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SUPPLEMENT ARTICLE

Feeding a diet contaminated with ochratoxin A for chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 1. Effects on growth and slaughter performance, haematological and serum traits

L. Pozzo[1], G. Salamano[2], E. Mellia[2], M.S. Gennero[2], L. Doglione[2], L. Cavallarin[3], M. Tarantola[1], G. Forneris[1] and A. Schiavone[1]

1 Dipartimento di Scienze Veterinarie, Università di Torino Grugliasco, Turin, Italy
2 Istituto Zooprofilattico Sperimentale (IZS) del Piemonte Liguria e Valle d'Aosta Turin, Italy, and
3 Istituto di Scienze delle Produzioni Alimentari (ISPA) CNR Grugliasco, Turin, Italy

Summary

The European Commission Recommendation 2006/576/EC, suggests that the maximum level of Ochratoxin A (OTA) in poultry feeds should be set at 0.1 mg OTA/kg. Thirty-six one-day-old male Hubbard broiler chickens were divided into two groups, a Control (basal diet) and an Ochratoxin A (basal diet + 0.1 mg OTA/kg) group. The growth and slaughter performance traits were recorded. The liver, spleen, bursa of Fabricius and thymus weights were measured. The erythrocyte and leukocyte numbers were assayed in blood samples, and the heterophils to lymphocytes (H/L) ratio was determined. Alpha-1-acid glycoprotein (AGP), lysozyme, the total protein and the electrophoretic pattern were evaluated in serum samples. Liver enzymes (alanino aminotransferase, ALT and aspartate aminotransferase, AST) and kidney function parameters (uric acid and creatinine) were quantified. The results revealed that feeding a 0.1 mg OTA/kg contaminated diet to chicks caused a decrease in the absolute thymus weight ($p < 0.05$) and a lower total protein ($p < 0.01$), albumin ($p < 0.01$), alpha ($p < 0.05$), beta ($p = 0.001$) and gamma ($p = 0.001$) globulins serum concentration in the Ochratoxin A group. Moreover, the albumin-to-globulin (A/G) ratio of the OTA-treated animals resulted to be higher ($p < 0.05$). Feeding broiler chickens, a diet contaminated with the maximum level admitted by the European Commission Recommendation (0.1 mg OTA/kg), did not affect the animal performance, slaughter traits, organ weights, haematological parameters, liver enzyme or renal function parameters concentrations but had an overall immunosuppressant effect, with reduction in the thymus weight and of the total serum protein, albumin, alpha, beta and gamma globulins concentration.

Keywords broiler chicken, Ochratoxin A, growth performances, slaughter traits, haematological, serum biochemical traits

Correspondence: A. Schiavone, Dipartimento di Scienze Veterinarie, Università degli Studi di Torino. Via L. da Vinci, 44, 10095 Grugliasco (TO), Italy. Tel: +39 011 6709208; Fax: +39 011 6709240; E-mail: achille.schiavone@unito.it

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Introduction

Ochratoxin A (OTA) is a mycotoxin that is produced by a number of mould genera, including *Aspergillus* and *Penicillium* (Bayman et al., 2002; Castella et al., 2002).

The most important economic problems encountered when chicks were fed OTA-contaminated diets were a reduction in the growth rate and feed consumption, poor feed efficiency (Huff et al., 1988; Mohiuddin et al., 1993; Raju and Devegowda, 2000; Santin et al., 2003; Stoev et al., 2004; Elaroussi et al., 2006, 2008) and an increase in mortality (Elaroussi et al., 2006), although some other specific damage to many tissues has also been noted (Huff et al., 1975; Dwivedi and Burns, 1984a; Stoev et al., 2000, 2002a,b).

Ochratoxin A causes atrophy and decrease in relative weights of the lymphoid organs including bursa of Fabricius, thymus and spleen, but it causes also bone marrow depression (Stoev et al., 2000, 2004; Kumar et al., 2004). This reduction is characterized by depression of antibody response and a relative increase in heterophils, severe lymphocytopenia,

erythrocytopenia and to a lesser extent monocytopenia (Chang et al., 1979; Ayed et al., 1991; Mohiuddin et al., 1993; Stoev et al., 2000; Elaroussi et al., 2006) with alteration of cytokine production (Politis et al., 2005).

