Residue of ochratoxin A in chicken tissues-risk assessment

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SUMMARY

Background: *Toxicological investigations of tissues of normally slaughtered chickens were carried out to provide preliminary evaluation of the incidence of OTA in chicken tissues (n=90). Majority of tissue samples were not found to contain measurable amounts of OTA, while in general, the OTA levels found in the analyzed tissue were low.*

Methods: *The presence of OTA in tissue samples was determined by HPLC-FL after liquid-liquid extraction procedure. Method validation was performed according to the Commission Decision 2002/657/EC.*

Results: *Of the 90 liver, kidney and gizzard samples originating from chicken farms located in the different agricultural areas of Serbia, OTA was reported in 23 (38.33%), 17 (28.3%) and 16 (26.6%) samples, respectively, with levels ranging from 0.14 to 3.9 ng/g in liver, 0.1 to 7.02 ng/g in kidneys and 0.25 to 9.94 ng/g in gizzard. None of the tissue samples contained more than the maximum level (10 ng/g) recommended by the European Commission.*

Conclusion: *Low OTA results also suggested that chicken meat available in the retail market is unlikely to pose an adverse health risk to the consumers in respect to OTA toxicity.*

Key words: *Ochratoxins; Chickens; Risk Assessment; Food Analysis*

INTRODUCTION

Modern industrial poultry production is the fastest and the most efficient way of obtaining high quality products for human consumption of all categories, such as poultry meat and eggs (1). Poultry meat is known as a highly valued food due to its biological and nutritional value primarily for the high protein content with a satisfactory amino acid composition and low energy value. Modern consumers of food require much more than just good taste (2). The most important values have become: quality, health and safety – because it does matter what we put on our plate (3). A healthy diet is safe, nutritious, energy-balanced and gastronomically acceptable and in accordance with age, gender and health status (4).

Toxic substances such as mycotoxins are almost ubiquitous in the environment. Their common occurrence in food and feed poses a threat to health of humans and animals. Ochratoxin A (OTA) is a wide spread mycotoxin produced by several species of the *Aspergillus* and *Penicillium* genera and is classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer (IARC) (5). Long-term exposure to OTA has been implicated in Balkan Endemic Nephropathy (BEN) and associated with urinary tract tumors because of rather high OTA levels detected in food samples and in blood or urine from affected persons (5, 6). OTA enters the food chain either through cereals, oilseeds, fruit (7) and their derived products or through products of animal origin when animals such as swine and poultry are fed OTA contaminated diets (8). OTA has been detected in pigs with a tissue distribution that follows the pattern blood > kidney > liver > muscle > adipose tissue (9–11). Studies have shown that pork products, especially sausages and products that include blood and kidneys, may contain OTA (12,13) and that production procedures such as seasoning and storage have no effect on the reduction of OTA levels (14). Human intake and absorption of OTA were confirmed through the detection of OTA residues in human blood serum, milk and kidney (15). OTA was last evaluated by the Scientific Committee on Food (SCF) when it concluded that OTA possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. The SCF estimated that the mean dietary intake ranged from 0.7 to 4.6 ng/kg BW per day. By combining the average OTA contamination levels in food which is to be consumed with 95% of probability, the JECFA estimated a dietary exposure of approximately 90 ng/kg BW per week corresponding to about 13 ng/kg BW per day.

Poultry meat comprises a substantial portion of the Serbian diet. In fact, the consumption of poultry has increased steadily and now leads in front of pork (17.4 kg and 16 kg, per capita, respectively).

Therefore, the aim of this paper is to bring up to date the current status of OTA contamination of the chicken meat intended for human consumption. In addition, as reports on OTA presence in chicken meat are limited and based on the analysis of OTA in 90 samples of chicken liver, kidneys and gizzard, the risk for the consumer is also assessed.

MATERIAL AND METHODS

Samples collection

In the slaughterhouse which processes approximately 200,000 to 250,000 broilers per week, at the end of the 5th, 6th or 7th fatting week, by random method (5 per farm), chickens for slaughtering were selected. A total of 90 livers, kidneys and gizzards of corresponding animals, were collected. The liver and kidneys were removed and weighed. After cutting pieces of kidney for histological examination, the rest of the sample was homogenized and stored at -20 °C before analysis.

