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Assessment of Ochratoxin (OTA) residues in meat and their biochemical effects in commercial farmed and backyard chicken

Rehab E. M. Gaafar*, Samah A. Abd El Hafez and Haidy E. Mohamed****

*Food Hygiene Lab., **Biochemistry Lab., Animal Health Research Institute, Ismailia Laboratory, Agriculture Research Center, Egypt

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ABSTRACT

In Egypt, the practice of raising chicken in farms as well as backyard is widespread. Ochratoxin A (OTA) is one of the most common mycotoxins in chicken feed and their meat, which poses a concern for both animal and human health. The study aimed to investigate OTA residues by using ELISA technique in meat, liver and kidney samples. Additionally, it aimed to evaluate total protein, albumin, globulin, functions and antioxidant state of both liver and kidney, in addition to assess some meat quality parameters of both farm-raised and backyard chicken. A total of 60 chicken (30 each of farm-raised and backyard chickens) were collected across different regions of Ismailia City, Egypt. The results revealed that incidence of OTA residues was significantly higher in all farm-raised chicken samples than in all backyard chicken samples, with significant higher concentration in kidney samples, followed by liver samples then breast and thigh meat samples. According to the Egyptian and International Standards, neither breast and thigh meat nor liver samples exceeded the permissible limits in both breeding systems. Moreover, farm raised chicken revealed noteworthy decline ($P<0.05$) in serum total proteins and albumin. Conversely, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea exhibited significant increases. A significant reduction in total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) within kidney and liver. Furthermore, there were no significant differences between backyard and farmed raised chicken meat in pH values, moisture%, protein% and ash %. However, significantly higher fat % and thiobarbeturic acid reactive substances (TBARS) content in farm raised chicken meat than backyard ones. In conclusion, OTA residues were higher in farm-raised chicken, inversely affecting their biochemical and oxidative capacity with no differences in meat quality except their higher fat content and higher meat oxidation. Regular monitoring for OTA residues in chickens and their feed with effective management and control strategies are essential for all breeding systems.

Corresponding author: Haidy E. Mohamed, Biochemistry Lab., Animal Health Research Institute, Ismailia Laboratory, Agriculture Research Center, Egypt .
Email: haidyvet_2000@yahoo.com
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INTRODUCTION

A recent trend in chicken meat consumption is gaining popularity. Consumers now seek meat from production systems that prioritize animal welfare, environmental responsibility, enhanced sensory quality and health benefits **(Singh et al. 2022)**. Poultry meat originates from various production systems, each of which can have distinct impacts on carcass and meat quality **(Baéza et al. 2022)**. In Egypt, around 70% of broilers are reared by medium to large-scale commercial farms, while the remaining 30% come from small-scale village farms **(Fahmy et al. 2015)**.

The traditional backyard chicken production system typically involves indigenous breeds that are well-suited to free-range backyard environments. This practice is followed by around 80% of the global rural population **(Singh et al. 2022)**. Consumers view birds raised in a free-range environment or with outdoor access as natural, environmentally sustainable and supportive of animal welfare. Despite the higher price associated with this rearing method, consumers believe it enhances animal welfare and yields products with unique flavor characteristics **(Chen et al. 2013)**. The meat they produce possesses desirable sensory characteristics. It is darker, firmer, and has a more pronounced flavor compared to the meat from commercial broilers **(Jayasena et al. 2014)**. However, the farm-raised chicken production sector, predominantly focused on indoor rearing of fast-growing chickens, has experienced significant growth **(Sharma et al. 2023)**. The success of the farm-raised chicken production sector depends on effective nutritional programs and modern practices, which have greatly enhanced meat production. However, these advancements have led to compromised meat quality due to various challenging factors, such as the consumption of mycotoxins **(Choi et al. 2023)**.

Mycotoxins have the potential to contaminate poultry feed, thereby posing a risk of contamination. Multiple studies have documented cases of human and animal poisoning attributed to the consumption of feed and food contaminated with mycotoxins **(Tatfo Keutchatang et al. 2022)**. Ochratoxins, recognized mycotoxins, are secondary toxic byproducts primarily synthesized by various fungal species, notably those within the genera *Aspergillus* and *Penicillium*. They have the capability to manifest in a diverse array of agricultural commodities during cultivation and storage stages, with occurrences observed globally **(Stoev, 2022)**. Ochratoxins exist in three forms Ochratoxin A (OTA), Ochratoxin B (OTB) and Ochratoxin C (OTC) that have been frequently identified in meat products. Among these, Ochratoxin A (OTA) poses the greatest risk **(Awad et al. 2019)**. The contamination of meat, edible offal, and meat products with OTA is primarily associated with the feed consumption containing Ochratoxins **(Sharafi, et al. 2023)**.