The European Commission (2006) issued the recommendation 2006/576/EC on the presence of Ochratoxin A in products intended for animal feeding. A maximum tolerable level of 0.1 mg OTA/kg was established for poultry feeds. A previous study on the occurrence of OTA in feeds and sera collected in poultry farms of Northern Italy showed that all the feed samples were contaminated by OTA, with values ranging from 0.04 to 6.50 μg/kg (Schiavone et al., 2008).

Previous studies on the feeding of OTA-contaminated diets were conducted with highly contaminated OTA feeds much over the levels usually found in poultry feeds in farm conditions (Beg et al., 2006; Schiavone et al., 2008; Yildiz, 2009).

The aim of this study was to evaluate the effect of feeding a diet contaminated with 0.1 mg OTA/kg, the maximum level fixed by the European Commission (2006), on the performance and slaughter traits, organ weights, haematological parameters, liver and kidney functions of broiler chickens.

Materials and methods

The experimental plan was designed according to the guidelines of the Italian law for care and use of experimental animals (Ministero della Salute, 1992).

Birds and diets

Thirty-six one-day-old male Hubbard broiler chickens were reared from day 1 to day 35 of age and randomly divided into two groups of eighteen birds each, three replicates per group. The birds were distributed over six pens (0.9 m wide × 1.50 m long), each equipped with a feeder, a drinker and rice hulls as litter. In the first two weeks, heating was provided to keep the room temperature according to the standard breeding practices. The temperature and relative humidity were recorded 24 h/24 h by means of a termoigrometer.

A commercial basal diet, based on barley, wheat, and soybean meal (Table 1) and formulated to meet or exceed National Research Council requirements (National Research Council (NRC), 1994), was adopted. The two groups received two different diets: the Control group received the basal diet and the Ochratoxin A group received an OTA-contaminated diet (0.1 mg OTA/kg added to the basal diet). Pure crystalline OTA (Sigma-Aldrich, Madrid, Spain) was dissolved in absolute ethanol (1 mg/ml), and the solution was sprayed over 500 g of the ground control diet. The treated feed was left overnight at room temperature for the solvent to evaporate. It was then mixed in the basal diet to obtain the desired level of 0.1 mg OTA/kg of diet (Denli et al., 2008). The Ochratoxin A concentration in the diets was analyzed by means of high-performance liquid chromatography (HPLC) to confirm the OTA concentrations in the experimentally contaminated diets (Pozzo et al., 2010). In this study, a total of 12 feed samples (taken at the end of the feed mixing, from the finished feed and from each of the the batches used for feeding each birds group) were analysed in duplicate. Data were analysed using ANOVA. The Levine's *F*-test indicated that differences in the corresponding variances were not significant ($p > 0.05$), thus all materials can be considered as homogeneous. Control diet was naturally contaminated at a level of 0.27 ± 0.09 μg OTA/kg.

Table 1 Ingredients and chemical composition of the basal diet

	Starter/grower	Finisher
Ingredients (g/kg)		
Wheat	274.0	308.0
Corn	253.5	252.4
Soybean meal	376.7	332.4
Animal fat	56.0	68.0
Dicalcium phosphate	13.0	12.4
Calcium carbonate	11.5	11.2
Sodium chloride	2.3	2.2
Sodium bicarbonate	1.3	1.5
DL-methionine	3.9	3.8
L-lysine	2.0	2.0
Treonin	0.8	1.1
Vitamin–mineral premix	5.0*	5.0†
Analyzed composition (g/kg)		
Dry matter	904.9	907.4
Crude protein	230.5	195.0
Ether extract	96.6	95.7
Crude fibre	30.4	27.5
Ash	52.9	50.4
Metabolizable energy (MJ/kg)‡	13.1	13.5

*supplied per kilogram of diet: vitamin A, 12500 IU; vitamin D3, 5000 IU; vitamin E, 30 mg; iron, 10 mg; Endo-Beta-1,4-Xylanase, 20000 BXU; Endo-Beta-1,3-Glucanasi, 5000 BU.

