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Tetracycline Residues in Intensive Broiler Farms in Upper Egypt: Hazards and Risks

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ABSTRACT

Antibiotics are used in poultry farms to enhance growth, feed efficiency and reduce diseases. Additionally, prophylactic treatment is common during periods of stress. Tetracyclines are the most commonly used antimicrobials in food-producing animals. In Egypt veterinary antibiotics are easily accessible with the increased intensive poultry production therefore, this study was conducted to estimate tetracyclines' residue in poultry tissues collected from broiler farms in Upper Egypt. Four members of tetracycline group; Oxytetracycline (OTC), Tetracycline (TC), Doxycycline (DOC) and Chlortetracycline (CTC) were evaluated in 282 samples (muscle, liver and kidney samples)using HPLC. OTC residue was detected in 50, 100 and 93 % of muscle, liver and kidney samples, respectively, while TC residue could be detected in 69, 72 and 86 %, respectively. For DOC and CTC, none of the analyzed samples showed value below their corresponding limit of detection. OTC, DOC and TC highest levels were recorded in liver; 7.23, 20.43 and 1.06µg/g, respectively followed by that of the kidney samples. The residues in all muscle and liver samples were higher than the maximum residue limits of both FAO and FDA reports for DOC. Furthermore, muscle and liver samples exceeded these guideline limits for CTC and OTC, respectively. Health risk of tetracyclines' residue for Egyptian population was estimated to ensure the hygienic fitness for secure consumption. In both adults and kids, the assessed risk for DOC in muscle, liver and kidney was distinctively. The assessed risk of OTC residue in liver and CTC in muscle in adults was also distinctively. Moreover, the assessed risk of TC in Kids for muscle and liver was considerable. So, the potential health hazard for Egyptian population was high from consumption of poultry edible especially liver containing high level of DOC and OTC.

Keywords: Intensive farms, Antibiotics, Residue, Oxytetracycline, Tetracycline, Doxycycline, Chlortetracycline.

INTRODUCTION

Antibiotics are used by the poultry producers and veterinarians to enhance growth and feed efficiency and reduce diseases as they are able to kill or inhibit the growth of microorganisms. Antibiotics usage has facilitated the efficient production of poultry and enhanced the health and well- being of poultry, allowing the consumer to purchase at a reasonable cost, high quality meat. Although these benefits all involved unfortunately, consumer perceptions are that edible poultry tissues could be contaminated with harmful concentration of drug residues (Donoghue, 2003).

Currently, approximately 80% of all food-producing animals receive medication for a part or most of their lives. The most commonly used antimicrobials in food-producing animals are tetracyclines, β-lactams, aminoglycosides, lincosamides, macrolides, and sulfonamides (Lee et al., 2001). Tetracyclines are broad-spectrum antibiotics, widely used in veterinary practice to treat and control a variety of bacterial infections and as growth promoters (De Ruyck et al., 1999). Tetracyclines display an antibacterial action on gram-positive and gram-negative bacteria as well as mycoplasmas, chlamydiae, rickettsia, spirochetes, actinomycetes, and some protozoa (Sundin, 2003). The bacteriostatic action of tetracyclines inhibits protein synthesis by binding to the bacterial 30S ribosomal subunit and present attachment of aminoacyl-tRNA to the ribosomal receptor site (Chopra et al., 1992; Roberts, 1996). Tetracyclines given to birds orally or parentally raise possibility for residues which, remain in edible tissues, particularly when the birds are slaughtered without the observance of withdrawal period (Donoghue and Hairston, 2000; Kan and Petz, 2000). Such residues may pose public health hazards to consumers including toxicological, microbiological, immunological and pharmacological disorders depending on the type of food and the amount of residue present (Heeschen, 1992; Oka et al., 2000). Additionally, the use of antibiotics in related food may lead to resistance in bacterial populations that do not respond to treatment commonly used for human illnesses (Lee et al., 2000). Disorders in the intestinal flora, and possible occurrence of allergic symptoms in people who are allergic to these drugs could make them

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very sick or even cause death in some individuals (Roberts, 1997; Cerniglia and Kotarski, 2005). As stated by the World Health Organization (WHO), the increasing emergence of antibiotic resistance in human pathogens is a special concern, not only for treating infectious disease but also for other pathologies in which antibiotic prophylaxis is needed for avoiding associated infections (WHO, 2000).The spread of antibiotic- resistant bacteria showed serious results: mortality rates doubled in cases when resistant infections are present; furthermore, length of treatments increased often requiring the use of more expensive antibiotics or antibiotic cocktails (WHO, 2007).

