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## OCHRATOXIN A RESIDUES IN MEAT AND EDIBLE OFFALS OF MARKETED BROILERS AND HENS

(With 4 Tables)

By

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بقايا أوكراتوكسين-أ في لحوم الدجاج وفضلات مذبوحاته الصالحة

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لمواجهة متطلبات الزيادة السكانية في مصر من توفير للبروتين الحيواني، كان إنشاء مزارع الدواجن أمراً حيوياً فكانت ثورة صناعة الدواجن لإنتاج كميات وفيرة من لحوم الدجاج. وتعتبر علائق وأعلاف الدواجن الركيزة الأساسية لإقامة تلك الصناعة، حيث تتكون من حاصلات زراعية عديدة من المعتاد إصابتها وتلوثها بالأعفان السامة التي يمكنها إفراز سموم فطرية كثيرة أشدها سميّة وأعظمها إنتشاراً هو الأوكراتوكسين - أ خاصة في ظل الظروف البيئية المصرية الملائمة لنمو العترات السامة التي يمكنها إفراز الأوكراتوكسين-أ بكميات كبيرة في تلك العلائق ليختزن جزء منه في لحوم وفضلات الدواجن الذي ينتقل بعدها إلى جوف ودم المستهلكين ليُلحق بهم مخاطر صحية جسيمة أهمها الإعتلال الكلوي والمناعى. لذلك أجريت تلك الدراسة على ثلاثين عينة من كل من عضلات ودهون وقوانص وأكباد وكلاوى ثلاثين دجاجة أشتريت من محلات الدواجن المختلفة في مدينة الزقازيق، حيث تم تحليل تلك العينات لوجود بقايا الأوكراتوكسين-أ وقد أسفر التحليل الكروماتوجرافى ذو الطبقة الرقيقة عن وجود هذا السم الفطرى في ١٩ (٦٣,٣%) عينة من العضلات ، ٢٣ (٧٦,٧%) من الدهون، ٢٤ (٨٠%) من القوانص، ٢٧ (٩٠%) من الأكباد و ٣٠ (١٠٠%) عينة من الكلاوى بكميات تراوحت متوسطاتها ما بين ٥,٩٤ إلى ٩,٦٦ ، ٩,٥١ إلى ١٧,٣٩ ، ٨,٤٥ إلى ١٥,٦٩ ، ٨,٩٧ إلى ١٤,٠ و ١٢,٩٠ إلى ١٨,٨٤ ميكروجرام لكل كيلوجرام على التوالى. وعند مقارنة نتائج العينات المختبرة بحد الأوكراتوكسين-أ المسموح به عالمياً، تبين أن عينة واحدة من العضلات المختبرة (٣,٣%)، ١١ (٣٦,٧%) من الدهون، ٨ (٢٦,٧%) من عينات كل من القوانص والأكباد و ١٢ (٤٠%) من الكلاوى قد تجاوزت كميات السم بها عن الحد المسموح به (٢٠ ميكروجرام لكل كيلوجرام). كما تناولت الدراسة أيضاً مدى تأثير المعاملة الحرارية على ثبات بقايا الأوكراتوكسين-أ في لحوم الدواجن الملوثة من خلال طهى لحوم الدجاج ذات السلالة الأجنبية، الدجاج ذات السلالة المحلية ودجاج الأمهات ذات السلالة الأجنبية في ماء مغلى لمدة ١ ، ١,٥ ، ٢ ساعة، فكانت النتيجة هي إختفاء بعض بقايا هذا السم بنسب متويزة تراوحت من ١٨,٢% إلى ٢١,٦% ؛ ٢٣,١% إلى ٢٧,٥% و ٣٦,٣%

إلى ٤٧,٤ % على التوالي. وقد تمت مناقشة الأهمية الصحية لتواجد بقايا الأوكراتوكسين-أ في لحوم الدجاج والفضلات الصالحة لمذبوحاته وكذلك وضع الحلول لهذه المشكلة الحيوية.