†supplied per kilogram of diet: vitamin A, 12500 IU; cholecalciferol, 2000 IU; vitamin B_1, 1.5 mg; riboflavin, 3.0 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 15 μg; dl-α-tocopheryl acetate, 75 IU; niacin, 25.0 mg; d-pantothenic acid, 8.0 mg; cobalt, 0.2 mg; iron, 30.0 mg; iodine, 1.4 mg; manganese, 80 mg; copper, 1.5 mg; zinc, 30.0 mg.

‡Based on the National Research Council (NRC) (1994) ingredient composition.

Growth performances

Mortality was recorded daily. Body weight and feed consumption per pen were recorded weekly (n = 3). The daily gain and feed conversion ratio (FCR) per pen were calculated weekly (n = 3).

Slaughter performances and organ mass

At 35 days of age, nine chickens per diet were humanely killed, bled and dissected to measure the live weight and chilled carcass, breast, thigh, liver, spleen, bursa of Fabricius and thymus weights (n = 9). Dressing percentages were then calculated.

Haematological and serum parameters

At the end of the experiment, blood samples were collected (5 ml) from the femoral vein and centrifuged for 15 min at 3000× g. The serum samples were stored at −20°C until use.

A blood smear was prepared from a droplet without anticoagulant. The total red and white cell counts were determined in an improved Neubauer haemocytometer after mixing with a Natt-Herrick solution in a 1 to 200 ratio (Natt and Herrick, 1952). The blood smears were stained with May-Grünwald and Giemsa –Romanowski stains. One hundred white blood cells were evaluated (n = 12) per smear to determine heterophils to lymphocytes (H/L) ratio, and the number of blood cell type was determined according to Campbell (Campbell, 1995).

The serum alpha-1-acid glycoprotein (AGP) concentration (μg/ml) was assayed (n = 12) using a commercially available radial immunodiffusion tray (Cardiotech Services, Inc.). The serum lysozyme assay (n = 12) employed *Micrococcus lysodeikticus* cells as the substrate for the lysozyme, using the Osserman and Lawlor method (Osserman and Lawlor, 1996).

The total proteins were quantified (n = 11) by means of the 'biuret method' (Bio Group Medical System kit); the serum electrophoretic patterns were obtained (n = 10) using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys®).

The alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid and creatinine serum concentrations were measured with enzymatic methods on a clinical chemistry analyzer (Screen Master Touch, Hospitex diagnostics) (n = 12).

Statistical analysis

The statistical analyses were performed with SPSS 17 for Windows (SPSS, Chicago, IL, USA). Before testing for group differences, normality of the 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 ± s.d. All data obtained were statistically analysed through an independent sample t-test. The results were considered statistically significant when associated with a probability lower than 5%. The results with a probability lower than 1% were considered highly significant.

Results

Growth performances

The mean body weight of the OTA-treated chickens resulted to be significantly lower than that of the Control group at 8 days ($p < 0.05$) and 15 days ($p < 0.01$). No statistically significant differences in body weight values were found between groups during the rest of the breeding cycle period (Fig. 1).

Feeding the OTA-contaminated diet caused a lower average daily gain (g/d) during both the first week (days 1–8) ($p < 0.05$) and the second week (days 8–15) ($p < 0.01$). No differences were found between groups during the remaining weeks (Fig. 2).

The daily feed intake of the OTA group resulted to be significantly lower than the Control group during the 1–8 day and 8–15 day periods ($p < 0.05$). No statistically significant differences in daily feed intake were found between groups in the following weeks (Fig. 3).

Besides, the chickens fed the OTA diet showed a significant lower FCR than the Control group during the 22–28 day period ($p < 0.05$). Moreover, FCR of the OTA-treated birds tended to be lower than the Control group over the 1–8 day and 8–15 day periods and during the entire period (1–35 day) ($p < 0.10$) (Fig. 4).

