www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 353-356

www.thepharmajournal.com
Received: 07-05-2022
Accepted: 11-06-2022

V Sureshkumar
Associate Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Theni, Tamil Nadu Veterinary and Animal Sciences University, India.

G Sarathchandra
Professor and Head, Pharmacovigilance Laboratory for Animal Feed and Food Safety, Directorate of Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu, India.

Corresponding Author
V Sureshkumar
Associate Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Theni, Tamil Nadu Veterinary and Animal Sciences University, India.

Evaluation of stability of enrofloxacin and ciprofloxacin antibiotic residues in broiler chicken tissues exposed to various cooking processes

V Sureshkumar and G Sarathchandra

Abstract
The effect of temperature on stability of drug residues in foods is imperative from consumer safety point of view. Hence, the effect of various cooking processes like boiling, roasting, microwaving and grilling on enrofloxacin and its metabolite ciprofloxacin residues in broiler chicken tissues was evaluated. Enrofloxacin and ciprofloxacin residues in control, raw and cooked tissues were analysed by a validated High performance thin layer chromatography-Fluorescent densitometry method. All the cooking processes resulted in significant reduction (p<0.01) in total enrofloxacin residues when compared to that of raw tissues. The highest decrease in residue levels was observed in boiled tissues; however the lost residues were detected in boiling fluid used for cooking. Among other cooking methods, roasting showed better reduction in residues followed by grilling and microwaving. In spite of that, the residue levels remained high in all the cooked tissues exceeding the maximum residue limits prescribed by European Union and Japan. The present study suggests that various cooking processes cannot completely deplete the enrofloxacin residues but it can only decrease their amounts.

Keywords: Cooking processes, enrofloxacin, ciprofloxacin, residues, broiler tissues

Introduction
Enrofloxacin, a fluoroquinolone developed exclusively for veterinary use is advocated in poultry in large-scale for treatment of chronic respiratory disease, colibacillosis, salmonellosis and fowl cholera (Anderson *et al*., 2003) [1]. After administration, enrofloxacin is metabolised in the liver via de-ethylation into pharmacologically active metabolite ciprofloxacin (Prescott *et al*., 2000) [2]. Ciprofloxacin is one of the most commonly advocated antimicrobial in human medicine worldwide.

The widespread use of fluoroquinolones as therapeutic and prophylactic agents, particularly in intensive poultry production, has become a great concern in recent years due to the identification of resistant *Campylobacter spp*. (Humphrey *et al*., 2005) [3], *Escherichia coli* (Khan *et al*., 2005) [4] and *Salmonella serovars* (Zhao *et al*., 2006) [5] in meat and possible transfer to humans via the food chain (Petrovic *et al*., 2009) [6]. This resistance has led to lowered therapeutic efficacy of these compounds in human infections (WHO, 1998; Norstrom *et al*., 2006) [7, 8].

In cognizance to this, since 2005 the U.S. Food and Drug Administration (FDA) has banned the use of enrofloxacin in poultry / food animals (U.S.FDA, 2005) [9]. Different maximum residue limits (MRLs) have been established by regulatory agencies in countries where the use of enrofloxacin has not been forbidden. MRLs of 100 µg/Kg and 200 µg/Kg for enrofloxacin and its metabolite ciprofloxacin have been established by the European Union (EU) for poultry muscle and liver respectively (EMEA, 2002) [10]. Whereas, Japan has established much lower MRLs (10 µg/Kg) in chicken muscle and liver (Ministry of Health and Welfare, Japan, 2005) [11].

Although most edible animal products are consumed after cooking, none of the required studies for veterinary drug licensing includes the effect of cooking on the amount and nature of the potentially present residues. Most information about drug residues in edible animal products and government regulatory considerations are directed towards raw products (Botsoglou and Fletouris, 2001) [12]. Information about the effect of temperature on the stability of drug residues in foods of animal origin is also crucial from the toxicological and food safety point of view. Only relatively few studies have addressed concerning the effect of these cooking processes on drug residues.

Hence, the effect of various cooking processes like boiling, roasting, microwaving and grilling on enrofloxacin and its metabolite ciprofloxacin residues in broiler chicken tissues was evaluated in the present study.

