end of the feeding trial, the efficacy of yeast was evaluated by measuring the following performance parameters: average daily weight gain (ADWG) (g/bird/day), average daily feed consumption (ADFC) (g/bird/day), and feed conversion ratio (FCR) calculated by the ratio between ADFC and ADWG, from day 1 to day 22. Furthermore, carcass weight (CW), dead weight (DW), biochemical parameters, macroscopic and microscopic changes in the liver, and AFB_1 residues in the liver and excreta of broilers were determined.

Pathological examination

When broilers reached an age of 22 days, the feeding trial was terminated and five broilers from each replicate of each treatment were selected at random and killed by cervical dislocation. Selected animals were weighed before euthanasia. A detailed necropsy was then conducted. The livers were removed and weighed. Blood samples (5 ml, with anticoagulant) were collected before euthanasia from the subclavian-axillary vein and centrifuged, and the plasma was separated and stored at −20 °C until analyzed. Biochemical determinations, including total protein, albumin (ALB), and globulin (GLOB) concentrations, and ALB/GLOB ratio in plasma for each broiler group were calculated on day 22. These concentrations were determined with a clinical chemistry analyzer (Commercial Kit, Wiener Laboratory, Rosario, Argentina) according to the procedure recommended by the manufacturer (Wiener Laboratory 2000). Plasma biochemical values were grouped and expressed as mean ± pooled sample standard error.

Histopathology

After pathological examination, livers (n = 20) from each treatment (5 from each replicate) were selected 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 (OM). Microscopically, hepatocellular degeneration of livers was graded as follows: slight (degree 1): mild hepatocellular swelling caused by hydropic degeneration and fatty changes only in centrilobular areas; moderate (degree 2): clear hepatocellular swelling in centrilobular and mid zonal areas; and severe (degree 3): diffuse and severe hepatocellular swelling, cytoplasmic paleness, and rupture (Ortatatli et al. 2005). The liver tissue samples from the different treatments were then removed and frozen at −20 °C for subsequent AFB_1 residual analysis.

Extraction for the analysis of AFB_1 residue in chicken excreta and liver tissue

The AFB_1 concentration in excreta was quantified as an indirect measure to evaluate the effect of the addition of *P. kudriavzevii* to broiler diets as a bioadsorbent. The excreta were collected manually with a small shovel from the collector trays under the cages. On the end of each week during the experiment, the excreta from each treatment were collected. A 50-g sample was taken from each treatment, and at the end of the experiment, all samples from each treatment were pooled and homogenized and a 25-g subsample was extracted, cleaned up with immunoaffinity columns (IAC), and analyzed for AFB_1 by HPLC-fluorescence. Briefly, 25 g of excreta was extracted with 100 ml methanol/water (60:40 *v*/*v*), 80 ml hexane, and 2 g NaCl. The mixture was shaken for 30 min in an orbital shaker and filtered through Whatman No. 4 filter paper. Twenty-five milliliters of the methanol/water phase of the filtrate were extracted twice with 25 and 15 ml chloroform in a

Table 2 Effects of aflatoxin B_1 and *Pichia kudriavzevii* on production parameters of 160 broilers

Treatments		Production parameters		
		ADWG (g/bird/day)	ADFC (g/bird/day)	Feed conversion ratio (g feed/g gain)
T1	Basal diet	115.80 ± 0.32^{b}	170.90 ± 1.07^{b}	1.48 ± 0.05^{a}
T2	Yeast	114.14 ± 0.77^{b}	169.87 ± 0.48^{b}	1.47 ± 0.05^{a}
T3	AFB_1	103.78 ± 0.66^{a}	159.29 ± 1.26^{a}	1.53 ± 0.09^{a}
T4	Yeast + AFB_1	113.65 ± 0.66^{b}	170.52 ± 0.58^{c}	1.50 ± 0.07^{a}

Values in columns with no common superscripts are significantly different ($P \leq 0.05$) according to Fisher's protected least significance test (LSD test). Yeast 0.1% and AFB_1 (100 μg/kg)

ADWG average daily weight gain, *ADC* average daily feed consumption

separatory funnel. The chloroform phase was then vacuum-dried in a rotatory evaporator. The dried extract was redissolved in 32 ml 10% methanol in PBS solution and passed through an AflaStar™ Immunoaffinity Column (COIAC 1000, Romer Labs, 3430 Tulln, Austria). The column was then washed with 5 ml distilled water and AFB_1 was eluted with 1 ml HPLC grade methanol. The extracts were evaporated to dryness under N_2 stream and redissolved in 500 μl mobile phase acetonitrile–methanol–water (17:17:66, *v*/*v*).

Livers ($n = 20$) from each treatment (5 from each replicate) were selected. Aflatoxin B_1 in the liver tissue was extracted and partially purified according to the method described by Tavčar-Kalcher et al. (2007). Briefly, the ground liver samples (50 g each) were mixed thoroughly with 5 ml of a 20% aqueous citric acid solution and diatomaceous earth (10 g). The mixture was extracted with 100 ml of dichloromethane by shaking for 30 min. The organic phase was filtered through Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, UK). Water was eliminated by adding 5 g Na_2SO_4. The filtrate was filtered for a second time and an aliquot (20 ml) was evaporated to dryness. Then, the concentrate was mixed with 20 ml acetonitrile/H_2O (75:25, *v*/*v*) and 10 ml hexane and centrifuged, and then, a 10-ml aliquot of the acetonitrile/H_2O (75:25, *v*/*v*) phase was taken and evaporated to dryness in a rotary evaporator. The residue was redissolved in methanol/water (80:20, *v*/*v*) and cleaned up. For cleanup, the dried extract was redissolved in 10 ml methanol/H_2O (80:20 *v*/*v*), added 90 ml distilled water, and passed through OASIS®, HLB, 6 cm^3 (200 mg) SPE cartridges (Waters Corporation, Milford, MA, USA), previously conditioned with methanol and water according to the methodology described by Sørensen and Elbæk (2005). Solid phase extractions were performed in a Vac Elut 20 position Manifold SPE extractor (Agilent Technologies Inc., Santa Clara, CA, USA). The toxin was eluted with 7 ml methanol, evaporated to dryness, and stored at −20 °C until analysis.

