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**ABSTRACT**

The aim of the present study was to evaluate the carry-over of fumonisin B<sub>1</sub> (FB<sub>1</sub>), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) residues and its metabolites (M<sub>1</sub>, B<sub>2a</sub> and aflatoxicol) into eggs of laying Japanese quails (*Coturnix coturnix japonica*) fed mycotoxin-contaminated rations. To this end, 288 quails were randomly distributed into 6 experimental groups (48 birds per group) and given rations containing AFB<sub>1</sub> ( g/kg) + FB<sub>1</sub> (mg/kg) at the following levels during 5 months: (0+0), (0+10), (50+0), (50+10), (200+0) and (200+10). Thirty-two eggs per treatment were collected each period of 30 days and analyzed for aflatoxins B<sub>1</sub>, M<sub>1</sub>, B<sub>2a</sub>, aflatoxicol and FB<sub>1</sub> by high performance liquid chromatography. No detectable residues of FB<sub>1</sub> were found in the eggs of quail receiving 10 mg FB<sub>1</sub>/kg feed. Total residues of AFB<sub>1</sub> (AFB<sub>1</sub> + AFM<sub>1</sub> + AFB<sub>2a</sub> + aflatoxicol) increased in the eggs of all groups receiving AFB<sub>1</sub>-contaminated rations (50 and 200 ng/g) along 5 months of exposure, at levels that ranged from 0.38 to 1.43 ng/g. The results indicate that the carry-over of AFB<sub>1</sub> from feed to eggs of laying quail increases after long-term exposure, emphasizing the importance of controlling aflatoxin levels in rations of laying quails.

**RESUMO**

O objetivo do presente trabalho foi avaliar a excreção de resíduos de fumonisina B<sub>1</sub> (FB<sub>1</sub>), aflatoxina B<sub>1</sub> (AFB<sub>1</sub>) e derivados metabólicos (M<sub>1</sub>, B<sub>2a</sub> e aflatoxicol) em ovos de codornas poedeiras (*Coturnix coturnix japonica*) alimentadas com ração contendo as toxinas isoladas ou associadas. Para isto, foram constituídos 6 grupos de 48 codornas, as quais foram alimentadas durante 5 meses com rações contendo AFB<sub>1</sub> ( g/kg) + FB<sub>1</sub> (mg/kg) nas concentrações: (0+0), (0+10), (50+0), (50+10), (200+0) e (200+10). A determinação de resíduos de AFB<sub>1</sub>, AFM<sub>1</sub>, AFB<sub>2a</sub>, aflatoxicol e FB<sub>1</sub> nos ovos foi realizada através da técnica de cromatografia líquida de alta eficiência, mediante a análise de 2 amostras de 32 ovos/tratamento/mês, totalizando 60 amostras. O nível de FB<sub>1</sub> empregado na ração (10 mg/kg) não foi capaz de originar resíduos detectáveis de FB<sub>1</sub> nos ovos. Os níveis de AFB<sub>1</sub> utilizados (50 e 200 ng/g) originaram resíduos totais (AFB<sub>1</sub> + AFM<sub>1</sub> + AFB<sub>2a</sub> + aflatoxicol) crescentes ao longo dos 5 meses de exposição, cujos valores variaram de 0,381,43 ng/g. Os resultados indicaram que a excreção de resíduos de aflatoxinas nos ovos de codornas aumenta continuamente com a exposição das aves, ressaltando a importância do controle desta toxina nas rações de codornas poedeiras.

**PALAVRAS-CHAVE**

KEY WORDS

AFB<sub>1</sub>. FB<sub>1</sub>. Residues. Eggs. Contamination.

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## 1. INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including men (COULOMBE, 1991). Exposure to toxins occurs predominantly by the ingestion of contaminated food, especially cereals and grains, such as corn, wheat, and peanut, among others (CHU, 1991). The most common toxigenic fungi found in cereals in Brazil include species from the genera *Aspergillus* and *Fusarium* (RODRIGUEZ-AMAYA, 2001).

