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Detection of Some Antibiotics Residues in Chicken Meat and Chicken Luncheon

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Abstract

A total of 40 random fresh chicken breast and chicken luncheon samples (20 of each) from different retail markets in Ismailia governorate, Egypt were collected and examined using high performance liquid chromatography (HPLC) to evaluate the antibiotics residues of oxytetracycline and ciprofloxacin. The obtained results indicated that the mean values of oxytetracycline were 0.5 ± 0.1 and 1.4 ± 0.2 µg / g in chicken breast and chicken luncheon respectively, while the mean values of ciprofloxacin were 0.02 ± 0.01 and 0.03 ± 0.01 µg/g., respectively. Oxytetracycline Residues were found to be higher than the maximum residual limits and Ciprofloxacin residue is lower than MRL. The obtained results confirmed widespread misuses of antibiotics especially oxytetracycline in farms and lack of application of recommended withdrawal times. The public health significance as well as some recommended measures to improve the quality of such food articles were discussed.

Key words: chicken meat, chicken luncheon, antibiotics residues, oxytetracycline, ciprofloxacin, HPLC.

Introduction

The chicken meat and further processed ready to eat chicken luncheon are not only tasteful, easy quick to prepare and the cheapest of all meats but also provides a unique well balanced source of proteins with essential amino acids , minerals and vitamins needed and required by all ages . Chicken meat and chicken luncheon represent a high regular percent from poultry products in Egyptian diet.

Antibiotics are widely used as therapeutic, prophylactive , growth promoting agents and nutritive purposes in poultry production (**Donoghue, 2003 and Jinap *et al.*, 2010).**

This wide spread use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which

include; carcinogenicity, mutagenicity, bone marrow toxicity and allergy **(Nisha, 2008)** as well as appearance of a resistant strains of pathogenic bacteria **(Hussein and Khalil , 2013).**

Oxytetracycline (OTC) is one of the most commonly prescribed antibiotics in veterinary medicine because of its broad spectrum bacteriostatic activity , low cost and more easily use by oral administration through drinking water or feed. It is a natural tetracycline compound that is derived from the fungus *Streptomyces rimosus* or synthetically produced, which is poorly metabolized in the body and excreted in its parent form, due to its high water solubility **(Slana and Dolenc, 2013).** However, overuse and insufficient lengths of withdrawal of OTC antibiotic in poultry production resulted in the presence of residues that may endanger human health **(Centikaya *et al*., 2012)** reach to teratogenic malformation to the fetus, hypoplasia in developing teeth when administered to infants **(Senyuva *et al.*, 2000)**.

Ciprofloxacin is a synthetic fluoroquinolone (FQ), with extensive utilization in animal and human (+ 18 years old) as mentioned by **(Brown, 1996).** It is a broad spectrum antibiotic acts by damaging the bacterial DNA and can enter cells easily. Therefore, is often used to treat intracellular pathogens. Ciprofloxacin related to the increased prevalence of resistant bacteria especially campylobacter spp. **(Jennifer *et al.*, 2007)**. Chondrotoxic effects and tendon rupture can be induced by fluoroquinolone **(Petrovi *et al.*, 2006)** which emphasis the importance of prevention in children and young people.

High performance liquid chromatography (HPLC) which employed in our study was not tasked for measuring concentration of antibiotic residues, but for detection their presence in chicken meat and chicken luncheon. This technique is simple, exact, detect the lowest residual limit and can be executed easily**.**

Healthy safe food must be free of antibiotics residues or their limits below the maximum residual limits (MRL) recommended by the international Codex Alimentarius Commission (CAC) and Egyptian Organization of Standardization **(EOS).**

The aim of this work is to ensure food safety and protect public health by a reliable screening analysis to determine the residuals level of oxytetracycline and ciprofloxacin as common veterinary antibiotics in chicken meat and chicken luncheon.

Material and Methods

1- Collection of samples: - : A total of 40 fresh chicken breast and chicken luncheon (20 of each) were collected from different retails markets in Ismailia governorate. The collected samples were transported to animal health research

institute (AHRI), Giza, Egypt under chilling for further examination by HPLC **(Agilent Technologies 1200 Series)**.

2- **Chemical examination (HPLC) -:** The collected samples divided into two groups. Oxytetracycline group and Ciprofloxacin group each group contained chicken breast meat and chicken luncheon (20 of each).

Oxytetracycline group:-

Extraction: (**Şenyuva *et al.*, 2000)** 2 g of sample was homogenized in a blender for 2 min and then 0.1 g citric acid, 1 ml nitric acid (30%), 4 ml methanol and 1 ml deionized water were added respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and then centrifuged for 10 min at 4000 rpm, Filtered through a 0.45 µm nylon filter.

