

Short communication. Total aflatoxin and ochratoxin A in the liver, kidneys and plasma of experimentally contaminated chickens

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Abstract

Mycotoxicosis, particularly that caused by aflatoxins and ochratoxins, is a serious problem for the poultry production industry. The aim of this study was to determine the total aflatoxin and ochratoxin A levels in liver and plasma, and kidneys and plasma, respectively, of chickens for fattening fed experimentally contaminated diets, and to assess the impact of these toxins on body weight increase and the feed conversion ratio (FCR). Forty eight 21 day-old Ross breeder chickens ($n = 12 \times$ four treatments) were fed diets containing different levels of mycotoxins: grower diet + ochratoxin A (200 mg kg^{-1} of feed) + total aflatoxin in different concentrations (T1 = $60 \text{ } \mu\text{g kg}^{-1}$ of feed; T = $50 \text{ } \mu\text{g kg}^{-1}$; T3 = $30 \text{ } \mu\text{g kg}^{-1}$). Control chickens (T4) were fed only the grower diet. No significant differences were seen in the weight increase of chickens subjected to the different treatments. However, lower FCRs were seen in those exposed to T1 and T2. In general, the amount of total aflatoxin and ochratoxin A found in the liver, kidneys and plasma (determined by ELISA) were directly related to the amounts added to the experimental diets and feed consumption. Plasma ochratoxin A was always higher than kidney levels; this relationship was not seen for total aflatoxin.

Additional key words: chickens for fattening, ELISA, micotoxicity.

Resumen

Comunicación corta. Aflatoxina y ochratoxina A totales en hígado, riñones y plasma de pollos contaminados experimentalmente

Las micotoxicosis, en particular las aflatoxicosis y ocratoxicosis, son un serio problema sanitario en la producción avícola. El objetivo de este trabajo fue determinar el nivel de aflatoxinas y ochratoxinas A en hígado y plasma, y riñones y plasma, respectivamente, de pollos alimentados con dietas experimentalmente contaminadas e investigar el impacto de estas toxinas en el peso corporal y el cociente de conversión del alimento (FCR). Se alimentaron 48 pollos Ross de 21 días de edad ($n = 12 \times$ cuatro tratamientos) con dietas conteniendo diferentes niveles de micotoxinas: una dieta de crecimiento + 200 mg kg^{-1} de ochratoxina A + aflatoxina total en diferentes concentraciones (T1 = $60 \text{ } \mu\text{g kg}^{-1}$; T2 = $50 \text{ } \mu\text{g kg}^{-1}$; T3 = $30 \text{ } \mu\text{g kg}^{-1}$). Lo pollos control (T4) fueron solamente alimentados con la dieta de engorde. No hubo diferencias significativas en el aumento de peso de los pollos sometidos a los diferentes tratamientos; sin embargo, se detectaron FCRs menores para los expuestos a T1 y T2. En general, la cantidad de aflatoxina total y ochratoxina A detectadas por ELISA en hígado, riñones y plasma fueron directamente proporcionales a los niveles de las micotoxinas añadidas a las dietas experimentales. La ochratoxina A en plasma fue siempre superior a la encontrada en riñón; esta relación no se detectó para la aflatoxina total.

Palabras clave adicionales: ELISA, micotoxicidad, pollos de engorde.

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The mycotoxins include a complex group of chemical substances that are toxic for animals (Schwerdt *et al.*, 2007). Foods with visible mould growth are rejected for human but not for animal consumption, leading to the possible intake of both toxigenic moulds and pre-synthesized mycotoxins (Quezada *et al.*, 2000).

Owing to the characteristics of mould growth, foodstuffs (mainly grains and animal feed) may commonly contain more than one mycotoxin. Synergistic action between them can enhance their toxic effects and alter the target organs affected (Farfan, 2000; Zinedine *et al.*, 2006).

Mycotoxicosis, particularly that caused by aflatoxins and ochratoxins, is a serious problem for the poultry production industry, negatively affecting many production variables [e.g. meat and egg production and the feed conversion ratio (FCR)] and causing immunosuppression leading to an increase in the incidence of infections (Quezada *et al.*, 2000). In industrial poultry production, chicken feed containing mycotoxins—even in small amounts—can cause a 10% reduction in chicken body weight (Abbas *et al.*, 2006).

Some of the greatest difficulties in detecting mycotoxins in animal feed include their heterogeneous distribution in the raw and finished products and the need to be able to detect low levels of these contaminants. In contrast, their direct detection and quantification in the birds themselves (in the plasma, liver, kidneys and muscles) confirms the existence of the problem and minimizes the errors involved in feed sampling and analysis (Furlan *et al.*, 2001).

The aim of this study was to determine the total aflatoxin and ochratoxin A levels in liver and plasma, and kidneys and plasma, respectively, of chickens for fattening fed for a short time with experimentally contaminated diets, and to assess the impact of these treatments on weight increase and FCR.