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is an hepatocarcinogen metabolite produced by strains of *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* during growth on several food and feed materials (MOSS, 1998). The occurrence of AFB<sub>1</sub> in food products is a public health concern because of its possible involvement in the aetiology of human liver cancer (COULOMBE, 1991).

AFB<sub>1</sub> is primarily biotransformed in the liver by cytochrome P-450 associated enzymes, which generate hydroxylated metabolites such as aflatoxins M<sub>1</sub> (AFM<sub>1</sub>) and B<sub>2a</sub> (AFB<sub>2a</sub>) (HSIEH & ATKINSON, 1991). Aflatoxicol (AFL) can also be formed by the reduction of AFB<sub>1</sub> by an NADPH-dependent cytoplasmic enzyme present in the soluble fraction of liver homogenates (BIEHL & BUCK, 1987). However, these compounds are also a potential human health risk because they can be carried-over into animal derived food products and some of them can still induce toxic effects in experimental models (KUIPER-GOODMAN, 1991). In addition, residual AFB<sub>1</sub> may be also present in edible products intended for human consumption, including liver and eggs (PARK & POHLAND, 1986).

Nowadays, 16 molecular structures are called fumonisins (AH-SEO & WON LEE, 1999), however the predominant toxin produced by *Fusarium moniliforme* strains is fumonisin B<sub>1</sub> (FB<sub>1</sub>). FB<sub>1</sub> is hepatocarcinogenic in rats, and is also associated to oesophageal cancer in humans, in some South Africa regions (THIEL et al., 1992).

There is very little information on the carry-over of aflatoxins into eggs of laying Japanese quail. BINTVIHOK et al. (1993) observed only AFB<sub>1</sub> at levels below 0.01 g/kg in the eggs after 12 weeks administration of rations with 50, 100 and 200 g AFB<sub>1</sub>/kg. OLIVEIRA et al. (2003) fed laying quails with rations containing 25, 50 and 100 g AFB<sub>1</sub>/kg feed and detected aflatoxins in eggs at levels that ranged from 0.01 to 0.08 g/kg (AFB<sub>1</sub>), 0.03 to 0.37 g/kg (AFM<sub>1</sub>), 0.01 to 1.03 g/kg (AFB<sub>2a</sub>), and 0.01 to 0.03 g/kg (AFL). However, no previous report on the transmission of FB<sub>1</sub> alone or in combination with other mycotoxins from feed to eggs was found in the literature. Therefore, the aim of the present work was to evaluate the transmission of residual FB<sub>1</sub>, AFB<sub>1</sub> and its metabolites (AFM<sub>1</sub>, AFB<sub>2a</sub> and AFL) into eggs of laying Japanese quail fed rations containing low levels of these toxins.

## 2. MATERIALS AND METHODS

Two hundred eighty-eight laying Japanese quail (*Coturnix coturnix japonica*) were purchased from a local commercial grower at 5 weeks of age. Birds were placed in three batteries of eight wire cages each (twelve birds per cage) and allowed to consume feed prepared according to specifications of the NATIONAL RESEARCH COUNCIL (1994) and water ad libitum. Each treatment group consisted of four replicate pens, each containing 12 birds. Dietary treatments were: 1) 0 mycotoxins (control); 2) 10 mg FB<sub>1</sub>; 3) 50 g AFB<sub>1</sub>; 4) 50 g AFB<sub>1</sub> + 10 mg FB<sub>1</sub>; 5) 200 g AFB<sub>1</sub>; and 6) 200 g AFB<sub>1</sub> + 10 mg FB<sub>1</sub>/kg feed. This final schedule was maintained throughout the remainder of the study. The treatment rations were fed ad libitum for 140 days (five 28-d laying periods).