For AFB_1 recovery analysis, AFB_1-free finely ground liver samples (50 g) obtained from commercial broilers were weighed into a 250-ml Erlenmeyer flasks and spiked with a standard solution of AFB_1 at 100 μg AFB_1/g liver. Analysis of fortified samples and their respective blanks were performed in duplicate. The extraction of AFB_1 in liver samples was done according to the protocol described above. The detection and quantification of AFB_1 in liver and excreta samples were done according to the protocol described below.

Quantification of AFB_1 residue in chicken excreta and in liver tissue

Aflatoxin B_1 in the extracts were detected and quantified by HPLC-fluorescence according to Trucksess et al. (1994). Dried extracts were redissolved in mobile phase [acetonitrile/H_2O (20:80, *v*/*v*)] and injected into the HPLC instrument. An aliquot (200 μl) was derivatized with 700 μl trifluoroacetic acid–acetic acid–water (20:10:70, *v*/*v*). The HPLC system consisted of a Hewlett Packard model 1100 pump (Palo Alto, CA, USA) connected to a Hewlett Packard 1100 Series variable wavelength fluorescence detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a silica Luna™ Phenomenex C18 reversed-phase column (150 × 4.6 mm, 5 μm particle size, Phenomenex Inc., Torrance, California, USA) connected to a SecurityGuard™ Phenomenex guard column (4 × 3.0 mm, 5 μm particle size). The mobile phase was pumped at a flow rate of 1.5 ml/min. The fluorescent detector was set at 360 nm excitation 440 nm emission wavelengths. Injection volume was 50 μl and the retention time of AFB_1 was 4.4 min. A calibration curve for the quantification of the toxin was performed with external standard solutions of 130, 65.03, 32.52, 16.25, and 8.15 μg/kg AFB_1. The limit of detection (LOD) was 5 μg/kg of sample and the limit of quantification (LOQ) was 8.15 μg/kg. Two inter-days recovery assays were done by spiking excreta with 130 μg/kg AFB_1 and showed a mean recovery percentage of 101.9%. The percentage of recovered AFB_1 for liver samples was 99 ± 13%.

Table 3 Comparative studies of the dead weight and carcass weight of 20 broilers

Treatments		Production parameters	
		Dead weight (g)	Carcass weight (g)
T1	Basal diet	1313.6 ± 43.2^{c}	900.6 ± 22.9^{c}
T2	Yeast 0.1%	1248.8 ± 59.1^{b}	883.8 ± 46.4^{bc}
T3	AFB_1 (100 μg/kg)	1004.8 ± 41.7^{a}	691.4 ± 41.6^{a}
T4	Yeast 0.1% + AFB_1 (100 μg/kg)	1198.6 ± 26.9^{b}	850.5 ± 12.4^{b}

Values in columns with no common superscripts are significantly different ($P \leq 0.05$) according to least significant difference Fisher's protected least significance tests (LSD test)

Statistical analysis

The data were analyzed by general linear and mixed model (GLMM) using InfoStat (version 2.03 for Windows 2012; University of Cordoba, Argentina) software. The data were analyzed by analysis of variance (ANOVA). Means and standard error (SEM) were compared using the Fisher's protected least significant test (LSD) ($P < 0.05$ and 0.0001). The significance $P < 0.05$ was used for the production parameters, dead weight, and carcass weight, and the significance $P < 0.0001$ was used for the biochemical parameters, liver weight, and AFB_1 residues in the livers.

Results

The production parameters evaluated showed significant differences among treatments (Table 2). Broilers fed the control diet (T1) had the highest ADWG value, while broilers fed the 100 μg/kg AFB_1 diet (T3) showed the lowest ADWG. Broilers fed the AFB_1 + yeast diet (T4) showed a significant increase in ADWG *(P* < 0.05), being similar to the control (T1) and demonstrating the adsorptive effect of the yeast. Broilers fed the AFB_1-contaminated diet (T3) showed the poorest ADC values, while broilers fed AFB_1 + yeast (T4) showed values similar to control birds (T1). Broilers did not show a significantly poorer *(P* < 0.05) value for this parameter on FCR, among different treatments.

Broilers fed the AFB_1-contaminated diet (T3) showed the lowest DW and CW values compared with the control group (Table 3). Broilers receiving the AFB_1 + yeast diet (T4) had CD and CW values similar to those observed in broilers fed the yeast treatment (T2).

The effects of AFB_1 and *P. kudriavzevii* supplemented diets on the biochemical parameters and liver weight of broilers are presented in Table 4. No significant differences ($P \leq 0.0001$) among the different treatments were observed in the evaluated biochemical parameters (PT, ALB, GLOB, and ALB/GLOB) and on liver weights.

The liver of broilers fed the AFB_1 diet (T3) showed a slightly lighter coloration compared with controls (Fig. 1). The liver of broilers fed the AFB_1 + yeast diet (T4) had similar appearance to controls, suggesting *P. kudriavzevii* exerted a protective effect against aflatoxicosis. The liver of broilers fed the yeast-only diet (T2) also had similar appearance to controls, demonstrating that the yeast has an inert and non-toxic effect on broilers.

