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Assessment of Doxycycline and Oxytetracycline Residues in Broiler Meat

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ABSTRACT
This study aims to detect the residues of Doxycycline, Oxytetracycline in broiler meat and giblet, using high performance liquid chromatography (HPLC), Doxycycline (DOC) residues were detected in 25%, 55 % and 70 % from the examined samples of meat, liver and kidney, respectively. Moreover, the levels of DOC residues (µg/kg) ranged from 3.4 to 15.9 µg/kg with a mean value 8.39 ± 0.14 µg/kg for meat,13.3 to 2753.5 µg/kg with a mean value 1142.85 ± 21.09 for liver and from 25.1 to 3661.6 µg/kg with a mean value 1305.59 ± 27.68 µg/kg for kidney. Oxytetracycline residues were detected in 25%, 60% and 45% of meat, liver and kidney, respectively. Moreover, the levels of oxytetracycline residues (µg/kg) ranged from 0.2 to 128.1 µg/kg with a mean value 37.52 ± 0.41 µg/kg for meat, 34.5 to 1248.0 µg/kg with a mean value 435.31 ± 12.86 µg/kg for liver and 26.8 to 412.7 µg/kg with a mean value 162.23 ± 5.35 µg/kg for kidney. Also this study revealed that frying is more efficient than boiling in decrease the residue limit of these two antibiotics.

1. INTRODUCTION

Chicken is the most common type of poultry in the world. The term broiler is applied to chickens that have been bred for meat production.

Veterinary drugs are widely used in the poultry to treat and prevent diseases. Despite obvious benefits, extensive use of these drugs can lead to residues in poultry meat products.

Antibiotics may be given periodically for several days during the life cycle of the broiler as growth promoters to increase growth-rate and productivity. The use of growth promoters is characterized by administration of very low-dose of antibiotics on a regular basis, mostly over a lifetime of the food-producing animal and given through feed. Antibiotic growth promoters are known to suppress the gut bacteria leaving more nutrients for chicken to be absorbed (Mathur and Agarwal, 2014).

The antibiotic given for animal treatment presents toxic residue for human health. These residues may be in the form of initial compounds or may change to be as metabolites with a modified structure (Wolton, 1993).

Tetracyclines (TCs) are among the most commonly used compounds in livestock production, including oxytetracycline (OTC), tetracycline (TTC), chlortetracycline (CTC) and doxycycline (DOC).

The non-observance of withdrawal periods, unregulated use of drugs and lake of awareness on the proper use of antibiotics lead to the presence of the antibiotic residues (Shitandi, 2004).

The antibiotic residues in meat that presented in human food may cause breast cancer, teratogenic effect, hypersensitivity and bactrial resistance (Thomas, 1994).

So the aim of this study to detect the residues of Doxycycline, Oxytetracycline in broiler meat and giblet, using high performance liquid chromatography (HPLC) and study the effect of different cooking methods (boiling and frying) on the residues of these two antibiotics.

2. MATERIALS AND METHODS

2.1. Determination of antibiotic residues in broiler meat using HPLC: (Senyuva et al., 2000):

2.1.1 Collection of samples:

A total of 60 random samples of fresh broiler meat "thigh meat", liver and kidney (20 of each) were collected from different traditional poultry shops located at different markets in Menoufia governorate. All collected samples were separately put in clean sterile polyethylene bag and transferred in an ice box without undue delay to the laboratory (residues analysis unit, Reference laboratory for quality control on poultry production, Animal Health Research Institute, Dokki) for determination of their levels of antibiotics residues.

2.1.2 Reagents and Chemicals required for HPLC analysis:

Water, acetonitrile, and methanol are all HPLC grade. The standards and other chemicals were purchased from Sigma-Aldrich (USA). Phosphate buffer (pH = 8.5), 0.05 mol/L: 8.7 g of potassium phosphate dibasic were dissolved in HPLC grade water to make 1 liter. Standard stock solutions (1 mg/mL) were made daily fresh in methanol. Spiking solutions were made by appropriate dilution of the stock solutions in phosphate buffer.

Principle:

In liquid chromatography (LC), a moving liquid (the mobile phase) carries the sample across a stationary phase (the solid support found within a LC column). The sample components separate based on their differing affinity with stationary phase. Every liquid chromatography usually includes the following key components: a pump system for solvent delivery, a sample injector, a column or columns, detectors and a data handling system.