In intensive poultry production, one of the most critical problems is keeping birds confined in overcrowded conditions and they are bred and managed for maximum yield. These conditions compromise their health and their immune responses and encourage infectious diseases to develop and spread easily. Without the aid of drugs for disease prevention, it would not be possible to keep the birds productive in the intensive conditions. Behavioral practices such as uncontrolled uses of antibiotics and lack of understanding about drug residues also contribute to food contamination. In the European Union (EU) in the 1990s, a total use of 5 million kg of antibiotics has been reported of these, 3.5 million kg were used for therapeutic purposes (Zhao et al., 2010) while the remaining were used as feed additives for growth promotion (Kemper, 2008). Additionally, many livestock producers use sub-therapeutic doses of antibiotics to prevent diseases which lead to antibiotic residues entering the human food chain (Darwish et al., 2013). According to Food and Drug Administration (FAD, 2014)the amount of antibiotics sold for use in animals raised for meat grew by 16 percent from 2009 to 2012.In Africa, in parallel to the incautious use of antibiotics in human medicine, agricultural sectors consume a large portion (50%) of antibiotics in animal farming (Miller et al., 2003). However, there is no clear regulation controlling antibiotic contamination of feedstuffs in many African countries. In Egypt, chicken is the staple product of livestock production, where its production reached 800,000 tons in 2006, accounting for 50.1% of total meat production (Ministry of Agriculture and Land Reclamation, 2007). Despite the large scale of chicken consumption in Egypt and the potential exposure to antibiotic residues, there is a little information about the presence of TC residues in chicken products (Salem, 2003; Salama et al., 2011). Despite the positive effects of antibiotics, poultry edibles may be contaminated with high concentrations of antibiotic residues.

In order to safeguard human health, internationally recognized organizations such as the WHO, the Food and Agriculture Organization (FAO/WHO, 1996) have set tolerance or maximum residue limits (MRLs) of antimicrobial agents prior to marketing. The increasing demand of food safety has forced research regarding the risk associated with food consumption contaminated by antibiotic residue. The basic step of risk estimation of the antibiotic levels in our food is that the drug concentrations in the edible products are not at levels greater than those established as safe that is referred to as the maximum residue level (MRL) (Riviere, 1999). Recently, the acceptable daily intake (ADI) has been reported to represent the quantitative risk assessment of tetracycline based on the amount of drug residues that can be safely consumed daily for a lifetime (Vragovic et al., 2011).

In Egypt veterinary antimicrobials are easily accessible and under low levels of control. From the safety standpoint, this study was conducted to determine the concentrations of tetracyclines in poultry edibles (muscles, liver and kidney) collected from broiler farms in Upper Egypt. Furthermore, risk assessment of tetracyclines' residue in poultry tissues for Egyptian population was evaluated to ensure the hygienic suitability of broilers edibles for safe consumption.

MATERIALS AND METHODS

Sample collection

A total of 282 samples were collected from broiler edibles in Upper Egypt as following: muscle, liver and kidney samples (70 samples each) were collected from large governmental broiler farms in Qena as well as muscles, liver and kidney samples (24 samples each) were collected from small private broiler farms in Assiut with a history of tetracyclines' application. Both governmental and private farms are intensive system; bird density was 25-30 kg/m^2, with deep litter floor type. All tissue samples were homogenized and stored at −20°C until analysis.

Sample Preparation

The extraction method was performed according to McDonald et al. (2009). In 50 ml centrifuge tube, 200 µl of 0.1M EDTA was added to two grams of tissue sample, then subsequently shaken at 2000 rpm for 2 min. 1000 µl of water was added to each sample and re-shaken for a further 5 min and allowed to stand in the dark at 4ºC for 30 min. Addition of 10 ml acetonitrile (HPLC grade, Honil Limited, London, UK) to each sample was followed with a further 10 min shake and centrifugation at 15000 rpm for 10 min. The supernatant was collected in 15 ml tube and evaporated to dryness under a stream of nitrogen and reconstituted in 1 ml of water. Each sample was then sonicated for 10 min and subsequently centrifuged at 16000 rpm for 10 min. The resulting supernatant was filtered with a 0.45 µm filter and 20 µl was injected onto the HPLC system. Four members of tetracyclines group; Oxytetracycline (OTC), Tetracycline (TC), Doxycycline (DOC) and Chlortetracycline (CTC) were selected as target compounds.

Chromatographic conditions and analysis of the samples

Quantitative evaluation for determination of four members of tetracyclins; OTC, TC, DOC and CTC was performed using Agilent Technologies 1200 Series, G1315D DAD HPLC system at the Analytical Chemistry Unit, Faculty of Science, Assiut University, Egypt. The separation and detection of OTC, TC, DOC and CTC were performed using Zorbax Extend C18 (4.6 × 150mm, 5 Micron, Agilent) analytical column with Diode-array detectors (DAD) at 260 nm. Mobile phase (Isocratic elution) composition varied with the samples' type. Acetonitrile was used for both mobile phase B and strong wash solution. In case of liver samples mobile phase A was Sodium dihydrogen phosphate buffer 25 mM, pH 2.5 and the ratio was: 90% (A): 10% (B). For kidney samples mobile phase A was Sodium dihydrogen phosphate buffer 25 mM, pH 4.5 and the ratio was: 83% (A): 17% (B). Finally, for muscle samples, the phase A was Sodium dihydrogen phosphate buffer 25 mM, pH 2.5 and the ratio was: 83% (A): 17% (B).The flow rate of the eluent was 1 ml/min. The temperature of the column was 35°C. The total run time for each injection was 10, 12 and 15 min for liver, kidney and muscle samples, respectively. The sample injection volume was 20 µl.