Aflatoxin B<sub>1</sub> used in the experiment was produced using a toxigenic strain of *Aspergillus flavus* IMI-190 (International Mycology Institute, London), as described by LIN & DIANESE (1976). Fumonisin B<sub>1</sub> was produced from *Fusarium verticillioides* M-1325 cultured in whole shelled corn (WEIBKING et al., 1993). Aflatoxin B<sub>1</sub> test concentrations were obtained using sterile maize oil as the diluent and appropriate amounts of these solutions were added to the basal diet, so as to obtain the required levels of AFB<sub>1</sub>. To guarantee a balanced diet for all treatments, AFB<sub>1</sub> oil mixtures were substituted for maize oil (1% v/w) in the feeds. Fumonisin B<sub>1</sub> culture material was added directly to the feed. Final mixtures were homogenized in a horizontal/helicoidal mixer (Marconi).

Thirty-two eggs per treatment were randomly collected on days 28, 56, 84, 112 and 140 of the intoxication period, and submitted to analysis for residues of FB<sub>1</sub>, AFB<sub>1</sub>, AFM<sub>1</sub>, AFB<sub>2a</sub>, and AFL. Extraction and quantification procedures followed the method recommended by the Association of Official Analytical Chemists (SCOTT, 1990), including modifications as previously described by OLIVEIRA et al. (2003). Final extract was divided in two portions for determination of aflatoxins, one for AFB<sub>1</sub> and the other for AFM<sub>1</sub>, AFB<sub>2a</sub> and AFL analysis. Fifty  $\mu$ l of trifluoroacetic acid (TFA) was added to the vial containing the AFB<sub>1</sub> portion to convert it to AFB<sub>2a</sub>. The two portions were evaporated to near-dryness and diluted in 0.5 ml of benzene-acetonitrile (98:2, v/v). Identification and quantification of the aflatoxin residues was achieved by high performance liquid chromatography (HPLC), using a Shimadzu 10VP liquid chromatograph with a 10 AXL fluorescence detector (excitation at 365 nm and emission above 420 nm). A Shim-Pack CLC-ODS Sil column (6 X 150 mm) and a Shim-Pack precolumn (4 X 10 mm CLC G-ODS, 5  $\mu$ m) were used. Calibration curves of aflatoxins were prepared using standard solutions of AFB<sub>1</sub>, AFM<sub>1</sub>, AFB<sub>2a</sub> and AFL (Sigma) previously evaluated according to SCOTT (1990). AFB<sub>1</sub> working solutions were prepared with TFA similarly to the sample extracts. Twenty  $\mu$ l of sample extracts were injected for determination of AFB<sub>2a</sub> from TFA derivatization of AFB<sub>1</sub> or for AFM<sub>1</sub>, AFB<sub>2a</sub> and AFL, respectively. The mobile phase consisted of water/methanol (1:1) with a flow rate of 0.5-0.7 ml/min (adjusted for optimum separation). Under these conditions, the retention times were approximately 11 min for AFB<sub>2a</sub>, 20 min for AFM<sub>1</sub> and 32 min for AFL. The concentration of AFB<sub>1</sub> in samples

was estimated from the difference between the AFB<sub>2a</sub> content in TFA and non-treated TFA fractions.

Analysis of FB<sub>1</sub> was performed according to the method of SMITH & THAKUR (1996), with some modifications, as follows. A 5-g aliquot of the sample was placed in a erlenmeyer containing 35 ml acetonitrile-water (1:1). Samples were placed in an orbital shaker (Tecnal) for 30 minutes and centrifuged at 2.000g for 15 minutes. Clean up of the extract (20 ml of the supernatant) was performed in solid phase extraction columns (Bond-Elut SAX), previously conditioned with 10 ml of methanol and 10 ml of methanol-water (3:1). After the initial washing with 8 ml of methanol-water (3:1) and 4 ml of methanol, the extract was eluted with 15 ml of methanol-acetic acid (95:5), collected in a vial and evaporated at 50°C under N<sub>2</sub>, until dryness.