OTA has diverse effects on the health of both humans and animals. It functions as a nephrotoxin and has demonstrated hepatotoxic, teratogenic, immunotoxic and carcinogenic properties across various species **(Stoev, 2022)**. Furthermore, the International Agency for Research on Cancer (IARC) has classified it as a potential human carcinogen, belonging to Group 2B **(Claeys et al. 2020)**. OTA has been identified as the primary factor contributing to the occurrence of "Balkan Endemic Nephropathy" in human **(Awad et al. 2019)**. In comparison to mammals, poultry are often seen as exhibiting a higher level of sensitivity to OTA **(Zhai et al. 2021**). The presence of OTA remains a subject of ongoing discussion, with reports indicating its potential as a carcinogenic, toxic, immunotoxic, and teratogenic agent in poultry (**Koszegi and Poor, 2016)**. Consequently, OTA produces changes in biochemical and hematologic profile of chicks **(Khan et al. 2023)**.

The exposure of livestock animals, including poultry, to OTA via their dietary intake may result in the presence of harmful residues in the final meat products. This contributes to human intake of OTA through indirect transmission **(Zhao et al. 2015)**. This mycotoxin is recognized for its ability to accumulate in the meat and organs of animals due to its high bioavailability and extended half-life, particularly in certain monogastric farm animals **(Pleadin et al. 2021)**. The concentrations of OTA in

many tissues mainly depend on factors such as the duration of exposure, the dosage administered and the route of entry **(Pfohl - Leszkowicz and Manderville, 2007)**. Resistance to heat treatments and tolerance to low pH conditions are notable characteristics of OTA. Common heat treatment methods applied to meat, such as baking, boiling, frying, and roasting, typically, these factors do not lead to substantial decreases in OTA levels **(Sueck et al. 2019)**.

Moreover, OTA residues pose a threat to food safety and security, as elevated levels in animal products can lead to economic losses due to border rejection in both global and local markets. It has been established that Ochratoxin A (OTA) poses as the major meat contaminant than other mycotoxins **(Pleadin et al. 2019)**.

Numerous analytical methods have been employed for the detection and quantification of OTA in feeds and foods. However, the most prevalent techniques include high-performance liquid chromatography (HPLC), Enzyme-Linked Immunosorbent Assay (ELISA), and thin-layer chromatography (TLC) **(Malir et al. 2016)**. The ELISA method offers several advantages over other methods. It enables quick, easy, economical, specific, and sensitive detection of samples **(Widiyanti and Maryam, 2023)**.

Therefore, the aim of this study was to prefer between farm-raised and backyard chicken in respect to the incidence of OTA residues in their meat, liver and kidney, comparing them with the standard's permissible limits, as well as their effects on chicken meat quality, in addition to OTA residues effects on the serum biochemical parameters and antioxidant state of kidney and liver of chicken.

MATERIALS and METHODS

Ethical Approval:

The care and procedure used for broiler chickens of the current trial were permitted by Institutional Animal Care and Use Committee (ARC-IACUC, Ethical approval number ARC-AHRI-19-24).

Collection of chicken:

A total of 60 alive marketed chickens were collected from different regions in Ismailia City, representing 30 commercial farm-raised chickens and 30 commercial backyard chickens.

Blood samples:

Blood samples were taken from wing vein of each chicken of each group and placed in plain tubes for serum separation. After clotting for 15 minutes, the samples were refrigerated for 3 hours. Subsequently, centrifugation at 3000 rpm for 20 minutes separated the serum, which was then stored at -20°C for biochemical analysis.

Tissue samples:

Previously collected chickens were slaughtered; meat (breast and thigh), liver and kidney samples were taken under good hygienic conditions from each chicken. Collected samples were transferred to the laboratory for examination of antioxidant parameters, ochratoxin A, and some quality parameters of meat.

Homogenization of liver and kidney tissues

The liver and kidney tissues were cleaned with ice-cold normal saline, dried with filter papers, and individually wrapped in aluminum foil before being stored at -20°C. Each tissue sample, weighing 1 gram, was then manually homogenized with 4 mL of cold saline solution (0.9% NaCl). Following homogenization, the samples underwent centrifugation at 3000 rpm for 15 minutes at 10°C. Subsequently, the collections were stored separately at -20°C for the assessment of liver and kidney antioxidant parameters.