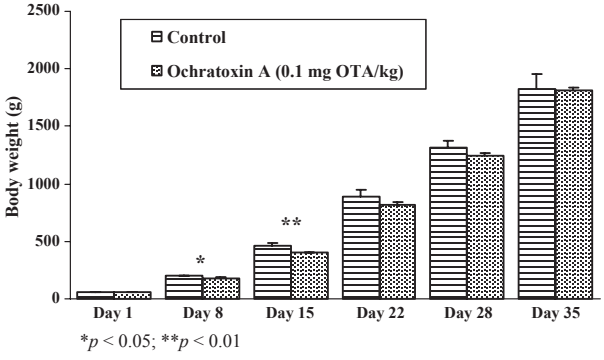

Fig. 1 Body weight (g) of broiler chickens

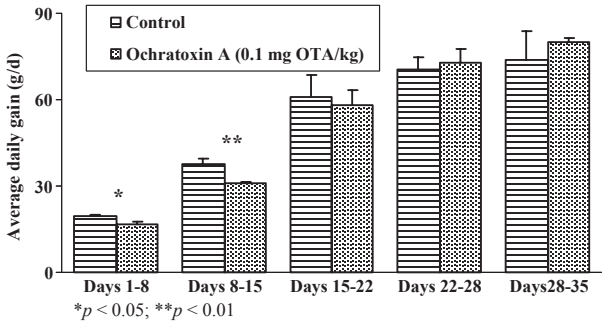

Fig. 2 Average daily gain (g/d) of broiler chickens.

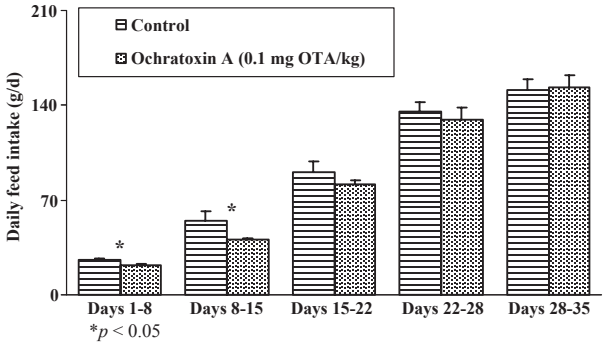

Fig. 3 Daily feed intake of broiler chickens (g/d).

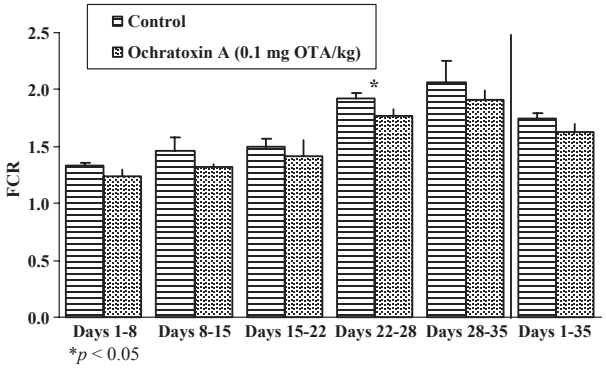

Fig. 4 Feed conversion ratio (FCR) of broiler chickens.

Slaughter performances and organ mass

The slaughter performances of broiler chickens were not affected by the diet type (Table 2).

Organ mass of the broiler chickens did not differ between groups, except for the absolute thymus weight that resulted to be significantly lower in broiler chickens of Ochratoxin A group ($p < 0.05$) (Table 2). However, the birds in the Ochratoxin A group showed a numerically lower relative thymus weight ($p < 0.10$) and a numerically lower absolute spleen weight ($p < 0.10$) than those in the Control group (Table 2).

Table 2 Slaughter performance traits of broiler chickens (mean ± s.d.)

