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SUPPLEMENT ARTICLE

Feeding a diet contaminated with ochratoxin A for broiler chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 2. Effects on meat quality, oxidative stress, residues and histological traits

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Summary

The European Commission Recommendation 2006/576/EC indicates that the maximum tolerable level of ochratoxin A (OTA) in poultry feeds is 0.1 mg OTA/kg. Thirty-six 1-day-old male broiler chicks were divided into two groups, a control (basal diet) and an OTA (basal diet + 0.1 mg OTA/kg) group. The OTA concentration was quantified in serum, liver, kidney, breast and thigh samples. The thiobarbituric acid reactive substances (TBARS) content were evaluated in the liver, kidney, breast and thigh samples. The glutathione (GSH) content, and catalase (CAT) and superoxide dismutase (SOD) activity were measured in the liver and kidney samples. Histopathological traits were evaluated for the spleen, bursa of Fabricius and liver samples. Moreover, the chemical composition of the meat was analysed in breast and thigh samples. In the OTA diet-fed animals, a serum OTA concentration of 1.15 ± 0.35 ng/ml was found, and OTA was also detected in kidney and liver at 3.58 ± 0.85 ng OTA/g f.w. and 1.92 ± 0.21 ng OTA/g f.w., respectively. The TBARS content was higher in the kidney of the ochratoxin A group (1.53 ± 0.18 nmol/mg protein vs. 0.91 ± 0.25 nmol/mg protein). Feeding OTA at 0.1 mg OTA/kg also resulted in degenerative lesions in the spleen, bursa of Fabricius and liver. The maximum tolerable level of 0.1 mg OTA/kg, established for poultry feeds by the EU, represents a safe limit for the final consumer, because no OTA residues were found in breast and thigh meat. Even though no clinical signs were noticed in the birds fed the OTA-contaminated diet, moderate histological lesions were observed in the liver, spleen and bursa of Fabricius.

Keywords broiler chicken, ochratoxin A, residues, lipid peroxidation, oxidative stress, histological lesions

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Introduction

Ochratoxin A (OTA) is one of the most commonly occurring mycotoxins and is produced by several species of *Aspergillus* (Van der Merwe et al., 1965) and *Penicillium* (Van Walbeek et al., 1969).

Ochratoxin A is a natural contaminant of animal feedstuffs (Binder et al., 2007; Schiavone et al., 2008; Pozzo et al., 2010), and documented field outbreaks of ochratoxicosis, as well as experimental feeding trials with OTA-contaminated feeds, have indicated its detrimental effects on chicks (Santin et al., 2002; Stoev et al., 2002; Elaroussi et al., 2008; Hanif et al., 2008) and its potential risk for the poultry industry (Zaghini et al., 2007; Biró et al., 2002). Due to its affinity with the ATP-dependent transporters, localized in the luminal membranes of the intestines, liver and kidney (Schrickx et al., 2006), and to its long half-life of 850 h in humans (Studer-Rohr et al., 2000), 72–120 h in swine and 4.1 h in chickens (Galtier et al., 1981), OTA has been detected in human milk (Breitholtz-Emanuelsson et al., 1993; Jonsyn et al., 1995) and swine (Aoudia et al., 2009; Milićević et al., 2009) and poultry tissues (Biró et al., 2002; Denli et al., 2008; Zaghini et al., 2007). OTA has been demonstrated to be immunosuppressive (Creppy et al., 1983), genotoxic (Wei and Sulik, 1993) and nephrotoxic (Purchase and Theron, 1968) and has been

declared a possible human carcinogen by the IARC classification in group 2B (IARC, 1993). OTA is also pro-oxidant, which may lead to an increase in the oxidative damage that contributes to its toxicity (Omar et al., 1990; Baudrimont et al., 1994).

The climate in southern Europe, with its warm temperatures and high humidity associated with wrong practices of grain storage, favours OTA synthesis (Pardo et al., 2004; Van Egmond et al., 2007) by *Aspergillus verrucosum*, while *Penicillium verrucosum* is the dominant OTA-producing contaminant of food and feeds in temperate regions, such as northern Europe (Frisvad et al., 1999; Larsen et al., 2001).