Biochemical analysis:

In serum : Total protein was measured according to the Biuret method explained by **Zheng et al. (2017)**. Albumin was measured according to **Kouzuma et al. (2002).** Globulin level determined by substracting albumin from total protein level. Creatinine and uric acid were measured according to the method of **Cholongitas et al. (2007)**. Alanine aminotransferase (ALT) and aspartate ami-

notransferase (AST) were assessed by the method of **Huang et al. (2006)**.

In tissues homogenate:

The Total Antioxidant Capacity (TAC) was assessed using a commercially accessible kit (catalog number: TA2513) from Bio diagnostic company, Egypt. The measurement involved utilizing colorimetric techniques to quantify the remaining H_2O_2 through an enzymatic process. This method relies on the interaction between antioxidants present in the sample and externally supplied H_2O_2, allowing for the estimation of TAC (liver and kidney) according to **(Koracevic et al. 2001)**. Antioxidant enzymes including: superoxide dismutase (SOD) activity was determined according to **(Nishikimi et al. 1972)** and glutathione peroxidase (GPx) according to **(Paglia and Valentine, 1967)**.

Quantification of ochratoxin A residues in meat, liver and kidney samples by using ELISA technique:

Previously collected chickens were slaughtered; meat (breast and thigh), liver and kidney samples were taken under good hygienic conditions from each chicken. Collected samples were transferred to the laboratory to be examined. Part of meat samples were examined fresh (zero day) and the other parts were frozen for examination later at constant time interval (at 30 and 60 days).

For ELISA analysis: The samples were thawed directly before analysis. ELISA determinations were conducted using a commercially available kit **(Ridascreen Ochratoxin A; R-Biopharm GmbH, Darmstadt, Germany)**. Sample extraction followed the instructions provided by the ELISA kit manufacturer. In summary, half of the sample was homogenized, and a 10g aliquot of the homogenate was mixed with 17.5ml of 1M hydrochloric acid and extracted with 20ml of dichloromethane. This mixture was shaken for 20 minutes and then centrifuged for 10 minutes at 6000 rpm. The aqueous phase was removed, and the organic phase was back extracted with 15ml of NaHCO3 (pH 8.1). After centrifugation at 6000 rpm for 15 minutes, the aqueous phase was collected. Subsequently, 7.5ml of 1M HCl and 12.5ml of dichloromethane were added, followed by another centrifugation at 6000 rpm for 15 minutes. The organic layer was withdrawn and evaporated to dryness using a rotavapor at 40°C. The resulting residue was reconstituted in 2.5ml of NaHCO3 and directly applied to the ELISA plates **(Matrella et al. 2006)**.

Assessment of some quality parameters of farm-raised and backyard chicken meat samples:

pH measurement of breast and thigh meat samples

The ultimate pH values of breast and thigh meat samples were measured after 24 hours from slaughtering as described by **Karunanayaka et al. (2016)**.

Proximate analysis of meat

The proximate analysis of breast and thigh meat samples were performed according to AOAC guidelines **(AOAC, 2016)**. The moisture contents were quantified by air oven drying method according to AOAC Official Method 990.19. Protein contents were determined by using Kjeldahl Method based on the standard procedure in AOAC Official Method 973.48. Soxhlet extraction method was used to determine fat content according to AOAC Official Method 960.39. Ash content was determined using the dry ashing method AOAC Official Method 999.11.

Determination of thiobarbeturic acid (TBA)

TBA was determined in breast and thigh meat samples on 0, 30 and 60 days of freezing according to Egyptian Organization for Standardization and Quality Control **(EOS, 63-10/2006)**.

Statistical analysis

The obtained findings were statistically analyzed by application of the T- test using **SPSS software, version 19.0 (SPSS Inc., Chicago, IL, USA)**. Statistical significance was determined at $p < 0.05$.

RESULTS

The data in **table 1** showed the results of some biochemical parameters of serum samples of farm- raised and backyard chickens. Additionally, results of some liver and kidney antioxidant activities of farm-raised and backyard chickens were revealed in **table 2.** Moreover, the data in **table 3** presented the incidence of ochratoxin A residues (ppb) in breast and thigh meat, liver and kidney samples of farm- raised and backyard chickens and their acceptability. Finally, the data in **table 4** showed some quality parameters of breast and thigh meats in farm- raised and backyard chickens.

Table 1. Serum biochemical parameters of farm- raised and backyard chickens samples.

Biochemical Parameters	Farm Raised chickens (n =30)	Backyard chickens (n =30)
ALT(U/µl)	30.07±0.99[a]	23.50±1.69[b]
AST(U/µl)	71.05±2.14[a]	56.18±3.5[b]
Total Protein (g/dl)	4.20±0.07[b]	5.02±0.11[a]
Albumin (g/dl)	2.49±0.05[b]	3.14±0.12[a]
Globulin (g/dl)	1.71±0.08[b]	1.88±0.04[a]
urea(mg/dl)	2.14±0.08[a]	1.71±0.09[b]
Creatinine (mg/dl)	0.47±0.01[a]	0.40±0.02[b]

Different superscript letters within the same row show the means are statistically different at P<0.05.