The livers of broilers fed with basal diet (T1) showed a microvacuolar diffuse fat degeneration all over the organ (Fig. 2a), while livers of broilers fed the 100 μg/kg of AFB_1 diet (T3) showed hepatocytes with lesions of degrees 1 and 2 (i.e., hydropic degeneration and fat microvacuoles) which are typical histopathological alterations caused by AF (Fig. 2c).

Table 4 Effects of aflatoxin (AFB_1) and *Pichia kudriavzevii* on biochemical parameters and liver weight of 20 broilers

Treatments	Biochemical parameters				Liver weight (g)
	PT	ALB	GLOB	ALB/GLOB	
T1 basal diet	2.93 ± 0.06^{a}	1.38 ± 0.11^{a}	1.55 ± 0.17^{a}	0.90 ± 0.14^{a}	27.46 ± 1.85^{a}
T2 (yeast 0.1%)	2.56 ± 0.12^{a}	1.25 ± 0.05^{a}	1.31 ± 0.27^{a}	0.96 ± 0.11^{a}	26.32 ± 2.83^{a}
T3 (AFB_1 100 μg/kg)	2.54 ± 0.09^{a}	0.97 ± 0.21^{a}	1.57 ± 0.14^{a}	0.67 ± 0.26^{a}	25.44 ± 0.94^{a}
T4 (yeast 0.1% + AFB_1 100 μg/kg)	2.81 ± 0.19^{a}	1.23 ± 0.05^{a}	1.58 ± 0.23^{a}	0.80 ± 0.16^{a}	26.76 ± 2.36^{a}

PT total protein, ALB albumin, GLOB globulin (g/100 ml),ALB/GLOB ratio. Liver weight in grams. Values with common superscripts were not significantly different ($P \leq 0.0001$), according to Fisher's protected least significance test (LSD test)

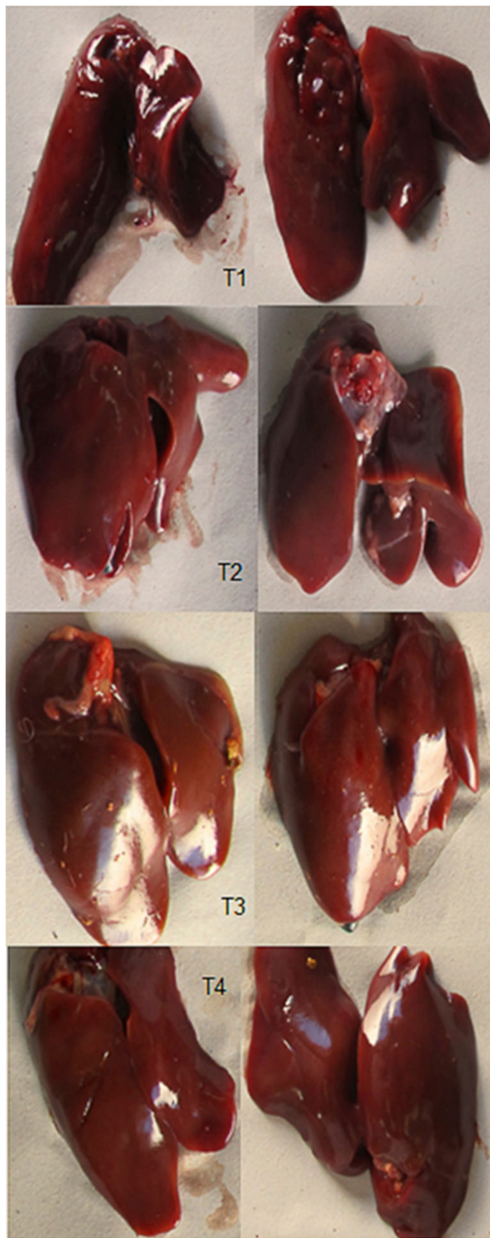

Fig. 1 Representative livers from broilers (22 days old) fed different treatments (T). *T1*: basal diet (B); *T2*: B + yeast; *T3*: B + aflatoxin B_1 (AFB_1); *T4*: B + yeast + AFB_1; *T5*: AFB_1 (100 μg/kg); and *T6*: yeast (0.1%)

Livers of broilers fed the yeast-only diet (T2) and the AFB_1 + yeast diet (T4) did not show signs of diffuse fat degeneration in hepatocytes (Fig. 2b–d). Moreover, T4 showed the ameliorating effects of the yeast on the AFB_1-associated lesions. Lesions of degree 3 (i.e., severe) were not observed in any of the treatments.

Table 5 shows AFB_1 concentrations (μg/g) found in broiler livers. In the AF-free treatments (T1 and T2), no AFB_1 residues were detected, while broilers fed the AFB_1-contaminated diets (T3 and T4) showed detectable AFB_1 levels. Aflatoxin B_1 concentration in livers from T3 was significantly higher (1.26 μg/g) ($P \leq 0.0001$) compared with levels found in T4 (1.09 μg/g). Low AFB_1 concentrations were detected in the excreta (1.64 to 1.90 μg/kg) and no significant differences were observed among treatments (data not shown).

Discussion

Aflatoxin contamination is a constant hazard to the poultry industry that results in substantial economic losses to producers due to sublethal but toxic effects of AFB_1. In the present study, the efficacy of an autochthonous *P. kudriavzevii* strain isolated from broiler feedstuff to prevent the toxic effects of aflatoxin in broilers fed diets containing natural contamination levels of AFB_1 was evaluated. Performance and biochemical parameters, macroscopic and microscopic changes, and residual levels of AFB_1 in the liver and excreta were also determined.