Quality control

Standard solutions were prepared for each compound at concentrations of 2.5, 5, 10, 30 and 50µg/ml and calibration curves were prepared by plotting the response factor (the ratio of peak area of analyteversus peak area of internal standard) as a function of the analyte concentration (McDonald et al., 2009). Calibration curves for all analytes were linear in the given range with a correlation coefficient of at least 0.99.

The recovery percentage was estimated to be 91.7%, 118.3%, 104.1% and 108.2% for OTC, TC, DOC and CTC, respectively. The Limit of Detection (LOD) µg/ml for muscle samples was 0.01, 0.2, 1.0 and 0.5, for OTC, TC, DOC and CTC, respectively and the corresponding values for kidney samples were 0.01, 0.1, 0.05, and 0.01 but in case of liver was 0.05, 0.1, and 0.25 for OTC, TC and DOC, respectively. A control positive was carried out for tetracyclines in selected muscle, liver and kidney samples by spiking analyzed samples with different concentrations of tetracycline's standard solution and then the samples were reanalyzed. In order to determine the reliability of instruments, a known standard were run after every 10 samples.

Calculation of Estimated Daily Intake

The estimated risk assessment from consumption of poultry edibles contaminated with antibiotic residue were represented by the Estimated Daily Intake (EDI) which was calculated by the equation given by Juan et al. (2010) with some modification according to the 66th meeting of JECFA (2006) that decided to use the median of the residue instead of the mean, in order to reflect a better estimation of chronic dietary intake.
EDI was determined using the following equation that described by Juan et al. (2010)

$$EDI = \frac{C \; X \; FIR}{BW}$$

EDI (µg /person/day); C is the median concentration of antibiotic in poultry (µg/kg); FIR is the food ingestion rate (kg/day);BW is the average adult body weight (70 kg for adults and 20 kg for kids)

Results obtained were compared with the Acceptable Daily Intake (ADI) and the level of achievement percentage was calculated according to Vragovic et al. (2011) with the following equation:

$$\text{Level of achievement} = \frac{EDI \; X100}{ADI}$$

Acceptable daily intake of tetracycline was 0–0.25 mg/kg body weight (FDA, 2014). Based on the estimated intake and comparison with the acceptable daily intake, the antibiotic risk was assessed as the ‘‘level of achievement’’ (Vragovic et al., 2011)

Statistical Analyses

Statistical analyses were performed usingthe SPSS version 16.0 for windows. The data were described using means, and standard deviations as descriptive statistics. The tissue distribution of antibiotics was compared by General Linear Model (GLM) and Tukey's post hoc test. Statistical difference with P<0.05 was considered as significant.

RESULTS

Tetracyclines could be detected in broilers' muscle, liver and kidney in this study. Figure 1 showed the typical chromatogram of Tetracyclines (OTC, TC, DOC and CTC). While in case of liver the used condition was successful for separation and detection of only OTC, TC and DOC.

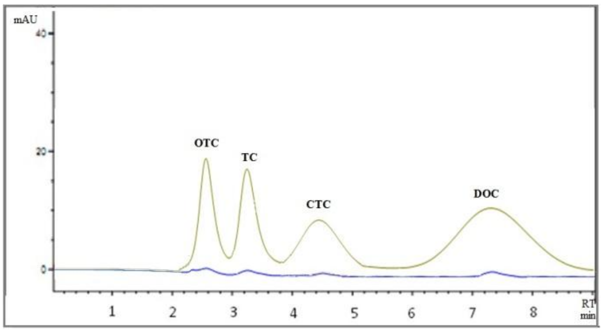

Figure 1: HPLC Chromatogram of sample spiked with a standard mixture of Oxytetracycline (OTC), Tetracycline (TC), Chlortetracycline (CTC) and Doxycycline (DOC)at 50µg/ml; RT: retention time in minutes; AU: absorbance units

The mean concentration of TCs residues in muscles, liver and kidney of broiler was presented in figure 2. As shown in figure 2(a) the highest OTC mean concentration was detected in liver (7.23µg /g) while the lowest was detected in muscle (0.073µg /g). There was a significant difference (P<0.01) between OTC residue in liver and that of muscle and kidney. OTC residue was detected in 50, 100 and 93 % of muscle, liver and kidney samples, respectively Levels of TC residue in the analyzed samples are presented in figure 2(b). TC residue could be detected in 69, 72 and 86% of muscle, liver and kidney samples, respectively. It was found a significant difference (P<0.01) between level of

TC in liver and that of muscle while the difference between TC level in liver and kidney was not significant. It was observed that liver has the highest level of TC residue (1.06µg /g) in comparison to other samples. The data presented in figure 2(c) shows the level of the estimated DOC residue in muscle, liver and kidney samples. As in OTC and TC the highest DOC level was recorded in liver (20.43µg /g) followed by that of kidney and the lowest was recorded in muscle samples. Assessment of tissue distribution of antibiotics represented that CTC concentration in muscle was significantly higher compared to kidney as shown in figure 2 (d). CTC residue level in muscle was 1.94µg /g while that in kidney was 0.69µg /g. For DOC and CTC, none of the analyzed sample showed value below their corresponding limit of detection.