The European Commission has recently issued a recommendation that sets guidelines regarding mycotoxin contamination for poultry feeds. A maximum tolerable level of 0.1 mg OTA/kg has been established, considering a feed moisture content of 120 g/kg (EC, 2006a).

The aim of this study was to evaluate the effect of feeding a diet contaminated with 0.1 mg OTA/kg on OTA tissue residues, meat chemical composition, oxidative stress and tissue histology of broiler chickens.

Materials and methods

Birds, diets and sample collection

Thirty-six 1-day-old male Hubbard broiler chicks were randomly divided into two groups (three replicates per group) and reared from day 1 to day 35.

The two groups received two different diets, and three replicates per diet were used: the control group received the basal diet (a soybean meal, wheat- and corn-based diet; first period: metabolizable energy: 13.1 MJ/kg and crude protein = 230.5 g/kg; second period: metabolizable energy = 13.5 MJ/kg and crude protein = 195.0 g/kg), while the ochratoxin A group received an OTA-contaminated diet (0.1 mg OTA/kg added to the basal diet). The contamination of feed and the results of homogeneity test are described in Part 1 of the present study (Pozzo et al., 2013). Control diet was naturally contaminated at a level of 0.27 ± 0.09 μg OTA/kg. At the end of the experiment, blood samples were collected (5 ml) from the femoral vein and centrifuged for 15 min at 3000 *g* for OTA determination in serum samples ($n = 6$). The serum was removed and stored at −20°C until use. At 35 days of age, nine chickens per diet were sacrificed by CO_2 gassing followed by neck-cutting, bled and then dissected. Thigh and breast meat samples were kept at −20°C for chemical composition analysis ($n = 6$) and thiobarbituric acid reactive substances (TBARS) evaluation ($n = 6$). The liver and kidney samples were kept at −80°C for OTA quantification ($n = 6$). Liver and kidney aliquots were homogenized in 5 volumes of a 0.1 M potassium phosphate buffer (pH 7.4) and stored at −80°C until the assay for thiobarbituric acid reactive substances (TBARS), glutathione (GSH) contents and catalase (CAT) and superoxide dismutase (SOD) activity ($n = 6$). Material was taken from the spleen, bursa of Fabricius and liver for the histopathological investigations and fixed in 0.05 M neutral buffered formalin ($n = 6$).

Meat traits: chemical composition and lipid peroxidation

Dry matter (method no. 934.01 and 950.46), crude protein (988.05 and 928.08), ether extract (920.39 and 991.36) and ash (942.05 and 920.153) quantification were performed for the breast and thigh meat according to AOAC guidelines (AOAC, 2000).

Susceptibility to lipid oxidation of the breast and thigh muscle, by means of thiobarbituric acid reactive substances (TBARS) evaluation, was measured according to the iron-induced procedure described by Huang and Miller (1993). The iron-induced TBARS assay was performed at 0, 15, 30, 60 min of incubation with $FeSO_4{\cdot}7H_2O$ (final concentration, 1 mM Fe^{+3}) as the oxidative agent and absorbance was read at 532 nm. Liquid malonaldehyde bis (diethyl acetal) (MDA) (Aldrich Chemical, Dorset, England) was used as the standard to determine the linear standard response and recovery. The TBARS values were calculated by multiplying the absorbance by a constant coefficient K (23.58) combining standard response, recovery (93.4%), molecular weight of the MDA and sample weight. The TBARS values are expressed as mg MDA/kg fresh meat.

Oxidative stress assays in the liver and kidney

TBARS were measured in the liver and kidney by means of the Ohkawa et al. (1978) method. Fluorimetric absorbance was assessed at 515 nm for λ_{ex} and at 553 nm for λ_{em} and compared with those obtained from MDA standards. The results were expressed as nmol MDA/mg of protein.