Broilers' performance was affected by AFB_1 and the addition of 0.1% yeast to diets contaminated with 100 μg/kg of AFB_1 showed ameliorative effects on the production parameters evaluated. However, the biochemical parameters were not improved. Our results agree with those reported by Afzal and Saleem (2004) who used a mycotoxin detoxifier containing oxyquinol, dichloro-thymol, and micronized yeast and observed a significantly improved body weight gain and feed efficiency in broilers fed diets containing the detoxifier when compared to diets contaminated with 78, 80, and 170 μg/kg AFB_1. Similarly, Celik et al. (2001) and Juan-Juan et al. (2010) demonstrated that the addition of yeast cell extracts to a diet containing 100 μg/kg of AFB_1 had ameliorative effects on broilers' performance and biochemical parameters. Hashmi et al. (2006) reported an efficient decrease of AF's toxic effects by supplementing yeast sludge (1%) and mannanoligosaccharide (0.26%) to 100, 200, and 300 μg/kg AFB_1-contaminated broiler diets. Similar results were also observed by Motawe et al. (2014) who used a mix of probiotic bacteria and yeast and demonstrated lower ADWG, lower ADC, and impaired FCR in broilers fed 0.5 and 1 mg/kg AFB_1-contaminated diets.

The effects of AFB_1 in broiler's liver are well known since this is AF's target organ. One of these effects is the significant increase in liver absolute weight (Ortatatli and Oğuz 2001; Aravind et al. 2003; Ortatatli et al. 2005; Magnoli et al. 2011a). In the present study, no significant differences in liver weight were detected probably due to the low levels of AFB_1 consumed. In the macroscopic examination, livers of broilers fed the basal diet plus yeast showed a slightly darker color, whereas livers of broilers fed the AFB_1-contaminated diet showed a typical color pattern (pale yellow) related to subclinical aflatoxicosis. The liver histopathology of broilers fed AFB_1 showed a typical pattern of subclinical aflatoxicosis. The present results agree with other authors who reported

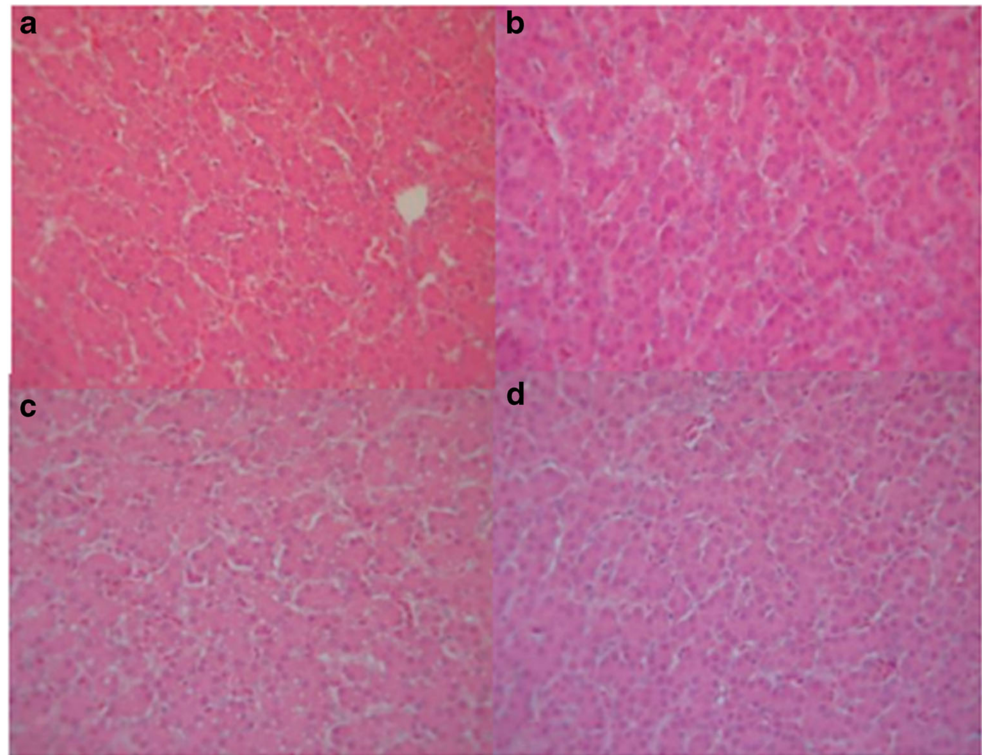

Fig. 2 Photomicrographs (optical microscopy) of hematoxylin and eosin-stained broiler liver sections from different treatments. **a** T1 = basal diet (B). **b** T2 = B + yeast. **c** T3 = B + AFB_1. **d** T4 = B + yeast+ AFB_1

microscopic lesions in broilers fed 100 μg/kg levels of dietary AFB_1 (Ortatatli and Oğuz 2001; Kemal et al. 2003; Ortatatli et al. 2005; Magnoli et al. 2011a, 2011b). Moreover, there were no signs of liver aflatoxicosis in broilers fed the basal diet plus yeast. Azizpour and Moghadam (2015) also reported that the addition of yeast glucomannan mitigated the negative effects of aflatoxin on the liver histopathology. In this study, residual AFB_1 was detected in the liver of broilers fed 100 μg/kg of AFB_1. On the other hand, the levels of residual AFB_1 in liver decreased when yeast was added to the diet. Other authors reported that different levels of AFB_1 in diets (50 to 100 and 3000 μg/kg) resulted in similar levels of residual AFB_1 in liver (0.13 to 0.15 ng/g) (Bintvihok 2002; Bintvihok and Kositcharoenkul 2006). However, the levels detected in the present study were ten times greater than the results reported by those authors.

