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HPLC DETERMINATION OF CIPROFLOXACIN RESIDUES IN CHICKEN MEAT AND ITS PRODUCTS

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ABSTRACT

This study consisted of two main parts; the first part is an experimental part consisted from 55 antibiotic-free 35-days chickens that treated with therapeutic dose (5 mg/kg body weight) in drinking water for three successive days to study the withdrawal time and the effect of freezing and heat treatment on the drug residues. The second part is a survey consisted from 100 samples include fresh, frozen chickens, chicken burgers and chicken luncheon, 25 samples from each (breast, thigh muscles and liver) randomly collected from markets and analyzed by HPLC analysis. Findings revealed that the 9th day after administration is the withdrawal day of ciprofloxacin residues. Freezing had a little effect on degradation of residues, boiling is the most efficient method in reduction of residues than roasting and frying. In survey, all samples of fresh, frozen chicken and chicken products were positive for ciprofloxacin residues and have levels exceed the MRL. Ciprofloxacin was widely used in broiler farms and found in nearly all samples in violative levels in chicken meat and its products. Withdrawal time of 9 days after treatment must be noticed and use of boiling in processing is more preferable.

Key words: Ciprofloxacin, chicken meat, HPLC, detection, freezing, boiling, roasting, frying.

INTRODUCTION

Ciprofloxacin is one of the second generation flouroquinolone antibiotics and has a broad spectrum effect against gram positive and gram negative bacteria and mycoplasma infection by inhibition of DNA gyrase enzyme that is essential for bacterial chromosome replication (Amjad *et al.,* 2005, Xiao *et al.,* 2014)). It is commonly used in human medicine, in animal production and in poultry farms for treatment and prophylaxis of bacterial infections. Also, it is used as growth promoters to stimulate growth and increase food intake and body gain (Donoghue, 2003). Its bioavailability and tissue distribution is excellent after oral administration (Papich, 1998). Ciprofloxacin is a primary metabolite of enrofloxacin in several animal species, including chicken and both enrofloxacin and ciprofloxacin are found in the muscle and tissue of chicken receiving enrofloxacin (Dimitrova *et al.,* 2007).

Violative concentrations of ciprofloxacin residues in foods destined for human consumption can occurs due to the administration of ciprofloxacin to boiler without an adequate withdrawal time, use higher doses than prescribed, using for longer period than recommended duration and using non-indicated route of administration (Mitchell *et al.,* 1998). These drug residues causes several hazards to human health, such as formation of bacterial resistance, changes in intestinal microflora and hypersensitivity reactions (Fabrega *et al.,* 2008). The use of ciprofloxacin doses lower than therapeutic dose in poultry industry is the main cause of acquisition of bacterial resistance (i.e. Campylobacter spp., Salmonella spp. and Escherichia coli) which adversely affects human medicine through reduction in the efficacy of such compounds in treating infections (WHO, 2011). Ciprofloxacin, even in low doses, strongly suppress facultative anaerobic human intestinal microflora which adversely affect digestion and vitamin synthesis (Cerngila and Kotarski, 1999). Several health risks such as antibiotic resistance, teratogenicity, carcinogenicity, hepatic and renal failure can threaten the consumers from consumption of chicken meat containing ciprofloxacin residues. Control of withdrawal times of antibiotics in poultry farms and post-mortem inspection of slaughtered carcasses for antibiotic residues can reduce the incidence of antibiotic residues and improve the safety of chicken meat (Karmi, 2014). The effect of cooking on ciprofloxacin residues should be studied to determine consumer's exposure to these drugs and any breakdown metabolites (Rose *et al.,* 1999). Cooking temperature and duration greatly affecting the level of antibiotic residues in meat. Also, the chilling and freezing can lower the residue levels in stored meat (Haagsma, 1993).

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Many methods of high sensitivity and specificity were optimized and validated for detection and determination of different antibiotic residues in animal meat and poultry such as High Performance Liquid Chromatography (HPLC), Liquid Chromatography (LC), Liquid Chromatography-Mass Spectrometry (LC-MS), Thin Layer Chromatography (TLC), Enzyme-Linked Immunosorbent Assay (ELISA) and Microbiological Assays which used for qualitatively and quantitative determination of these antibiotic residues in food samples (Shareef *et al.*, 2009). HPLC is the most preferable and most common method used in the determination of ciprofloxacin in poultry meat due to its higher sensitivity, higher specificity, short time and good performance (Gigosos *et al.*, 2000, Garica-Ovando *et al.*, 2004).

MATERIALS AND METHODS

Samples

This study consisted of two main parts; the first part is an experimental part consisted from 55 antibiotic-free 35-days chickens that treated with therapeutic dose (5 mg/kg body weight) in drinking water for three successive days to study the withdrawal time and the effect of freezing at -20 ℃ for one month and heat treatment (boiling (at 100 ℃ for 9 minutes for liver and 24 minutes for muscles), roasting (at 200 ℃ for 25 minutes) and frying (in sunflower for 10 minutes)) on the drug residues. The second part is a survey consisted from 100 samples including fresh, frozen chickens, chicken burgers and chicken luncheon, 25 samples from each, were randomly collected from markets. Breast, thigh muscles and liver were taken from each chicken carcass for analysis.

