



Enrofloxacin residues in chicken meat and giblete

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ABSTRACT

It is very important to achieve the safety and sanitary quality of the meat as the presence of antibiotic residues in food stuff can cause hazards to human so that the aim of this study is evaluation the level of enrofloxacin residues in chicken meat and giblets and the effect of different cooking methods on reduction of these levels. One hundred and twenty random samples of chicken breast, thigh, gizzard and liver (30 of each) were collected from different chicken slaughter shops located in Menoufia government for evaluation of their enrofloxacin residues. The collected samples were examined by using microbiological inhibition technique (MIT) and High Performance Liquid Chromatography (HPLC) in addition to the effect of heat treatment (boiling, grilling and frying) on these residues was studied. The obtained results revealed that the incidence of enrofloxacin residues in chicken breast, thigh, gizzard and liver were 13.33%, 16.67%, 26.67% & 40% by MIT and 13.33%, 20%, 30% & 43.33% by HPLC, respectively. The results indicated that 85% of the examined samples not exceeded the MRL of enrofloxacin residues. The reduction% of enrofloxacin levels in thigh muscle samples was 28.3%, 17.8% & 11.2% by boiling, 57.4%, 43.6% & 35.2% by frying and 69.5%, 62.4% & 47.7% by grilling of such samples. The public health significance and some recommendations to control such antibiotic residue in chicken tissues for human safety were discussed.

Keywords: chicken meat, gizzard, liver, enrofloxacin, MIT, HPLC.

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1. INTRODUCTION

In poultry, antibiotics are widely used as growth promoters in low doses in poultry feed for increasing growth rates, reduce mortality of growing chickens and increasing body weight gain with improving feed conversion, while for prophylaxis or therapy much higher dose levels for prevention and control of diseases as well as growth stimulants, therefore antibiotic usage had facilitated their efficient production and also enhanced the health and well-being of poultry by reducing the incidence of

diseases, that allowing the consumer to purchase at a reasonable cost, high quality meat and eggs (Donoghue, 2003 and Elnasri et al., 2012).

Presence of antibiotic residues in food have been linked to growing public health concerns over the spread of antibiotic resistant microorganisms, human allergic reactions and imbalances in intestinal microflora (Hsieh et al., 2011). Antibiotic residues are pharmacological active substances either active principle or their metabolites which remain in food stuffs

above MRL. Thus, WHO and FAO establish tolerances (MRLs) for drugs in the relevant tissues of food producing animals. The tolerance is the tissue concentration below which a marker residue for the drug or chemical must fall in the target tissue before that animal edible tissues are considered safe for human consumption (Nisha, 2008).

Cooking time and temperature play major role among the various agents affect antibiotic residues after cooking process. Generally, cooking processes do not assure a full break-down of different antibacterial drug residues present in food originated animals and they can only decrease their amounts. Stability of antibacterial drug residues under cooking conditions is an important research field, which provides valuable information related to health safety aspects and is very important from a safety and toxicological point of view (Heshmati, 2015).

Enrofloxacin residues are heat stable compounds that don't be affected by cooking processes, there is a decrease occur when they were lost by exudation into the water fluid, but when lost in water fluid may be increased in the fluid (Lolo et al., 2006).

So this study aimed to determine the most effective cooking method that reduce the enrofloxacin residues concentration in chicken meat and giblets, Frying proved to be the most effective method according to the results.

2. MATERIALS & METHODS:

2.1 Collection of samples:

A grand total 120 random samples of chicken meat including breast, thigh, gizzard and liver (30 of each) were collected from different chicken slaughter

shops located in Menoufia government. Each sample was wrapped in a plastic bag and put in an ice box then transferred to the laboratory for determination of Enrofloxacin residues as well as study the effect of different cooking methods (boiling, frying and grilling) on such residues. The antibiotic residues were determined either qualitatively by microbiological technique or quantitatively by High Performance Liquid Chromatography (HPLC).