2.2. Determination of DOC and OTC. (Senyuva et al., 2000):

2.2.1 Extraction of the drug from the samples:

All samples were finely diced with scissors after trimming of the external fat and fascia. Two grams of each sample to be analyzed were weighed using digital balance and then cut into very small pieces and subsequently ground into fine powder using Sartorius mincer. Then the powder was homogenized in a blender for 2 min. and 0.1 gm of citric acid was added. One ml of nitric acid (30%), 4 ml methanol and 1 ml deionized water were added to this mixture, respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min. and centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 um nylon filter, 20 µl of solution was injected into HPLC for analysis.

2.2.2 Chromatographic condition:

A mobile phase of methanol and formic acid 0.1% using a gradient method with a flow rate of 1.5 ml/ min. at 25°C were carried out. The separation was done on Hypersil gold C18 (10um, 100×4.6 mm) columns with mobile phase as described above. Detection was performed with UV detector set at 350 nm wave length. Quantification of residues in samples was obtained and calculated from areas under curves extrapolated automatically by the software (Chromo Quest 5).

2.2.3 Calibration curve:

The curve was prepared by using concentrations of 10, 20, 30, 40, 50 and 60 µg /L of DOC and OTC in eluent. These standards were prepared from the daily prepared stock solution and treated as 100 mg of DOC and OTC standard was accurately weighed and put in a 100 ml volumetric flask, the powder was dissolved in 100 ml of methanol to make a stock solution. The detection limit for DOC and OTC was 0.01 ppm, while the retention time was 4.7 minutes for DOC and 3.9 minutes for OTC.

The concentration of each antibiotic residue in the samples was calculated with reference to a calibration curves obtained from work solutions of DOC or OTC. For the preparation of the work solutions, DOC or OTC hydrochloride (Sigma Aldrich USA) stock solutions (1mg/ ml in methanol) of the antibiotics were diluted to several concentrate ions by using methanol as diluent.

The collected eluate was evaporated to dryness under a nitrogen stream at 30°C in a water bath. The sample was reconstituted in 1ml of dipotassium hydrogen phosphate buffer. The sample was mixed and filtered through 0.45 µm filters before injection into the LC system.

2.2.4 Liquid chromatography operating conditions:

Injection volume, 50 µl; flow rate, 1 mL/min; wave length, 232 nm; column temperature, 35°C; stop time, 15 min; post time, 6 min.; mobile phase 0.05 M phosphoric acid : acetonitrile = 75:25 (PH 3.0 v/v).

2.2.5 Quantization of the amount of antimicrobial residue by peak height or peak area analysis:

Spiked samples were prepared by adding 500 µl spiking solution to 5 g of each antibiotic as well as control sample (free of such antibiotics) to obtain spiking levels equal to1µg/ml. Accordingly, the retention time estimated by which the concentrations of these antibiotics could be measured. Calculation of the exact concentration of antibiotic residue in the examined sample in correlation to the standard curve for each antibiotic was applied by using 4 known standard solutions of such antibiotics.

Part 2: Experimental Part (Heat treatment): ***Javadi et al. (2011)***

The main purpose of the work is studying the effect of some cooking methods on the concentration of antibiotic residues. Accordingly, 3 positive samples of broiler meat and liver containing low, medium (around permissible limit) and high concentrations of DOC and OTC were subjected to the various cooking methods used at home. Sample weight 10g, thickness 2.5 cm and core of temperature 71.5°C.

Heat treatment as boiling at 100°C for 30 minutes and frying at 200°C for 15 minutes was applied on the positive samples which proved to contain DOC and OTC residues to determine the efficacy of each cooking method on the stability of such serious residues.

3. RESULTS AND DISCUSSION

3.1. The incidence of doxycycline residues in the examined samples using High Performance Liquid Chromatography (HPLC):

High-performance liquid chromatography (HPLC) is the analytical technique which widely used in pharmacokinetic and drug residues studies. It becomes the most powerful method for the confirmation of antibiotic residues in different matrix. Concerning HPLC methods are relatively easy to develop and are very suitable for the analysis of small-molecular-weight compounds. The methods can easy transfer to different laboratories without modification Moats (1997).

Results achieved in table (1) recorded that the DOC residues were detected in 25%, 55 % and 70 % from the examined samples of meat, liver and kidney, respectively. Moreover, the levels of DOC residues (µg/kg) ranged from 3.4 to 15.9 µg/kg with a mean value 8.39 ± 0.14 µg/kg for meat,13.3 to 2753.5 µg/kg with a mean value 1142.85 ± 21.09 for liver and from 25.1 to 3661.6 µg/kg with a mean value 1305.59 ± 27.68 µg/kg for kidney .

According to Egyptian Organization of Standardization "EOS" No. 3692 (2008), which tabulated the MRLs of DOC residues in meat, liver and kidney is 200,600 and 1200 µg/kg, respectively 35% and 30 % of the examined liver and kidney samples, respectively were unaccepted. In contrast, the meat were accepted within the permissible limit Table (2).