Materials and Methods

Twelve one-day-old broiler chicks (Broiler strain B_1) obtained from Institute of Poultry Production and Management, Madhavaram Milk Colony, Chennai-600 051 were used for the study. Necessary approval was obtained from Institutional Animal Ethics Committee, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, for conducting the experiment. Chicks were randomly divided into control and treatment group. Treatment group was administered with enrofloxacin at recommended therapeutic dose 10 mg/kg body weight through drinking water for five consecutive days from 43rd to 47th day of age, while control group received non-medicated water. Six birds from each group were sacrificed ethically 24 h after the last dose. Muscle (breast and thigh) and liver tissues were collected in duplicate, following standard protocol. One set of samples were retained as uncooked raw tissues, while another set were subjected to various cooking processes.

Boiling, roasting and microwaving procedure was followed as described by Javadi *et al*. (2011) [13]. For grilling of muscle (breast and thigh) samples, the protocol previously described by Lolo *et al*. (2006) [14] was employed.

Enrofloxacin and ciprofloxacin in muscle and liver tissues were quantified by a validated High performance thin layer chromatography (HPTLC) - Fluorescent densitometry assay as described by us earlier (Sureshkumar and Sarathchandra, 2018) [15]. Data were subjected to one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) [16].

Results and Discussion

All the cooking processes resulted in significant reduction ($p<0.01$) in total enrofloxacin residues when compared to that of raw tissues. The present study revealed that the change in enrofloxacin and its metabolite ciprofloxacin residue levels in the tissues studied is dependent on the type of cooking (Table 1). Cooking by boiling showed the highest reduction in enrofloxacin residues in all the tissues when compared to that of microwaving, roasting and grilling methods. Indeed, the lost amount of analytes in the boiled tissues was found in the boiling fluid used for cooking. This is in agreement with Lolo *et al*. (2006) [14], who also observed that there was apparent decrease in quinolone concentration in the breast and whole leg of chicken, when boiling at 100^0C for 10 minute and the lost amount of residue from the tissue was found in the liquid used for cooking. Papapanagiotou *et al*. (2005) [17] also reported comparable observation while heating processes of piglet muscle, after allowance for weight loss and migration of sulphamethazine into the surrounding fluid has been made; there was a considerable reduction in the quantity of sulphamethazine in piglet muscle during boiling, autoclaving and microwaving.

Table 1: Effect of various cooking processes on enrofloxacin residues (µg/Kg) in broiler chicken tissues, Mean±SE (n=6)

	Raw tissue	Boiled	Boiling Fluid	Roasted	Microwaved	Grilled
Muscle-Breast	337.23^a ±4.97	222.13^c ±8.35	109.60^d ±1.62	283.37^b ±8.02	307.54^b ±4.19	296.02^b ±8.87
Muscle-Thigh	286.12^a ±4.95	188.58^c ±11.87	94.42^d ±1.63	237.70^b ±7.10	258.46^b ±4.10	251.52^b ±6.40
Liver	830.93^a ±25.31	538.58^c ±18.21	286.42^d ±15.70	681.17^b ±10.72	747.93^b ±13.84	-

Means bearing different superscript (a, b, c, d) within the row differ significantly, $p<0.01$

Few studies have addressed the effect of cooking or heat treatments on the fluoroquinolone residues in different samples. Rose *et al*. (1999) [18] demonstrated that residues of veterinary drugs have varying degrees of stability during cooking and, therefore, the cooking influences the level of risk posed by such residues. Since most of the meat derived from food animals are always cooked before consumption, the variations in enrofloxacin residue levels in the tissue are dependent on the type of cooking (Lolo *et al*., 2006) [14].

On the other hand, there was no significant difference among microwaving, roasting and grilling methods on enrofloxacin residue levels in all the tissue samples studied. However, microwave cooking method showed the lowest reduction in enrofloxacin residues next to grilling and roasting in all the cooked samples. The descending order of reduction in enrofloxacin residues observed irrespective of the tissue analyzed in the present study is boiling>roasting>grilling>microwaving. This observation is in accordance with Javadi *et al*. (2011) [13], who showed that, most reduction of enrofloxacin residues in cooked muscle samples related to boiling and roasting processes for cooked liver samples and the highest detectable amount of residues belonged to microwaving process in all cooked samples. In contrast, Lolo *et al*. (2006) [14] avowed that the amount of residue increased in case of roasting and grilling because the minor moisture content of the treated tissues caused the apparent concentration of the quinolone residues.

