Antibiotic use in food animals: determination of enrofloxacin residue in chicken tissue

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ABSTRACT

Background: Poultry farmers in Nigeria employ the use of various antibiotics with or without the guidance of veterinarians, to promote growth and prevent infections in poultry. Although antibiotics benefit most of its uses, this has led to the accumulation of toxic antibiotic residues in edible poultry products destined for human consumption.

Objective: The present study was aimed at determining the residual amounts of enrofloxacin in chicken muscle samples.

Method: Forty birds were obtained from commercial poultries in Lagos State and randomly allotted into 7 groups. They were humanely sacrificed, dissected and 10.0 g of muscle tissue was sectioned from each drumstick and homogenized in a porcelain mortar. 5.0 g of the homogenate was weighed and transferred into a 5 ml plain sample bottle and processed for analysis by high performance liquid chromatography to determine the amount of enrofloxacin in the tissues.

Results: Enrofloxacin was detected in all samples from the four farms. The mean concentration of enrofloxacin ranged from 23.5 to 88.1 µg/g in all the 7 samples analyzed; these values were higher than the maximum residue level of 0.1 µg/ug in Europe and 0.3 µg/ug in the United States of America.

Conclusion: This study confirmed misuse of enrofloxacin in poultry farms and emphasizes the need for stricter regulation regarding antibiotic use in poultry as well as the screening of chicken for residues before sale.

Keywords: Enrofloxacin, HPLC, Maximum residue limit, Poultry, Residue

Usage d'antibiotiques chez les animaux destinés à l'alimentation humaine: Détermination du résidu d'enrofloxacine dans le tissu de poulet

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Résumé

Contexte: Les éleveurs de volailles au Nigeria utilisent divers antibiotiques, avec ou sans les conseils des vétérinaires, pour promouvoir la croissance et prévenir les infections chez les volailles. Bien que les antibiotiques bénéficient de la plupart de ses usages, cela a conduit à l'accumulation de résidus toxiques d'antibiotiques dans les produits comestibles de volaille destinés à la consommation humaine.

Objectif: La présente étude visait à déterminer les quantités résiduelles d'enrofloxacine dans les échantillons de muscle de poulet.

Méthode: Quarante oiseaux ont été obtenus auprès d'élevages de volailles commerciaux dans l'État de Lagos et répartis au hasard en 7 groupes. Ils ont été humainement sacrifiés, disséqués et 10,0 g de tissu musculaire ont été sectionnés de chaque pilon et homogénéisés dans un mortier de porcelaine. On a pesé 5,0 g de l'homogénat et on l'a transféré dans un flacon d'échantillon brut de 5 ml et on l'a analysé par chromatographie liquide à haute performance pour déterminer la quantité d'enrofloxacine dans les tissus.

Résultats: L'enrofloxacine a été détectée dans tous les échantillons des quatre fermes. La concentration moyenne d'enrofloxacine variait de 23,5 à 88,1 µg/g dans tous les 7 échantillons analysés; Ces valeurs étaient supérieures au niveau maximal de résidus de 0,1 µg/ug en Europe et de 0,3 µg/ug aux États-Unis d'Amérique.

Conclusion: Cette étude a confirmé l'usage abusif de l'enrofloxacine dans les élevages de volailles et souligne la nécessité d'une réglementation plus stricte concernant l'usage des antibiotiques chez les volailles ainsi que le dépistage des résidus de poulet avant la vente.

Mots clés: Enrofloxacine, CLHP, Limite maximale de résidus, Volaille, Résidus

INTRODUCTION

Poultry farmers use antibiotics to enhance the health and productivity of poultry by boosting growth and feed efficiency[1] and as prophylactics.[2] Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase high quality meat and eggs at a reasonable price.[1]

The antibiotics are usually administered in sub-therapeutic doses, usually in water or feed, to protect animals against disease and to promote growth.[3,4] Exposing chickens to drugs through illegal or extra-label uses of drugs and use of mislabeled feed containing antibiotics may cause accumulation of veterinary drug residues in poultry meat.[5,6]