Table 2. Liver and kidney antioxidant activities of farm-raised and backyard chickens samples.

Sample type	Quality parameters	Farm Raised chickens (n =30)	Backyard chickens (n =30)
Liver	SOD (U/g tissue)	199.5±7.55[b]	214.9±8.38[a]
	GPx (U/g tissue)	7.9±0.47[b]	10.2±0.54[a]
	TAC (Mm/g wet tissue)	0.543±0.04[b]	0.749±0.05[a]
Kidney	SOD (U/g tissue)	87.32±3.57[b]	119.13±9.97[a]
	GPx (U/g tissue)	8.4±0.44[b]	10.5±0.67[a]
	TAC (Mm/g wet tissue)	0.558±0.04[b]	0.753±0.05[a]

Different superscript letters within the same row show the means are statistically different at P<0.05.

Table 3. Incidence and acceptability of ochratoxin A residues (ppb) in meat (breast and thigh), liver and kidney samples of farm- raised and backyard chickens:

Sample type	Quality parameters	Farm Raised chickens (n =30)	Backyard chickens (n =30)
Breast meat	Number of Positive Samples (%)	9 (30)	4 (13.3)
	Number of negative Samples (%)	21 (70)	26 (86.7)
	Min. detected	0.01	0.01
	Max. detected	1.98	1.09
	Mean value concentration	$0.47\pm0.12.^{aC}$	0.14 ± 0.06^{bC}
	Number Within MPL (%)	30 (100)	30 (100)
	Number Exceeded MPL (%)	0 (0)	0 (0)
Thigh meat	Number of Positive Samples (%)	9 (30)	4 (13.3)
	Number of negative Samples (%)	21 (70)	26 (86.7)
	Min. detected	0.01	0.01
	Max. detected	1.14	0.57
	Mean value concentration	0.25 ± 0.07^{aC}	0.05 ± 0.03^{bC}
	Number Within MPL (%)	30 (100)	30 (100)
	Number Exceeded MPL (%)	0 (0)	0 (0)
Liver	Number of Positive Samples (%)	26 (86.7)	10 (33.3)
	Number of negative Samples (%)	4 (13.3)	20 (66.7)
	Min. detected	0.01	0.01
	Max. detected	3.75	3.42
	Mean value concentration	2.54 ± 0.21^{aB}	1.04 ± 0.27^{bB}
	Number Within MPL (%)	30 (100)	30 (100)
	Number Exceeded MPL (%)	0 (0)	0 (0)
Kidney	Number of Positive Samples (%)	28 (93.3)	13 (43.3)
	Number of negative Samples (%)	2 (6.7)	17 (56.7)
	Min. detected	0.01	0.01
	Max. detected	4.57	3.89
	Mean value concentration	3.59 ± 0.21^{aA}	1.52 ± 0.33^{bA}
	Number Within MPL (%)	30 (100)	30 (100)
	Number Exceeded MPL (%)	0 (0)	0 (0)

MPL: Maximum permissible limits.
Different lowercase superscript letters within the same row show the means are statistically different at $P<0.05$.
Different uppercase superscript letters within the same column show the means are statistically different at $P<0.05$.

Table 4. Some meat quality parameters of farm- raised and backyard chickens.

Quality parameters	Sample type	Farm Raised chickens (n =30)	Backyard chickens (n =30)
pH	Breast Meat	5.82±0.02[a]	5.78±0.03[a]
Moisture content		74.42±0.05[a]	74.18±0.04[a]
Protein Content		23.69±0.08[a]	23.89±0.02[a]
Fat Content		1.46±0.06[a]	1.15±0.02[b]
Ash Content		1.13±0.01[a]	1.11±0.2[a]
$TBARS_{0\ day}$ (mg/kg)		0.158±0.002[a]	0.132±0.001[b]
$TBARS_{30\ days}$ (mg/kg)		1.183±0.003[a]	1.142±0.006[b]
$TBARS_{60\ days}$ (mg/kg)		2.081±0.005[a]	2.029±0.009[b]
pH	Thigh Meat	6.02±0.02[a]	5.97±0.03[a]
Moisture content		75.67±0.04[a]	75.43±0.06[a]
Protein Content		20.36±0.06[a]	20.69±0.08[a]
Fat Content		3.81±0.18[a]	3.16±0.21[b]
Ash Content		1.02±0.01[a]	0.99±0.01[a]
$TBARS_{0\ day}$ (mg/kg)		0.270±0.014[a]	0.229±0.005[b]
$TBARS_{30\ days}$ (mg/kg)		1.362±0.005[a]	1.327±0.003[b]
$TBARS_{60\ days}$ (mg/kg)		2.173±0.006[a]	2.127±0.002[b]

Different superscript letters within the same row show the means are statistically different at $P<0.05$.