Total GSH content in the kidney and liver samples was determined with the method of Mitchell et al. (1973). Absorbance was recorded at 412 nm. The results were expressed as nmol GSH per mg of protein.

Catalase activity was determined according to the method described by Goth (1991). Yellow complex of molybdate and the absorbance of H_2O_2 were measured at 405 nm in the spectrophotometer against a blank. CAT activity was expressed as U/mg protein.

The SOD activity assay involves the inhibition of nitroblue tetrazolium (NBT) reduction, with xanthine –xanthine oxidase used as a superoxide generator (Sun et al., 1988). Formazan production was determined at 560 nm. The SOD activity was expressed as % of inhibition with respect to the activity control. The% inhibition of the rate of increase in absorbance was calculated as follows: % Inhibition = (Slope of Activity Control – Slope of Activity Sample) × 100. One unit of SOD is defined as the amount of protein that inhibited the NBT reduction rate by 50%.

The total protein in tissue homogenates of the kidney and liver was determined by means of the Bradford standard protein assay using bovine serum albumin as the standard (1976).

Histological investigations

Liver, bursa of Fabricius and spleen samples were collected for histological examination and fixed in 10% neutral buffered formalin ($n = 6$). The tissues were routinely embedded in paraffin wax blocks, sectioned at a thickness of 5 μm and stained with haematoxylin and eosin (H-E). Additional liver and bursa of Fabricius sections were submitted to periodic acid-Schiff (PAS) staining to evaluate the presence of glycogen.

The liver samples were evaluated by three qualified different operators who adopted the following score: 1, slight vacuolar degeneration with small vacuoles; 2, moderate vacuolar degeneration; 3, widespread vacuolar degeneration; 4, severe widespread vacuolar degeneration; and 5, hydropic degeneration with foci of necrosis.

The mean numbers of apoptotic cells were counted in 10 follicles (40X) for the spleen samples.

Analysis of ochratoxin A

Ochratoxin A extraction from serum and from kidney and liver samples was carried out according to the methods developed by Pozzo et al. (2010) and Vettorazzi et al. (2008), respectively.

Ochratoxin A concentrations were quantified in samples according to Visconti et al.'s (2000) analysis method. The HPLC apparatus consisted of a Dionex P680 pump (Dionex, Sunnyvale, CA, USA) equipped with a Rheodyne Model 7725 injection valve (Rheodyne, Rohnert Park, CA, USA), a Dionex RF-2000 fluorimetric detector ($\lambda_{ex} = 333$, $\lambda_{em} = 460$), a Dionex thermostatted column compartment TCC-100, a Dionex ASI100 autosampler series (Dionex) and a Chromeleon®6 data handling system (Dionex). A Luna C18(2) analytical column was used (150 × 4. 6 mm, 5 μm particles) (Phenomenex, Torrance, CA, USA), preceded by an Analytical Guard Cartridge System (Phenomenex).

The OTA concentration in the serum is reported as ng/ml. The OTA concentration in the tissues is reported as ng/g of fresh weight (f.w.).

Validation study

Recovery experiments were performed by spiking blank liver, kidney, breast and thigh samples with OTA at the concentration of 7.5 ng/g. The spiked samples were then extracted according to the previous mentioned protocol, and the OTA recovery value (%) was calculated in all the matrices by dividing the experimental OTA concentration by the expected theoretical OTA level, assuming an OTA recovery rate of 100%.

The calculated OTA recovery rate was 86.1% for the liver, 81.5% for the kidney, 73.5% for the breast and 78.3% for the thigh (Table 1).

The calculated limit of detection (LOD) (*s/n* 3/1) for the feed was 0.1 ng/g and the limit of quantification (LOQ) (*s/n* 10/1) was 1 ng/g. The LOD for the serum was 0.03 ng/g and the LOQ was 0.1 ng/g.

The calculated LOD (*s/n* 3/1) for the liver was 1.5 ng/g and the LOQ (*s/n* 10/1) was 5 ng/g. The LOD for the kidney, breast and thigh was 2 ng/g and the LOQ was 6 ng/g.