In order to promote growth and prevent disease in poultry, sub-therapeutic antibiotics (STAs) are often administered, which act by improving nutrient absorption and by depressing the growth of organisms that compete for nutrients.[7,8] There is however, growing concern among health workers and the public about the decreasing effectiveness of many antibiotics to treat human and animal diseases. In particular, the widespread use of antibiotics encourages the growth of antibiotic resistance in pathogen populations.[9] Consequently, there is concern that the widespread use of antibiotics, including STAs in animals, could promote development of drug-resistant bacteria that could pass from animals to humans, thus posing a danger to human health.[8] There is a worrisome world-wide trend of increased resistance to the these agents among bacteria responsible for both hospital and community acquired infection including methicillin-resistant Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcesens, Escherichia coli, Salmonella spp, Campylobacter spp and Neisseria gonorrhoeae.[10] In response to these concerns, the European Union (EU) has banned the use of antimicrobial drugs for growth promotion, and the FDA has concluded plans to phase out the use of medically important antibiotics in livestock for food production purposes.[11]

Some of the commonly used antibiotics today in poultry industries in developing countries include tylosine, neomycin, gentamycin, tetracyclines (chlortetracycline, oxytetracycline), sulfonamides (sulfadimethoxine, sulfamethazine, sulfathoxazole), penicillin (ampicillin), arsenicals (roxarsone), enrofloxacin, erythromycine.[12] Long-term use of these antibiotics results in their accumulation in tissues and organs at different concentrations.

Enrofloxacin, a second-generation fluoroquinolone administered to pigs, turkeys and chickens, has a broad-spectrum antibacterial activity, good bioavailability after oral administration and good to excellent tissue distribution.[13]

Poultry farming contributes to antibiotic use, though relatively little attention has been paid to how antibiotics are used in farm animals by regulatory bodies. The Food and Agriculture Organization (FAO) has reported that despite the fact that antibiotic use is beneficial to animal health, they should be used carefully to reduce the emergence of drug-resistant bacteria, with the resultant decrease in risk to public health risks. In addition to helping to protect the health and welfare of food animals, they are also conducive to meeting the increasing global demand for safe food of animal origin, including milk, meat and eggs.

Presence of drugs or antibiotic residues in food producing animals above the Maximum Residue Level (MRL) is recognized worldwide by various public health authorities as being illegal.[14] Their consumption could result in public health hazards including: antibiotic-resistant bacteria,[15] allergies, carcinogenic effects and potential harmful effects on human intestinal micro flora.[16,17] Despite these concerns, some researchers have reported little convincing scientific evidence that the use of antibiotics in food-producing animals is contributing to the antibiotic resistance issues that are relevant to human medicine.[18,19,20] Some researchers also reported in another study that antibiotic use in food-producing animals in a worst case scenario contributes less than 1% to the overall antibiotic resistance problem confronted by the medical profession.[21] This low figure should not however, remove existing valid reasons that point to the human health impact of antibiotic use in food-producing animals.

In Nigeria, the so-called "backyard" poultry farmers, who have easy access to veterinary drugs over the counter, are involved in a substantial proportion of poultry farming. They often use antibiotics without prescription by a veterinarian and with little or no supervision/control.[22] This indiscriminate use of antibiotics and the occurrence of antibiotic residues in food animal tissues is a clear pointer to the global problem.[4]

A search of available literature has revealed that several pathological defects in man have occurred due to man's consumption of toxic levels of antibiotic residues in food animals.[23,24] Because of the potential of these antibiotics to affect human health, consumption of food animals treated with antibiotics needs to be delayed for specific withdrawal period. This is to ensure that all residues in the affected animals are depleted to safe levels before

human consumption.[25.26]
The aim of this study was to determine the levels of enrofloxacin residue using High Performance Liquid Chromatography, considering the risk and public health significance of consumption of edible poultry containing antibiotic residues.

MATERIALS AND METHODS

Enrofloxacin and tinidazole (internal standard) were obtained from Central Research Laboratory of College of Medicine, University of Lagos. Chemicals used in this study were obtained from the following sources; Acetonitrile (HPLC grade, Sigma-Aldrich), methanol (Sigma-Aldrich), syringe filters 0.2µm (Acrodisc), centrifuge (Model 800D) and Agilent® 1200 series, homogenizer, vortex mixer, chickens (average weight 20 kg, purchased from commercial poultries).

Preparation of working concentrations of enrofloxacin and tinidazole (reference standard)

Enrofloxacin and internal standard stock solutions (1 mg/mL) were separately prepared by weighing 25 mg each into 25 mL volumetric flask using methanol as diluent. Gradient concentrations (2.5-60 µg/mL) were prepared in methanol in the presence of 25 µl of 10 µg/ml internal standard.