HPLC analysis

Ciprofloxacin was extracted from tissues with dichloromethane (Sigma-Aldrich) and HPLC analysis of the samples was conducted using C18 column (250 mm x 4.6 mm, 5µm) and detected with photodiode-array detector at 280 nm wave length. The mobile phase consists of mixture of 0.1 M orthophosphoric acid at pH 3.5 and acetonitrile (15:85, v/v).

Calculation

Standard curve of ciprofloxacin standard solutions (concentration versus peak area) was made and from measured peak area of test samples, ciprofloxacin concentration calculated by using linear equation as follows: $y = m x + b$

Where: y: peak area, x: ciprofloxacin concentration (ng/g), m: slope of curve, b: intercept of y

RESULTS

Results revealed that the 9th day after administration is the withdrawal day of ciprofloxacin residues where the detected level of ciprofloxacin in all chicken tissues (breast, thigh and liver) was lower than the MRL and at day 11th no residues were detected. Freezing of meat at -20 ℃ for one month had a little effect on degradation of residues, the reduction percentage were 4.7%, 3.9% and 5.8% in breast, thigh and liver tissues, respectively. Boiling of tissues at 100 ℃ for 9 minutes for liver and 24 minutes for muscles have a dramatic effect where the reduction percentage of residues were 79.7%, 80.4% and 80.1% in breast, thigh and liver, respectively. Frying of chicken tissues in sunflower for 10 minutes showed reduction percentage, 53.5%, 57.8% and 43.6% in breast, thigh and liver, respectively. Roasting of meat in oven at 200 ℃ for 25 minutes for liver and 40 minutes for muscles causing reduction in residue levels in percentage of 41%, 39.9% and 32.4% in breast, thigh and liver, respectively. In survey study, about 76%, 80%, 88%, 92% and 80% of fresh chicken meat (breast and thigh), fresh liver, frozen chicken meat (breast and thigh), chicken burger and chicken luncheon were positive for the presence of ciprofloxacin residues, respectively. Comparing results of ciprofloxacin residues in chicken meat with EU MRLs revealed that about 64%, 68%, 76%, 72% 64%, 80% and 64% of fresh breast, fresh thigh, fresh liver, frozen breast, frozen thigh, burger and luncheon samples have residue levels above MRLs, respectively (Table 1).

Table 1 Incidence and number of ciprofloxacin residues in chicken meat and its products.

Sample	Number	Percentage of positive samples		Percentage of violative samples		EU MRLs (ng/g)
		No.	%	No.	%	
Fresh breast	**25**	**19**	**76**	**16**	**64**	**100**
Fresh thigh	**25**	**19**	**76**	**17**	**68**	**100**
Fresh liver	**25**	**20**	**80**	**19**	**76**	**200**
Frozen breast	**25**	**22**	**88**	**18**	**72**	**100**
Frozen thigh	**25**	**22**	**88**	**16**	**64**	**100**
Burger	**25**	**23**	**92**	**20**	**80**	**100**
Luncheon	**25**	**20**	**80**	**16**	**64**	**100**

N: number of examined samples

Table 2: Effect of freezing and heat treatments on the levels of ciprofloxacin residues in fresh chicken meat.

Sample		Reduction percentage of residues			
		Freezing	Boiling	Roasting	Frying
Fresh chicken (n=25)	Breast	4.7	79.7	41	53.5
	Thigh	3.9	80.4	39.9	57.8
	Liver	5.8	80.1	32.4	43.6