	Control	Ochratoxin A*	p-value
Live weight (g)	1950.0 ± 352.7	1872.2 ± 309.3	NS
Chilled carcass	1421.0 ± 248.1	1347.3 ± 211.4	NS
Chilled carcass (%)†	73.0 ± 2.3	72.0 ± 1.6	NS
Breast (g)	374.2 ± 96.2	342.0 ± 83.5	NS
Breast (%)‡	26.3 ± 4.7	25.1 ± 2.5	NS
Thighs (g)	366.2 ± 69.4	364.4 ± 70.6	NS
Thighs (%)‡	25.9 ± 2.7	26.9 ± 1.3	NS
Liver (g)	51.8 ± 7.3	50.9 ± 7.8	NS
Liver (%)§	3.7 ± 0.5	3.8 ± 0.4	NS
Spleen (g)	3.0 ± 0.9	2.4 ± 0.6	NS
Spleen (%)§	0.2 ± 0.1	0.2 ± 0.0	NS
Bursa Fabricii (g)	3.2 ± 1.1	2.9 ± 1.3	NS
Bursa Fabricii (%)§	0.2 ± 0.1	0.2 ± 0.1	NS
Thymus (g)	10.3 ± 1.9	7.7 ± 1.9	< 0.05
Thymus (%)§	0.7 ± 0.1	0.6 ± 0.1	NS
Liver (g)	51.8 ± 7.3	50.9 ± 7.8	NS

*0.1 mg OTA/kg feed.
†chilled carcass/live weight.
‡organ weight/chilled carcass.
§organ weight/live weight.
NS = $p > 0.05$.

Haematological and serum parameters

Erythrocyte, leucocyte and H/L ratio were not affected by the diet (Table 3).

Also, the albumin to globulin ratio (AGP) and lysozyme serum concentration did not differ between groups. The concentration of total protein ($p < 0.01$), albumin ($p < 0.01$) and alpha ($p < 0.05$), beta ($p = 0.001$) and gamma ($p = 0.001$) globulin serum concentration were reduced by feeding the diet contaminated with OTA, while the A/G ratio of the OTA-treated animals resulted to be significantly higher than the Control group ($p < 0.05$) (Table 3).

No statistically significant differences were found between groups concerning the hepatic enzymes concentrations (ALT and AST) and the renal function parameters (creatinine and uric acid) (Table 3).

Discussion

The OTA contamination represents a real problem in poultry farms because OTA-contaminated feeds may cause economic losses inducing a reduction in weight gain and feed consumption (Raju and Devegowda, 2000; Santin et al., 2003; Stoev et al., 2004; Elaroussi et al., 2006) and increase in the mortality rate (Kumar et al., 2003; Elaroussi et al., 2006).

Table 3 Haematological and serum biochemical traits of broiler chickens (mean ± s.d.)

	Control	Ochratoxin A*	p-value
Erythrocyte (10^6 cells/μl)	2.40 ± 0.33	2.24 ± 0.26	NS
Leucocyte (10^3 cells/μl)	14.10 ± 4.59	12.71 ± 3.74	NS
H/L ratio	0.42 ± 0.12	0.53 ± 0.28	NS
Lysozyme (mg/ml)	3.50 ± 0.88	3.75 ± 2.08	NS
AGP (mg/ml)	0.38 ± 0.12	0.36 ± 0.15	NS
Total protein (g/dl)	3.88 ± 0.80	2.64 ± 0.90	< 0.01
Albumin (g/dl)	1.43 ± 0.31	1.04 ± 0.25	< 0.01
Alpha globulin (g/dl)	1.00 ± 0.22	0.74 ± 0.21	< 0.05
Beta globulin (g/dl)	0.60 ± 0.18	0.35 ± 0.12	0.001
Gamma globulin (g/dl)	0.81 ± 0.17	0.55 ± 0.15	0.001
A/G	0.59 ± 0.04	0.64 ± 0.04	< 0.05
AST (UI/l)	170.23 ± 19.84	167.41 ± 30.74	NS
ALT (UI/l)	6.04 ± 1.96	6.20 ± 1.40	NS
Uric acid (mg/dl)	15.57 ± 2.00	15.30 ± 2.43	NS
Creatinine (mg/dl)	0.39 ± 0.03	0.37 ± 0.02	NS

H/L, heterophil/lymphocytes; AGP, alpha-1-acid glycoprotein; A/G, albumin/globulin; AST, alanino aminotransferase; ALT, aspartate aminotransferase.
*0.1 mg OTA/kg feed.
NS = $p > 0.05$.

