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Research Article
Evaluation of Oxytetracycline Residue in Muscles and Organs of Broiler Birds in Sokoto Metropolis

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Abstract

Background and Objective: The broad-spectrum antibacterial drugs are commonly used in food-producing animals. Suspected deposits of these drugs in products of these animals may have negative effects on human health. The present study aimed to determine the oxytetracycline (OTC) residues in broiler's muscles and organs (heart, kidney and liver) samples. **Materials and Methods:** A total of 288 broiler birds were randomly allotted to four treatments T_1 (20 mg kg^{-1}), T_2 (40 mg kg^{-1}), T_3 (60 mg kg^{-1}) and T_4 (control) in a Completely Randomized Design (CRD). The treatments consisted of 72 birds per treatment, divided into six replicates, with 12 birds in each replicate. Detection of oxytetracycline (OTC) residues in muscles and organs was performed by high-performance liquid chromatography with an ultraviolet detector. **Results:** The result at 7 days withdrawal period traces of oxytetracycline found on muscles and organs analysis were within the acceptable limit. **Conclusion:** During 7 days withdrawal period, traces of oxytetracycline in muscles and organs (heart, kidney and liver) of broiler birds sampled for HPLC analysis were within the acceptable limits and it is recommended that, a withdrawal period of at least 7 days should be adhered to (depending on the drug) before slaughtering the birds.

Key words: Drug residue, broiler meat, oxytetracycline, Sokoto metropolis, muscles

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have been used in poultry production for both therapeutic and prophylactic purposes. Omeiza *et al.*[1], reported that most poultry farmers and livestock marketers/herd owners have employed the use of various antibiotics with or without the guidance of an expert in some states of Nigeria. Although antibiotics are beneficial, the illegal use of these drugs had led to the accumulation of toxic antibiotic residue in edible poultry products destined for human consumption in Nigeria. This poses a major threat hazard to the public that could be a toxicological or immunological consequence. Concerns were raised that the use of antibiotics as therapeutics and for growth promotion could lead to a problem of increasing resistance in bacteria of human and animal origin. Especially regarding resistance in Gram-negative bacteria (*Salmonella* spp. and *Escherichia coli*).

The indiscriminate use of antibiotics in animal production is an important factor contributing to the menace of antibiotic resistance. Even though the World Health Organization (WHO) identified antibiotic resistance as one of the major health challenges[2]. In developing countries Nigeria inclusive, the use of antibiotics in the animal production sector remains unregulated, leading to inappropriate use of the drugs and a widespread increase in antibiotics resistance. Mostly, antibiotics are administered for the treatment of infections, prevention of diseases and growth promotion[3].

According to Manyi-Loh *et al.*[4], some of the commonly used antibiotics today in poultry industries in developing countries like Nigeria that need to be studied on drug residue concentration in various organs and tissues of animal bodies are Tylosin, Neomycin, Gentamicin, Tetracyclines, (Chlortetracycline, Oxytetracycline), Sulfonamides, Penicillin (Ampicillin), Arsenicals (Roxarsone), Enrofloxacin and Erythromycin. However, it is highly recommended that whichever type of antibiotics a farmer uses, care should be taken to abide by its withdrawal period. According to Prestinaci *et al.*[2], the acceptable Maximum Residue Limit (MRL) for oxytetracycline (OTC) as recommended by the joint FOA/WHO expert committee on food additives is 0.2, 0.6 and 1.2 μg g^{-1} for liver, lung and kidney tissues, respectively.

Thus, the production practices of the poultry farmers particularly those that make indiscriminate use of antibiotics need to be investigated. Maximum residue level and drug withdrawal period as well as the level of antibiotics usage ought to be stringently adhered to in treated birds before products (meat or eggs) are approved for human utilization.

This research dwelled on analysing residue in meat and organs of broiler birds administered below, normal and above recommended dosages of OTC drugs.

MATERIALS AND METHODS

Study area: The study was carried out at the Poultry Production Unit (PPU) of the Teaching and Research Farm in the Department of Animal Science, Faculty of Agriculture, Usmanu Danfodiyo University, Sokoto between July, 2019 to September, 2021. Sokoto is located between latitudes 12° and 13°N, Longitudes 4° and 6°E in the Northern part of Nigeria and lies at an altitude of 350 m above sea level[5]. The state falls within the Sudan Savannah vegetation zone with alternating short rainfall and long dry season. A dry period (harmattan) occurs between October and February[6]. The annual rainfall is about 700 mm. The rainy season starts from June to early October with a peak in August, potential evapotranspiration has been reported to be 162 mm. A maximum temperature of 45 has been reported in April and a minimum of 13.2 on January[7]. The state is one of the largest livestock-producing areas in Nigeria. Among the livestock produced in the area are camel, cattle, donkey, sheep, goats and poultry to a large extent[5].