From our study it was found that OTC level from liver (8.48µg /g) in samples from small farms in Assiut was higher than large farms in Qena (6.88µg /g). While liver samples from large farms showed a higher DOC residue level (24.24µg /g) however, the differences between the levels in small and large farms were not significant. Kidney samples collected from large farms showed higher levels of DOC, TC and CTC. For muscle samples from both large and small farms, residue levels were nearly the same for TC, CTC and DOC but samples from small farms exhibited OTC level lower than the detection limit. Finally, it was observed that the highest antibiotic residue was recorded for DOC in liver and kidney representing 20.43, 14.4 µg /g, respectively.

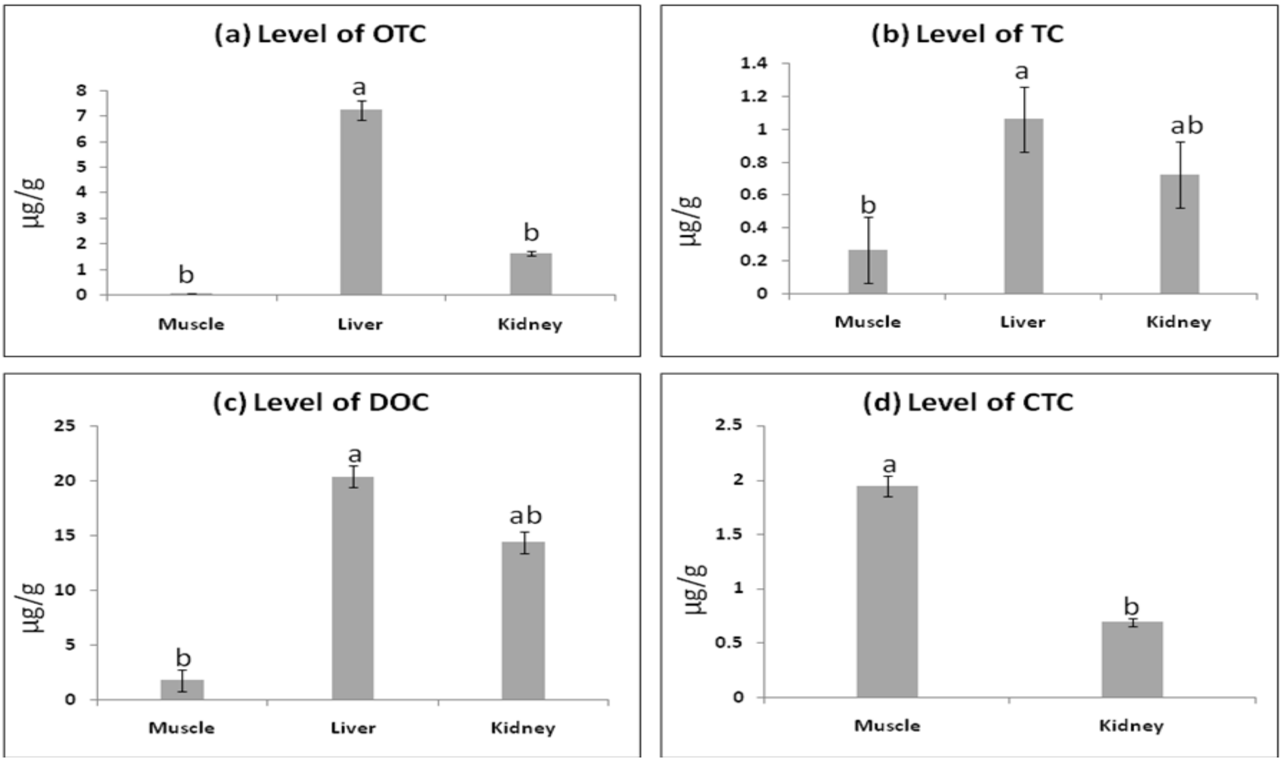

Figure 2. Level of Tetracycline group (OTC, TC, DOC and CTC) expressed as µg/g in muscle, liver and kidney samples from broiler farms in Upper Egypt; abc: means with different letters are significantly different (P<0.05)