In the present study, there were no significant differences between AFB_1 residual levels in excreta among the different treatments. It was expected that the yeast would bind the toxin reducing its bioavailability and, hence, increase its excretion. However, the low percentage of AFB_1 recovered from the excreta could be due to the low levels of toxin administered to animals (simulating a chronic aflatoxicosis). In addition, there could be another mechanism of mycotoxin reduction such as degradation by microorganisms of the intestinal biota (Vanhoutte et al. 2016). However, the adsorption of AFB_1 to the yeast and the consequent reduction of its bioavailability could also be explained by the amelioration observed in the AFB_1 plus yeast treatment. It is predicted that, when more aflatoxin molecules are absorbed by feed additives in the gastrointestinal tract, there are less negative effects on the target organs. The adsorbing effects of yeast are attributed to its ability to selectively bind to aflatoxin molecules. The yeast cell wall consists of a network of β-1,3 glucan backbone with β-1,6 glucan side chains, which in turn are attached to highly glycosylated mannan proteins, which make the external layer (Yiannikouris et al. 2004). In this way, a toxin-yeast complex is formed which is eliminated by the excreta decreasing the concentration of the toxin in blood. From the intestines, toxins

Table 5 Aflatoxin B_1 residue in the livers of 20 broilers fed dietary treatments from d 1 to 22

Treatments	AFB_1 (μg/g)[1]	
T1	Basal diet (AFB_1 3.2 μg/kg)	ND
T2	Yeast 0.1%	ND
T3	AFB_1 (100 μg/kg)	1.26 ± 0.04^a
T4	Yeast 0.1% + AFB_1 (100 μg/kg)	1.09 ± 0.10^b

Values with different superscripts were significantly different ($P \leq 0.0001$) according to Fisher's protected least significance test (LSD test)

T1 basal diet (AFB_1 3.2 μg/kg), *T2* yeast (0.1%), *T3* AFB_1 (100 μg/kg), *T4* yeast 0.1% + AFB_1 100 μg/kg, ND no detectable levels (detection limit 0.001 μg/g)

[1] Mean levels of AFB_1 in the liver (μg/g) were obtained from five liver samples

enter the liver through the liver's portal blood supply and then spread to most soft tissues. However, most toxins accumulate in the liver and kidneys where biotransformation occurs (Leeson et al. 1995).

In the liver, AFB_1 is bioactivated by cytochrome P450, a group of enzymes abundant in the liver and related with the bioactivation and metabolism of different kinds of xenobiotics and endogenous compounds (Diaz et al. 2010). These compounds are excreted in the urine although a small part can remain in tissues as residues. These residual levels of AFB_1 in liver were analyzed in the present study. Biotransformation of AFB_1 in mammals is relatively well understood; however, studies in poultry are still needed (Savlík et al. 2007). Diaz et al. (2010) have reported differences in the rate of AFB_1 biotransformation into its epoxide and dihydrodiol forms in commercial poultry species including ducks, chickens, turkeys, and quail (Lozano and Diaz 2006). Future studies should be performed to search for AFB_1-biotransformation derivatives in animal tissues.

Concluding, the addition of *P. kudriavzevii* to a broiler diet containing AFB_1 was shown to be effective in ameliorating the toxin's adverse effects on productive parameters and residual AFB_1 levels in livers. The macroscopic and histopathological aspect of the livers reflected the effect the low levels of AFB_1 in broilers and could be indicating a beneficial effect of yeast when supplemented to aflatoxin-contaminated diets in poultry production. Also, *P. kudriavzevii* by itself did not demonstrate any adverse effects (i.e., diarrhea). As a consequence of all these facts, productive parameters of broilers fed with the native yeast strain were similar to those encountered in the control broilers. This work makes a contribution on using native yeasts from broilers' environment for controlling chronic aflatoxicosis in avian production.

Acknowledgments The authors are grateful to the SECyT (Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto) and Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET), which supported this study through grants. L.R.C and S.M.C held positions at CONICET. We thank Dra. Ana Maria Dalcero for the collaboration and contribution in this work.

Compliance with ethical standards

Funding This study was funded by Secretaría de Ciencia y Técnica, Universidad Nacional de Río Cuarto SECyT; Consejo Nacional de Investigación Científica y Técnica, Argentina (CONICET); and Agencia Nacional de Promoción Científica y Tecnológica (Fondo para la Investigación Científica y Tecnológica, FONCyT).

Conflict of interest None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Declarations Availability of data and material: databases and all relevant raw data are freely available to any scientist wishing to use them.

References

Afzal M, Saleem Z (2004) Effects of addition of a mycotoxin detoxifier in poultry feed containing different levels of aflatoxins on the performance of broilers. Asian-Australian J Anim Sci 17:990–994

Aravind KL, Patil VS, Devegowda G, Umakantha B, Ganpule SP (2003) Efficacy of esterified glucomannan to counteract mycotoxicosis in naturally-contaminated feed on performance, serum biochemical and haematological parameters in broilers. Poult Sci 82:570–576

Armando MR, Dogi CA, Pizzolitto RP, Escobar F, Peirano MS, Salvano MA, Sabini LI, Combina M (2011) *Saccharomyces cerevisiae* strains from animal environmental with aflatoxin B1 binding ability and antipathogenic bacteria influence in vitro. World Mycotoxin J 4:59–68

Armando MR, Pizzolitto RP, Dogi CA, Cristofolini A, Merkis C, Poloni VL, Dalcero AM, Cavaglieri LR (2012a) Adsorption of ochratoxin A and zearalenone by potential probiotic *Saccharomyces cerevisiae* strains and its relation with cell wall thickness. J Appl Microbiol 113(2):256–264