## SUMMARY

A thirty samples from each of muscle, fat, gizzard, liver and kidney taken from 30 broilers and hens purchased from different poultry shops at Zagazig city, were analysed for occurrence of Ochratoxin A residues. Thin layer Chromatographic technique can detect the toxin in 19 (63.3%) muscle; 23 (76.7%) fat; 24 (80%) gizzard; 27 (90%) liver and 30 (100%) kidney samples, with mean  $\pm$  SE values ranged from  $5.94 \pm 2.21$  to  $9.66 \pm 2.42$ ;  $9.51 \pm 2.94$  to  $17.39 \pm 3.57$ ;  $8.45 \pm 2.93$  to  $15.69 \pm 4.24$ ;  $8.97 \pm 2.83$  to  $14.00 \pm 2.54$ ; and  $12.90 \pm 3.30$  to  $18.84 \pm 3.30$  micrograms / kilogram, respectively, in foreign breed broilers, native breed broilers, 2<sup>nd</sup> foreign breed hens. Of the examined samples, 1 (3.3%) muscle; 11 (36.7%) fat; 8 (26.7%) from each of gizzard and liver; and 12 (40%) kidney samples were contaminated with Ochratoxin A levels more than the permissible limit (20  $\mu$ g/kg). Effect of heat treatment on the stability of the ochratoxin A residues, was completed by traditional cooking (in boiling water) of toxin - contaminated muscles of foreign breed broilers for 1 hour; of native breed broilers for 1.5 hour; and of foreign breed hens for 2 hours, and resulted in disappearance of some residues ranged from 18.2% to 21.6%; 23.1% to 27.5%; and 36.3% to 47.4%, respectively. Public health significance of ochratoxin A residues in meat and edible offals of marketed broilers and hens, as well as the suggestive measures for resolving such problem were also discussed.

*Keywords: Ochratoxin, Residues, Edible Offals.*

## INTRODUCTION

Mycotoxins are considered unavoidable contaminants in foods and feeds because agronomic technology has not yet advanced to the stage at which preharvest infection of susceptible crops by fungi can be eliminated (Wood, 1992). Intensive production of a huge number of poultry farms in Egypt, necessitates a great storage of poultry feed and its ingredients for unlimited periods. Bad storage conditions (higher temperature and moisture) provides favourable circumstances for



## **MATERIALS and METHODS**

### ***1. Collection and preparation of the samples:***

A total of thirty, apparently healthy, farm broilers and hens were purchased from different poultry shops at Zagazig city {ten from each of foreign breed broilers (about 2 kgm live body weight and 50 days - old); native breed broilers (about 1.25 kgm live body weight and 100 days-old) and foreign breed hens (about 3 kgm live body weight and 2 years-old), were slaughtered and dressed at the same shops of purchase. Each carcass with its edible offals were wrapped and marked in individual plastic bag, after passing meat inspection, then transferred to the laboratory of Food Control Department/Faculty of Veterinary Medicine / Zagazig University, where chilled, prepared and analysed as soon as possible. Fifty grams from each of muscle and fat, in addition to all of gizzard, liver and both kidneys were taken as a representative samples from each carcass. Each sample was prepared as fine mince by using sharp knife- on the wooden cutting board. The remainder of each carcass was retained frozen, for few days, at home refrigerator, until the estimation of ochratoxin A residues in the muscle sample was completed. For studying the effect of heat treatment on the stability of ochratoxin A residue, the remainder of retained frozen carcass corresponded with toxin - contaminated muscle sample, was traditionally cooked (in boiling water) for 1 hour, 1.5 hour and 2 hours in cases of foreign breed broiler, native breed broiler and foreign breed hen carcasses, respectively. Fifty grams of ready - to - eat muscle sample was finely minced and analysed for ochratoxin A residues which remained after cooking.

### ***2. Estimation of ochratoxin A residues in prepared samples:-***

The analytical procedures were completed according to Nesheim (1973); AOAC Official Methods of Analysis (1984) and Nesheim *et al.*, (1992). Such procedures can be categorized into 4 steps: Extraction of the ochratoxin A from the contaminated tissues; Purification of the extract; Concentration of the purified extract; and Qualitative and quantitative estimation of the toxin.