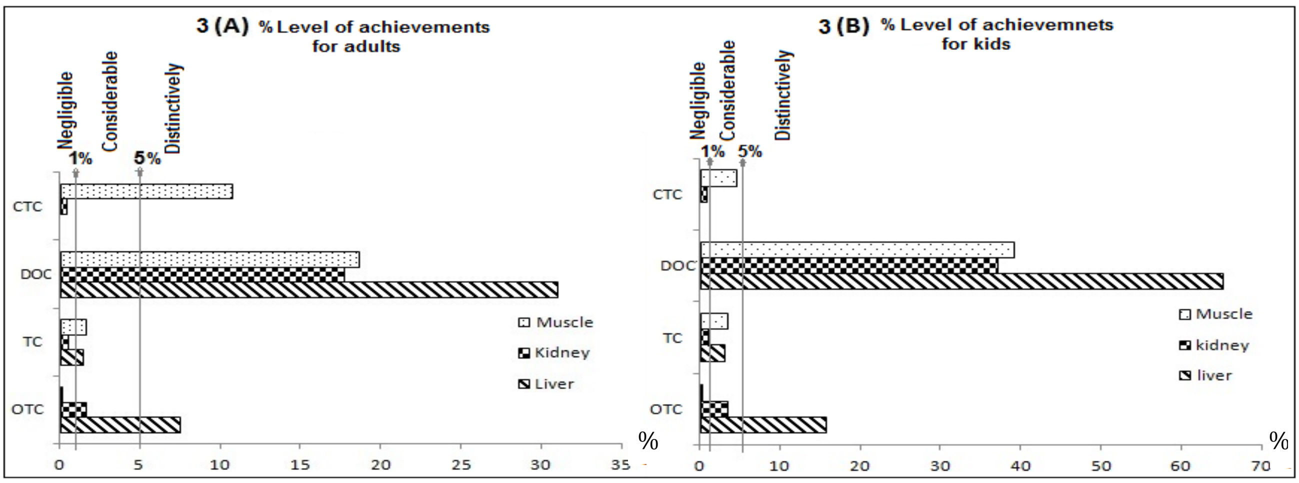

Figure 3. Level of achievement percentage of tetracyclines' residues in muscle, kidney and liver samples of broilers (33-40 days old)

Table 1. The frequency of poultry samples containing tetracycline residues higher than the maximum residue limits recommended by the FAO/WHO and FDA for tetracycline group

		Muscle			**Kidney**			**Liver**		
		Large	**Small**	**Total**	**Large**	**Small**	**Total**	**Large**	**Small**	**Total**
OTC	FAO/WHO	16.7%	0%	12.5%	54.5%	100%	64.3%	100%	100%	100%
	FDA	33.3%	0%	25%	90.9%	100%	92.9%	100%	100%	100%
TC	FAO/WHO	66.7%	75%	68.8%	18.2%	0%	14.3%	71.4%	75%	72.2%
	FDA	66.7%	75%	68.8%	45.5%	0%	35.7%	71.4%	75%	72.2%
DOC	FAO/WHO	100%	100%	100%	90.9%	100%	92.9%	100%	100%	100%
	FDA	100%	100%	100%	90.9%	100%	92.9%	100%	100%	100%
CTC	FAO/WHO	100%	100%	100%	9.1%	0%	7.1%	-	-	-
	FDA	100%	100%	100%	45.5%	0%	35.7%	-	-	-

The FAO/WHO limits for tetracyclines are 200, 1200 and 600 µg/kg for muscle, kidney and liver, respectively; The FDA limits for tetracyclines are 100, 600 and 300 µg/kg for muscle, kidney and liver, respectively.

Table 2. Comparison of tetracyclines' residues in poultry edibles (µg/g) with the levels reported by other researchers

Location	**OTC**			**TC**			**DOC**			**CTC**			**Reference**
	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	Muscle	Liver	Kidney	
Egypt	**0.07**	**7.23**	**1.63**	**0.27**	**1.06**	**0.73**	**1.76**	**20.4**	**14.4**	**1.94**		**0.69**	**Our study**
El-Sharkia, Egypt	0.394												Hussein and khalil (2013)
Giza, Egypt	0.11-1.089	2.93		0.25-0.311			0.13-6.0	0.18-5.26		0.15-1.67	0.211-8.15		Salama et al. (2011)
Croatia				0.002									Vragovic et al. (2011)
Iran				0.039									Ehsani et al. (2010)
Mexico	0.083-2.05			2.56									Miranda et al. (2009)
China							0.005-0.008						Shen et al. (2010)
Pakistan	0.03-0.085	1.27	2.12										Shahid et al. (2007)
Iran	0.088	0.576	0.517										Salehzadeh et al. (2006)
Assiut, Egypt				≥ 0.1	≥ 0.1								Salem (2003)
Assiut, Egypt	17.1-21.4												Karmy (2001)
Saudai Arabia	0.96	1		0.72	0.84		0.77	0.36		0.82	0.88		Al-Gamdi et al. (2000)
Belgium							0.01-1.68						De Wasch et al. (1999)
Mexico				0.48-1.84									Vázquez -Moreno et al. (1990)

The results obtained in this study were compared with the Maximum Residue Limits (MRL) for TCs. MRL according to both FAO/WHO (1999; 2004) and Canadian limit (2015) was 200, 600 and 1200µg /kg for muscle, liver and kidney, respectively. While the corresponding limits according to FDA (2004) and EC (2010) were 100, 300 and 600µg /kg. The frequency of the samples containing TCs residues higher than the MRL is shown in Table 1. It can be noticed that all muscle and liver samples were higher than the MRL of both FAO and FDA for DOC. Meanwhile, muscle and liver samples exceeded the guideline limits for CTC and OTC, respectively. However, muscle and kidney samples from the small farms in Assiut showed mean concentration below FAO and FDA limit for OTC and CTC, respectively.