Relatively higher results were obtained by Hosnia et al. (2015) who recorded that, highest levels of DOC residues in liver was 20.43 µg/kg. However, lower results were reported by Cetinkayaa et al. (2012) where the levels of DOC residues (µg/kg) ranged from 19.9 to 35.6 µg/kg in chicken meat samples.

The use of HPLC method for detecting the antibiotic residues in this study was confirmed by Salisbury et al. (1990), MecCracken et al. (1995) and Croubles et al. (1996) who used microbial assay and HPLC methods for detection of the antibiotic residues. Moreover, FAO/ WHO (1998) and Jevinova et al. (2003) mentioned that HPLC could identify the antibiotic at level below the MRL.

The health risk of tetracycline residue for Egyptian population was estimated to ensure the hygienic fitness for consumption. In both adults and kids. The assessed risk of DOC in meat, liver and kidney was distinctively, as well as for OTC residue in liver and CTC in muscle of adults. 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 edible poultry especially liver containing high level of DOC and OTC residues (Hosnia et al. 2015).

1.1. The incidence of oxytetracycline residues in the examined samples by HPLC:

The results given in table (3) revealed that Oxytetracycline residues were detected in 25%, 60% and 45% of meat, liver and kidney, respectively. Moreover, the levels of oxytetracycline residues (µg/kg) ranged from 0.2 to 128.1 µg/kg with a mean value 37.52 ± 0.41 µg/kg for meat, 34.5 to 1248.0 µg/kg with a mean value 435.31 ± 12.86 µg/kg for liver and 26.8 to 412.7 µg/kg with a mean value 162.23 ± 5.35 µg/kg for kidney.

The MRLs of OTC in meat, liver and kidney are 200,600 and 1200 ug/kg, respectively, According to "EOS" No. 3692 (2008), so table (4) revealed that 15% of the examined liver samples were unaccepted.

In contrast, the examined samples of meat and kidney were accepted where their OTC residue was within the permissible l

Table (1): Statistical analytical results of doxycycline residues (ppb) by HPLC in the examined broiler̛s meat and giblets samples (n=20)

Chicken tissues	+ve samples No	+ve samples %	Min.	Max.	Mean ±S.E*
Meat	5	25	3.4	15.9	8.39 ± 0.14
Liver	11	55	13.3	2753.5	1142.85 ± 21.09
Kidneys	14	70	25.1	3661.6	1305.59 ± 27.68

S.E*= Standard error of mean

Table (2): Acceptability of the examined broiler̛s meat and Giblet samples according to their contents of doxycycline residues (n=20).

Chicken tissues	Maximum Residual Limit (ppb)*	Accepted samples No	Accepted samples %
Meat	200	20	100
Liver	600	13	65
Kidneys	1200	14	70
Total (60)		47	78

*** Egyptian Organization of Standardization "EOS" No. 3692 (2008)**

Table (3): Statistical analytical results of oxytetracycline residues (ppb) in the examined broiler̛s meat and giblets samples (n=20).

Chicken tissues	+ve samples No	+ve samples %	Min.	Max.	Mean ±S.E*
Meat	5	25	0.2	128.1	37.52 ± 0.41
Liver	12	60	34.5	1248.0	435.31 ± 12.86
Kidneys	9	45	26.8	412.7	162.23 ± 5.35

S.E*= Standard error of mean

Higher results were obtained by Al-Ghamdi et al. (2000), Salehzadeh et al. (2006) who recorded that the positive samples showed MRLs 27.77%, 95.55% and (18.88%) in muscles, liver and kidney samples, respectively, Olatoye and Ehinmowo (2009), Hussein and Khalil (2013) , Hosnia et al. (2015) and El-Bagory et al. (2016) who recorded that the positive samples showed 55%,70% and 70% of the examined samples of local broilers meat, liver and kidneys were positive However, lower results were reported by Sattar et al. (2014), Sewond et al. (2014) and Lara et al. (1992) who recorded that the levels of OTC residues (ug/g) ranged from 0.012 - 0.014µg ⁄ g in chicken meat samples.

The residues of OTC may pose a health threat to consumers, depending on the type of food and amount of residues. Also the human health problems resulting from intake of subchronic exposure level of OTC include gastrointestinal disturbances (Barker & Leyland, 1983).

Oxytetracycline residues have a teratogenic risk to the foetus with allergic reaction (Schenck and

Callery, 1998). Also, OTC residues help in development of resistant pathogens for human and animals (Van de Bogaard and Stobberingh, 2000).