A calibration curve was obtained using the linear least-squares regression procedure of absorbance versus concentration. OTA linearity, in the working standard solutions at two determinations of six concentration levels of between 0.5 and 10 ng/ml, was excellent, as shown by the correlation coefficient ($r^2 = 0.999$).

Statistical analysis

The statistical analyses were performed with SPSS 17 for Windows (SPSS, Chicago, IL, USA). Before testing for group differences, the normality of the

Table 1 Recoveries from the method used to determine ochratoxin A in the tissues

Source	Spiking level (ng/g)	Recovery ± SD (%)*	RSD (%)†
Liver	7.5	86.1 ± 3.3	4.0
Kidney	7.5	81.5 ± 4.5	5.2
Breast	7.5	73.5 ± 1.7	2.3
Thigh	7.5	78.3 ± 4.5	5.7

*SD = standard deviation ($n = 4$ replicates);
†RSD = relative standard deviation.

data distribution was assessed in the two groups (control group and ochratoxin A group) using the Shapiro–Wilk test. The results are presented as the mean value ± standard deviation (SD). The homogeneity of variance assumption was assessed by means of Levene's test. All the obtained data were statistically analysed with an independent sample *t*-test (control group vs. ochratoxin A group).

The results were considered statistically significant when associated with a lower probability than 5%. Results were considered highly significant at 1%.

Results

Meat traits: chemical composition and lipid peroxidation

Table 2 shows the chemical composition and the TBARS content (mg MDA/kg fresh meat) of the breast and thigh. No statistically significant differences between groups were found for the dry matter, crude protein, ether extract and ash content of breast; however, the ether extract of breast and thigh of OTA-treated chickens tended to be numerically lower than the control group ($p < 0.10$).

As far as the TBARS content produced during the induced lipid peroxidation in the breast and thigh is concerned, no statistically significant differences were found between groups at different measuring times, except for the breast TBARS content at the beginning of the reaction, when the TBARS content was higher in the control group ($p < 0.05$). The mean TBARS concentration in the breast at T_0 was 0.52 mg MDA/kg fresh meat in the control group and 0.21 mg MDA/kg fresh meat in the ochratoxin A group (Table 2).

Oxidative stress assays in the liver and kidney

Table 3 shows the TBARS and GSH concentrations and CAT and SOD activities in the liver and kidney. Feeding the OTA-contaminated diet did not affect TBARS and GSH content or CAT and SOD activities in the liver and kidney, except for the TBARS concentration in the kidney, which was significantly higher in the ochratoxin A group ($p < 0.01$). TBARS kidney concentration was 1.53 nmol/mg protein for the ochratoxin A group and 0.91 nmol/mg protein for the control group.

Histological investigations

Spleen samples of chickens fed with OTA showed an increase in apoptosis in the follicles (Fig. 1a,b). The number of apoptotic cells was significantly higher in samples from the ochratoxin A group than the control group ($p < 0.01$), with a mean value of 5.2 ± 2.4 and 1.7 ± 0.6 apoptotic cells/follicle respectively. Intra/interfollicular cysts of different sizes (Fig. 1c,d) containing PAS-positive material were found in the bursa of Fabricius samples of the OTA-treated animals.

Five of six liver samples from animals in the ochratoxin A group showed degenerative lesions; of these, three showed vacuolar degeneration with small vacuoles and the other two hydropic degeneration with foci of necrosis (Fig. 1e,f). The PAS stain was negative in all the samples.

Three of the six liver samples from the control group showed minimal degenerative lesions, but none of them showed hydropic degeneration.

