BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

The Carry-over of Aflatoxin B_1 and Fumonisin B_1 Residues from Feeds to Eggs of Laying Quails

Transferência de Resíduos de Aflatoxina B_1 e Fumonisina B_1, da Ração para Ovos de Codornas

AUTORES
AUTHORS

✉ **Carlos Augusto Fernandes OLIVEIRA[1*]**
Daniela de Lara PEDROSO[1]
Roni OGIDO[2]
Ricardo de ALBUQUERQUE[2]
Benedito CORRÊA[3]

[1]Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brasil;
[2]Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Duque de Caxias Norte, 225, 13635-900, Pirassununga, SP, Brasil
Fone: 55-19-35654173, Fax: 55-19-35654114;
[3]Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil.
*e-mail: carlosaf@usp.br

ABSTRACT

The aim of the present study was to evaluate the carry-over of fumonisin B_1 (FB_1), aflatoxin B_1 (AFB_1) residues and its metabolites (M_1, B_{2a} 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_1 (g/kg) + FB_1 (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_1, M_1, B_{2a}, aflatoxicol and FB_1 by high performance liquid chromatography. No detectable residues of FB_1 were found in the eggs of quail receiving 10 mg FB_1/kg feed. Total residues of AFB_1 (AFB_1 + AFM_1 + AFB_{2a} + aflatoxicol) increased in the eggs of all groups receiving AFB_1-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_1 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_1 (FB_1), aflatoxina B_1 (AFB_1) e derivados metabólicos (M_1, B_{2a} 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_1 (g/kg) + FB_1 (mg/kg) nas concentrações: (0+0), (0+10), (50+0), (50+10), (200+0) e (200+10). A determinação de resíduos de AFB_1, AFM_1, AFB_{2a}, aflatoxicol e FB_1 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_1 empregado na ração (10 mg/kg) não foi capaz de originar resíduos detectáveis de FB_1 nos ovos. Os níveis de AFB_1 utilizados (50 e 200 ng/g) originaram resíduos totais (AFB_1 + AFM_1 + AFB_{2a} + 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

AFB1. FB1. Residues. Eggs. Contamination.
AFB1. FB1. Resíduos. Ovos. Contaminação.

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_1 (AFB_1) 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_1 in food products is a public health concern because of its possible involvement in the aetiology of human liver cancer (COULOMBE, 1991).

AFB_1 is primarily biotransformated in the liver by cytochrome P-450 associated enzymes, which generate hydroxylated metabolites such as aflatoxins M_1 (AFM_1) and B_{2a} (AFB_{2a}) (HSIEH & ATKINSON, 1991). Aflatoxicol (AFL) can also be formed by the reduction of AFB_1 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_1 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_1 (FB_1). FB_1 is hepatocarcinogenic in rats, and is also associated to oesophagic 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_1 at levels below 0.01 g/kg in the eggs after 12 weeks administration of rations with 50, 100 and 200 g AFB_1/kg. OLIVEIRA et al. (2003) fed laying quails with rations containing 25, 50 and 100 g AFB_1/kg feed and detected aflatoxins in eggs at levels that ranged from 0.01 to 0.08 g/kg (AFB_1), 0.03 to 0.37 g/kg (AFM_1), 0.01 to 1.03 g/kg (AFB_{2a}), and 0.01 to 0.03 g/kg (AFL). However, no previous report on the transmission of FB_1 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_1, AFB_1 and its metabolites (AFM_1, AFB_{2a} 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_1; 3) 50 g AFB_1; 4) 50 g AFB_1 + 10 mg FB_1; 5) 200 g AFB_1; and 6) 200 g AFB_1 + 10 mg FB_1/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_1 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_1 was produced from Fusarium verticillioides M-1325 cultured in whole shelled corn (WEIBKING et al., 1993). Aflatoxin B_1 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_1. To guarantee a balanced diet for all treatments, AFB_1 oil mixtures were substituted for maize oil (1% v/w) in the feeds. Fumonisin B_1 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_1, AFB_1, AFM_1, AFB_{2a}, 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_1 and the other for AFM_1, AFB_{2a} and AFL analysis. Fifty l of trifluoroacetic acid (TFA) was added to the vial containing the AFB_1 portion to convert it to AFB_{2a}. 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 m) were used. Calibration curves of aflatoxins were prepared using standard solutions of AFB_1, AFM_1, AFB_{2a} and AFL (Sigma) previously evaluated according to SCOTT (1990). AFB_1 working solutions were prepared with TFA similarly to the sample extracts. Twenty l of sample extracts were injected for determination of AFB_{2a} from TFA derivatization of AFB_1 or for AFM_1, AFB_{2a} 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_{2a}, 20 min for AFM_1 and 32 min for AFL. The concentration of AFB_1 in samples

was estimated from the difference between the AFB_{2a} content in TFA and non-treated TFA fractions.