A survey conducted about the occurrence of OTA in poultry farms of Northern Italy showed that OTA is widespread in poultry feed, even with low levels, ranging from 0.04 to 6.50 μg/kg (Schiavone et al., 2008). The wide diffusion of OTA-contaminated poultry feeds, even at low concentrations, drove us to evaluate whether feeding a diet contaminated with 0.1 mg OTA/kg, the maximum level fixed by the European Commission Recommendation (2006/576/EC) (European Commission, 2006) for poultry feeds, may have led to consequences on the performances and the immune system response during broiler chickens breeding.

The results of the performance traits of the present study demonstrated that a diet contaminated with 0.1 mg OTA/kg did not affect the final body weight, overall weight increase, overall feed consumption or total FCR. However, the body weight and the average daily gain of the OTA-treated birds resulted to be significantly lower than the birds of the Control group during the first two weeks, making equal between groups for the rest of the experiment. Moreover, during the 1–8 day and 8–15 day periods, the feed consumption of the OTA-treated birds resulted to be significantly lower than the Control group. These results confirm that young chickens are more sensitive to an OTA-contaminated diet than adult chickens (Marquardt and Frohlich, 1992; Dortant et al., 2001). As a whole, the similar final body weight of the OTA and Control groups, coupled with the early reduction in feed intake and live body weight and the subsequent improvement in FCR for the Ochratoxin A group, could be explained by the compensatory growth phenomenon, as reported by some studies in restricted-refed broilers (Zubair and Leeson, 1996), and by a possible adaptation effect of the animals, after feeding OTA-contaminated diet for 35 days. Hassan et al. (2010) fed a diet contaminated with different OTA levels (0–0.1–0.5–1–3–5–10 mg/kg) to laying hens for 21 days. The diet contaminated with 0.1 mg OTA/kg, which is the same concentration used in the present study, did not affect the body weight or feed consumption during the three weeks of dietary treatment, but caused a worsening of the feed conversion during the third week of treatment. Sakhare et al. (2007) demonstrated that broiler chickens fed an OTA-contaminated diet (0.2 mg OTA/kg) showed a lower weight increase and worse FCR, than a Control group. Similar results were obtained by El Barkouky et al. (2010) with a diet contaminated with 0.2 mg OTA/kg fed to broiler chickens for 5 weeks. During a study of Elaroussi et al. (2006), birds fed contaminated diets (0.4 and 0.8 mg OTA/kg) displayed an OTA dose-dependent decrease in feed consumption and body weight and a dose-dependent worsening of feed conversion. Hanif et al. (2008) showed that a feed contaminated with 0.5 mg OTA/kg did not affect feed consumption and caused a weight decrease during the second breeding period in OTA-treated broiler chickens, compared with a Control group. During the same study, a diet contaminated with 1 mg OTA/kg caused a reduction in feed consumption over the entire period, and a lower body weight of the OTA-treated broiler chickens compared with the non-treated ones. Similar results were presented by other authors (Prior et al., 1980; Manning and Wyatt, 1984; Huff et al., 1988; Hoehler et al., 1996; Raju and Devegowda, 2000; Stoev et al., 2002a,c; Garcia et al., 2003; Santin et al., 2003; Verma et al., 2004; Sawale et al., 2009). Huff et al. (1975) demonstrated that, after feeding a diet contaminated with 0–0.5–1.0–2.0–4.0 and 8.0 mg OTA/kg for three weeks, OTA started to cause an inhibition of weight increase at an OTA contamination of 2 mg OTA/kg. The low values of body weight and growth depression in OTA-treated chicks showed to be due to the protein synthesis disorders (Bunge et al., 1978; Creppy et al., 1979) and the feed refuse (Elaroussi et al., 2006; Hanif et al., 2008) provoked by OTA. Only a few studies in literature have shown that OTA did not affect body weight or feed intake. In a study conducted by Biró et al. (2002), a diet contaminated with 0.354 mg OTA/kg fed for 28 days had no negative effects on the live

weight of OTA-treated broiler chickens. During a study by Politis et al. (2005), animals fed a diet contaminated with 0.5 mg OTA/kg during 42 days did not show different body weight gain or feed intake values.

In the present study, the diet contaminated with OTA caused an improved FCR, during the 22–28 day period. It is possible to hypothesise that this effect is due to a reduced feed ingestion, which may be associated with the increased digestibility (Nelson et al., 1982).