DISCUSSION

The emergency concern for both animal and human health is the presence of OTA in animal feed **(Yang et al. 2020)**. Numerous nations have verified the existence of OTA in animal feed; the percentage and concentration of the contamination with OTA in chicken complete feeds were determined to be 38% and 75% , respectively, according to monitoring done in Pakistan **(Sherazi et al. 2015)**. In Egypt, **Hegazy et al. (2022)** detected ochratoxin in feed of broiler chicken in Dakahlia, Sharkia and Domiate governorates. **Ghada and Emtenan, (2023)** found OTA in chicken feed samples from Beni-Suef Governorate, Egypt which were varied from 2.50 to 11.90 ppb in the winter and from 3.50 to 9.90 ppb in the summer. The examination in Ismailia city, Egypt, conducted by **Hassan et al. (2012)** found that the concentration of OTA varied across various types of rations and feed ingredients. The analysis showed that rations formulated by poultry producers within the farm had notably higher concentrations compared to the main ingredients used in those rations. The amounts of OTA in samples taken from the Egyptian countryside ranged from 18 to 421 µg/kg in wheat **(Azza and Abou-Baker, 2006)**.

The existence of OTA in backyard hens intended for self-consumption is still currently unknown. **Guerrini et al. (2020**) demonstrated that while OTA can be present in backyard hens, it is less common than in farm-raised broilers whose only source of OTA exposure is feed. The backyard birds were raised in a manner very similar to free-range, but because they were allowed to roam around freely, peck and scratch in the grass as they pleased, and eat kitchen scraps, it's possible that they were exposed to less OTA because of their low daily feed intake of both cereal grains and mash.

Serum biochemical parameters

Feeding OTA-contaminated food to bird species causes serious health issues such as stunted development, low feed conversion ratio,

and notable deterioration of essential organs such as the liver and kidneys **(Khatoon et al. 2016)**.

Liver and kidney functions serve as sensitive biochemical indicators of mycotoxin poisoning **(Schiavone et al. 2008)**. In our study the serum ALT and AST, showed a substantial increase ($P < 0.05$) in the farm-raised chickens compared to the backyard chickens. These findings were in consistent with **Awais et al. (2022)** and **Khan et al. (2023)** who had further reported alterations in liver function due to increased ALT and AST values. These increases attributed to hepatocytes damage by toxin and release the enzymes into bloodstream (**Fan et al. 2015)**.

Farm-raised chickens in our investigation had significantly decrease serum total protein, albumin and globulin levels. These results are consistent with those of other studies **Elaroussi et al. (2008); Khan et al. (2023)**. The decline in serum proteins levels observed in birds exposed to OTA may result from inhibition of hepatic protein synthesis occurring post-transcriptionally through competitive inhibition of phenylalanine-tRNA-synthesis. This inhibition halts amino-acylation and peptide elongation processes. One significant consequence of OTA binding to albumin is the delay in its elimination, as it restricts OTA transfer from the bloodstream to hepatic and renal cells, thus prolonging its half-life (**Eid et al. 2022)**.

The results showed that farm-raised chickens' serum, urea and creatinine levels were considerably higher ($P < 0.05$) than backyard chickens. Serum creatinine and urea were frequently used to assess kidney impairment in vivo **(Qing et al. 2021)**. The findings were in line with **Elaroussi et al. (2008)** and **Khan et al. (2023)** as they noted that alterations in renal function, evidenced by elevated levels of creatinine and urea, suggest impaired renal function, affirming the kidney as a principal and primary target of OTA toxicity. Elevated levels of BUN(blood urea nitrogen) can be attributed to inflammatory and degenerative alterations in the kidney, known as nephrotoxicity, induced by OTA **(Anand et al. 2018)**.

Liver and kidney activities of antioxidant enzymes

Despite numerous in vivo and in vitro investigations into the nephrotoxic and hepatotoxic effects of OTA, the exact mechanisms and the influence of oxidative stress on the detrimental impacts of these mycotoxins remain uncertain **(Damiano et al. 2020)**.

OTA has been linked to an imbalance between oxidant and antioxidant parameters in both the kidney and liver **(Palabiyik et al. 2013)** and initiates free radical formation both in the kidney and liver, but not in blood **(Kövesi et al. 2019)**.