Table 2 Chemical composition and TBARS* of the breast and thigh meat of broiler chickens (mean ± SD)

	Breast			Thigh		
	Control	Ochratoxin A†	p-value	Control	Ochratoxin A†	p-value
Chemical composition						
Dry matter (%)	26.1 ± 3.5	25.8 ± 0.4	NS	26.0 ± 1.2	25.1 ± 1.1	NS
Crude protein (%)	23.2 ± 3.3	23.0 ± 0.67	NS	18.4 ± 0.5	18.5 ± 0.8	NS
Ether extract (%)	0.4 ± 0.1	0.3 ± 0.1	NS	1.5 ± 0.4	1.2 ± 0.2	NS
Ash (%)	0.3 ± 0.1	0.3 ± 0.0	NS	1.3 ± 0.0	1.3 ± 0.0	NS
Iron-induced TBARS (mg MDA‡/kg fresh meat)*						
0 min	0.5 ± 0.2	0.2 ± 0.2	<0.05	1.3 ± 0.3	1.0 ± 0.4	NS
15 min	0.7 ± 0.1	0.7 ± 0.2	NS	1.7 ± 0.6	1.5 ± 0.4	NS
30 min	1.1 ± 0.5	0.7 ± 0.1	NS	2.2 ± 0.7	1.8 ± 0.6	NS
60 min	0.7 ± 0.2	0.7 ± 0.1	NS	2.2 ± 0.8	1.5 ± 0.5	NS

NS = p > 0.05;
*thiobarbituric acid reactive substances;
†0.1 mg OTA/kg basal diet;
‡malonaldehyde.

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Table 3 Oxidative stress in the liver and kidney of broiler chickens (mean ± SD)

	Liver			Kidney		
	Control	Ochratoxin A*	p-value	Control	Ochratoxin A*	p-value
Oxidative stress						
TBARS (nmol MDA/mg protein)†‡	0.2 ± 0.0	0.2 ± 0.1	NS	0.9 ± 0.3	1.5 ± 0.2	<0.01
GSH (nmol/mg protein) §	2.2 ± 0.7	2.3 ± 0.9	NS	0.5 ± 0.1	0.5 ± 0.1	NS
CAT (U/mg protein)¶	540 ± 118	502 ± 110	NS	545 ± 60.7	560 ± 27.3	NS
SOD (U/mg protein)**	21.1 ± 2.0	20.4 ± 6.1	NS	22.6 ± 3.5	25.6 ± 3.2	NS

NS = p > 0.05;
*0.1 mg OTA/kg basal diet;
†thiobarbituric acid reactive substances;
‡malonaldehyde;
§lutathione;
¶catalase;
**superoxide dismutase.

Ochratoxin A in the serum and tissue samples

The OTA serum concentration was higher in the ochratoxin A group, with a mean value of 1.15 ng/ml in the ochratoxin A group and 0.16 ng/ml in the control group. The liver and kidney of the control group did not show any OTA residues. The OTA concentration in liver and kidney samples from the ochratoxin A group was 1.92 and 3.58 ng OTA/g fresh weight respectively. OTA residues were not found in the breast and thigh samples in either group (Table 4).

Discussion

The European Commission Recommendation 2006/576/EC sets the maximum tolerable level for OTA contamination in poultry feeds at 0.1 mg OTA/kg feed (European Commission, 2006a). A number of studies have been conducted to assess the level of OTA residues in tissues, the effect on oxidative stress and the occurrence of histopathological lesions when animals are fed an OTA-contaminated diet (Hoehler and Marquardt, 1996; Hoehler et al., 1997; Biró et al., 2002; Bozzo et al., 2008; Elaroussi et al., 2008). However, most of the studies were carried out assaying diets contaminated with OTA at levels higher than the limit set by the EU for poultry feeds (European Commission, 2006a) and also higher than the levels of contamination by OTA commonly found in farm conditions (Beg et al., 2006; Schiavone et al., 2008; Yildiz, 2009). The aim of the present study was to evaluate whether feeding broiler chickens with a diet contaminated with 0.1 mg OTA/kg may affect the quality and safety of edible tissues and the level of oxidative stress in the animals.

The results indicate that an OTA-contaminated diet at the maximum OTA level allowed by the EC fed to broiler chickens did not affect the chemical composition of breast or thigh meat. However, the ether extract of breast and thigh of OTA-treated chickens tended to be numerically lower than the control group, probably because chickens of the OTA group grew up slowly and collected a minor quantity of fat content, which is the last tissue to develop (Hossner, 2005).