2.2. Microbiological Antibiotic Technique (Okerman et al., 2001 & Ferrini et al., 2006):

2.2.1. Test organisms:

Escherichia coli: used for detection of enrofloxacin antibiotic residues using standard II nutrient agar at pH 8.0.

2.2.2. Preparation of test organisms:

E. coli was prepared on nutrient broth incubated at 35°C for 24 hours. The culture was diluted to obtain density of 10⁷ cells/ml and kept in refrigerator.

2.2.3. preparation of test plates:

Standard II nutrient agar (Merck Art. Nr.7883) + 0.1 KH₂PO₄+ test agar (Merk Art. Nr.10663) at pH 8. The media were sterilized by autoclaving for 15 minutes at 121°C under 1.5 atmospheric pressure. Plate contained 100 ml of standard II nutrient agar adjusted at pH 8.0 seeded with *E. coli* strain for detection of Enrofloxacin.

2.2.4. Interpretation of results:

The result was indicated by measuring the diameter of inhibition zones of the growth of the *E. coli* cells around samples. A zone more than or equal to 2 mm was recorded as positive result, while a zone from 1 to less than 2

mm was recorded as suspicious one and a zone less than 1 mm will be considered as negative result according to Heitzman (1994).

2.3. Determination of enrofloxacin residues using HPLC (McEvoy, 2002 and Samanidou et al., 2007):

2.3.1. Samples preparation:

Five grams of the sample homogenate were accurately mixed into a polypropylene centrifuge tube with 10 ml of chloridrich acid 0.15 molar and centrifuged at 4400 rpm for 20 min at 4°C. The exaction step was repeated twice and supernatants were pooled and finally filtered by 0.2 µm syringe filter.

2.3.2. Solid-phase extraction:

Enrofloxacin determination performed by means of an HPLC system consisting of a waters prep LC 4000 (USA) and a Spectroflow 783 UVV is detector (WATERS tm 486, tunable absorbance, USA) a 125 mm×4mm i.d.LiChrospher 100°C 18 HPLC column (5 m) from waters was used. All the data analysed by personal computer software (millennium v 12.15).

2.3.3. Liquid chromatography operating conditions:

The mobile phase used, was water- HPLC grade acetonitrile (CAN) -triethylamine (TEA) (83:14:0.45 v/v), pH adjusted to 2.3 with 85% H₃PO₄ before adding CAN. The flow-rate was 1 ml/min. Injection volume, 20µl; flow rate, 1 mL/min; wave length, 220 nm; column temperature, 35°C; run time, 10 min.; retention time, 7 min.; mobile phase A, 0.1% Aqueous Formic acid; mobile phase B, 0.1% Formic acid in ACN.

2.4. Effect of cooking methods on Enrofloxacin content:

Accurately, 9 positive samples of chicken thigh containing low, medium (around permissible limit) and high residue concentrations were subdivided into control (without heat treatment) and those subjected to ordinary cooking methods (boiling, frying or grilling). The boiling of the tested samples was applied by immersion of chicken thigh into a water bath preheated to 100 C and boiled for 30 minutes then removed and allowed to cool (Javadi et al.,2011). The frying treatment of thigh was carried out in a pan containing suitable amount of cotton seed oil at 200 C for 10 minutes, however, the grilling was performed by putting of the chicken thigh on grill for 7 minutes to be grilled over medium to high flames with the lid closed to retain heat (Lolo et al., 2006).

3. RESULTS:

Table (1) shows the incidence of enrofloxacin residues in different chicken muscles and giblets by MIT were 13.33% in breast, 16.67% in thigh, 26.67% in gizzard and 40% in liver. Concerning HPLC, 13.33% in breast, 20% in thigh, 30% in gizzard and 43.33% in liver were positive.

Table (2) indicated that the mean level of Enrofloxacin residues were 72.6 ± 4.2 in breast , 95.1 ± 6.7 in thigh , 163.7 ± 10.3 in gizzard & 208.5 ± 14.6 in liver.