The total dietary exposure levels of TCs (OTC, TC, DOC and CTC) determined in this study were compared with the ADI to assess the potential health risk faced by consumers (adults and kids) as shown in Figure 3.We assumed that the average daily consumption for adults was 100, 20 and 20 g for muscle, liver and kidney, respectively. However, for kids the average consumption was 60, 12 and 12 for muscle, liver and kidney, respectively. Acceptable Daily Intake of tetracycline is 0–0.25 mg/kg body weight (FDA, 2014). Based on the estimated intake and comparison with the acceptable daily intake, the risk was assessed as negligible when the ''level of achievement'' was less than 1% of acceptable daily intake (<1% of ADI), as considerable if ''level of achievement'' was 1–5% of acceptable daily intake (1–5% of ADI) and as distinctively if ''level of achievement'' was greater than 5% of acceptable daily intake (>5% of ADI) (Vragovic et al., 2011). Figure 3 (A) and (B) presented the level achievements of TCs calculated according to the median value for adult and kids, respectively. The level of achievement for DOC in muscle, liver and kidney was >5% of ADI in both adults and kids. However, the level of achievement for OTC residue in liver and CTC in muscle in adults was >5% of ADI. Finally, the level of achievement for Kids was 1–5% of ADI for TC in muscle and liver as well as OTC in kidney.

DISCUSSION

Levels of antibiotic residue in poultry edibles

Antibiotics have been used in animal feed for about 50 years not only as an anti-microbial agent, but also as a growth-promoting and performance improvement agent. Tetracyclines are common additives in feed for livestock and poultry. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005; Niewold, 2007). The reasons to use growth promoter include a more efficient conversion of feed to animal products, an increased growth rate and a lower morbidity/mortality rate. In poultry, growth promoters and control Clostridium perfringens infections, which are potentially fatal. It is estimated that this translated into an improvement of 1.5per cent, with added economic benefits from the reduction of Clostridium perfringens infections (JETACAR, 1999).

Although many trails have been done to recognize CTC in liver samples, it was difficult to obtain a separate chromatogram which might be due to the matrix or analyte effect on the chromatogram or their rapid elimination from the body. This situation was reported by Tyrpenou and Xylouri-Frangiadaki (2009) that OTC and TC residues were detected and identified more easily with HPLC than CTC. Explanation was suggested that TCs pose the same physicochemical properties and CTC is the most active one**.** Its stability is low in strong acid or basic conditions resulting in the reverse formation of the epimers (4-epi-tetracyclines) in pH 3.0 (Søeborget al., 2004). Furthermore, matrix effect on the chromatographic determination of antibiotics including alteration in the chromatogram and the effect on the analyte behavior was also suggested by Codony et al. (2002). CTC residues may be eliminated rapidly from broilers' tissues and its residue concentration in liver after the last medication day was found to be very low as reported by Tyrpenou and Xylouri-Frangiadaki (2009) that liver was essentially free of CTC residues two days after withdrawal**.**

Residue of TCs was estimated in poultry edible consumed by Egyptian in Qena and Assiut Provinces. The levels of TCs members analyzed in this study were compared with the levels previously reported by other researchers obtained from the open literature as reported in table 2. It can be observed that the levels of OTC in muscle samples were lower than that reported by Karmy (2001) and Hussein and Khalil (2013), this may be due to the high degree of bacterial resistance to OTC specially gram negative bacteria so the poultry producer prefer to use another TC member such as DOC which reflect the importance of continuous monitoring of poultry edibles for the drug residue.

It was clear from this study that DOC showed the highest level in liver (20.4µg /g) and kidney (14.4µg /g) followed by OTC. This result was in disagreement with that reported by Salama et al. (2011); who found that OTC was the highest followed by DOC. This may be attributed to the great affinity of DOC to the lungs and it is therefore useful for treatment of bacterial respiratory infections. DOC is the preferred agent among the tetracycline members however; its price in the Egyptian market is high compared with OTC but due to its high antibacterial efficiency the rate of its use is increasing in the broiler farm especially with the high degree of bacterial resistance to the other members of TCs group. DOC acts effectively against many Gram-positive and Gram-negative bacteria like Bordetella, Campylobacter, E. coli, Haemophilus, Pasteurella, Salmonella, Staphylococcus and Streptococcus spp. as well as Chlamydia, Mycoplasma and Rickettsia spp. in addition, DOC is rapidly and well absorbed from gastrointestinal tract and it has a longer half- life (15-22 hours). Following absorption DOC is widely distributed through the body with the highest level in the kidney and liver and in bone and dentine (Anadón et al., 1994). Laczay et al. (2001) indicated that DOC has a high relative liposolubility (5 to 10 fold increases in relation to older tetracyclines) which readily compensates for the high protein binding and it is eliminated slowly from tissues. The effective dose of DOC is 20 mg/kg/day for 4 days in drinking water and its effective withdrawal period is 7 days (Anadón and Martınez-Larranaga, 1999). Briefly, higher DOC level in broilers may be due to increase administration rate, as well as the unpaid attention to its withdrawal period.