Ochratoxin A is known to cause oxidative damage in tissues, to stimulate lipid peroxidation and to change the activity of antioxidant enzymes and GSH tissue concentrations (Omar et al., 1990; Baudrimont et al., 1994). During the present study, feeding a diet contaminated with 0.1 mg OTA/kg did not affect the lipid peroxidation or oxidative stress parameter concentrations in broiler chicks. These results agree with literature data, which reports no effects of an OTA dietary treatment (2.5 mg OTA/kg) on MDA concentrations in chicken breast muscle (Hoehler et al., 1997). The only effects of feeding OTA on oxidative stress levels were lower breast TBARS concentration in the ochratoxin A group at the beginning of the reaction and higher kidney TBARS content in the OTA fed animals. The lower TBARS values from breast tissues in OTA fed birds could be related to the numerically lower ether extract content found in the breast of the ochratoxin A group. The higher level of kidney TBARS content found in the OTA fed animals could be explained by the evidence that the kidney is the target organ in which OTA accumulates (Aoudia et al., 2009; Milićević et al., 2009). OTA also accumulated in liver samples of OTA-treated chickens, even at lower OTA concentrations, but, in our experiment, this did not lead to lipid peroxidation. Previous studies

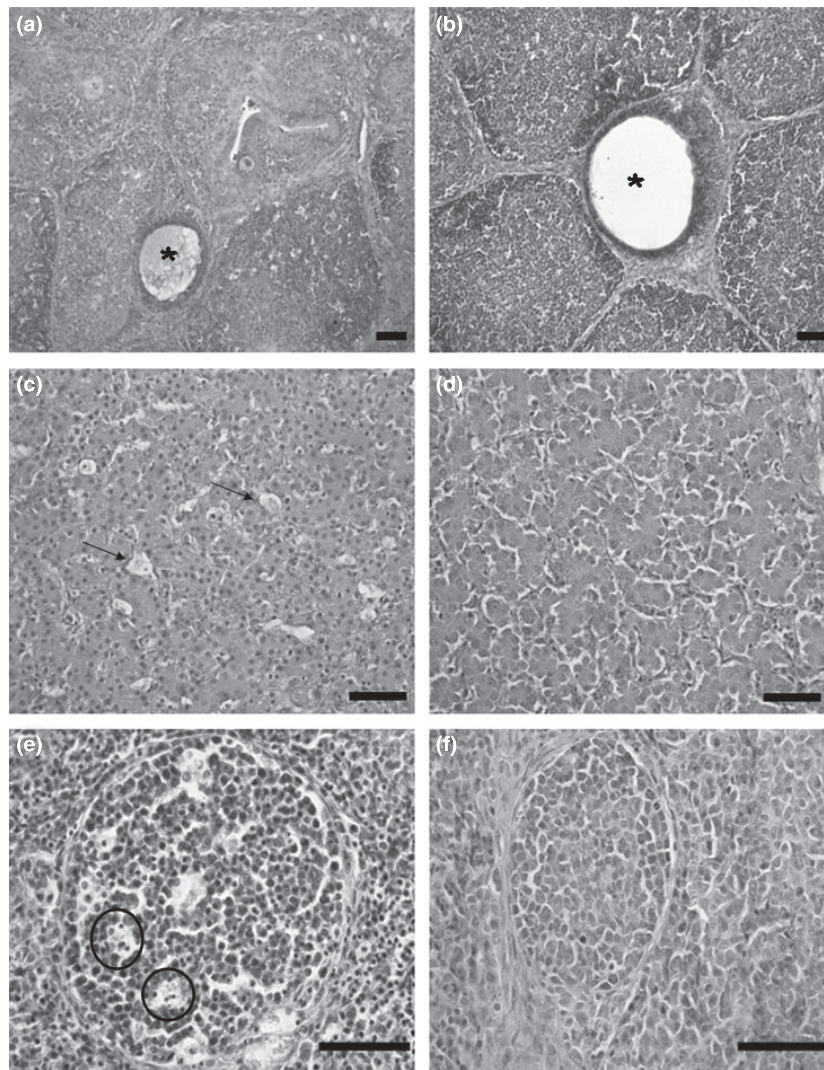

Fig. 1 Histological investigations. (a) Spleen sample of the OTA group showing follicles with several apoptotic cells. (b) Spleen sample of the control group with a normal follicle. (c) Bursa of Fabricius sample of the OTA group with intra/interfollicular cysts containing PAS-positive material. (d) Bursa of Fabricius sample of the OTA group with an intrafollicular cyst. (e) Liver sample of the OTA group with vacuolar degeneration. (f) Liver sample of the control group. (a,b,d–f) Haematoxylin and eosin; (c) PAS staining. Bars (a–f) 50 μm.

reported an increase in liver MDA content in OTA-treated animals, when a diet contaminated at 2.5 mg OTA/kg was fed to broiler chickens (Hoehler and Marquardt, 1996; Hoehler et al., 1997). Therefore, adding 0.1 mg OTA/kg to the diet of broiler chicks did not alter the antioxidant response in liver and kidney and did not cause lipid peroxidation in liver, but it did induce lipid peroxidation in the kidney of OTA-treated chicks.