Analysis of FB_1 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_2, 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-phthaldialdehyde (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_{18} 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_1. Quantification was performed based on the peak area corresponding to each toxin, compared with the standard (Sigma).

3. RESULTS AND DISCUSSION

Residues of FB_1 were not detected in the eggs of any quail fed FB_1-contaminated rations, alone or in combination with AFB_1. The limit of quantification for FB_1 was 20 µg/kg, as considered by the minimum amount of toxin that could

Tabela 1 Concentrations (g/kg) of residues of AFB_1, AFM_1, aflatoxicol, AFB_{2a} and total aflatoxins found in quail eggs during 140 days of exposure.

Treatment	Day of Intoxication	AFB_1	AFM_1	Aflatoxicol	AFB_{2a}	Total aflatoxins
50 g AFB_1/kg	28	0.02 [a]	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_1/kg + 10 mg FB_1/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_1/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_1/kg + 10 mg FB_1/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

[a] 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_1, AFM_1, AFB_{2a} and AFL were detected in the eggs of all groups receiving AFB_1-contaminated rations. The control egg pools showed no fluorescent peak near the aflatoxin retention times. The concentrations of AFB_1 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_1 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_1 in eggs from quail exposed to rations containing 50-200 g AFB_1/kg feed during 84 days.

Residues of AFM_1 were detected in eggs of intoxicated quail from day 28, except for the group receiving 50 g AFB_1/kg alone. The concentrations of AFM_1 were in the range of 0.02-0.39 g/kg and the highest levels were found in eggs from treatment 200 g AFB_1/kg, alone or in combination with FB_1, particularly after 56 days of continuous ingestion of the contaminated diet. Our results indicate that the excretion of AFM_1 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_1/kg. These results agree with previous data reported by MICCO et al. (1987), who found a marked rapid conversion of AFB_1 into AFL in laying hens after 2-7 days of treatment with 30 g/kg body weight/day. Additionally, the incorporation of FB_1 to the AFB_1-contaminated rations apparently did not modify the metabolism to AFL and its excretion into eggs, as observed for AFB_1 and AFM_1.

Higher concentrations of AFB_{2a} were also observed in eggs from quail fed 200 g AFB_1/kg, ranging from 0.07 to 0.86 g/kg. For laying hens, however, previous studies reported relative lower levels of AFB_{2a} in the eggs (WOLZAC et al. 1995; MICCO et al. 1987). Since AFB_{2a} is considered to be less toxic than AFB_1 (NEAL et al. 1981), our results suggest that the formation of this metabolite represents a major detoxification pathway for AFB_1 in the laying quail, as previously reported by OLIVEIRA et al. (2003). Coherently, the total aflatoxin content ($AFB_1 + AFM_1 + AFB_{2a} + AFL$) 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_1 residues is relatively most probable to occur in quail when the birds are continuously exposed for long periods to low levels of AFB_1 in the diet. This fact may be related to the lower capacity of quail in detoxifying AFB_1 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_1 residues into eggs of laying Japanese quail after long-term administration of FB_1 in rations is negligible. However, the AFB_1 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_1 contamination in rations of laying quail is recommended in order to avoid the occurrence of aflatoxin metabolites in quail eggs intended for human consumption.

4. REFERENCES

AH-SEO, J., WON LEE, Y. (1999). Natural occurrence of the C series of fumonisins in moldy corn. Applied Environmental Microbiology, v. 65, n. 3, p. 1331-1334. 1999.

BIEHL, M.L., BUCK, W.B. Chemical contaminants: their metabolism and their residues. Journal of Food Protection, v. 50, n. 12, p. 1058-1073, 1987.

BINTVIHOK, A., THIENGININ, S., PATCHIMASIRI, T., THUMMABOOD, S., SHOYA, S., OGURA, Y., KUMAGAI, S., DOI, K., INGKANINUN, P., POOMVISES, P. Toxic effects of dietary aflatoxin and its residue in tissues and eggs in laying quails. In: Proceedings of the 11th International Symposium of the World Association of Veterinary Food Hygienists, Thailand: Bangkok, 1993. p. 299-307.