Samples were re-diluted with 200 µl of acetonitrile-water (50:50) and filtered using a PVDF membrane with 0.22 µm pores (Millipore). A 100-µl aliquot was placed in a test tube. After that, 200 µl of o-phthalaldehyde (OPA) reagent (prepared using 40 µg of OPA diluted in 5 ml of sodium tetraborate solution 0.1 M and 50 µl of 2-mercaptoethanol)

were added. After 2 minutes, 20 µl were used for quantification of the toxins in the same HPLC equipment as previously described, provided with a fluorescence detector (335 and 440 nm for excitation and emission, respectively) and a reverse phase C<sub>18</sub> column (150 x 4.6 mm, particle size 5 µm Phenomenex, Torrance, USA) kept at constant temperature of 30°C. Mobile phase was made up of acetonitrile-water-acetic acid (50:50:1) at the constant flow equal to 1 ml/minute. The retention time was approximately 9 minutes for FB<sub>1</sub>. Quantification was performed based on the peak area corresponding to each toxin, compared with the standard (Sigma).

### 3. RESULTS AND DISCUSSION

Residues of FB<sub>1</sub> were not detected in the eggs of any quail fed FB<sub>1</sub>-contaminated rations, alone or in combination with AFB<sub>1</sub>. The limit of quantification for FB<sub>1</sub> was 20 µg/kg, as considered by the minimum amount of toxin that could

**Tabela 1** Concentrations (g/kg) of residues of AFB<sub>1</sub>, AFM<sub>1</sub>, aflatoxicol, AFB<sub>2a</sub> and total aflatoxins found in quail eggs during 140 days of exposure.

Treatment	Day of Intoxication	AFB <sub>1</sub>	AFM <sub>1</sub>	Aflatoxicol	AFB <sub>2a</sub>	Total aflatoxins
50 g AFB <sub>1</sub> /kg	28	0.02 <sup>a</sup>	ND	0.14	0.23	0.39
	56	ND	0.08	0.13	0.17	0.38
	84	ND	0.09	0.16	0.15	0.40
	112	0.03	0.02	0.15	0.20	0.40
	140	0.04	0.12	0.17	0.17	0.50
50 g AFB <sub>1</sub> /kg + 10 mg FB <sub>1</sub> /kg	28	ND	0.04	0.13	0.21	0.38
	56	ND	0.12	0.13	0.21	0.46
	84	0.01	0.08	0.11	0.20	0.40
	112	0.02	0.22	0.27	0.16	0.67
	140	0.06	0.33	0.18	0.12	0.69
200 g AFB <sub>1</sub> /kg	28	ND	0.13	0.11	0.18	0.42
	56	0.08	0.37	0.14	0.07	0.66
	84	0.04	0.30	0.30	0.22	0.86
	112	0.05	0.20	0.16	0.15	0.56
	140	0.04	0.32	0.23	0.48	1.07
200 g AFB <sub>1</sub> /kg + 10 mg FB <sub>1</sub> /kg	28	ND	0.12	0.20	0.12	0.44
	56	0.05	0.39	0.31	0.10	0.85
	84	0.02	0.20	0.22	0.18	0.62
	112	0.08	0.13	0.36	0.86	1.43
	140	0.08	0.28	0.22	0.50	1.08

<sup>a</sup>Mean of duplicate samples analyzed.  
ND not detected.

generate a chromatographic peak five times higher than the height / noise rate of the baseline.