Histological investigations showed an increase in apoptosis in the follicles of the spleen, intra/interfollicular cysts of different sizes containing PAS-positive material in the bursa of Fabricius and vacuolar degeneration in the liver of the OTA-treated chicks. These results suggest that the maximum level of OTA set by the European Recommendation for poultry feed did not completely prevent histopathological lesions in the immune organs of broiler chicks. These results agree with the data obtained in the first part of the study (Pozzo et al., 2013) that, for OTA-treated chicks, reported a decrease in thymus weight and in total protein, albumin, alpha, beta and gamma globulin serum concentration. However, Kozaczynski showed that long-term (20 week) administration of 0.2 mg OTA/kg in feed, at twice the concentration used in the present study, caused

Table 4 OTA in the serum and tissues of broiler chickens (mean ± SD)

	Control	Ochratoxin A*	p-value
Serum (ng OTA/ml)	0.2 ± 0.1	1.2 ± 0.4	<0.001
Liver (ng OTA/g f.w.)	ND†	1.9 ± 0.2	-
Kidney (ng OTA/kg f.w.)	ND†	3.6 ± 0.9	-
Breast (ng OTA/g f.w.)	ND‡	ND‡	-
Thigh (ng OTA/g f.w.)	ND‡	ND‡	-

*0.1 mg OTA/kg basal diet;
†not detected – OTA ≤ LOD (=1.5 ng OTA/g);
‡not detected – OTA ≤ LOD (=2 ng OTA/g).

no histopathological lesions (Kozaczynski, 1994). During a study by Bozzo et al. (2008), two feeds sampled in two laying hen farms contaminated with 0.255 and 0.285 mg OTA/kg caused not only vacuolar degeneration, as in the present study, but also necrotic cells with lipid infiltration. Biró et al. (2002) fed a diet contaminated with 0.354 mg OTA/kg to broiler chickens and observed discrete focal fibrosis in the parenchyma around the bile ducts, a modest epithelial proliferation of biliary caniculi in the liver and lymphocyte depletion and increased numbers of reticulocytes in the spleen. Elaroussi et al. (2008) showed that two diets contaminated with 0.4 and 0.8 mg OTA/kg caused marked degenerative changes in the bursa of Fabricius and mononuclear cell infiltration in the liver, which were more evident for the higher OTA level and with prolongation of the experimental period. Hanif et al. (2008) fed two diets contaminated with 0.5 and 1 mg OTA/kg for 42 days and observed areas of necrosis and vacuolar degeneration in the liver and necrosis of most parts of the follicular cells, connective tissue proliferation around the follicles and atrophy of some follicles in the bursa of Fabricius of animals fed the diet contaminated with 1 mg OTA/kg. Santin et al. (2002), feeding a diet contaminated with 2 mg OTA/kg to broiler chickens, observed vacuolar degeneration and megalocytosis of the hepatocytes with accompanying hyperplasia of the biliary epithelium in the liver and lower numbers of mitotic cells and lymphofollicular depletion in the bursa of Fabricius. Dwivedi and Burns (1984), feeding a diet contaminated with 2 and 4 mg OTA/kg for 20 days to broiler chickens, found vacuolar degeneration, as in the present study, glycogen accumulation in the hepatocytes and a reduction in lymphocytes in the immune system organs.