Armando MR, Dogi CA, Rosa C, Dalcero AM, Cavaglieri LR (2012b) *Saccharomyces cerevisiae* strains reduce *Aspergillus parasiticus* growth and aflatoxin B_1 production at different interacting environmental conditions. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 29(9):1443–1449

Association of Official Agricultural Chemists (AOAC) (1994) Official methods of analysis 979.22 14th ed. see column chromatographic procedure. Gaithersburg

Association of Official Agricultural Chemists (AOAC) (1995) Official methods of analysis, 16th edn. 990.33 AOAC Int., Gaithersburg

Association of Official Agricultural Chemists (AOAC) (2000) Official Methods Sampling of Aflatoxins, Preparation of sample 977.16 17th ed. Washington, DC

Azizpour A, Moghadam N (2015) Effects of yeast glucomannan and sodium bentonite on the toxicity of aflatoxin in broilers Brazilian Journal of Poultry Science ISSN 1516-635X Oct - Dec 2015 / Special Issue. Nutrition - Poultry feeding additives / 007–014

Baptista AS, Horii Y, Calori-Domingues MA, Da Gloria EM, Salgado JM, Vizioli MR (2004) The capacity of manno-oligosaccharides, thermolysed yeast and active yeast to attenuate aflatoxicosis. World J Microbiol Biotechnol 20:474–481

Basmacıoglu H, Oguz H, Ergul M, Col R, Birdane YO (2005) Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin Czech. J Anim Sci 50(1):31–39

Bhat R, Rai RV, Karim A (2010) Mycotoxins in food and feed: present status and future concerns. Comprehensive Rev Food Sci Food Saf 9:57–81

Bilgic HN, Yesildere T (1992) Renal lesions on experimental aflatoxicosis in chickens. I U Vet Fak Derg 18:102–108

Bintvihok A (2002) New insights to controlling mycotoxin danger in ducks. Anim Feed Sci Tech 6:28–29

Bintvihok A, Kositcharoenkul S (2006) Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon 47:41–46

Bovo F, Franco LT, Kobashigawa E, Rottinghaus GE, Ledoux DR, Oliveira CAF (2015) Efficacy of beer fermentation residue containing *Saccharomyces cerevisiae* cells for ameliorating aflatoxicosis in broilers. Poult Sci 94(5):934–942

CAST (2003) Mycotoxins: risks in plant, animal, and human systems. Richard JL, Payne GA, editor. Ames, IA: Council for Agricultural Science and Technology (CAST) Council for Agricultural Science and Technology (CAST) Task Force Report No. 139

Celik K, Denli M, Erturk M, Ozturkcan O, Doran F (2001) Evaluation of dry yeast (SCE) in the feed to reduce AFB1 residues and toxicity to japonica quails. J Appl Anim Res 20:245–250

Chiacchiera SM, Magnoli CE, Astorga P, Miazzo R, Combina M, Dalcero AM, Kikot E, Basaldella E (2000) Use of synthetic zeolites to adsorb different mycotoxins, prevention of mycotoxicoses. Actualidad Fisicoquímica Organica 12:218–236

Daković A, Matijasevic S, George E, Rottinghaus D, Ledoux R (2008) Aflatoxin B_1 adsorption by natural and copper modified montmorillonite. Colloids Surf B: Biointerfaces 66:20–25

Denli M, Okan F (2006) Efficacy of different adsorbents in reducing the toxic effects of aflatoxin B1 in broiler diets. South African J Anim Sci 36:222–228

Denli M, Okan F, Doran F (2004) Effect of conjugated linoleic acid (CLA) on the performance and serum variables of broiler chickens intoxicated with aflatoxin B1. South African J Anim Sci 34:97–103

Diaz GJ; Murcia HW, Cepeda SM (2010) Cytochrome P450 enzymes involved in the metabolism of aflatoxin B1 in chickens and quail. Poult Sci 89,11:2461-2469, ISSN 0032-5791

Dixon J, Kannewischer BI, Tenorio Arvide MG, Barrientos Velazquez AL (2007) Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: an introductory plan. Appl Clay Sci 40: 201–208

Dos Anjos FR, Ledoux DR, Rottinghaus GE, Chimonyo M (2015) Efficacy of adsorbents (bentonite and diatomaceous earth) and turmeric (*Curcuma longa*) in alleviating the toxic effects of aflatoxin in chicks. Brit Poult Sci 56(4):459–469

El-Nezami H, Kankaanpaa P, Salminen S, Ahokas J (1998) Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B_1. Food Chem Toxicol 36:321–326

El-Nezami H, Mykkanen H, Kankaakpaä P, Salminen S, Ahokas J (2000) Ability of *Lactobacillus* and *Propionibacterium* strains to remove aflatoxin B, from the chicken duodenum. J Food Protect 63:549–552

Fraga ME, Curvello F, Gatti MJ, Cavaglieri LR, Dalcero AM, Da Rocha Rosa CA (2007) Potential aflatoxin and ochratoxin A production by *Aspergillus* species in poultry feed processing. Vet Res Commun 31: 343–353

Freimund S, Sauter M, Rys P (2003) Efficient adsorption of the mycotoxins zearalenone and T-2 toxin on a modified yeast glucan. J Environ Sci Heal B 38(3):243–255

González Pereyra ML, Dogi C, Torres Lisa A, Wittouck P, Ortíz M, Bagnis G, Yaciuk R, Poloni L, Torres A, Dalcero AM, Cavaglieri LR (2014) Genotoxicity and cytotoxicity evaluation of probiotic *Saccharomyces cerevisiae* RC016: a 60-day subchronic oral toxicity study in rats. J Appl Microbiol 117(3):824–833