Table (4): Acceptability of the examined samples of broiler̀s meat and giblets according to their contents of Oxytetracycline residues (n=20).

Chicken tissues	Maximum Residual Limit (ppb)*	Accepted samples	
		No	%
Meat	200	20	100
Liver	600	17	85
Kidneys	1200	20	100
Total (60)		57	95

*** Egyptian Organization of Standardization "EOS" No. 3692 (2008)**

Table (5): Effect of different cooking methods on the doxycycline residues (ppb) in broiler̀s meat and liver.

Trial	Control		Boiling				Frying			
			Content		Reduction %		Content		Reduction %	
	meat	liver	meat	liver	meat	liver	meat	liver	meat	liver
1	3.4	191.6	0	0	100	100	0	0	100	100
2	8.7	628.5	1.4	73.8	83.9	88.2	0	9.4	100	98.5
3	15.9	2051.3	4.6	519.6	71.1	74.7	2.1	42.7	86.8	97.9
Mean	9.33	957.1	2	197.8	85	87.6	0.7	17.4	95.6	98.8

Table (6): Effect of different cooking methods on the oxytetracycline residues (ppb) in broiler̀s meat and liver.

Trial	Control		Boiling				Frying			
			Content		Reduction %		Content		Reduction %	
	meat	liver	meat	liver	meat	liver	meat	liver	meat	liver
1	8.1	58.8	0	0	100	100	0	0	100	100
2	29.3	945.2	10.7	151.6	63.4	84.0	0	21.5	100	97.7
3	128.0	1135.4	33.1	307.2	74.2	72.9	14.6	46.9	88.5	95.8
Mean	55.1	713.3	14.6	152.9	79.2	85.6	4.9	22.8	96.2	97.8

Effect of different cooking methods on antibiotic residues (ppb) in broiler meat and liver.

The obtained results in table (5) revealed that the mean of reduction percentage of DOC residues in broiler meat sample containing the drug after boiling and frying were 85% and 95.6%, respectively, as it not exceed the permissible limits according to EOS. No. 3692 (2008).

Also, the obtained results in table (5) record that the mean of reduction percentage of DOC residues in broiler liver sample containing the drug after boiling and frying were 87.6% and 98.8% , respectively, as it not exceed the permissible limits according to EOS. No. 3692 (2008).

While the obtained results in table (6) declared that the mean of reduction percentage of OTC residues in broiler meat sample containing the drug after boiling and frying were 79.2% and 96.2%, respectively, as it not exceed the permissible limits according to EOS. No. 3692 (2008).

Finally the obtained results in table (6) declared that the mean of reduction percentage of OTC residues in broiler liver sample containing the drug after boiling and frying were 85.6% and 97.8% , respectively, as it not exceed the permissible limits according to EOS. No. 3692 (2008).

The present results nearly agree with those recorded by Hussein and Khalil (2013) who showed that frying significantly diminish the percentage of OTC residues (95.7 %). While lower result recorded by Hsu (1997) who showed that boiling and stir-frying degraded 39-48 % of the OTC in chicken muscle tissues,also lower result recorded by Marouf and Bazalou (2005) they showed that the effect of cooking method as frying process on oxytetracycline residues and the reported reduction percentage was 85.71 %. In contrast the higher results were obtained by El-Bagory et al. (2016) who revealed that application of various cooking methods; boiling, frying of chicken muscles indicated that cooking had an effect in reducing the concentration of OTC residues with mean reduction % of 84.52% and 93.62% after boiling and frying, respectively. .

From these results, it was concluded that cooking methods (boiling and frying) have strong effects on the OTCand DOC residues in broiler meat and liver in total and partial degrading of these residues. So, application of heat treatment like boiling, and frying may lead to destroying of all drug residues. Therefore, using a higher temperature and longer time can lead to great reduction in antibiotics residues in meat and liver and it can give a margin of safety for consumers El-Bagory et al. (2016). .

Amount of TC residues is significantly affected by cooking where by both cooking time and methods were found to be important. Though all cooking methods are effective, microwaving exhibited significantly fast reduction of all the four types of TC. Reduction in TC concentrations during boiling and deep-frying was due to migration of the TC from the meat to the cooking medium (water and oil) while during the microwave ing process, reduction was due to juice exuding out from the tissue. The overall loss of TC residues was due to denaturation of protein-TC compounds. The observed findings may be helpful in confirming and selecting the ideal method for cooking so as to effectively reduce TC. (Nguyen et al, 2013).

Cooking methods for long time, high temperature and small thickness of meat can lead to great reduction in antibiotics residues as temperature easily reach the core of meat. Frying can lead to great reduction in antibiotics residues in meat and liver than boiling.

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