To assess the OTA level in the breast, thigh, liver and spleen, the analytical methods used in the present study were first validated for these tissues, because some of them had been developed for different tissues or for different animal species. The methods chosen were not modified and proved to be appropriate for the analysis of OTA in breast, thigh, liver and spleen samples, as shown by the recovery levels found in the validation study. The overall recoveries found for OTA fulfilled the EU performance criteria concerning the official control of the level of mycotoxins in foodstuffs (EC, 2006b).

In the present study, OTA residues were found in the kidney, liver and serum, but not in the breast or thigh samples of chickens fed a diet contaminated with 0.1 mg OTA/kg. The OTA concentrations were 3.58 ng OTA/g f.w. in the kidney, 1.92 ng OTA/g f.w. in the liver and 1.15 ng OTA/ml in the serum respectively. These results are very similar to the findings of some other authors (Prior et al., 1980; Zaghini et al., 2007; Bozzo et al., 2008; Denli et al., 2008). A similar OTA distribution was shown by Zaghini et al. (2007) during a study carried out on laying hens. Unlike the present study, Zaghini et al. (2007) found OTA residues in meat. Different results were also reported by Kozaczynski (1994), who showed that feeding a diet contaminated with 0.2 mg OTA/kg, that is, twice the concentration added in the present study, for 20 weeks, did not cause OTA accumulation in the kidney, liver, breast or thigh of broiler chicken. OTA accumulation in chicken tissues is due to its long half-life of 4.1 h (Galtier et al., 1981). Moreover, OTA is a substrate for ATP-dependent transporters, which are localized in the canalicular membranes of the liver and that makes another of the major cause of OTA liver accumulation (Schrickx et al., 2006). As far as kidney is concerned, it is thought that the excretion of OTA into the urine is mainly by tubular secretion, presumably via the organic anion transporters (OAT) system, and that this may play an important role in OTA accumulation and in the development of nephrotoxicity (Anzai et al., 2010).

Biró et al. (2002) fed a diet contaminated with 354 ng OTA/g to broiler chickens and the mycotoxin was distributed in the following manner: liver > kidney > plasma > muscle.

The accumulation of OTA residues in tissues, especially in the liver and kidney, is probably a result of the OTA elimination route via the kidney and partly via the liver, which exerts a direct toxic effect in these organs (Petzinger and Ziegler, 2000; Ringot et al., 2006). While these OTA concentrations are not a threat to humans, in some cases the liver may be used in recipes for local dishes and the kidney may remain in the chicken carcass and enter into the pet food production line.

Conclusion

The OTA dietary treatment did not alter the antioxidant response in liver and kidney and did not cause lipid peroxidation in liver, but it induced lipid peroxidation in kidney. Although the consumption of OTA-contaminated feed at 0.1 mg/kg did not cause clinical signs in the birds, moderate histological lesions were observed in immune organs.

In conclusion, our research shows that the maximum acceptable level of OTA contamination in poultry feed set by European Recommendation 2006/576/EC (0.1 mg OTA/kg) is a safe limit for the final consumer, because no OTA residues were found in the breast and thigh meat. Additionally, no effects were observed on the chemical composition or lipid peroxidation of meat. However, low OTA residues were found in the liver and kidney, even though these OTA levels do not represent a threat to humans. Monitoring OTA contamination in poultry feed is necessary to ensure chicken meat quality and safety.

Conflicts of interest

The authors have no conflicts of interest to declare.

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