Chromatographic condition: Mobile phase was distal water / acetonitrile (85:15 v/v)

Column (C18 – 150 x 4.6 mm –5 µm), Temp. of column 25ºc, Flow rate 1.5 ml/min.

Fluorescence detection wave length 360 nm. Detection time (4-6 min), Injected volume: 25µl.

Ciprofloxacin group:-

Extraction: (Verdon *et al.*, 2004) 2 g of sample was weighed in a 50 ml polypropylene centrifuge tube, homogenized for 2 min and then 8 ml of trichloro acetic acid 5% (TCA) was added and vortex for 1 min., rotary agitated for 10 minutes , then centrifuged for 5 minutes at 14000 rpm, filtered through a 0.45 µm nylon filter.

Chromatographic condition Mobile phase was 0.01 M phosphoric acid / acetonitrile (80:20 v/v), Column(c 18 – 250 x 4.6 mm –5 µm) Flow rate: 0.3 ml/min.

Fluorescence detection wave length (excitation 280 nm – emission 450 nm)

Detection time (10-12 min) Injected volume: 25µl.

Quality control :- Standard solutions were prepared for oxytetracycline and ciprofloxacin (Sigma –USA) 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 analyte versus 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.

3- **Statistical analysis:** The results are expressed as mean ± standard Error (SE). The antibiotic residues were expressed as part per million (ppm). Data were statistically analysed using Microsoft excel and statistical analysis system software **(SAS version 9.1, SAS Institute, Inc., 2003).**

Results and Discussion

The antibiotic residues in poultry products especially chicken breast and ready to eat chicken luncheon is an important problem worldwide **(Bertini *et al.*, 2003)**.

The results in **table (1)** express the oxytetracycline residuals level which detected in chicken breast which ranged from not detected to 2.5 µg/g with mean value 0.5 ± 0.1 µg/g while in chicken luncheon the results ranged from not detected to 2.9 µg/g with mean value 1.4 ± 0.2 µg/g this level is higher than MRL approved by **(EOS 3692/2008** and **Hussein and Khalil, 2013 and Swafy *et al.*, 2015).** The results not agreed with **(Hussein *et al.*, 2016)** as he couldn't detect oxytetracycline residues in chicken luncheon and this could be attributed to the effect of heat treatment during processing. In **table (3)** the accepted limits represented only 30%, 15% on chicken breast and chicken luncheon respectively, with 70 %, 85% Un-accepted samples where the **EOS** had set the MRLs for oxytetracycline to be 0.2 ppm for chicken muscle. Many authors indicated that the sufficient heating temperature, cooking method and time can reduce some antibiotics residues but it does not generally provide an additional margin of safety for consumers **(Fathy *et al.*, 2015 and Hussein *et al.*, 2016).** As shown in **figure (1)** calibration curve for spiked standard oxytetracycline were up to 0.99 and the Chromatographic determination of the oxytetracycline residues in chicken breast.

The results in **table (2)** represented the ciprofloxacin residual level which ranged from not detected to 0.1 µg/g with mean value 0.02 ± 0.01 µg/g and from not detected to 0.09 µg/g with mean values 0.03 ± 0.01 µg/g in Chicken breast and Chicken luncheon respectively. These results agreed with **(Petrovi *et al.*, 2006 and Sattar *et al.*, 2014)** that recorded the lowest residue level of ciprofloxacin in chicken muscles than chicken organ especially liver**.** But lower results were recorded by **(Omotoso and Omojola, 2015).**

In **table (3**), the accepted samples were 90 and 100% while un-accepted were 10% and zero % of chicken breast and chicken luncheon respectively. These results emphasize the true of unnecessary exposure of ciprofloxacin with a low dose for a long period has its drawbacks on consumers' health **(Khan *et al.*, 2015).** The withdrawal period allowed for the ciprofloxacin concentration to decrease below MRLs level in the meat is four days **(Petrovi *et al.*, 2006 and Maisa and Nashwa, 2011)**. This withdrawal

time increased in poultry treated with ciprofloxacin according to the hepatic and or renal function which increases protein binding of the drug leading to an increased withdrawal time period up to 23 days **(Khan *et al.*, 2015).**

Codex Alimentarius Commission **(CAC)** and the **EOS** does not determine MRL for fluoroquinolones including ciprofloxacin but MRL established by the European Union (**EC /2010)** is 100 µg/ kg **(Tavakoli *et al.*, 2015)**. As shown in **figure (2)** calibration curve for spiked standard Ciprofloxacin were up to 0.98 and the Chromatographic determination of ciprofloxacin residues in chicken breast.