Gutleba AC, Caloni F, Girauda F, Cortinovis C, Pizzo F, Hoffmanna L, Bohna T, Pasquali M (2015) Detection of multiple mycotoxin occurrences in soy animal feed by traditional mycological identification combined with molecular species identification. Toxicol Rep 2: 275–279

Hashmi I, Pahsa TN, Jabbar MA, Arkam M, Hashmi AS (2006) Study of adsorption potential of yeast sludge against AF in broiler chicks. J Anim Pl Sci 16:12–14

Juan-juan L, De-cheng S, Xiao-ou S (2010) Binding capacity for AFB1 by different adsorbents. Agri Sci China 9:449–456

Kemal C, Muzaffer D, Türker S, (2003) Reduction of toxic effects of aflatoxin B1 by using baker yeast (Saccharomyces cerevisiae) in growing broiler chicks diets. R Bras Zootec 32:615-619

Khadem AA, Sharifi SD, Barati M, Borji M (2012) Evaluation of the effectiveness of yeast, zeolite and active charcoal as aflatoxin absorbents in broiler diets. Global Vet 8:426–432

Kumar TAC, Balachandran C (2014) Pathological effect of citrinin and aflatoxin in broiler chicken. Int J Life Sci Pharma Res 4:1–15

Kurtzman CP, Robnett CJ (2003) Phylogenetic relationships among yeasts of the *Saccharomyces* complex determined from multigene sequence analyses. FEMS Yeast Res 3:417–432

Lamelas K, Mair G, Beczkowski G (2010) Evolución del sector Avícola año 2009, perspectiva 2010. Boletín avícola 58:1–26 Ministerio de Agricultura, Ganadería, Pesca y Alimentación. Accessed Nov. http://www.minagri.gob.ar

Leeson S, Dias GJ, Summers JD (1995) Tricothecenes. In: Poultry Metabolic Disorders. Guelph, Ontario, Canada, 190–226

Lozano MC, Diaz GJ (2006) Microsomal and cytosolic biotransformation of aflatoxin B1 in four poultry species. Br Poult Sci 47:734–741

Magnoli AP, Monge MP, Miazzo RD, Cavaglieri LR, Magnoli CE, Merkis CI, Cristofolini AL, Dalcero AM, Chiacchiera SM (2011a) Effect of low levels of aflatoxin B1 on performance, biochemical parameters, and aflatoxin B1 in broiler liver tissues in the presence of monensin and sodium bentonite. Poult Sci 90:48–58

Magnoli AP, Texeira M, Rosa CAR, Miazzo RD, Cavaglieri LR, Magnoli CE (2011b) Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens. Poult Sci 90:352–357

Magnoli AP, Alonso VA, Cavaglieri LR, Dalcero AM, Chiacchiera SM (2013) Effect of monogastric and ruminant gastrointestinal conditions on *in vitro* aflatoxin B_1 adsorption ability by a montmorillonite. Food Addit Contam Part A 30:743–749

Magnoli AP, Copia P, Monge MP, Magnoli CE, Dalcero AM, Chiacchiera SM (2014) Negligible effects of tryptophan on the aflatoxin adsorption of sodium bentonite. Food Addit Contam Part A 31(12):2063–2070

Magnoli AP, Rodriguez MC, Poloni VL, Rojo MC, Combina M, Chiacchiera SM, Dalcero AM, Cavaglieri LR (2016) Novel yeast isolated from broilers' feedstuff, gut and faeces as aflatoxin B1 adsorbents. J Appl Microbiol ISSN 1364–5072

Manafi M, Hedayati M, Yari M (2014) Aflatoxicosis and herbal detoxification: the effectiveness of thyme essence on performance parameters and antibody titers of commercial broilers fed aflatoxin B1. Res Zool 4(2):43–50

Miazzo R, Rosa RCA, Carvalho De Queiroz EC, Magnoli C, Chiacchiera SM, Palacio G, Saenz M, Kikot A, Basaldella E, Dalcero A (2000) Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poult Sci 79:1–6

Miazzo R, Peralta MF, Magnoli C, Salvano M, Ferrero S, Chiacchiera SM, Carvalho ECQ, Rosa CA, Dalcero A (2005) Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poult Sci 84:1–8

Monge MP, Dalcero AM, Magnoli CE, Chiacchiera SM (2013) Natural co-occurrence of fungi and mycotoxins in poultry feeds from Entre Ríos, Argentina. Food Addit Contam Part B: Surveillance 6:168–174

Motawe HFA, Abdel Salam AF, El Meleigy KHM (2014) Reducing the toxicity of aflatoxin in broiler chickens' diet by using probiotic and yeast. Int J Poult Sci 13(7):397–407

Nacional Research Council, (NRC). (1994) Nutrient requirements of chickens. En: Nutrient requirements of poultry. 8th rev. ed. National Academy Press, Washington DC 11–15

Nemati Z, Janmohammadi H, Taghizadeh A, Maleki H, Nejad Mogaddam GH, Arzanlou M (2014a) Occurrence of aflatoxins in poultry feed and feed ingredients from north western Iran. Eur J Zool Res 3:56–60

Nemati Z, Janmohammadi H, Taghizadeh A, Moghaddam GH, Maleki Nejad H (2014b) Effect of bentonite supplementation to the contaminated diets with aflatoxin B1 on broiler performance. presented at the 6th Iranian congress on animal science, Tabriz

Nemati Z, Karimi A, Besharati M (2015) Impact of aflatoxin contaminnated feed and yeast cell wall suplementation on immune system in broiler chickens Intl Conf. International Conference on Innovations in Chemical and Agricultural Engineering (ICICAE'2015) Feb. 8–9, 2015 Kuala Lumpur