Chemicals and reagents

Ochratoxin A crystalline material was purchased from Sigma (St. Louis, MO, USA). Stock concentrated solution was prepared in toluene–acetic acid (99:1 v/v) at a final concentration of 1 mg/mL and kept under safe conditions at

-20 ºC and wrapped in aluminum foil, because OTA gradually breaks down under ultraviolet (UV) light. The OTA working solution was prepared by diluting the stock solution with toluene–acetic acid (99:1 v/v) to ~10 ng/ml. The actual concentration of OTA was calculated using a UV spectrophotometer set at 333 nm (e 5550). After suitable dilutions in water–methanol–acetic acid (50:49:1 v/v/ v), the working solution was used to prepare the external calibration curve. A working standard OTA for HPLC was prepared daily just before starting the injection of a series of samples. Other reagents were HPLC grade. All other chemicals were reagent grade or chemically pure.

Extraction and cleanup for ochratoxin analyses from the kidney, liver and gizzard

Kidney, liver and gizzard analyses were performed by the method of Matrella et al., (10), which briefly includes a double extraction with acidic ethyl acetate. The organic phase was removed and extracted with 0.5 M $NaHCO_3$, pH 8.4. The aqueous extract was adjusted to pH 2.5 with 7 M H_3PO_4. OTA was finally back extracted into ethyl acetate (3 ml). The organic phase was evaporated to dryness under N_2 steam and reconstituted in 150-μL mobile phases and a 20 μL aliquot was injected.

Chromatographic conditions (HPLC)

An aliquot of 20 μL for serum samples and 50 μL for kidneys, liver and gizzard samples were injected onto a Waters Symmetry Shield RP (Reversed Phase) 18, high pressure liquid chromatography column (length and inner diameter 150×4.6 mm, particle size 5 μm) on a Waters Alliance 2695 HPLC system. The column was eluted with 4% acetic acid and acetonitrile (32:68 v/v) at 25 °C and a flow rate of 1 mL/min. Measurements were performed by fluorescence detection at wavelengths of 334 nm (excitation) and 460 nm (emission) gains 10. A volume of 20 μL was injected for the standards and 20 μL for the samples. For more accuracy, 40 μL was re-injected in the case of the samples with an amount of OTA near the detection limit.

Statistical analysis

Descriptive statistics of the data set were performed with a standard program and descriptive data were presented as means, standard deviation (SD), and range, for continuous variables. Statistical differences in the mean levels of OTA contamination across the three groups of positive samples were determined by one-way ANOVA ($p < 0.05$). Significance was set at $p < 0.05$.

RESULTS

Sample analysis

The performances of the employed analytical methods complied with the acceptable range set by the Commission Decision (EC) 2002/657 (16). A total of 270 tissue samples subjected on the OTA presence were analyzed. The incidence of OTA contamination in liver, kidneys and gizzard is summarized in Tables 1 and 2. None of 90 tissue samples originating from the chicken farms located in the central agricultural area of Serbia, contained OTA. Of the 90 liver, kidneys and gizzard samples originating from the chicken farms located in the northern agricultural area of Serbia, OTA was reported in 23 (38.33%), 17 (28.3%) and 16 (26.6%) samples, respectively. The data in Table 2 show that majority (124) of these samples did not contain ochratoxin A and the rest largely had concentrations close to the detection limit. It should be noted that the samples from both zones were not collected at the same time. OTA levels in liver were slightly higher than those in kidneys and gizzard, but this difference was not statistically significant (p < 0.05). The average level of OTA in liver was 0.58 ± 1.04 ng/g (range 0.14-3.9 ng/g), whereas OTA average values in kidneys and gizzard were 0.51 ± 1.38 ng/g (range 0.1 to 7.02 ng/g), and 0.51 ± 1.75 ng/g (range 0.25-9.94 ng/g) respectively. High values of the standard deviation is due to two samples in kidneys, and one in gizzard that showed a concentration of OTA up to 7.02 ng/g and 9.5 ng/g, respectively. Although there was obvious difference in the mean value of contamination in samples from different locations, it was not statistically significant. Generally, OTA level in tissues was related to location of the farm.

All the analyzed tissue samples were below the limit established by JECFA (Joint FAO/WHO Expert Committee on Food Additives) (10 ng/g) (17). Only one sample of gizzard contained OTA (9.94 ng/g), which is close to the maximum permissible toxin level defined in Serbia, including those proposed by the JECFA.