Forty eight 21 day-old male Ross breeder chickens were obtained from a commercial hatchery. Birds were chosen that showed a good size, weight (average 778 g, SE \pm 13.74) and which looked generally healthy. All had been housed in an electrically heated ($28 \pm 2^\circ\text{C}$) compartment with continuous lightning and fed a commercial starter feed until 21 days old. The starter and grower diets provided as part of the experimental diets contained nutrient levels (mineral, vitamins, proteins, fat, MJ ME kg^{-1}) according to the recommen-

dations of Rostagno *et al.* (2005). The starter diet was subjected to ELISA analysis for residual total aflatoxin and ochratoxin A before being provided.

The birds were divided at random into four treatment groups (n = 12 each) that would receive different levels of dietary mycotoxins, the concentrations of which reflected those most commonly detected in corn grains and animal feed according to the *Divisão de Toxicologia, Laboratório Central de Controle de Qualidade e Toxicologia (LACCQSA), Delegacia Federal de Agricultura de Minas Gerais, Minas Gerais, Brazil* (unpublished data): T1) grower diet + total aflatoxin at $60 \mu\text{g kg}^{-1}$ of feed + ochratoxin A at 200mg kg^{-1} of feed; T2) grower diet + total aflatoxin at $50 \mu\text{g kg}^{-1}$ of feed + ochratoxin A at 200mg kg^{-1} of feed; T3) grower diet + total aflatoxin at $30 \mu\text{g kg}^{-1}$ of feed + ochratoxin A at 200mg kg^{-1} of feed; and T4) control; grower diet alone. Total aflatoxin was applied in the proportion 7 to 1.89 to 1.12 to 0.28 for aflatoxin B₁, B₂, G₁ and G₂ respectively. Total aflatoxin and ochratoxin A (Sigma Chem. Co., Saint Louis, MO, USA) were obtained from LACCQSA. *Ad libitum* access to feed and water was allowed.

Feed intake and body weight were recorded at pre-established intervals (3, 6 and 9 days). Weight increase (g) and the FCR were calculated at the end of the treatments (i.e., after 9 days of exposure) following the method of Figueiredo *et al.* (2006). The mycotoxin levels in the plasma, liver and kidneys were also determined after 3, 6 and 9 days of treatment. Four chickens from each group were slaughtered on each test date by cervical dislocation following the taking of blood samples by heart puncture (7 mL). One mL of blood was dissolved in 5 mL of 70% (v/v) methanol and homogenized for 30 s using a vortex before centrifuging at $2,700 \text{g}$ for 15 min. The supernatant was collected and a portion subjected to mycotoxin analysis (Hirano *et al.*, 1991; Sarimehmetoglu *et al.*, 2004). Liver and kidney samples (1 g) were aseptically macerated, added to 5 mL of 70% (v/v) methanol, homogenized in stomacher for 2 min, and also centrifuged at $2,700 \text{g}$ for 15 min. As above, the supernatant was collected and a portion subjected to mycotoxin analysis.

Total aflatoxin and ochratoxin A in the starter diet, plasma, liver and kidneys were determined by ELISA (Ridascreen Fast, R-biopharm, Germany). This test works best when total aflatoxin in the analysed material

is in the range 1.7-45 $\mu\text{g kg}^{-1}$ and ochratoxin A is in the range 5.0-40.0 mg kg^{-1} .

A sufficient number of microtitre wells for all standards and samples were inserted into a microwell holder. Standard solutions (100 μL) and prepared samples were added to separate wells and incubated for 60 and 120 min at room temperature in the dark for aflatoxins and ochratoxin A respectively. The liquid was poured off the wells and the microwell holder tapped vigorously three times against absorbent paper while upside down to ensure the complete removal of all liquid from the wells. All the wells were washed by filling them with 250 μL of distilled water and emptied as above. Monoclonal antibody conjugate (100 μL) was then added and incubated for 60 and 120 min at room temperature in the dark for total aflatoxins and ochratoxin A respectively. The washing sequence was then repeated three times. Substrate (50 μL) and chromogen (50 μL) were added to each well and mixed thoroughly and incubated for 30 and 15 min at room temperature for aflatoxins and ochratoxin A respectively. Stop reagent (50 μL) was then added to each well, mixed, and the absorbance measured at 450 nm.

The mean absorbance values of the standards and samples were analysed using the RI.DAVIN.EXE computer program (R-Biopharm). Data obtained for the different treatments were subjected to ANOVA (in complete randomised blocks), followed by a multiple

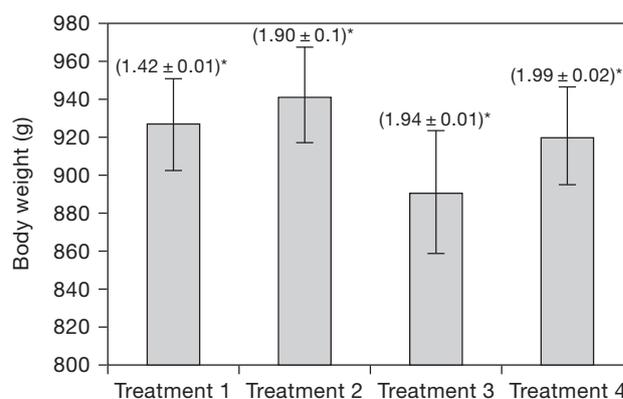


Figure 1. Increase in body weight and food conversion ratio (in parenthesis)* in experimentally contaminated chickens for fattening after 9 days of treatment.

comparison Tukey test. Significance was set at $p < 0.05$. All calculations were made using the Sigma Stat 3.1 program.