Extraction of enrofloxacin in spiked chicken

Each of the gradient concentrations of enrofloxacin standard was spiked into macerated and homogenized chicken muscle (5.0 g) of a local bird (that had not been treated with any drug); 25 µl of internal standard (10 µg/mL) was added. The mixture was vortex mixed and 5mL of chilled acetonitrile was added to deproteinize and extract enrofloxacin from the samples. The resulting mixture was centrifuged at 3000 rev/min for 10 minutes. The clear supernatant was filtered through syringe filter (0.2 µm) and 20 µl of the filtrate was injected into the HPLC machine for analysis.

HPLC Chromatographic conditions and analysis

The chromatographic elution was performed under isocratic conditions at 25°C. An Agilent® HPLC fitted with rheodyne valve injector (20 µl loop) coupled with a variable wavelength UV/Visible detector (294 nm), and data analyzed by chem.-station software. The column was a reversed-phase (250 x 4.6 mm); SMTC18, 5 µm. The mobile phase was acetonitrile: 25mM potassium dihydrophosphate buffer (20:80). The flow rate was set at 1 mL/minute.
The peak area ratios (enrofloxacin/internal standard) obtained were plotted against the gradient concentrations prepared. The resulting calibration regression equation was used to evaluate the amount of enrofloxacin in all the samples.

Sample treatment

Forty (40) birds were obtained from commercial poultries in seven local government areas of Lagos State and randomly allotted into 7 groups. They were humanely sacrificed, dissected and 10.0 g of muscle tissue was sectioned from each drumstick and homogenized in a porcelain mortar. 5.0 g of the homogenate was weighed and transferred into a 5 ml plain sample bottle. 25 µl of internal standard was added and the resulting mixture was subjected to extraction procedure as above.

Recovery studies

Three different concentrations (40, 60 and 80 µg/mL) were prepared in methanol and blank chicken muscle. Recovery rates were calculated from the peak area ratios of obtained by direct injection of enrofloxacin in methanol and that extracted from plasma.

Data analysis

Data obtained were analyzed using Microsoft® excel analytical software to determine the descriptive statistics such as mean values and standard variations.

RESULTS

Results show the concentration of enrofloxacin in the different samples of chicken meat sampled (Table 1). Various concentrations of enrofloxacin (from the lowest-23.5±0.16 to the highest- 88.1±7.92) were detected in all seven samples analyzed.

The concentration of enrofloxacin (µg/g) in the seven (7) samples analyzed ranged from the lowest, 23.5±0.16 to the highest. 88.1±7.92

Table 1: Concentration of enrofloxacin in chicken meat samples

Sample	Concentration (µg/g)
S1	49.3±5.64
S2	31.8±0.71
S3	30.4±0.16
S4	23.5±0.16
S5	88.1±7.92
S6	32.3±3.06
S7	36.4±0.75

Data expressed as Mean±SD n=40

At concentrations of enrofloxacin, 40, 60, 80 µg/mL recovery rate was 86.5, 70.0, and 91.9% respectively

Table 2: Enrofloxacin recovery rates

Conc. µg/mL	PAR in Neat Solvent	PAR In spiked Chicken Tissue	% Recovery
40	4.32	3.74	86.5
60	8.50	5.95	70.0
80	8.44	7.75	91.9

PAR = Peak Area Ratio

A plot of Peak Area Ratio of enrofloxacin versus concentration of enrofloxacin standard in methanol (fig. 1).