Therefore, this study assesses the activities of crucial antioxidant enzymes, including Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), alongside measuring the total Antioxidant Capacity (TAC) levels in liver and kidney tissues. These are vital components of the innate antioxidant defense mechanism and play a crucial role in maintaining the intracellular redox equilibrium **(He et al. 2018; Ma et al. 2018** and **Cheng et al. 2019)**.

The results showed in farm raised chickens that the activities of SOD, GPx and TAC were notably diminished in both liver and kidney tissues compared to backyard chicken. The SOD enzyme serves as the primary line of defense against reactive oxygen radicals by converting them into hydrogen peroxide (H_2O_2). Subsequently, the potent H_2O_2 is broken down into water and oxygen by the GPx enzyme. Inadequate levels of either or both enzymes could result in oxidative damage to cellular structures, highlighting their indispensable roles in maintaining cellular integrity **(Marczuk-Krynickaabcdef et al. 2003)**. The findings were in accordance with findings from prior research conducted by **Li et al. (2020)** who investigated the influence of OTA in the diet of broilers at a rate of 50 µg/kg. They noticed a decrease in the total antioxidant capacity (TAC) and significantly lower levels of SOD and CAT in the kidney compared to the control group. According to their

findings, OTA triggers the generation of reactive oxygen species, resulting in oxidative stress within the kidneys of chickens. The observed reduction in GPx activity, an enzyme responsible for converting hydrogen peroxide to water via glutathione (GSH), can be linked to the decline in selenium levels caused by OTA's interference with essential uptake mechanisms **(Palabiyik et al. 2013)**. **Damiano et al. (2021)** propose that OTA's toxic effects are partially associated with reduced antioxidant enzyme activities and partly with heightened free radical production.

Reactive oxygen species (ROS) play a partial role in the cytotoxic and genotoxic effects of OTA as reported by **Costa et al. (2016)**. Additionally ROS contribute to the reduction in antioxidant potential, affecting glutathione and vitamin E levels, activity of SOD, GPx and CAT of living organisms **(El-Shafie et al. 2015; Zhu et al. 2016** and **Abdel-Wahhab et al. 2017)**. Also **Bhatti et al. (2021)** revealed that a significant reduction in hepatic and renal TAC of the birds in experimental groups fed OTA. OTA present in tissues interacts with copper and zinc ions within SOD molecules, leading to the inhibition of the enzyme's activity **(Meki and Hussein, 2001)**. **Gan et al. (2015)** demonstrated that OTA significantly reduces GPx1 activity due to increased oxidative damage. Incorporating OTA into a broiler's diet, at concentrations of 3.2 or 6.4 mg/kg of food, led to a significant reduction in GPx and SOD across multiple organs, including the kidney and liver **(Hameed et al. 2017)**.

The research conducted by **Marin-Kuan et al. (2006)** provided evidence supporting the theory that oxidative stress induced by OTA may result from a decline in cellular antioxidant enzymes. This reduction could be attributed to the downregulation of genes responsible for antioxidant regulatory elements (ARE), ultimately contributing to OTA-related oxidative harm **(Cavin et al. 2007)**.

On the other hand, **Pozzo et al. (2013**) demonstrated that dietary OTA treatment did not alter the antioxidant response in the liver and kidney. Additionally, **Qing et al. (2022)** showed an elevation in TAC and GPx levels in the liver. This could be attributed to the prolonged exposure to OTA, which activated antioxidative reactions in chicks.

Incidence of ochratoxin A residues in edible tissues (breast, thigh meat and liver) of back yard and farmed raised chicken:

The present results revealed that OTA residues existence in both farm-raised and backyard chickens. The prevalence and concentration of OTA residues in both their liver samples were significantly higher than those in both their breast and thigh meat. This is because the liver, as the body's detoxifying organ, naturally accumulates these toxins as it filters them out of the bloodstream **(Awad et al. 2019)**.

Similar previous findings were recorded by **Iqbal et al. (2014)**, **Al Khalaileh (2018)**, **Awad et al. (2019)**, **Alaboudi et al. (2022)** and **Tatfo Keutchatang et al. (2022)**. However, **Murad (2015)** reported higher percentage of OTA residues in chicken meat than liver. The natural existence of mycotoxins residues in meat and meat products is mainly related to contaminated feed consumption. OTA is accumulated in the animals meat as well as organs because of its high level bioavailability and long life **(Tolosa et al. 2020)**.