As appear from the obtained results the higher tissue concentrations of DOC, OTC and TC were reported in liver and kidney than muscle samples. Similar findings were reported by Salehzadeh et al. (2006), Salama et al. (2011) and Sattar et al. (2014). This was anticipated because most antibiotic are eliminated from the body via the bile and the same extent via the kidney, moreover, the higher antibiotic residues in liver and kidney could be attributed to the role of liver in drug metabolism and detoxication by its microsomal enzymes as well as to the role of kidney in the filtration and clearance of blood from any undesirable constituents (Lin and Lu, 1997).

However, high residual level of OTC was recorded in liver followed by kidney. OTC is poorly metabolized in target animals and excreted practically in its parent form, due to its high water solubility (Slana and Dolenc, 2013). In addition, it is cheap and available as well as it is produced in different trade names and forms in many companies in Egypt. Also, it is used for control of infections and at sub-therapeutic levels for disease prevention and as growth promoting agent.

In our study the four members of TC group were evaluated in various poultry edibles collected from both large and small broiler farms. Similar findings were previously reported by Al-Ghamdi et al. (2000) and Salama et al. (2011). These obtained results reflect the extensive use of multiple TCs member in broiler farms. Multi-TC use is not clinically acceptable as these drugs share the same mechanism of action and cover the same microbial spectrum (Chopra and Roperts, 2001). Therefore, this practice may contribute significantly to the development of microbial resistance to the drugs (Al-Ghamdi et al., 2000). Many reports indicated that microbial resistance to antibiotics may arise as result of animal exposure to antimicrobial agents with the resistance being possible transferred to human pathogens (Al-Ghamdi et al., 1999). Antibiotics are used frequently in intensive farms at a rate of 2 to 50 grams per ton for improved performance in the animals and this rate is increased in times of stress as the prophylactic treatment is important during these periods.

Guideline limits and risk assessment for Egyptian consumers

Intensive breeding for food production depend on antimicrobials; that has led during recent decades to a considerable increase in the use of veterinary antibiotics for therapeutic, disease-preventive or growth promoting reasons (Anadón and Martínez-Larrañaga, 1999). As a consequence, the distribution and use of antibiotics in poultry have been the focus of major regulatory initiatives by the responsible agencies worldwide furthermore; health problems associated with antibiotic residues have raised concern when they still persist at unacceptable levels at slaughter (Anadón and Martínez-Larrañaga, 1999). Turner (2011) mentioned that there are different uses of antibiotics; treatment of disease, metaphylaxis, prophylaxis and growth promoter. Treatment of disease (therapeutic use) occurs at high doses for a relatively short period of time however, intensive poultry breeding could not allow treating individual bird so usually; the whole flock will be treated (metaphylaxis). For prevention of disease (prophylaxis) using low, sub-therapeutic doses of antibiotics, over a period of several weeks and sometimes longer, when thought that the birds are at a risk of infection, finally, for growth promotion very low sub-therapeutic doses of antibiotics are given to birds to increase their performance and productivity and it can last for a large part of the bird's life (Turner, 2011). Such practices will lead to antibiotic residues entering the human food chain (Darwish et al., 2013).

The frequency of the samples containing TC residues more than the MRL recommended by the FAO/WHO and FDA is shown in Table 1. All muscle and liver samples in this study contained DOC level higher than MRL of both FAO and FDA. As well as OTC in all liver samples was higher than MRL, meanwhile, CTC level in all muscle samples exceeded MRL. While, muscle and kidney samples from the small farms in Assiut showed mean concentration below these limit for OTC and CTC, respectively. It's clear from these results that antibiotic residues already exist in our food chain. These high levels of antibiotic residue may be attributed to the unpaid attention to the withdrawal period of the antibiotic and/ or because long-term administration of these drugs in feed that used for controlling infections, which is considered to be one of the most common causes of chicken morbidity (Al-Ghamdi et al., 2000). In previously reported studies in Egypt reported by Karmy (2001) and Hussein and Khalil (2013) OTC residues in all muscle samples exceeded the recommended MRL decided by FDA and EC (100 µg /kg). However, Salem (2003) reported that only 21.875 % and 31.25 % of muscle and liver samples, respectively contained TC exceeded the limit of FAO/WHO. And Salama et al. (2011) found that 8, 8.67 and 13.33 % samples of breast, thigh and liver, respectively, had TC residues above the MRL recommended by FAO/WHO. In addition, the level of TCs above the MRL, decided either by FAO/WHO or EC, have been reported in chicken products in many countries such as Mexico (Vazquez-Moreno et al., 1990), Belgium (De Wasch et al., 1998), Saudi Arabia (Al-Ghamdi et al., 1999), Iran (Salehzadeh et al., 2006) and Pakistan (Shahid et al., 2007). The recommended withdrawal time for TCs group is 7 days. The withdrawal periods are intended to ensure that no harmful residues remain in food products after slaughter and an adequate withdrawal period predicts that the drug concentrations in the edible tissues are below the MRLs (Anadón and Martínez-Larrañaga, 1999).