#### ***Extraction and purification:***

Each 50 gm of prepared samples was introduced into 1 litre flask, and shaken for 30 minutes in a mixture of chloroform and 0.1 M phosphoric acid (250 ml: 25 ml). Lower weights of prepared samples were shaken in proportional volumes of chloroform / 0.1 M phosphoric



acid mixture. The solid residue was eliminated by filtration. The filtrate was purified first by liquid - liquid partition in a separatory funnel, then on a column filled with bicarbonated diatomaceous earth. After washing with 40 ml of hexane and 75 ml of chloroform, the ochratoxin A was eluted with 75 ml of freshly prepared chloroform: formic acid (99:1). The eluate was concentrated with a rotating evaporator. The extract was transferred to a small vial; evaporated to dryness under gentle stream of nitrogen; redissolved in 1 ml of benzene: acetic acid mixture (99: 1) and subjected to thin layer chromatographic analysis.

***Qualitative and quantitative estimation of the toxin by TLC:***

Extracts and standards (1µg/ml) were spotted (10 µl) on silica plates. The plate was developed in a mixture of benzene - methanol - acetic acid (18+1+1), dried, and examined under ultra-violet light. Ochratoxin A exhibited green fluorescence for an Rf of 0.65. A confirmatory test involved a colour change in the fluorescence of the toxin from green to blue after spraying the plate with alcoholic sodium bicarbonate solution. Fluorescence intensities of ochratoxin A spots in sample were visually matched with those of standard spots, to obtain the level of toxin in sample spot and consequently in the tested sample as a whole by microgram / kilogram (µg / kg).

Standard ochratoxin A was purchased from Sigma Chemical Comp. St. Louis, MO.U.S.A.

The statistical analysis of the obtained results was made according to the methods of Snedecor (1971).

## RESULTS

**Table 1:** Numbers and percentages of ochratoxin A - contaminated muscle, fat, gizzard, liver and kidney samples of farm broilers and hens (N\* = 10 for each).

Farm chickens	Foreign breed broilers (about 50 days - old)	Native breed broilers (about 100 days -old)	Foreign breed hens (about 2 years - old)
Muscle	5 (50%)	6 (60%)	8 (80 %)
Fat	7 (70 %)	7 (70%)	9 (90 %)
Gizzard	7 (70 %)	8 (80%)	9 (90 %)
Liver	8 (80 %)	9 (90 %)	10 (100 %)
Kidney	10 (100 %)	10 (100 %)	10 (100 %)

N\* = number of examined samples.

**Table 2:** Mean  $\pm$  SE\*\* levels of ochratoxin A residues in muscle, fat, gizzard, liver and kidney samples of farm broilers and hens ( $\mu\text{g} / \text{kg}$ ).

Farm chickens	Foreign breed broilers	Native breed broilers	Foreign breed hens
Samples	(about 50 days - old)	(about 100 days -old)	(about 2 years - old)
Muscle	5.94 $\pm$ 2.21	7.41 $\pm$ 2.28	9.66 $\pm$ 2.42
Fat	9.51 $\pm$ 2.94	16.02 $\pm$ 4.08	17.39 $\pm$ 3.57
Gizzard	8.45 $\pm$ 2.93	15.69 $\pm$ 4.24	15.32 $\pm$ 3.33
Liver	8.97 $\pm$ 2.83	10.75 $\pm$ 3.07	14.00 $\pm$ 2.54
Kidney	12.90 $\pm$ 3.30	16.67 $\pm$ 3.86	18.84 $\pm$ 3.30

SE\*\* = Standard error.

**Table 3:** Numbers and percentages of muscle, fat, gizzard, liver and kidney samples of broilers and hens, contaminated with ochratoxin A residues, more than the permissible limit ( $20\mu\text{g} / \text{kg}$ )\* (N\*\* = 10 for each).