Experimental design: The study was carried out using a Complete Randomised Design (CRD), a total of 288 broiler birds were used, 4 treatments, (72 birds per treatment) divided into 6 replicates, with 12 birds in each replicate. Treatment 1 was (20 mg kg^{-1} OTC-half recommended dose), treatment 2 (40 mg kg^{-1} OTC recommended dose), treatment 3 (60 mg kg^{-1} with OTC above-recommended dose) and treatment 4 control (0% OTC) which were administered via drinking water.

Source of experimental birds: Day-old broiler chicks for this study were obtained from an Agrited farm in Ibadan Oyo State, Nigeria. The birds were transported to Sokoto under the cool hours of the evening through the night and arrived in the morning hours.

Feed compounding: Feed ingredients used for this experiment include, maize, groundnut cake (GNC), soya bean meal and bone meal-required crushing so that the particle size will suit the group of birds the feed is to be meant for. Feed

Table 1: Gross, calculated and analyzed chemical composition of experimental diets

Ingredient (kg)	Starter	Finisher
Maize	52.0	50.5
Soya beans meal	17.0	14.0
Groundnut cake	15.5	14.0
Fish meal	2.5	1.50
Wheat offal	8.0	11.0
Limestone	2.0	4.0
Bone meal	2.0	4.0
Premix	0.25	0.25
Salt	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Total	100 kg	100 kg
Calculated chemical composition		
Crude protein (%)	22	20.05
Energy (kcal kg^{-1})	3005	2852
Methionine	0.35	0.35
Lysine	1.0	0.9
Calcium	0.8	0.8
Phosphorus (average)	0.45	0.45
Fibre (%) (maximum)	6.0	6.0
Analysed chemical composition		
Crude protein	22	20
Energy (kcal kg^{-1})	3005	2850
Methionine	0.5	0.5
Lysine	1.0	0.9
Calcium	1.4	2.6
Phosphorous	0.6	0.8
Fibre	5.4	5.1

ingredients in powdery form were weighed and mixed with the crushed ones. The feed compounding was done on a clean concrete floor and thoroughly mixed with a shovel to a uniform mix. The gross, calculated and analysed chemical composition of experimental diets were presented in Table 1.

Antibiotic residue determination using High-Performance Liquids Chromatography (HPLC): Samples where heart, kidney and liver were collected from four birds in each treatment forming sixteen samples, which were used for the HPLC analysis of the residual presence of OTC. Samples were collected after 7 days withdrawal period of antibiotic administration, they were then enveloped in a self-sealing polythene bag and moved to Usmanu Danfodiyo University, Central Laboratory in a cool chain for preliminary screening of the samples for the presence of OTC residue. The muscles and organs samples collected were subjected to an HPLC machine for both qualitative and quantitative analysis of OTC as adopted[8].

Tissue extraction: Five grams of heart, kidney, liver and muscle samples were weighed and cut each separately into smaller sizes using a surgical blade and then transferred into falcon tubes and 5 mL of methanol was added. The mixture was homogenised for 60 sec, after homogenization, the samples were centrifuged and vortexed and filtered, the process of centrifuging and vortexing and adding 2 mL of acetonitrile (60%) HPLC grade was repeated until reasonable and clear sample content was gotten and was measured with Eppendorf pipette into HPLC vials for subsequent running in the HPLC analyser as adopted[8].

Data presentation results: Graphs were used to indicate OTC residue in samples examined.

RESULTS

High-Performance Liquid Chromatography (HPLC): Oxytetracycline residue quantification result using HPLC shows no residue in all the samples of the treatments. The least of the standard set for detection was 0.63 mg mL^{-1} as shown in Fig. 1, while Fig. 2-5 were samples of heart, kidney, liver and muscle, respectively taken from an above-normal dose of oxytetracycline without peak of detection.