Best part of the previously mentioned studies demonstrated that OTA-contaminated feeds have a dose-dependent negative effect on chickens growth performances and feed conversion. Some of these studies (Elaroussi et al., 2006; Sakhare et al., 2007; Hanif et al., 2008; El Barkouky et al., 2010) demonstrated that feeds contaminated with OTA concentrations at a level close to the maximum level admitted by the EC may decrease weight gain and feed consumption, whereas some others (Biró et al., 2002; Politis et al., 2005) demonstrated that almost the same concentrations do not cause any effects on chickens performances. The results of the present research indicated that a diet contaminated with 0.1 mg OTA/kg, corresponding to the maximum level established by the EC for poultry feed caused slight feed refuse and loss in weight gain in young chicks.

The present study shows that a diet contaminated with 0.1 mg OTA/kg did not affect either the slaughter performance traits of broiler chickens or the organ weights, except for the thymus weight, which was significantly lower in the Ochratoxin A group than the Control group. Moreover, the OTA-treated chickens showed a lower absolute spleen weight. These findings are in accordance with the histopathological observations presented in Part 2 of the present study (Pozzo et al., 2013) that showed an increase in apoptosis in the splenic follicles of OTA-treated chickens. It is known that the immunosuppressant activity of OTA is characterized by a reduction in size of the vital immune organs (Al-anati and Petzinger, 2006). Stoev et al. (2002b) showed that the relative weight of the lymphoid organs (thymus, bursa of Fabricius and spleen) decreased when feeding chickens with a diet contaminated with 1 and 5 mg OTA/kg. The same findings have been presented in many other studies (Dwivedi and Burns, 1984b; Huff et al., 1988; Sreemannarayana et al., 1989; Singh et al., 1990; Stoev et al., 2002c; Elaroussi et al., 2006, 2008). The present study showed that a diet contaminated with 0.1 mg OTA/kg did not cause the reduction in bursa of Fabricius weight, but it may have a slight immunotoxic effect because of the reduction in the thymus and spleen weight.

During the present study, OTA showed no effects on the haematological traits. Similar findings were observed after feeding broiler chickens with a diet contaminated with 0.2 mg OTA/kg (Sakhare et al., 2007). However, a significant reduction in the total number of erythrocytes and leucocytes in broiler chickens fed an OTA-contaminated diet (1 mg OTA/kg) was observed by Sawale et al. (2009). Similar results have been found in other studies (Ayed et al., 1991; Mohiuddin et al., 1993). Elaroussi et al. (2006) showed a reduction in the red blood and white blood cell numbers after feeding two diets contaminated with 0.4 and 0.8 mg OTA/kg to broiler chickens. Moreover, Stoev et al. (2002b) showed a decrease in the total number of erythrocytes and an increase in the number of leucocytes in birds fed an OTA-contaminated diet (5 mg OTA/kg).

Several studies have reported that H/L is affected by stressors and that it can be used as a sensitive haematological indicator of stress response in chicken populations (Gross and Siegel, 1983; Kowalski et al., 2006; Salamano et al., 2010). In the present study, the OTA-contaminated diet fed to the broiler chickens did not affect the H/L ratio. The study by Janaczyk et al. (2006) recorded an increase in percentage of heterophils, accompanied by a decreased percentage of lymphocytes, which may be translated as an increase in the H/L ratio, after ten days of feeding broiler chickens with an OTA-contaminated diet with 6 mg OTA/kg. Considering that feeding a diet contaminated with 0.1 mg OTA/kg did not cause effects on erythrocytes and leucocytes number, heterophils and lymphocytes included, it is possible to speculate that this OTA level did not cause suppression of hematopoiesis in the bone marrow.

Lysozyme is one of the principal factors of innate immunity in birds. It is a powerful antibacterial enzyme contained in the cytoplasmic granules of herterophils (Campbell, 2004a). The negative effect of stress upon the lysozyme concentration could be explained by a possible increase in blood cortisol concentrations. On the other hand, acute and short-time stress challenges induced an elevation of lysozyme concentrations instead of a decrease because the glucocorticoid concentrations are probably not or weakly modified during such stimuli (Yotova et al., 2004; Stoyanchev et al., 2007). Nevertheless, in our study, no effect on lysozyme concentration was observed in the OTA-treated group.