Farm chickens	Foreign breed broilers	Native breed broilers	Foreign breed hens
Samples	(about 50 days - old)	(about 100 days -old)	(about 2 years - old)
Muscle	-	-	1 (10%)
Fat	2 (20 %)	5 (50 %)	4 (40 %)
Gizzard	2 (20 %)	3 (30 %)	3 (30 %)
Liver	2 (20 %)	3 (30 %)	3 (30 %)
Kidney	2 (20 %)	5 (50 %)	5 (50 %)

\* Permissible limit of ochratoxin A in food as reported by Schuller and Van Egmond (1981).

\*\* N = Number of examined samples.

**Table (4):** Percentages range (minimum-maximum) of ochratoxin A residues disappeared on traditional cooking of contaminated muscle samples of foreign breed broilers, native breed broilers and foreign breed hens, in boiling water for 1 hour, 1.5 hour and 2 hours, respectively.

Farm chickens	Foreign breed broilers	Native breed broilers	Foreign breed hens
	(about 50 days - old)	(about 100 days -old)	(about 2 years - old)
Percentages range of disappeared ochratoxin A	18.2% - 21.6 %	23.1% - 27.5 %	36.3% - 47.4%



## DISCUSSION

Ochratoxin A is a nephrotoxin and its residues are most likely to be found in the blood and kidneys, but also in liver and muscle (Krogh *et al.*, 1974 and Rutqvist *et al.*, 1978).

Results in Table (1) showed that the ochratoxin A residues can be detected in 5 (50%), 6 (60%) and 8 (80%) muscle samples; 7 (70%), 7 (70%) and 9 (90%) fat samples; 7 (70%), 8 (80%) and 9 (90%) gizzard samples; 8 (80%), 9 (90%) and 10 (100%) liver samples; as well as in all kidney samples of foreign breed broilers, native breed broilers and foreign breed hens, respectively. The obtained results were in agreement with those reported by Krogh (1976); Prior and Sisodia (1978); Juskiewicz *et al.* (1982); Reichman *et al.* (1982); Micco *et al.* (1987) and Gracey and Collins (1992), also agreed with findings obtained by Abdelhamid *et al.* (1996) and El-Bagoury and Abdel-Khalek (1997) that revealed the occurrence of ochratoxin A in poultry feeds and related feedstuffs, marketed in Egypt, with high frequencies ranged from 50% to 100%. The highest number of toxin - contaminated samples was of hen's samples, followed by native breed broiler's samples, then foreign breed broiler's samples. These results were expected due to prolonged exposure of old-age chicken to ochratoxin A-contaminated feeds accompanied with difficult excretion of the ingested toxin, rendered the toxin residues in their meat and edible offals of high frequency (Krogh *et al.*, 1976 b and Leistner, 1984). Furthermore, the ochratoxin A-contaminated kidney samples were greater than that of liver, gizzard, fat, and muscle samples. These findings were nearly similar to those obtained by Prior and Sisodia (1978).

The mean  $\pm$  SE values and ranges (minimum - maximum) of ochratoxin A residues, recorded in Table (2), were  $5.94 \pm 2.21$  (0-17.06),  $7.41 \pm 2.28$  (0-17.34) and  $9.66 \pm 2.42$  (0-22.03) micrograms per kilogram ( $\mu\text{g}/\text{kg}$ ) in muscle samples;  $9.51 \pm 2.94$  (0-23.86),  $16.02 \pm 4.08$  (0-33.27) and  $17.39 \pm 3.57$  (0-39.03)  $\mu\text{g}/\text{kg}$  in fat samples;  $8.45 \pm 2.93$  (0-25.01),  $15.69 \pm 4.24$  (0-35.27) and  $15.32 \pm 3.33$  (0-34.83)  $\mu\text{g}/\text{kg}$  in gizzard samples;  $8.97 \pm 2.83$  (0-23.02),  $10.75 \pm 3.07$  (0-22.96) and  $14.00 \pm 2.54$  (0.95 - 24.05)  $\mu\text{g}/\text{kg}$  in liver samples; and  $12.90 \pm 3.30$  (1.08 - 29.14),  $16.67 \pm 3.86$  (0.28 - 31.72) and  $18.84 \pm 3.30$  (2.88 - 34.12)  $\mu\text{g}/\text{kg}$  in kidney samples, of foreign breed broilers; native breed broilers; and foreign breed hens, respectively. The lowest concentrations of the