Aflatoxin residues in the eggs during the feeding trial are presented in table 1. Residues of AFB<sub>1</sub>, AFM<sub>1</sub>, AFB<sub>2a</sub> and AFL were detected in the eggs of all groups receiving AFB<sub>1</sub>-contaminated rations. The control egg pools showed no fluorescent peak near the aflatoxin retention times. The concentrations of AFB<sub>1</sub> in individual egg samples ranged from 0.01 to 0.08 g/kg, which are similar to the results reported by OLIVEIRA et al. (2003), who fed quails with AFB<sub>1</sub> at levels of 25-100 g/kg, and slightly higher than those obtained by BINTVIHOK et al. (1993), who found levels below 0.01 g/kg of AFB<sub>1</sub> in eggs from quail exposed to rations containing 50-200 g AFB<sub>1</sub>/kg feed during 84 days.

Residues of AFM<sub>1</sub> were detected in eggs of intoxicated quail from day 28, except for the group receiving 50 g AFB<sub>1</sub>/kg alone. The concentrations of AFM<sub>1</sub> were in the range of 0.02-0.39 g/kg and the highest levels were found in eggs from treatment 200 g AFB<sub>1</sub>/kg, alone or in combination with FB<sub>1</sub>, particularly after 56 days of continuous ingestion of the contaminated diet. Our results indicate that the excretion of AFM<sub>1</sub> in quail eggs may occur especially under conditions of long-term exposure, which represents a relevant hazard to human health considering the carcinogenic potency of this toxin.

Aflatoxicol was detected at levels of 0.11-0.36 g/kg, and highest concentrations were observed in groups receiving 200 g AFB<sub>1</sub>/kg. These results agree with previous data reported by MICCO et al. (1987), who found a marked rapid conversion of AFB<sub>1</sub> into AFL in laying hens after 2-7 days of treatment with 30 g/kg body weight/day. Additionally, the incorporation of FB<sub>1</sub> to the AFB<sub>1</sub>-contaminated rations apparently did not modify the metabolism to AFL and its excretion into eggs, as observed for AFB<sub>1</sub> and AFM<sub>1</sub>.

Higher concentrations of AFB<sub>2a</sub> were also observed in eggs from quail fed 200 g AFB<sub>1</sub>/kg, ranging from 0.07 to 0.86 g/kg. For laying hens, however, previous studies reported relative lower levels of AFB<sub>2a</sub> in the eggs (WOLZAC et al. 1995; MICCO et al. 1987). Since AFB<sub>2a</sub> is considered to be less toxic than AFB<sub>1</sub> (NEAL et al. 1981), our results suggest that the formation of this metabolite represents a major detoxification pathway for AFB<sub>1</sub> in the laying quail, as previously reported by OLIVEIRA et al. (2003). Coherently, the total aflatoxin content (AFB<sub>1</sub> + AFM<sub>1</sub> + AFB<sub>2a</sub> + AFL) in egg samples ranged from 0.38 to 1.43 g/kg, which were similar to data obtained by OLIVEIRA et al. (2003), and increased along the 140 days of exposure. These data indicate that the carry-over of AFB<sub>1</sub> residues is relatively most probable to occur in quail when the birds are continuously exposed for long periods to low levels of AFB<sub>1</sub> in the diet. This fact may be related to the lower capacity of quail in detoxifying AFB<sub>1</sub> and the relative higher sensitivity of Japanese quail to the toxin, as reported in previous study (LEESON et al. 1995).

Results of the present study indicate that the excretion of FB<sub>1</sub> residues into eggs of laying Japanese quail after long-term administration of FB<sub>1</sub> in rations is negligible. However, the AFB<sub>1</sub> levels used in the experimental rations were

in the range of natural occurrence of the toxin in contaminated grains and cereals in Brazil and other countries (RODRIGUEZ-AMAYA, 2001). Thus, the aflatoxin metabolites found in the quail eggs may be of great importance for the public health because its toxic and carcinogenic effects have been demonstrated in many laboratory species (COULOMBE, 1991). Therefore, the control of AFB<sub>1</sub> contamination in rations of laying quail is recommended in order to avoid the occurrence of aflatoxin metabolites in quail eggs intended for human consumption.

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