Oğuz H (2011) A review from experimental trials on detoxification of aflatoxin in poultry feed. Eurasian J Vet Sci 27:1–12

Oğuz H, Kececi T, Birdane YO, Onder F, Kurtoglu V (2000a) Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during experimental aflatoxicosis. Res Vet Sci 69:89–93

Oğuz H, Kurtoglu V, Coskun B (2000b) Preventive efficacy of clinoptilolite in broiler during chronic aflatoxin (50 and 100 ppb) exposure. Res Vet Sci 69:197–201

Oğuz H, Hadimli HH, Kurtoglu V, Erganiş O (2003) Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Rev Med Vet B Aires 154:483–486

Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcero AM, Rosa CA (2006) Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia 162:355–362

Onwurah FB, Okejim J, Amaefula KU (2013) Effect of yeast as water additive in the management of litter in the production starter broiler. Asian J Nat Appl Sci 2:127–130

Ortatatli M, Oğuz H (2001) Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. Res Vet Sci 71:59–66

Ortatatli M, Oğuz H, Hatipoglu F, Karaman M (2005) Evaluation of pathological changes in broilers during chronic afla toxin (50 and 100 ppb) and clinoptilolite exposure. Res Vet Sci 78:61–68

Pizzolitto RP, Armando MR, Salvano MA, Dalcero AM, Rosa CA (2013) Evaluation of *Saccharomyces cerevisiae* as an antiaflatoxicogenic agent in broiler feedstuffs. Poult Sci 92:1655–1663

Raju MVLN, Devegowda G (2000) Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Brit Poult Sci 41:640–650

Rosa CAR, Miazzo R, Magnoli C, Salvano M, Chiacchiera SM, Ferrero S, Saenz M, Carvalho EC, Dalcero A (2001) Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult Sci 80:139–144

Rosa AP, Uttpatel R, Santurio JM, Sher A, Duarte V, Santos CB (2012) Performance of broilers derived from breeder hens fed with diets containing aflatoxins and esterified glucomannan as adsorbent. Braz J Anim Sci 41:347–352

Roto SM, Rubinelli PM, Ricke SC (2015) An introduction to the avian gut microbiota and the effects of yeast based prebiotic-type compounds as potential feed additives. Frontiers in Vet Sci 2:28

Santin E, Paulillo AC, Maiorka A, Satiko L, Macari M, Fischer Da Silva AV (2003) Evaluation of the efficacy of *Saccharomyces cerevisiae* cell wall to ameliorate the toxic effects of aflatoxin in broilers. Int J Poult Sci 2:341–344

Santin E, Paulillo AC, Nakagui LSO, Alessi AC, Maiorka A (2006) Evaluation of yeast cell wall on the performance of broiles fed diets with or without mycotoxins. Rev Bras Cienc Avic 8:221–225

Savlík M, Poláčkcová L, Szotáková B, Lamka J, Velík J, Skálová L (2007) Activities of biotransformation enzymes in pheasant (Phasianus colchicus) and their modulation by in vivo administration of mebendazole and ubendazole. Res Vet Sci 83:20–26

Schatzmayr G, Florian Z, Täubel M, Schatzmayr D, Klimitsch A, Loibner AP, Binder EM (2006) Microbiologicals for deactivating mycotoxins. Mol Nutr Food Res 50(6):543–551

Sørensen LK, Elbæk TH (2005) Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 820:183–196

Sur E, Celik I (2003) Effects of aflatoxin B1 on the development of the bursa of Fabricius and blood lymphocyte acid phosphatase of the chicken. Br Poult Sci 44:558–566

Tavčar-Kalcher G, Vrtač K, Pestevšek U, Vengust A (2007) Validation of the procedure for the determination of aflatoxin B1in animal liver using immunoaffinity columns and liquid chromatography with post-column derivatisation and fluorescence detection. Food Contr 18:333–337

Tessari ENC, Oliveria CAF, Cardoso ALSP, Ledoux DR, Rottinghaus GE (2006) Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Brit Poult Sci 47: 357–364

Trucksess MW, Stack ME, Nesheim S, Albert R, Romer T (1994) Multifunctional column coupled with liquid chromatography for determination of aflatoxins B1, B2, G1 and G2 in corn, almonds. Brazil nuts, peanuts, and pistachio nuts: collaborative study. J AOAC Int 77:1512–1521

U.E. (2006) Reglamento 401/2006 de la Comisión, de 23 de febrero de 2006, por el que se establecen los métodos de muestreo y de análisis para el control oficial del contenido de micotoxinas en los productos alimenticios. Diario Oficial de la Unión Europea L70 de 09/03/ 2006, pp.12–34

Vanhoutte I, Audenaert K, De Gelder L (2016) Biodegradation of Mycotoxins: Tales from Known and Unexplored Worlds. Front Microbiol 7:561.doi: 10.3389/fmicb.2016.00561

Wiener Laboratory (2000) Calorimetric method for determination of total protein, albumin and serum transaminase. Wiener Laboratory, Rosario

Yiannikouris A, Poughon L, Cameleyre X, Dussap CG, Francüois J, Bertin G, Jouany JP (2003) A novel technique to evaluate interactions between *Saccharomyces cerevisiae* cell wall and mycotoxins: application to zearalenone. Biotechnol Lett 25:783–789

Yiannikouris A, François J, Poughon L, Dussap CG, Bertin G, Jeminet G, Jouany JP (2004) Alkali-extraction of b-D-glucans from *Saccharomyces cerevisiae* cell wall and study of their adsorptive properties toward zearalenone. J Agr Food Chem 52:3666–3673

Zain ME (2011) Impact of mycotoxins on humans and animals. J Saudi Chem Soc 15:129–144