The longer the cooking time and higher the temperature applied, the more reduction of enrofloxacin residues occurred. This is evidenced in roasting process, in which tissues were exposed to 200 ^{0}C (25 min for liver; 40 min for muscle samples) resulted in 15.77%, 16.78% and 17.79% reduction in enrofloxacin residues in breast muscle, thigh muscle and liver respectively (Table 2). This is in corroborate with Ismail-Fitry *et al*. (2008) [19], who demonstrated that the longer the duration of deep-frying and higher the temperature applied, the more reduction of sulfonamide residues in chicken meat balls. However, over cooking could damage the food properties and taste. Therefore, appearance and taste of the food still need to be retained by controlling the time and temperature of cooking at its best, while the risk from antibiotic residues could be reduced at the same time.

Table 2: Percent reduction in enrofloxacin residues (µg/Kg) in broiler chicken tissues after various cooking processes, Mean±SE (n=6)

	Raw tissue	Boiled	%	Roasted	%	Microwaved	%	Grilled	%
Muscle-Breast	337.23 ±4.97	222.13 ±8.35	34.05	283.37 ±8.02	15.77	307.54 ±4.19	8.69	296.02 ±8.87	12.11
Muscle-Thigh	286.12 ±4.95	188.58 ±11.87	33.82	237.70 ±7.10	16.78	258.46 ±4.10	9.54	251.52 ±6.40	11.98
Liver	830.93 ±25.31	538.58 ±18.21	35.21	681.17 ±10.72	17.79	747.93 ±13.84	9.81	-	-

Even though the different cooking methods caused perceptible decrease in enrofloxacin residue levels inall the tissues studied, the residue levels remained high in cooked tissues and found to be far above the MRLs prescribed by European Union (100 µg/Kg for muscle; 200 µg/Kg for liver) and Japan (10 µg/Kg for muscle and liver). Different cooking methods could not bring down the residues to the safer level, leaving the meat unfit for human consumption. The results of the present study are consistent with earlier studies previously reported by other authors who studied the fate of drug residues. According to Al-Mustafa and Al-Ghamdi (2000) [20], norfloxacin residue in the liver and muscle samples of poultry in the eastern province of Saudi Arabia, from all positive raw samples with concentration of norfloxacin residue above MRL, 40.5% of muscle samples and 72.1% of liver samples had residue above MRL after cooking in water at 100°C for 20 min. Streptomycin in eggs subjected to boiling or frying did not change residue concentrations, whereas oxytetracycline levels were reduced to a minor extent by frying and roasting procedures (O'Brien *et al*., 1980) [21]. Other veterinary drugs such as sulphamethazine (Rose *et al*., 1995) [22] and oxytetracycline (Rose *et al*., 1996) [23] remained quite stable during cooking procedures.

Conclusion

The results of the present study suggest that the various cooking processes cannot annihilate the total amount of enrofloxacin residues but it can only decrease their amounts and most of the residues in boiling process are excreted from tissue to cooking fluid during the boiling process. Thus, exposure to residues may be reduced by discarding any juices which come from the edible tissues as they are cooked. Between the various agents affecting antibiotics residue after the cooking process, it was found that cooking time and temperature can play major roles about antibiotic residue reduction while cooking food.

Acknowledgement

The Authors are highly thankful to Drugs and Pharmaceutical Research Programme, Department of Science and Technology (DST), Government of India, New Delhi, for the financial assistance in conducting the experiment as part of the DST scheme entitled "A National Facility for Pharmacovigilance on Drug Residue and other Toxic Xenobiotics including Genetically Manipulated Organisms (GMOs) in Veterinary Products" at Pharmacovigilance Laboratory for Animal Feed and Food Safety, DCAHS, TANUVAS, Chennai.