ochratoxin A were found in young-age broiler's samples, while the highest levels were estimated in the samples of old-age hens, this attributed to the excessive accumulation of this toxin in muscles and organs of farm chickens due to prolonged ingestion of toxin - contaminated feed (Krogh *et al.*, 1976 b and Leistner, 1984). The kidney samples were contaminated with higher concentrations of ochratoxin A than found in each of the liver, gizzard, fat and muscle samples, these findings were similar to those recorded by Prior and Sisodia (1978); Reichmann *et al.* (1982) and Micco *et al.* (1987), also, the toxin levels in analysed fat samples were higher than that found in the muscle samples, such results, may be due to the ochratoxin A is a fat soluble and not readily excreted, so it accumulated in the fatty tissue with high levels (Pitt and Leistner, 1991). Nearly similar levels of ochratoxin A in contaminated chicken's muscle, gizzard, liver and kidney samples were obtained by Elling *et al.* (1975) and El-Bagoury and Abdel-Khalek (1997), while extraordinary high concentrations (114 & 131  $\mu\text{g} / \text{kg}$  approx.) in chicken's kidney and muscle samples, respectively, were determined by El-shewy *et al.* (1997). Of the examined samples in this study, only one (10%) hen's muscle sample; in addition to; 2 (20 %), 5 (50 %) and 4 (40 %) fat samples; 2 (20 %), 3 (30 %) and 3 (30%) from each of gizzard and liver samples; and 2 (20 %), 5 (50 %) and 5 (50 %) Kidney samples, of foreign breed broilers; native breed broilers; and foreign breed hens, respectively, were contaminated with ochratoxin A residues more than the permissible limit (20  $\mu\text{g} / \text{kg}$ ) described by Sculler and Van Egmond (1981) (Table, 3) It could be safely estimated that the high levels of ochratoxin A in the examined samples of chicken's meat and edible offals, may be attributed to the very high concentration of ochratoxin A (up to 5590  $\mu\text{g} / \text{kg}$ ) in the poultry feeds and its related feed stuffs which marketed in Egypt and respated by Abdelhamid *et al.* (1996); El-Bagoury and Abdelkhalek (1997) and El-Shewy *et al.* (1977). High levels of ochratoxin A in the examined samples of chicken's meat and edible offals, obtained in this study, could be estimated according to studies performed by Abdelhamid *et al.* (1996); El-Bagoury and Abdel Khalek (1997) and El Shewy *et al.* (1997) that determined very high concentrations of ochratoxin A (up to 5590  $\mu\text{g} / \text{kg}$ ) in the poultry feeds and its related feedstuffs which marketed in Egypt.

Concerning the effect of heat treatment on the stability of the ochratoxin A residues in contaminated muscles when cooking it in the



boiling water for 1 hour; 1.5 hour; and 2 hours, the results in Table (4) showed disappearance of the toxin by the percentages ranged from 18.2 % to 21.6 %; 23.1 % to 27.5 %; and 36.3% to 47.4 % in the muscle samples of foreign breed broilers; native breed broilers; and foreign breed hens, respectively. Higher losses of the toxin, ranged from 65 % to 87 % in cereals treated by autoclaving for 0.5 to 3 hours, and represented by 47 % in beans after blanching and heat processing for 1 hour, were reported by Trenk *et al.* (1971) and Harwing *et al.* (1974). On the other hand, the levels of ochratoxin A losses in this study may be agreed with that recorded by Schindler and Nesheim (1970); Trenk *et al.* (1971); Davis *et al.* (1972) and Moreau (1979) who described the ochratoxin A as a stable compound; has a melting point of 160 °c; and withstands autoclaving for 3 hours. The lost parts of the toxin may be converted to another, more or less toxic compound, as recorded by Rásonyi *et al.* (1997) who concluded that the ochratoxin A is partly converted to the 3S epimer form (3S - OTA), as a result of heating to temperatures comparable to cooking and roasting.