Moreover, the current results revealed that incidence of OTA residues found was significantly higher in all farm-raised chicken samples than in all backyard chicken samples. Similar previous results were indicated by **Iqbal et al. (2014)** and **Guerrini et al. (2020)**. At commercial farms, chickens are reared in controlled sheds, their only way to get OTA is from their feed **(Guerrini et al. 2020)**, which can be contaminated by fungi at various stages, including during production, transport, or improper storage. These poor conditions are the main culprits behind high levels of mycotoxins in feed, posing a health risk **(Fahmy et al. 2015)**. Moreover, some farmers might incorporate low price moldy grains in animal feed to reduce costs, pose a health risk for mycotoxin contamination in addition to lowering nutritive values of the grain **(Al Khalaileh, 2018)**. While, the backyard chickens were

raised on pastures as free-range systems, wander freely, peck at the ground, eat fresh fruits and potentially fruit waste, and might even consume molded kitchen wastes (bread). This diverse diet subjected them to potential OTA contamination beyond their daily feed, such as from moldy grains or fruits on the ground **(Guerrini et al. 2020)**.

Presence of mycotoxins residues in chicken meat and liver, as well as meat products were previously reported by several studies as **Tatfo Keutchatang et al. (2022)**, **Ouf et al. (2023)**, **Sharafi et al. (2023)** and **El Asuoty et al. (2023).** OTA is resistant to low acidity and heat treatments as boiling, frying, baking and roasting **(Sueck et al., 2019)**, as well as salting, drying and storage, causing no important changes in these mycotoxins reduction in the final meat products **(Pleadin et al. 2014)**.
Regarding the acceptability of meat and liver samples the concentration of detected OTA residues in all examined breast, thigh and liver samples did not exceeded the maximum permissible limits recommended by **The Egyptian Organization for Standardization (EOS, No. 7136/2010)**, **Commission Regulation No. 1881/2006 (European Commission, 2006)** and **FAO (2004)** which is 5 ppb. Similarly, **Murad (2015)**, **Al Khalaileh (2018)**, **Tatfo Keutchatang et al. (2022)**, **El Asuoty et al. 2023** and **Ouf et al. 2023** previously reported that all meat samples were within the maximum permissible limits.

This cleared the seriousness of potentiality of risks incidence resulted from the consumption of contaminated chicken meat or its offal with ochratoxin A. Consumers exposed to this ochratoxin A may subjected to serious health risks as the nephrotoxicity, hepatotoxicity, genotoxicity and the neurotoxicity, and it can lead to organs and tissues tumors **(Malir et al. 2016)**. It causes carcinogenic immunosuppressive and teratogenic effects; it acts as fertility inhibitor and mutagenic because it has the ability to pass placenta **(Stoev, 2022)**.

Some Quality Aspects of Breast and Thigh Meats of Backyard and Farm-raised chickens:

Many previous researchers reported that outdoor breeding as backyards could improve flavor and quality of meat and meat products when compared with the commercial indoor confined rearing **(Wang et al. 2009)**. However, these outdoor breeding impacts still controversial because quality traits of meat influenced by many several factors as age, genotype, density, nutrition and pasture intake **(Chen et al. 2013)**, as well as environmental factors **(Devatkal et al. 2018)**. Poultry meat and its products are gaining popularity by consumers because of their high nutritive values, low calories, low fat contents and desirable organoleptic attributes **(Michalczuk et al. 2017)**.

Concerning the pH of breast and thigh meat **(table 4)**, results revealed no significant ($P<0.05$) differences were found between backyard and farm-raised chickens. Meat pH has a significant role in preservation of meat and its stability, whereas high pH of meat results in less shelf life, which is preferred to bacterial growth. The decline rate of pH depends on glycolytic enzymes activity just prior to death, and ultimate pH is influenced by muscle initial reserves of glycogen **(Tong et al. 2014)**.

Relatively similar results were obtained by **Tong et al. (2014)**, **Michalczuk et al. (2017)** and **Li et al. (2017)**. Conversely, **Jiang et al. (2011)**, **Almasi et al. (2015)** and **Stadig et al. (2016)** reported lower ultimate pH of chickens breast meat with indoor access. This decline in postmortem pH is believed to be due to free-range access, which might influence muscle fibers size and density, which in turn could affect how quickly the meat becomes acidic after slaughter. Additionally, higher physical activity of outdoor access chickens may lead to the reduction of glycogen reserves in muscles **(Jiang et al. 2011)**. Conversely, several studies recorded lower meat pH in free-range access chickens than from indoor ones as **Ponte et al. (2008)** and **Sun et al. (2013)**, which is believed to be resulted from better ability of free range chicken to cope with stress leading to less stress pre-slaughter, leading to more muscles glycogen remaining **(Ponte et al. 2008)**.