n: number of examined samples

DISCUSSION

In this study, results revealed that at the 9th day after administration of last therapeutic dose of ciprofloxacin, the residue levels were below the EU MRLs and disappeared at day 11th. Also, higher ciprofloxacin residues were detected at zero day (6 hours after last dose) in breast, thigh muscles and in liver, the liver reached highest levels and depleted slower than muscles (EMEA, 2002, Ellis, 2004). It is observed that residue concentrations in breast muscles were higher than those in thigh muscles which agreed with similar findings (Reyes-Herrera *et al.,* 2005, Abou Elnile, 2006, Alestig, 1990). The withdrawal time is the period of time required after completion of treatment needed for tissues concentrations of the drug and/or its metabolites to deplete to less than the established MRLs, so that to deliver safe food for human consumption, the withdrawal time of drugs must be observed and the inappropriate withdrawal period is the most common cause for presence of illegal drug residues in meat that have risk on human health (Kukanich *et al.,* 2005, Paige *et al.,* 1999). Concerning the effect of freezing on reduction of ciprofloxacin residues showed that freezing has a very little effect and these findings in agreement with others who stated that freezing may act as a factor in reduction of antibiotic residues in frozen samples (Mahmoud and Mohsen, 2008, Haagsma, 1993, Eissa *et al.,* 1998). The antibiotic residues have a varying degrees of stability during cooking, therefore cooking influences the level of risk pose by such residues, also, study the effect of different methods of processing on residue levels become needed to accurately determine the risk for consumer (Moats, 1999, Rose *et al.,* 1999). Boiling is the most efficient method in reduction of residues then followed by frying and roasting. The reduction rate were up to 80%, 57% and 41% for boiling, frying and roasting, respectively. Cooking processes cannot remove the total amounts of ciprofloxacin residues but it can only decrease its amounts. Also, most of the residues in boiling process were excreted from tissues into cooking fluid; so that discarding any juices which come from the edible cooked tissues may be reduce exposure to antibiotic residues (Javadi *et al.,* 2011, Fathy *et al.,* 2015, Heshmati, 2015, Javadi, 2011). Other authors have another opinion who stated that cooking procedures do not reduce the enrofloxacin and its metabolite ciprofloxacin, as this residue retained its stability during heating, so that higher reduction rate by boiling while lower rate in case of water loss processing such as roasting and grilling (Lolo *et al.,* 2006, Botsoglou and Fletouris, 2001, Rose *et al.,* 1999, Steffenak *et al.,* 1994). In survey study, results showed that high percentages of chicken meat and its products were positive for ciprofloxacin residues (76-92%) and also, higher rates of them exceeded the established EU MRLs (64-80%). It is noticed, also, that chicken products (burger and luncheon) were higher than carcasses and frozen samples were higher than fresh and liver samples were higher than muscles. Increased demand for chicken meat, higher prices of red meats, need for more meat production and rapid breeding forces people to use more antibiotics for growth stimulation and weight promotion (Paryad and Mahmoudi, 2008), extensive non-therapeutic use of antibiotics and lack of adequate control of administration and withdrawal times increase the risk of accumulation in chicken meat and its products (Lemus *et al.,* 2008). Presence of antibiotic residues and their metabolites in chicken meat may cause several adverse effects for human such as direct toxicity, developing resistant bacteria, allergies, hypersensitivity reactions, harms intestinal microflora, affect digestion and vitamins synthesis, even in very low doses in contaminated foods (Kirbis, 2007, Myllyniemi, 2004, javadi, 2011), therefore many food agencies established maximum limits of drug residues (MRLs) which are the maximum amount of residues that could legally permitted to be in the food product without causing adverse effects to the consumers (Reyes-Herrera *et al.,* 2005). Veterinary drug residue concentrations in meat depend on various factors such as drug dosage, animal species, age, feeding, healthy status, poor management, extra-label drug use, withdrawal time and route of administration (Kaneene and Miller, 1997, Codex Alimentarius Commission, 2001).

CONCLUSION

Ciprofloxacin residues were very common in all chicken meat and chicken products and in violative levels higher than MRLs; this finding may be due to common use of such antibiotic in poultry farms either for treatment or as growth enhancer. Withdrawal time of 9-11 days after treatment must be observed and use

of boiling in processing is more preferable. Regular and routine monitoring of chicken meat and products for ciprofloxacin residue should be conducted.

AUTHORS' CONTRIBUTIONS

Author performs collection, preparation, processing, and analysis of samples, data acquisition, writing, preparation and revision of manuscript.

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تقدير متبقيات السبروفلوكساسين فى لحوم الدواجن ومنتجاتها باستخدام التحليل الكروماتوجرافى عالى الكفاءة

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تتكون هذه الدراسة من جزئين أساسيين: الجزء الأول تجريبى وتتكون من ٥٥ فرخة عمر ٣٥ يوم خالية من المضادات الحيوية ومعالجة بجرعة علاجية فى مياه الشرب لمدة ثلاثة أيام متتالية وذلك لدراسة فترة سحب السبروفلوكساسين من لحوم الدواجن وكذلك تأثير التجميد والمعاملات الحرارية على مستوى متبقيات السبروفلوكساسين فى لحوم الدواجن. أما الجزء الثانى فهو دراسة مسحية لمدى تواجد متبقيات السبروفلوكساسين و يتكون من عدد ١٠٠ عينة من لحوم الدواجن الطازجة والمجمدة وبعض منتجاتها مثل برجر ولنشون الدواجن وقد أظهرت النتائج أن فترة سحب السبروفلوكساسين من لحوم الدواجن كانت عند اليوم التاسع وأن التخزين بالتبريد عند درجة حرارة ٢٠ تحت الصفر لمدة شهر كان له تأثير ضعيف على تكسير المتبقيات وأن الطهى بالغليان عند درجة حرارة ١٠٠ لمدة ٢٤ دقيقة كان له التأثيرالأكبر فى تكسير المتبقيات. كذلك دلت نتائج المسح على أن جميع العينات كانت ايجابية لوجود متبقيات السبروفلوكساسين فى لحوم الدواجن ومنتجاتها بمستويات تفوق الحد الأقصى لها. تنصح الدراسة بضرورة مراعاة فترة سحب الأدوية البيطرية المستخدمة فى مزارع الدواجن قبل الذبح و أن يشمل الفحص الطبى البيطرى للحوم اختبار المتبقيات. كما تنصح الدراسة باستخدام الطهى بالغلى لأنها تعمل على تكسير المتبقيات وخفض مستواها.