From public health point of view, the present picture is very dull, for the detection of ochratoxin A with high percentages and high levels of contamination. Its occurrence in different tissues of apparently sound carcasses of farm broilers and hens, may be among factors responsible for the outbreak of nephritic failures in Egypt. Furthermore, the ochratoxin A possess considerable teratogenic and carcinogenic potency, which becomes apparent after the accumulation of lesions due to chronic ingestion of very low levels of toxin over long periods. Ochratoxin A also, produce serious lesions in liver, nervous system and immune system (Leitao *et al.*, 1992).

For providing the consumers with safe poultry meat and edible offals, free or nearly free from ochratoxin A residues, the following suggestive measures should be fulfilled:-

- In poultry feed factories: strict control measures should be applied to assure that its marketed feeds and feedstuffs which intended for poultry farms; are free or nearly free from contamination with ochratoxin A.

- In poultry meat plants: before mass slaughtering of any broiler's or hen's flock, meat and edible offal samples taken from a few representative number of apparently healthy broilers or hens in question, must be analysed for occurrence of ochratoxin A residues which if found



by levels more than 20 µg / kg, the slaughter should be delayed and the feeding of the flock on ochratoxin A - free diets is recommended for 3-4 weeks as a sufficient withholding period to clear the muscles and organs from the toxin (Krogh *et al.*, 1976 b) .

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mouldness, which accentuate the toxicity of the feed. The moulds producing Ochratoxins are widely distributed in soil and decaying organic matter and are associated with moulding feed. Ochratoxins are produced mainly by *Aspergillus ochraceus*; *Penicillium viridicatum* and *Penicillium verrucosum*, as well as by other *Aspergilli* and *Penicillia* (Banwart, 1989). Ochratoxin A is the most common and toxic member of this group of related toxins. In Egypt and other countries, this toxin can be estimated, with very high frequencies and levels in poultry feeds and its related feedstuffs (Shotwell *et al.*, 1968 & 1969; Scott *et al.*, 1972; Basil *et al.*, 1981; Patterson, 1983; El-Kady, 1986; Hassan, 1990; El-Far *et al.*, 1995; Hamad *et al.*, 1995; Abdelhamid *et al.*, 1996 and El-Shewy *et al.* (1997). When farm broilers and hens are exposed to ochratoxin A -contaminated feeds, a portion of the ingested toxin will be retained as residues in their organs, meat and fat, and not quickly eliminated from the tissues (Krogh *et al.*, 1976 b and Leistner, 1984). The only observable lesion in affected chickens is often evidenced as kidney damage, therefore, the remaining parts of carcass may pass meat inspection, and the transfer of ochratoxin A from poultry feed to human food is possible. Ochratoxin A residue, in meat and edible offals of chickens, was previously estimated by many researchers (Elling *et al.*, 1975; Prior and Sisodia, 1978; Juskiewicz *et al.*, 1982; Reichman *et al.*, 1982; Micco *et al.*, 1986; Micco *et al.*, 1987; El-Bagoury and Abdel-Khalek (1997) and El-Shewy *et al.*, 1997). Occurrence of ochratoxin A in human milk and blood have been reported in several European countries (Hald, 1991; Breitholtz-Emanuelsson *et al.*, 1992; Olsen *et al.*, 1992 and Hofmann and Müller, 1994) as well as in Egyptian's urine (Abdelhamid and Saleh, 1996). Since the ochratoxin A could be an environmental major factor for provoking human renal disease and cancer (Bacha *et al.*, 1996; Creppy, 1996 and Simon, 1996); induced carcinomas in male mice when orally administered with large doses (Kanisawa and Suzuki, 1978 and Bendele *et al.*, 1983); is strong immunosuppressive agent in low concentrations (Röschenthaler *et al.*, 1981); and is a relatively stable molecule (Chang and Chu, 1977), even the occurrence of low toxin residue in poultry tissue, becomes of public health significance.

Therefore, this study was planned to throw light on the safety of meat and edible offals of most popular farm broilers and hens marketed at Zagazig city, in relation to Ochratoxin A residues. Consumption of farm poultry meat has been expanding steadily over the last years in Egypt,



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