Table (3) revealed that the acceptability of the examined samples of chicken meat were 96.67% for breast and 90% for thigh , 80% in gizzard and 73.33% in liver based on their contents of enrofloxacin residues with total accepted samples 85%. The total unaccepted samples were 3.33% in breast, 10% in thigh, 20% in gizzard and 26.67% in liver according to European Union "EU" (2010).

Table (4) recorded the influence of various cooking methods on the Enrofloxacin residues in chicken thigh muscles reduced from 27.2 ppb to 19.5 ppb (28.3%), 11.6 ppb (57.4%) and 8.3 ppb (69.5%), from 93.6 ppb to 76.9

ppb(17.8%), 52.7 ppb (43.6%) and 35.1 ppb (62.4%) and from 151.8 ppb to 134.7 (11.2%), 98.3 ppb (35.2%) and 79.4 ppb (47.7%) after boiling, frying and grilling of samples, respectively .

Table (1): Incidence of enrofloxacin residues in the examined samples of chicken meat and giblets(n=30).

Technique	MIT*		HPLC**	
	No	%	No	%
Tissues				
Breast	4	13.33	4	13.33
Thigh	5	16.67	6	20
Gizzard	8	26.67	9	30
Liver	12	40	13	43.33
Total	29	24.17	32	26.67

MIT*= Microbiological Inhibition Test
HPLC**= High Performance Liquid Chromatography

Table (2): Levels of enrofloxacin residues (ppb) in the examined samples of chicken meat and giblets (n=30).

Chicken tissues	Min.	Max.	Mean ±S.E*
Breast	18.9	106.5	72.6 ± 4.2
Thigh	27.2	151.8	95.1 ± 6.7
Gizzard	53.7	294.9	163.7 ± 10.3
Liver	61.8	322.4	208.5 ± 14.6 ++

S.E*= Standard error ofmean
++ ANOVA test indicated high significant differences (P< 0.01) between examined samples.

Table (3): Acceptability of the examined samples of chicken meat and giblets based on their contents of enrofloxacin residues (n=30).

Chicken tissues	Maximum Residual Limit (ppb)*	Accepted samples		Unaccepted samples	
		No	%	No	%
Breast	100	29	96.67	1	3.33
Thigh	100	27	90	3	10
Gizzard	200	24	80	6	20
Liver	200	22	73.33	8	26.67
Total (120)		102	85	18	15

* European Union "EU" (2010)

Table (4): Influence of various cooking methods on the enrofloxacin residues in chicken thigh.

Control thigh samples	Boiling		Frying		Grilling	
	Content	Reduction %	Content	Reduction %	Content	Reduction %
27.2	19.5	28.3	11.6	57.4	8.3	69.5
93.6	76.9	17.8	52.7	43.6	35.1	62.4
151.8	134.7	11.2	98.3	35.2	79.4	47.7

4. DISCUSSION

The results indicated that the high incidence of enrofloxacin residues was in liver which agreed with Hegazy (2003) and Sattar et al. (2014) with incidence 40%. The lower incidence of enrofloxacin residues was recorded by Pena et al. (2010), Hussein and Khalil (2013) and Kim et al. (2013) with incidence 20.4%, 4% and 3.6% respectively. While Arnildo et al. (2014) and Saleh-Nehal (2017) could not detect enrofloxacin residues in any of the examined chicken meat and giblet samples.

The presence of antibiotic residues in food stuff can cause hazards to human health. These hazards are such as allergy and sensitivity to the antibiotics, in addition to intestinal micro flora disturbance and imbalance, antibiotic resistant strains of bacteria may also results (Mumtaz et al., 2000 and Myllyniemi et al., 2000).