Alpha-1-acid glycoprotein (AGP) is a 'positive' acute-phase protein (APP) in chickens (its serum con-

centration increases in response to a challenge), while albumin behaves as a 'negative' APP in both mammals and chickens (its concentration decreases in response to challenge) (Adler et al., 2001; Murata, 2007). Assessment of acute-phase protein concentrations might be a useful indicator of stress responses (Murata, 2007; Salamano et al., 2008, 2010). In the present study, a diet contaminated with 0.1 mg OTA/kg did not affect the AGP serum concentration. All the parameters of stress response considered during the present study, as well as H/L, lysozyme and AGP, were not affected by the diet contaminated with 0.1 mg OTA/kg.

A significant decrease in the albumin concentration, total protein and alpha, beta and gamma globulins, and an increase in the A/G ratio occurred in the Ochratoxin A group. Alpha and beta globulins are considered acute-phase proteins, while the gamma fraction is elevated in chronic conditions and includes immunoglobulins (Lumeij, 2008). The results of the present study suggest that OTA impairs hepatic protein synthesis in broiler chicks, as it has already been found (Mohiuddin et al., 1993; Stoev et al., 1999, 2000, 2002a), probably due to a decreased hepatic synthesis. In addition, a possible increased OTA-induced protein elimination mechanism through the kidneys could be involved (Elaroussi et al., 2008). Moreover, during the present study, the OTA-contaminated diet decreased the gamma globulin concentration, and it is possible to hypothesize that OTA may predispose broiler chickens to infectious diseases (Al-anati and Petzinger, 2006), even at the low concentration used in the present study. Sakhare et al. (2007) recorded a significantly decrease in the total serum protein concentration in chickens fed an OTA-contaminated diet (0.2 mg OTA/kg) after 21 days, but not after 42 days, and they did not observe any changes in the albumin serum concentration. Some authors have shown that an OTA-contaminated diet (1 mg OTA/kg) fed to broiler chickens may significantly decrease the total protein and albumin concentrations (Sreemannarayana et al., 1989; Raina et al., 1991; Sawale et al., 2009). The same findings were observed by Agawane and Lonkar (2004), who found a reduction in the total serum protein, serum albumin and serum globulin concentrations after feeding a 0.5 mg OTA/kg contaminated diet to broilers. Birds fed diets contaminated with 0.4 and 0.8 mg OTA/kg displayed a decrease in the total protein, albumin and globulin concentration (Elaroussi et al., 2006). Other studies have demonstrated similar findings (Manning and Wyatt, 1984; Raju and Devegowda, 2000). Decreased gamma globulin fractions may be indicative of immunodeficiency (Campbell, 2004b).

During the present study, the hepatic enzymes (AST and ALT) and renal function parameters (uric acid and creatinine) were not affected. Previous studies showed the increase in AST and ALT, when OTA was supplemented to broiler diet at 0.5–1 mg OTA/kg (Raina et al., 1991; Agawane and Lonkar, 2006). Furthermore, Kumar et al. (2004) demonstrated an increase in AST when chickens fed a diet contaminated with 2 mg OTA/kg. Dietary OTA contamination with 0.4 and 0.8 mg OTA/kg resulted in a significant increase in AST, ALT, uric acid and creatinine levels in the study conducted by Elaroussi et al. (2008).

Conclusion

In conclusion, feeding broiler chickens with an OTA-contaminated diet at the maximum contamination level admitted in poultry feeds by the European Commission Recommendation 2006/576/EC (0.1 mg OTA/kg) negatively affected the thymus weight and the total protein, albumin, alpha, beta and gamma globulins serum concentration. Nevertheless, the OTA contamination did not affect the performance traits, slaughter traits, organ weights, haematological traits or liver enzyme and renal function parameters. Monitoring OTA contamination in poultry feeds is necessary to guarantee animal health and to prevent the risk of decreased productions in poultry farms.

Conflicts of interest

The authors have not declared any potential conflicts.

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