Table 1. Incidence of OTA in tissue of slaughtered chicken from the central region

Locations of farms and period of sampling	Incidence of OTA					
	Liver (N=30)		Kidneys (N=30)		Gizzard (N=30)	
	positive samples (%)	$\overline{X} \pm SD$ (min-max)	positive samples (%)	$\overline{X} \pm SD$ (min-max)	positive samples (%)	$\overline{X} \pm SD$ (min-max)
A January	nd	nd	nd	nd	nd	nd
B January	nd	nd	nd	nd	nd	nd
C January	nd	nd	nd	nd	nd	nd
D February	nd	nd	nd	nd	nd	nd
E February	nd	nd	nd	nd	nd	nd
F February	nd	nd	nd	nd	nd	nd
TOTAL	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**

Table 2. Incidence of OTA in tissue of slaughtered chicken from the northern region

Locations of farms and period of sampling	Incidence of OTA					
	Liver (N=60)		Kidneys (N=60)		Gizzard (N=60)	
	positive samples (%)	$\overline{X} \pm SD$ (min-max)	positive samples (%)	$\overline{X} \pm SD$ (min-max)	positive samples (%)	$\overline{X} \pm SD$ (min-max)
A March	4 (80)	2.19 ± 1.69 (1.3-3.9)	3 (60)	0.23 ± 0.21 (0.35-0.4)	1 (20)	0.37 ± 0.83 (1.85)
B April	5 (100)	0.26 ± 0.09 (0.14-0.38)	5 (100)	3.36 ± 3.04 (0.71-7.02)	5 (100)	0.84 ± 0.53 (0.25-1.4)
C April	3 (60)	1.2 ± 1.42 (0.53-2.76)	3 (60)	1.09 ± 4.45 (0.1-4.74)	1 (20)	1.99 ± 4.45 (9.94)
D May	nd	nd	nd	nd	3 (60)	2.23 ± 4.09 (0.78-9.5)
A June	2 (40)	0.89 ± 1.34 (1.45-3.0)	3 (60)	0.45 ± 0.5 (0.4-1.2)	1 (20)	1.07 ± 2.39 (5.34)
E June	4 (80)	1.29 ± 1.17 (0.45-2.9)	3 (60)	1.34 ± 1.45 (1.0-3.1)	4 (80)	1.05 ± 1.12 (0.35-2.9)
F June	nd	nd	nd	nd	nd	nd
G July	nd	nd	nd	nd	nd	nd
H July	nd	nd	nd	nd	nd	nd
I September	3 (60)	1.16 ± 1.08 (1.67-2.25)	nd	nd	1 (20)	0.08 ± 0.18 (0.4)
A September	2 (40)	0.46 ± 0.64 (1.1-1.22)	nd	nd	nd	nd
J October	nd	nd	nd	nd	nd	nd
TOTAL	**23 (38.33)**	**0.58 ± 1.04 (0.14 - 3.9)**	**17 (28.33)**	**0.51 ± 1.38 (0.1 - 7.02)**	**16 (26.6)**	**0.51 ± 1.75 (0.25-9.94)**

N – total number of analyzed samples, nd – non-detectable, $\overline{X}$ – arithmetic mean (conc. below LOD are regarded as zero), SD – standard deviation

DISCUSSION

Currently, OTA is the most probable mycotoxin involved in the Balkan nephropathy (BEN) endemic and some renal tumours could be involved in the same pathologies in other European countries (6). Potential risks for animal and human health mainly depend on the duration and OTA-exposure level. Dietary exposure to OTA varies considerably depending on different factors, among which food-processing systems must be considered. These systems are often traditional and characteristic of the different geographical regions (18). Mycotoxins, in general, are stable compounds, and OTA, in particular, is a moderately heat stable molecule that can survive most food processing operations and, therefore, it appears in final and derived products (19).

The very limited data of spontaneous occurrence of ochratoxicosis in poultry in Serbia are available. Therefore, it was very difficult to compare our results with ones obtained with different analytical methodology. The results from ochratoxin A analysis of chicken tissues in this investigation are similar to the results from previous findings about natural occurrences of ochratoxicosis in poultry and those reported in the EFSA and the SCOOP reports.