References

1. Anderson AD, Nelson JM, Rossiter S, Angulo FJ. Public health consequences of use of antimicrobial agents in food animals in the United States. Microb Drug Resist. 2003;9:373-379.
2. Prescott JF, Baggot JD, Walker RD. Fluoroquinolones. In: Prescott JF, Baggot JD, Walker RD (ed) Antimicrobial therapy in veterinary medicine, 3rd edn. Iowa State University Press, Iowa, 2000, 315-339.
3. Humphrey TJ, Jorgensen F, Frost JA, Wadda H, Domingue G, Elviss NC, *et al*. Prevalence and subtypes of ciprofloxacin resistant Campylobacter spp. in commercial poultry flocks before, during and after treatment with fluoroquinolones. Antimicrob Agents Chemother. 2005;49:690-698.
4. Khan AA, Nawaz MS, Summage West C, Khan SA, Lin J. Isolation and molecular characterization of fluoroquinolones resistant Escherichia coli from poultry litter. Poult Sci. 2005;84:61-66.
5. Zhao S, McDermott PF, Friedman S, Qaiyumi S, Abbott J, Kiessling C, *et al*. Characterization of antimicrobial resistant Salmonella isolated from imported foods. J Food Prot. 2006;69:500-507.
6. Petrovic J, Baltic M, Stefanovic S, Milanov D, Ratajac R. Determination of flumequine residues in broiler chickens with HPLC and screening method.Acta Vet (Beograd). 2009;59:547-555.
7. WHO. Use of quinolones in food animals and potential impact on human health. Report of a WHO meeting, Geneva, Switzerland, 2-5 June, 1998, WHO/EMC/2DI/98.
8. Norstrom M, Hofshagen M, Stavnes T, Schau J, Lassen J, Kruse K. Antimicrobial resistance in *Campylobacter jejuni* from humans and broilers in Norway. Epidemiol Infect. 2006;134:127-130.
9. U.S. Food and Drug Administration. Enrofloxacin for poultry: Final decision of withdrawal of new animal drug application following formal evidentiary public hearing availability. Fed Regist. 2005;70:44105.
10. EMEA. The european agency for the evaluation of medicinal products, veterinary medicines and inspections. Committee for Veterinary Medicinal Products, Enrofloxacin (extension to all food producing species), Summary report (5). 2002, EMEA/MRL/820/02-FINAL.
11. Ministry of Health and Welfare, Japan. Specifications and standards for food, food additives. Notification no. 499. Ministry of Health and Welfare, Tokyo, 2005.
12. Botsoglou NA, Fletouris DJ. Drug residues in foods: Pharmacology, food safety and analysis. Marcel Dekker, Inc. New York, 2001.
13. Javadi A, Mirzaie H, Khatibi SA. Effect of roasting, boiling and microwaving cooking methods on enrofloxacin residues in edible tissues of broiler. African J Pharm Pharmacol. 2011;5:214-218.
14. Lolo M, Pedreira S, Miranda JM, Vazquez BI, Franco CM, Cepeda A, *et al*. The effect of cooking on enrofloxacin residues in chicken muscle. Food Addit Contam. 2006;23:988-993.
15. Sureshkumar V, Sarathchandra G. A HPTLCFluorescent densitometry assay for simultaneous detection of enrofloxacin and ciprofloxacin in broiler chicken tissues. Food Anal. Methods. 2018;11:1076-1085.

16. Snedecor GW, Cochran WG. Statistical Methods, 8th Edn., Iowa State University Press, Ames, 1994.
17. Papapanagiotou EP, Fletouris DJ, Psomas EI. Effect of various heat treatments and cold storage on sulphamethazine residues stability in incurred piglet muscle and cow milk samples. Anal Chim Acta. 2005;529:305-309.
18. Rose MD, Bygrave J, Sharman M. Effect of cooking on veterinary drug residues in food: Part 9. Nitroimidazoles. Analyst. 1999;124:289-294.
19. Ismail-Fitry MR, Jinap S, Jamilah B, Saleha AA. Effect of deep-frying at different temperature and time on sulfonamide residues in chicken meat-balls. J Food Drug Anal. 2008;16:81-86.
20. Al-Mustafa ZH, Al-Ghamdi MS. Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health. Int J Environ Health Res. 2000;10:291-299.
21. O’Brien JJ, Campbell N, Conaghan T. Antibiotic residues in meat: Cooking and cold storage effects. Vet Rec. 1980;106:365.
22. Rose MD, Farrington WHH, Shearer G. The effect of cooking on veterinary drug residues in food: Sulphamethazine (sulphadimidine). Food Addit Contam. 1995;12:739-750.
23. Rose MD, Bygrave J, Farrington WHH, Shearer G.The effect of cooking on veterinary drug residues in food. Oxytetracycline. Food Addit Contam. 1996;13:275-286.