CHU, F.S. Mycotoxins: food contamination, mechanism, carcinogenic potential and preventive measures. Mutation Research, v. 259, n. 3, p. 291-306, 1991.

COULOMBE, R. A. Aflatoxins. In: SHARMA, R.P. & SALUNKHE, D.K. (Ed). Mycotoxins and phytoalexins. Boca Raton: CRC Press, 1991. p. 103-143.

HSIEH, D.P.H., ATKINSON, D.N. Bisfuranoid mycotoxins: their genotoxicity and carcinogenicity. Advances in Experimental Medicine and Biology, v. 283, n. 4, p. 525-532, 1991.

KUIPER-GOODMAN, T. Risk assessment to humans of mycotoxins in animal-derived food products. Veterinary and Human Toxicology, v. 33, n. 4, p. 325-333, 1991.

LEESON, S., DIAZ, G.J., SUMMERS, J.D. Poultry Metabolic Disorders and Mycotoxins. Guelph: University Books, 1995. 352p.

LIN, M.T., DIANESE, J.C. A coconut agar medium for rapid detection of aflatoxin production by Aspergillus spp. Phytopathology, v. 66, n. 12, p. 1466-1469, 1976.

MICCO, C., BRERA, C., MIRAGLIA, M., ONORI, R. HPLC determination of the total content of aflatoxins in naturally contaminated eggs in free and conjugate forms. Food Additives and Contaminants, v. 4, n. 4, p. 407-414, 1987.

MOSS, M. O., 1998, Recent studies of mycotoxins. Journal of Applied Microbiology Symposium Supplement, v. 84, p. 62S-76S.

NATIONAL RESEARCH COUNCIL. Nutrient requirements of poultry. 9th ed. Washington, DC: National

Academy of Sciences, 1994. 155p.

NEAL, G.E., JUDAH, D.J., STIRPE, F., PATTERSON, D.S. The formation of 2,3-dihydroxy-2,3-dihydro aflatoxin B1 by the metabolism of aflatoxin B1 by liver microsomes isolated from certain avian and mammalian species and the possible role of this metabolite in the acute toxicity of aflatoxin B1. Toxicology and Applied Pharmacology, v. 58, n. 3, p. 431-437, 1981.

OLIVEIRA, C.A.F., ROSMANINHO, J.F., CASTRO, A.L., BUTKERAITIS, P., REIS, T.A., CORRÊA, B. Aflatoxin residues in eggs of laying Japanese quail after long term administration of rations containing low levels of aflatoxin B1. Food Additives and Contaminants, v. 20, n. 7, p. 648-653, 2003.

PARK, D.L., POHLAND, A.E. A rationale for the control of aflatoxin in animal feeds. In: STEYN, P.S., VLEGGAAR, R. (Ed). Mycotoxins and phycotoxins. Amsterdam: Elsevier Appied Science, 1986. p. 473-82.

RODRIGUEZ-AMAYA, D.B. Occurrence of mycotoxins and mycotoxin-producing fungi in Latin America. In: KOE, W.J., SAMSON, R.A., VAN EGMOND, H.P., GILBERT, J., SABINO, M. (Ed). Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. Wageningen: W. J. de Koe, 2001. p. 309-320.

SCOTT, P.M. Natural poisons. In: HELRICH, K. (Ed). Official methods of analysis of the Association of Official Analytical Chemists. 15th ed. Arlington: Association of Official Analytical Chemists (AOAC), 1990. p. 1184-1213.

SMITH, J.S., THAKUR, R.A. Occurrence and fate of fumonisins in beef. Advances in Experimental Medicine and Biology, v. 392, p. 39-55, 1996.

THIEL, P.G., MARASAS, W.F.O., SYDENHAM, E.W., SHEPHARD, G.S., GELDERBLOM, W.C.A. The implications of naturally occurring levels of fumonisins in corn for human and animal health. Mycopathologia, v. 117, n. 1, p. 3-9, 1992.

WEIBKING, T.S., LEDOUX, D.R., BERMUDEZ, A.J., TURK, J.R., ROTTINGHAUS, G.E. Effects of feeding Fusarium monilifome culture material, containing known levels of fumonisin B1, on the young broiler chick. Poultry Science, v. 72, n. 4, p. 456-466, 1993.

WOLZAK, A., PEARSON, A.M., COLEMAN, T.H., PESTKA, J.J., GRAY, J.I. Aflatoxin deposition and clearence in the eggs of laying hens. Food and Chemical Toxicology, v. 23, n. 12, p. 1057-1061, 1985.