An important parameter for meat quality determination is the proximate chemical com-

position. According to the proximate analysis of breast and thigh meat of backyard and farm-raised chickens, the results in **table 4** revealed no significant (P<0.05) differences in moisture and ash contents as reported by **Stadig et al. (2016)** and **Michalczuk et al. (2017)**. Conversely, **Bai et al. (2022)** reported moisture and ash contents were significantly (P<0.05) higher in backyard than farm-raised chickens. whereas, **Sun et al. (2013)** found free range chicken produced breast meat with significantly lower moisture content.

Moreover, according to protein content, no significant differences were found between the two rearing systems. The results were in concurrence with the findings of **Chen et al. (2013)**, **Stadig et al. (2016)**, **Michalczuk et al. (2017)** and **Bai et al. (2022)**. While, others previously reported that meat of free-range chicken which is similar to backyard had higher protein content than the indoors or farm raised ones as **Jiang et al. (2011)**, **Sun et al. (2013)** and **Sharma et al. (2023)**. However, others reported the protein content to be inferior as **Castellini et al. (2002)** and **Mikulski et al. (2011)**.

Whereas the current results of fat contents revealed significantly higher values in farm raised chickens than backyard ones. This result is consistent with previous studies revealing that rearing chicken with no confinement can reduce fat content of muscle **(Chen et al. 2013** and **Sun et al. 2013)**. The reason for this might be that the chickens move around more, as they have more space to roam. Doing more exercise, in addition to access to healthy and variable food sources, helps to prevent them from storing too much fat (lipogenesis) **(Castellini et al. 2002)**. However, **Stadig et al. 2016** and **Bai et al. 2022** reported that fat contents did not differ between the two breeding systems.

Furthermore, concerning the oxidative state of breast and thigh meat achieved in **table 4**, there were gradual increase in TBARS content under freezing storage, with more significant increase in farm-raised chicken than backyard ones. Oxidative reactions may take place during meat frozen storage targeted mainly proteins and lipids **(Pereira et al. 2022)**. Similar previous results were reported by **(Sharma et al. 2023)** who showed better (p<0.05) antioxidative state in the backyard meat, as well as **(Skřivan et al. 2015)** who showed that the rearing system of chicken affects significantly on TBA value in their meat. Conversely, opposite results were reported by **(Michalczuk et al. 2017)** where no significant differences between the rearing systems. Furthermore, **Ouf et al. (2023)** reported the increase in TBA values during chilling, resulting in about 30% unacceptable samples on the 6th day of chilling.

Numerous research have confirmed that a major way by which mycotoxins damage tissues is the buildup of oxidative stress in the body **(Zhao et al. 2017)**, which can produce high percentages of unstable molecules called reactive oxygen species (ROS), which ultimately lead to tissue injury by damaging proteins and nucleic acids, creating harmful by-products as malondahydes **(Cao et al. 2021)**. In meat, free radicals are one of the essential oxidizing agents lead to fats oxidation, which affects meat quality **(Malekinezhad et al. 2020)**. Fat oxidation of meat proceeds along its storage, representing one of the main spoilage processes which lead to meat quality deterioration, resulting in synthesis of volatile compounds low in molecular weight as aldehydes, leading to the generation of rancidity with unacceptable taste and aroma, which are refused by consumers **(Michalczuk et al. 2017)**. Poultry meat is highly susceptible to this oxidative spoilage because of its high level of unsaturated fatty acids **(Shahidi and Zhong, 2015)**. As oxidation occurs, oxidized meat undergoes significant chemical changes that are potentially harmful to consumers. This is the cause of rancid meat to be spoiled and unfit for consumption **(Ouf et al. 2023)**.

CONCLUSION

Based on the findings of the current study, it can be inferred that both farm-raised and backyard chickens contained OTA residues with higher percentages in farm-raised ones. Their percentages were the highest in kidney, followed by liver then meat. According to Egyptian and international standards, all examined samples were within permissible limits in both breeding systems. These

OTA residues inversely affected the serum biochemical parameters and antioxidant activities of liver and kidney enzymes of farm raised chicken. Concerning meat quality, no differences existed between both farm-raised and backyard chicken except the lower fat content and better oxidative state in backyard ones.

Recommendation

Necessity of increasing the awareness of mycotoxins' potential existence in chicken meat and feed, their financial losses and their potential health risks to both animals and consumers.

Monitoring of OTA residues regularly in chicken meat and feed with effective control and management strategies, are imperative for all breeding systems to ensure chicken meat quality and safety.

Meat quality assessment periodically is required for producing safe products to consumers and determine the best system of breeding.

Regulatory measures and safety protocols for consumers within all breeding systems in poultry industry

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