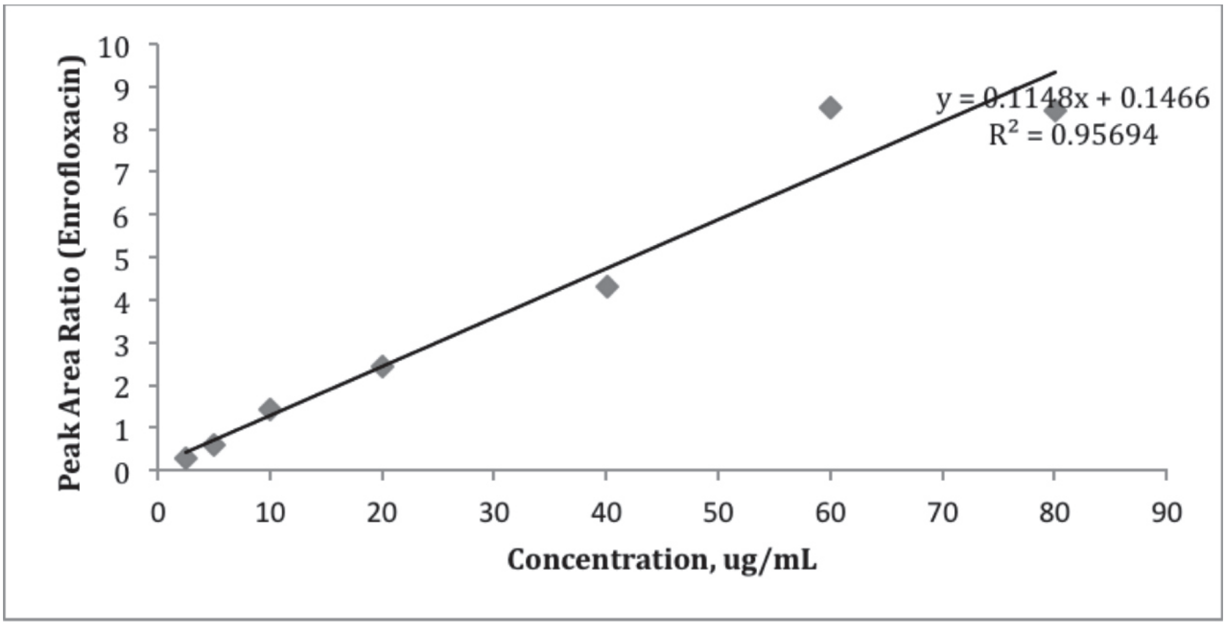

Figure 1: Plot of Calibration of Enrofloxacin Standard in Methanol

A plot of Peak Area Ratio of spiked enrofloxacin versus concentration of enrofloxacin detected in the blank chicken meat samples (fig. 2).

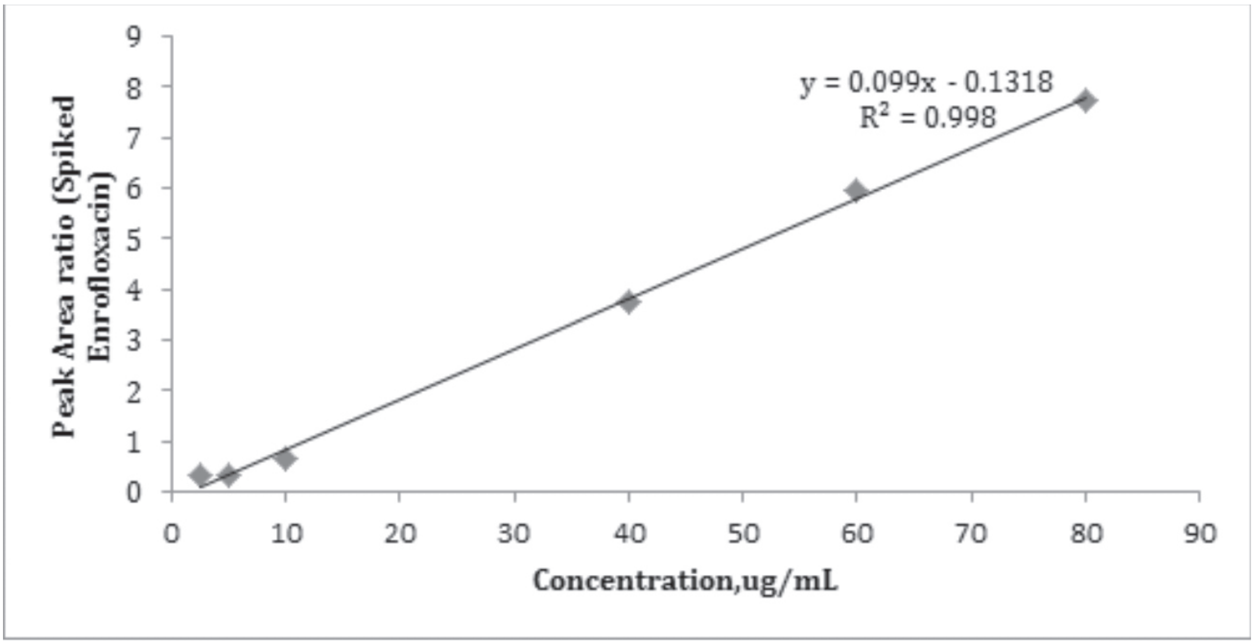

Figure 2: Plot of Peak Area Ratio (Spiked Enrofloxacin) versus Concentration.