Table (1): The Oxytetracycline residual levels expressed by (ppm) in chicken breast and chicken luncheon as compared with accepted limit by **EOS 2008/3692.** (n =20 of each)

Item	Chicken breast	Chicken luncheon	Limit ppm (µg/g)
Min.	**ND**	**ND**	**≤ 0.2**
Max.	**2.5**	**2.9**	
Mean ± SE	**0.5 ± 0.1**	**1.4 ± 0.2**	

(ND = not detected)

Table (2): The Ciprofloxacin residual levels expressed by (ppm) in chicken breast and chicken luncheon as compared with accepted limit by **EC/ 2010.** (n = 20 of each)

Item	Chicken breast	Chicken luncheon	Limit ppm (µg/g)
Min.	**ND**	**ND**	**≤ 0.1**
Max.	**0.1**	**0.09**	
Mean ± SE	**0.02 ± 0.01**	**0.03 ± 0.01**	

(ND = not detected)

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Table (3): Incidence of Oxytetracycline and Ciprofloxacin residual levels accepted and Un-accepted in chicken breast and chicken luncheon.

Item	Oxytetracycline				Ciprofloxacin			
	Chicken breast		Chicken luncheon		Chicken breast		Chicken luncheon	
	No.	%	No.	%	No.	%	No.	%
Accepted	6	30	3	15	18	90	20	100
Un-accepted	14	70	17	85	2	10	0	0

Figure (1): Chromatographic determination of oxytetracycline residues in chicken meat

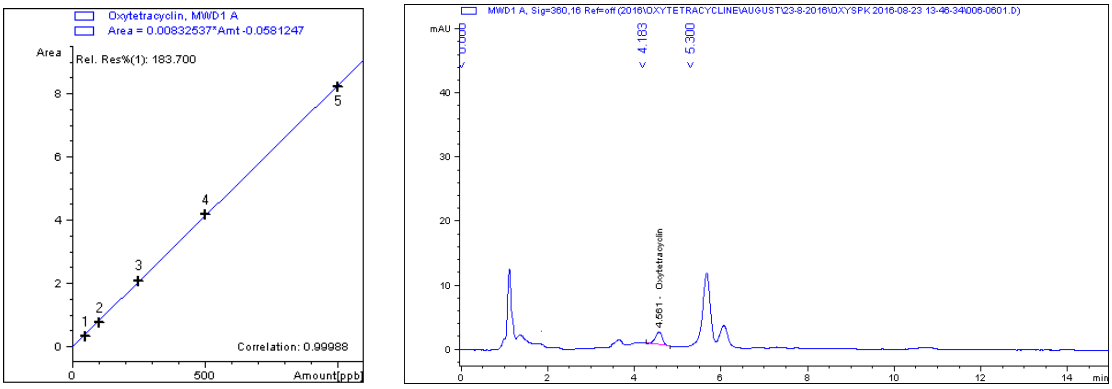

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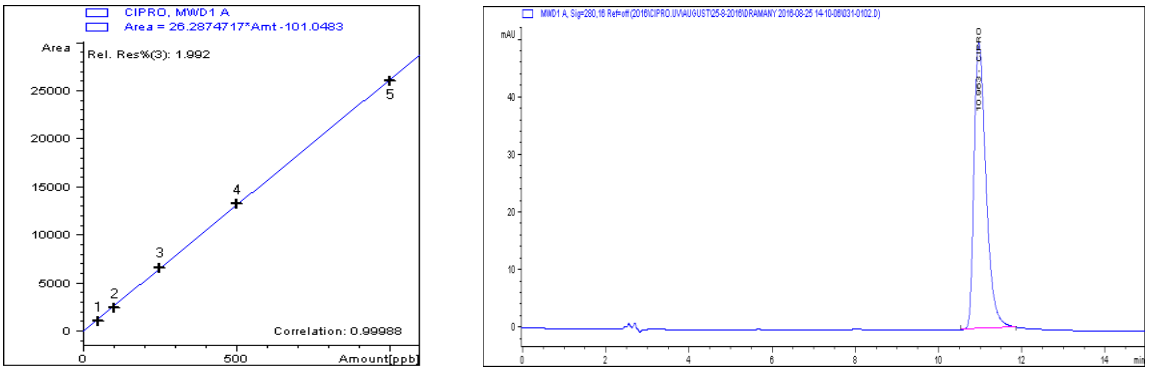
Figure (2): Chromatographic determination of ciprofloxacin residues in chicken meat



Conclusion and Recommendation

Results confirmed the presence of antibiotic residues of both oxytetracycline and ciprofloxacin in chicken breast and ready to eat chicken luncheon samples which pose a potential hazard to consumers. So, veterinary authorities should control the use of antibiotics in poultry farms and banned their use as growth promoter.

Rules should be taken to ensure the proper withdrawal periods before slaughtering and marketing. In addition, a monitoring policy should be implemented to ensure the conformity of poultry meat with international standards.

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