Microbiological inhibition screening tests is to rapidly verify the presence of antibiotics in samples that then may undergo further analyzed with more sophisticated immune chemical and chromatographic methods (Kiline and Ckli, 2008 & Javadi et al., 2009). The advantages of microbial methods which comprise cheap costs, easy to be used,

don't require expensive equipment and the laboratory staff could be efficiently effective (Javadi et al., 2009)

HPLC expanded its use in the 1990s due to the availability of columns, good performance, variety of available detectors and possibility of automation. HPLC is a separative technique, where the choice of the detection system is very important for selectivity and sensitivity. Relatively simple and rapid typical detections for the presence of multi-residues in tissue samples could be achieved by using HPLC method, requiring a preliminary clean-up through solid-phase extraction followed by filtration before injection into a reverse phase HPLC (Bergweff and Schloesser, 2003). HPLC technique is the most ideal method, it is a simple and rapid technique.

The results revealed that the thigh showed higher residual concentration than breast (95.1 ± 6.7 for thigh and 72.6 ± 4.2 for breast) due to the difference in protein, calories, fats and saturated fat content as chicken thighs tend to be more fatty and less protein content than breast.

The obtained results disagreed with results recorded by Schneider and Donoghue (2004) and Raphaella (2008) (95.16 for breast and 71.40 mg/kg for thigh). Lower residual concentration in liver

was detected by Anadón et al. (1995) with level 25ppb± 3ppb.

ANOVA test indicated high significant differences ($P < 0.01$) between the examined samples.

The most common public health problems that caused by antibiotics such as nausea, headache and abdominal discomfort, rashes, including hypersensitivity reactions and various central nervous system, adverse effects as depression, insomnia, nervousness, anxiety and euphoria. Moreover FDA classified ciprofloxacin (degradation product of enrofloxacin) as a pregnancy category “C” which may be harmful to unborn baby. Also, it passes into breast milk and may harm a nursing baby (Abo El-Enaen, 2008).

MRL in addition to number and percentage of accepted and unaccepted samples was noted, MRL was 100 ppb for each breast and thigh samples and 200 ppb for each gizzard and liver samples (EU/2010). Therefore the unaccepted enrofloxacin residue at thigh and breast samples was 1(3.33%) and 3(10%) respectively. Regarding gizzard and liver, the unfit samples were 6(20%) and 8(26.67%), respectively.

Actually, 4 days withdrawal period is the allowed time for the enrofloxacin concentration to decrease to an acceptable level in the meat and liver, prior to slaughter (Petrovic et al., 2006).

Withdrawal periods, ranging from a few days to a few weeks, were suggested for approved animal drugs. These times vary according to the drug used, dosage,

route of administration, and animal species and were defined as the time required for 99% of the animals in a population to be free of drug residues above the tolerance level. Failing to adhere to these recommended periods was reported to be the primary cause of violated levels of veterinary drugs in food (Kukanich et al., 2005).

The effect of different cooking methods (boiling, frying and grilling) by the enrofloxacin residues in chicken thigh muscles were 28.3%, 17.8% & 11.2% by boiling 57.4%, 43.6% & 35.2% by frying and 69.5%, 62.4% & 47.7% by grilling of such samples, frying proved to be the most effective method for reduction of antibiotic residues levels. Reduction of enrofloxacin residues by various cooking methods also recorded by Hegazy (2003), Atwa (2015) and Khan et al. (2016).

While Lolo et al. (2006) noted that grilling of the residue increase its concentration due to the decreases of the moisture content of the treated piece that caused by an apparent of the enrofloxacin residues.

Good cooking act as minimal safeguards in destroying the antibiotic residues present in edible tissues. Factors affecting on the degradation of antimicrobial drugs include drug formulation and pharmacodynamics, cooking temperature and duration as well as the shape and thickness of cooked tissue (Isidori et al., 2005; Hassani et al., 2008 and Javadi et al., 2011).

As conclusion, modern methods and technology of screening and detection of antibiotic residues as HPLC is considered

as an adequate instrument for successful screening of antibiotic residues in meat because it can be automated and computer-controlled. Heat treatment by cooking processes that have a higher temperature and longer time should be done to inactivate antibiotic and growth promoter residues in chicken meat and giblet. From obtained results, frying proved to be the most effective method for reduction of antibiotic residues levels.

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