DISCUSSION

The result of this present study showed that enrofloxacin was present in all samples in concentrations higher than the recommended MRLs set out by the European Agency for the Evaluation of Medicinal Products and the FDA. The highest concentration of enrofloxacin detected in this present study was 88.1ug/g a figure that is 881 times more than the 0.1 ug/g stipulated by the EMEA. These results confirmed that enrofloxacin was significantly used in poultry farms where samples were collected. The result from this current study is similar to that of Rokni,[27] who reported enrofloxacin residue in chicken tissues in concentrations higher than the stipulated levels. It is possible to therefore posit that the use of enrofloxacin and other antibiotics is a common practice among poultry farmers. Various researchers have reported that edible poultry tissues may be contaminated with harmful concentration of drug residues due to continuous and improper antibiotic usage in poultry industry.[1,23]

A few other studies on antibiotic residues in poultry animals performed in Nigeria all reported MRLs for the various antibiotics analyzed exceeding international standards.[16,22,26,28,29] In Nigeria and some other developing countries, food standards and regulations are inadequate and in some cases do not even exist to regulate and control the use of antibiotics and other medicines in food producing animals like cattle and poultry.[4,30]

The unsupervised or poorly supervised administration of antibiotics portends danger due to the possible adverse effects they impose on health of humans who consume such food animals. Human exposure to animal products containing considerable levels of antibiotic residues may induce and transfer resistance to human pathogens,[31] and cause several problems including disturbed immunological response and disorder of intestinal flora in susceptible individuals.

The Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medicinal Products have established Maximum Residue Limits (MRLs) for antibiotic residues, including enrofloxacin, in animal derived food. The European Agency for the Evaluation of Medicinal Products (EMEA) has set the MRLs of enrofloxacin at 0.10 ug/g in chicken muscle[32] and 0.030 ug/g according to FDA in different poultry tissues.[33]

The presence of enrofloxacin in such high concentrations in the chicken tissue from this study may suggest the indiscriminate use of antibiotics in poultry for various uses; it is possible that the recommended withdrawal time in the poultry was either not strictly applied or may be insufficient for this drug.[6,26,30] A study by Elkholy[34] determined that the withdrawal time of enrofloxacin in tissues of laying hens was 5 days. Nonga[35] observed the non-prudent use of antibiotics in Tanzania in a cross sectional survey study they did on use and occurrence of drug residues in poultry farms in Morogoro where 70% of the farms were positive for antimicrobial residues.

Enrofloxacin, a fluoroquinolone, is reported as having the ability to disrupt DNA replication and transcription process and also causes toxic effects in animals and humans including damage to the juvenile joint, the kidney, the eye, and the central nervous system.[36,37,38] Some antibiotic-induced allergic reactions have also been reported, in relation to quinolones.[39] These harmful effects of quinolones, including enrofloxacin on human health, makes it necessary to frequently monitor their usage in food animals and analyze such animal products for the presence of antibiotic residues. Enforcing regulations and controls related to use of antibiotics in food-producing animals will help ensure that they are not present in tissues above their safe Maximum Residue Limit (MRL) or if banned from use in veterinary practice, are not present at all.[28,30,40] Establishing and strengthening the regulatory system in terms of safety standards, proper monitoring, surveillance, testing and public education, will ensure the proper antibiotic use that safeguards public health and food safety.

A major limitation was that the poultry (chickens) sampled for this study were sourced within Lagos State only. The researchers did not sample chickens from other parts of the country, which could have different levels of the antibiotic.

CONCLUSION

This study suggested general misuse of enrofloxacin in poultry farms, with potential to cause hazards to humans consuming these antibiotic-containing food animals. The implications for public health are obvious, considering allergies and drug resistance that could develop in humans. In the poultry farms, proper supervision of drug administration, observance of the proper pre-slaughter withdrawal time and routine analysis of antibiotics in animal tissues before marketing should be enforced by the appropriate authorities.

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