Risk assessment (risk characterization) brings together hazard characterization and exposure assessment (Renwick et al., 2003; Woodward, 2008).

The standard approach to assess the safety of chemical contaminants in foodstuffs intended for human consumption is the ADI which represents the total drug residue that can be safely consumed daily throughout one's life (Anadón and Martínez-Larrañaga, 1999). The establishment of the ADI from the determination of a no observable effect level (NOEL) and application of an appropriate safety factor provide the hazard identification and characterization (Anadón and Martínez-Larrañaga, 1999). The total dietary exposure levels of TCs determined in this study were compared with the ADI to assess the potential health hazard faced by consumers (adult and kids) as presented in figure 3. From our study in both adults and kids, the assessed risk for DOC in muscle, liver and kidney was distinctively additionally, the assessed risk of OTC residue in liver and CTC in muscle in adults was also distinctively. Moreover, the assessed risk of TC in Kids for muscle and liver as well as OTC in kidney was considerable. From these obtained results we assume the potential health hazard for Egyptian population was high from consumption of poultry edible especially liver containing high level of DOC and OTC.

In general words a number of possible human hazards related to antibiotic residues have been reported including; allergic/toxic reactions, chronic toxic effects (microbiological, carcinogenicity, reproductive and teratogenic effects) occurring with prolonged exposure to low levels of antibiotics and development of antibiotic-resistant bacteria in treated animal (Gomes and Demoly, 2001; Raison-Peyron et al., 2001).

Potentially, microbiological effects are one of the major health hazards in human beings and the antibiotic residues present in edible tissues can produce bacterial resistance in the consumers which consider one of the major reasons of therapeutic failures amongst such peoples (Al-Ghamdi et al., 1999). Therefore, critical problems resulted from consumption of food with antibiotic residues especially in kids. TC group has been considered by Kloppenburg et al. (1995) as immunosupressive agent. Long-term exposure to tetracycline class during tooth development (last half of pregnancy, infancy and childhood to the age of 8 years) may cause permanent discoloration of the teeth (yellow-gray-brown) and enamel hypoplasia (Sánchez et al., 2004). Formation of a stable calcium complex in any bone-forming tissue and decrease in fibula growth rate has been observed in prematures due to tetracycline and animal studies indicated that tetracyclines cross the placenta causing toxic effects on the developing fetus that often related to retardation of skeletal development (Arora and Hans, 2007). In addition, benign intracranial hypertension which means abnormally high pressure inside the skull, manifested by headache, blurred vision, diplopia, vision loss and papilledemahas been associated with tetracyclines' exposure (Donnet et al., 1992; Weller et al., 1994). Photosensitivity manifested by an exaggerated sunburn reaction may be observed in some individuals (Smilack, 1999).

CONCLUSIONS

Concentrations of tetracyclines in poultry edibles (muscles, kidney and liver) collected from broiler farms in Upper Egypt and, risk assessment of tetracyclines' residue in poultry tissues for Egyptian population were evaluated to ensure the hygienic suitability of broilers edibles for safe consumption. This study reflects the abundant and improper usage of antibiotic (tetracyclines) in intensive poultry farms in Upper Egypt with great potential health risk for Egyptian population specially kids, consuming poultry edibles containing high levels of tetracycline residues. DOC and OTC in poultry edibles were much higher than the recommended guideline residue limit. Meanwhile, higher level of tetracyclines' residue usually observed in liver and kidney rather than in muscle due to their role in metabolism and excretion of antibiotics. Accordingly, consumption of internal organs of broiler is not recommended for human consumption.

Recommendation

Now, urgent practice should be applied in intensive poultry farms in order to reduce antibiotic application. Firstly, general improvement of broilers health conditions is the first guard through regulation of the bird's environment and avoid overcrowding to minimize the stress. In addition, supplements of natural products as probiotics, prebiotics, antioxidants and plant extracts have been reported as a candidate replacement for antibiotics and could used as growth promoter in broiler chicken (Capcarova et al., 2010). Appling a restrict bio-security strategy for prevention and control of disease in the farm. Vaccination could offer a good protection for the whole flock against wide range of pathogenic bacteria.

Public awareness of the antibiotic residue problem in food is not enough and more governmental efforts are needed to control this problem. The extensive use of antibiotic as growth promoter and for disease prevention rather than for treatment infection specially without following the proper withdrawal time of the antibiotic should be controlled and prohibited by the law. The responsibility for residue control and prevention cannot lie solely upon a governmental agency; rather the responsibility must be shared by the government, producers, veterinarians, marketing associations, and other interested parties, who must try for both healthy and efficiently grown animals as well as a safe food supply.

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