The data confirm those of the previous studies that some inhabitants have moderate to very high exposure to OTA that is, in part, reflected by high levels in blood and urine. However, there is no direct correlation between OTA in the blood or urine and OTA consumed, thus OTA in the blood or urine cannot be recommended as a biomarker of OTA exposure.

Convincing evidences indicate that exposure to OTA, results in DNA damage in the kidney, liver and testicles. OTA produces an inhibition of protein synthesis and lipid peroxidation by oxidative processes (20). These mechanisms may generate nephrotoxic, neurotoxic and immunotoxic effects (21-23). The mechanisms by which OTA is carcinogenic are not entirely elucidated and two hypotheses are still under discussion: (i) an indirect mechanism which would result in a classification as an epigenetic carcinogen (24, 25) or (ii) due to direct covalent binding of OTA on DNA, suggesting genotoxic mechanisms being involved in the carcinogenicity (26, 27). Although there is evidence for a time- and dose-dependent induction of DNA lesions in vivo when applying the 32P-postlabeling technique, the chemical identities of adducts and metabolites implicated, need to be elucidated (28).

Toxicokinetics are an important determinant in the toxicity of OTA (29). The toxic-kinetic parameters of OTA have not been well determined in humans, but in some animal studies OTA showed high availability after oral administration (30). OTA binds rapidly and with high affinity to plasma proteins with increased T1/2 values (31). The metabolism in humans is basically produced through cytochrome P-450 (32) generating less toxic metabolites (33) which are eliminated in urine and feces (34). Also, reabsorption of OTA from the intestine, enterohepatic recirculation, and

reabsorption in the kidney proximal and distal tubules favor its accumulation in the organism (30). In humans, OTA is rather persistent, with a serum half-life of about 35 days, due to unfavorable elimination kinetics (35). OTA can cross the placenta and is excreted in animal milk (36).

Risk assessment

The most recent international exposure assessments were performed respectively by the Scientific Committee on Food (SCF) and the JECFA (37). The SCF estimated that the mean dietary intake ranged from 0.7 to 4.6 ng OTA/kg BW per day. By combining the average contamination levels with the 95th percentile of food consumption the JECFA estimated a dietary exposure of approximately 90 ng/kg BW per week corresponding to about 13 ng/kg BW per day. Exposure seems to be associated predominantly with the consumption of contaminated plant-derived products, and only to a minor extent to foods of animal origin (37).

It has been shown that chicken meat and meat products are the minor contributors to OTA intake. Data on the exposure assessment of Serbian population to OTA from the consumption of chicken meat are estimated for the first time in this investigation. OTA daily intake estimated in this study is only for orientation purposes and the values obtained need to be checked in the future since number of samples investigated is limited. In Serbia, since the chicken meat consumption is 17.5 kg per year (i.e. 47.94 g, per day), and the average OTA levels in the liver, kidneys and gizzard are 0.58 ng/g and 0.51 ng/g, respectively, the estimated daily intake of OTA through chicken meat by a 60 kg adult is 0.46 ng kg-1 b.w. and 0.40 ng kg-1 BW, respectively. This value is below the OTA Tolerable Daily Intake (TDI) estimated by JECFA and EFSA and it can be concluded that there is no significant toxicological risk for Serbian consumers. Additionally, it should be mentioned that the studies were carried out in a region with a long tradition of excellent agriculture and that tissue samples represent a random population of chicken, that were believed to have been exposed to low doses of ochratoxin A.

CONCLUSION

The results found in this study suggest that, in general, OTA contamination in chicken meat originating from different parts of Serbia is low. Of 270 tissue samples tested, measurable amounts of OTA were found in 56 tissue samples, which accounts for 20.7% of all samples.

The actual concentration in chicken tissues is generally very low, and hence, for the consumer the contribution to the total intake of ochratoxin A from chicken products is very small compared with other sources. However, the daily intake of OTA through chicken meat depends on (1) the concentration in the food, (2) the amount consumed, (3) the frequency of consumption and (4) the consumers' preferences. To protect consumer health and to reduce economic losses, surveillance and control of mycotoxins in food and feed has become a major objective for producers, regulatory authorities and researchers worldwide.

Acknowledgements

Conflict of interest

We declare no conflict of interest.

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