Figure 1 shows the increase in body weight and FCR in the experimentally contaminated chickens after 9 days of treatment. No significant differences ($p < 0.05$) were seen in the weight increase of the chickens subjected to any of the treatments (although somewhat greater weight increases were seen in the T1 and T2 birds). Lower FCR values were recorded for the T1 and T2 chickens, the FCR of the T1 chickens being significantly different to that of all other groups ($p < 0.05$).

Table 1. Levels of total aflatoxin (B_1 , B_2 , G_1 and G) in the liver and plasma, and of ochratoxin A in the kidneys and plasma of experimentally contaminated chickens for fattening after 3, 5 and 9 days of treatment

Treatment	Total aflatoxin ($\mu\text{g kg}^{-1}$)					
	Liver			Plasma		
	3 d	6 d	9 d	3 d	6 d	9 d
T1	2.8 ^a	2.6 ^b	3.8 ^c	2.0 ^a	1.8 ^a	1.6 ^a
T2	2.5 ^a	2.3 ^b	2.0 ^b	1.6 ^{ab}	1.6 ^a	1.4 ^{ab}
T3	1.7 ^b	1.7 ^a	1.6 ^b	1.5 ^{ab}	1.5 ^a	1.4 ^{ab}
T4	1.5 ^b	1.2 ^a	1.4 ^b	1.2 ^{bc}	1.5 ^a	1.2 ^{bc}

Treatment	Ochratoxin (mg kg^{-1})					
	Kidney			Plasma		
	3 d	6 d	9 d	3 d	6 d	9 d
T1	4.0 ^b	11.0 ^a	13.6 ^a	31.8 ^a	19.2 ^b	33.0 ^a
T2	2.5 ^c	10.9 ^a	12.6 ^b	27.6 ^{ab}	17.3 ^b	19.7 ^b
T3	3.0 ^c	11.5 ^a	12.5 ^b	27.1 ^{ab}	16.9 ^b	19.3 ^b
T4	2.8 ^c	11.2 ^a	11.8 ^b	23.4 ^{bc}	15.2 ^c	14.3 ^c

Values followed by the same letter in the same column are not significantly different ($p < 0.05$) according to the Tukey test.

Quezada *et al.* (2000), who studied the weight increase and FCR in chickens for fattening fed a diet with 2.0 µg kg⁻¹ of aflatoxin B₁, noted no significant difference ($p < 0.05$) after 28 days of treatment compared to time zero. Similar findings were reported by Čelýk *et al.* (2003) in birds fed a diet with 200 µg kg⁻¹ of aflatoxin B₁ for 37 days.

Table 1 shows the levels of total aflatoxin in the liver and plasma, and of ochratoxin A in the kidneys and plasma, of the birds in the different treatment groups (mean coefficient of variation 20%). No significant differences were seen between the liver total aflatoxin levels of the T1 and T2 chickens until day 6. No differences were ever recorded for this variable between the T2 and T4 birds. After 9 days the highest total aflatoxin concentration (3.8 µg kg⁻¹) was seen in the T1 birds. Liver total aflatoxin levels were related to the amount added to the experimental diet. Determining the level of mycotoxins in the liver is a useful way of predicting the exposure of birds to mouldy feed; it also suggests that residues will be found in other tissues and organs (Quezada *et al.*, 2000).

The level of ochratoxin A in the kidneys of the birds was also directly related to the amount of mycotoxin added to the diet (Table 1). No significant differences were seen among the levels of ochratoxin A in the kidneys in the T2, T3 and T4 birds at any time point.

The levels of total aflatoxin and ochratoxin A in the liver and kidneys respectively of the T4 chickens gave testimony to a slight natural contamination before beginning the experimental treatments. Levels of total aflatoxin and ochratoxin A in the starter feed offered to the chickens in the pre-experimental interval showed values of 0.9 µg kg⁻¹ and 9.6 mg kg⁻¹ for total aflatoxin and ochratoxin A respectively (data not shown).

Table 1 shows the mean plasma total aflatoxin and ochratoxin A levels in the chickens of each treatment group. No significant difference were seen among the levels of plasma total aflatoxin in the T1, T2 and T3 chickens at any time point. No significant difference was seen in the plasma ochratoxin A levels in any treatment group after 6 days.

In all treatments and at all time points, the amount of ochratoxin A detected in plasma was always higher than that found in the kidneys. Ochratoxin A is widely distributed in birds, however it mainly pools in the kidneys; small amounts are found in the liver and muscles (Merquardt and Frohlich, 1992).

The present results suggest that a program to control the negative impact of mycotoxins on the poultry

industry might include assessing mycotoxin levels in bird tissues.

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