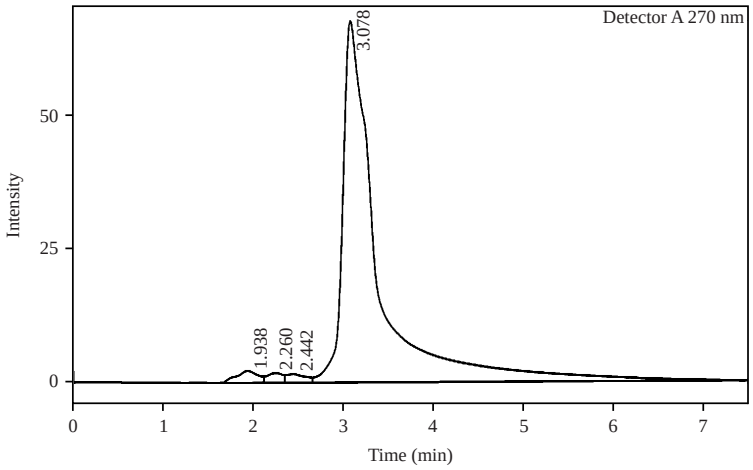

Fig. 1: HPLC chromatogram of oxytetracycline standard of 0.63 mg mL^{-1}

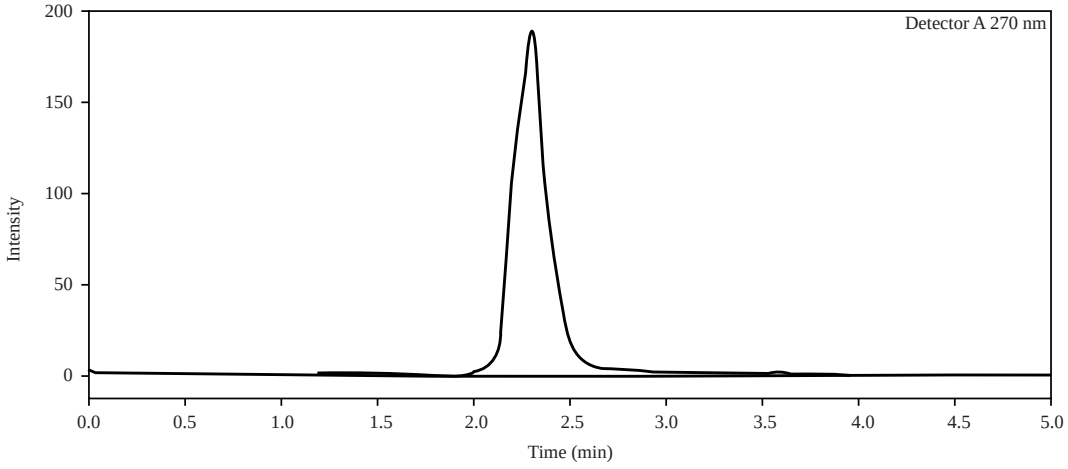

Fig. 2: HPLC chromatogram of (60 mg kg^{-1}) heart

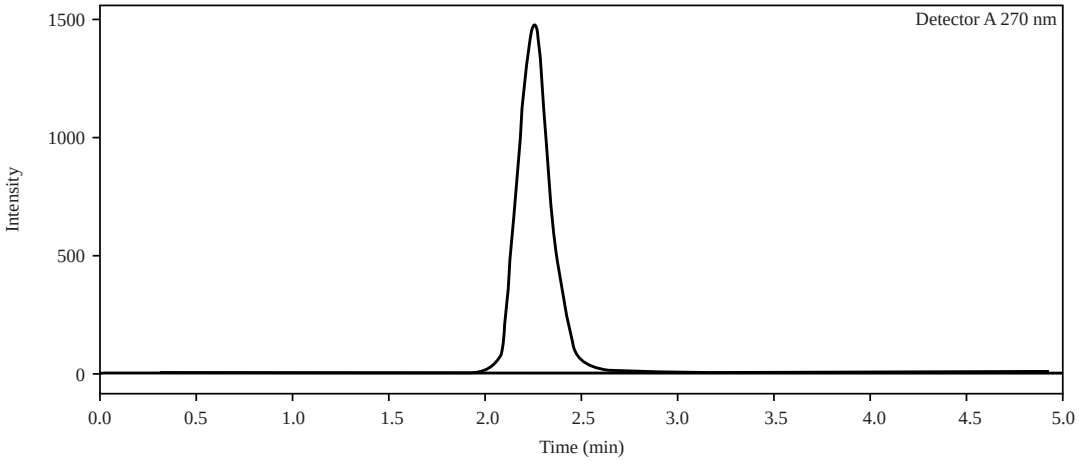

Fig. 3: HPLC chromatogram of (60 mg kg^{-1}) kidney

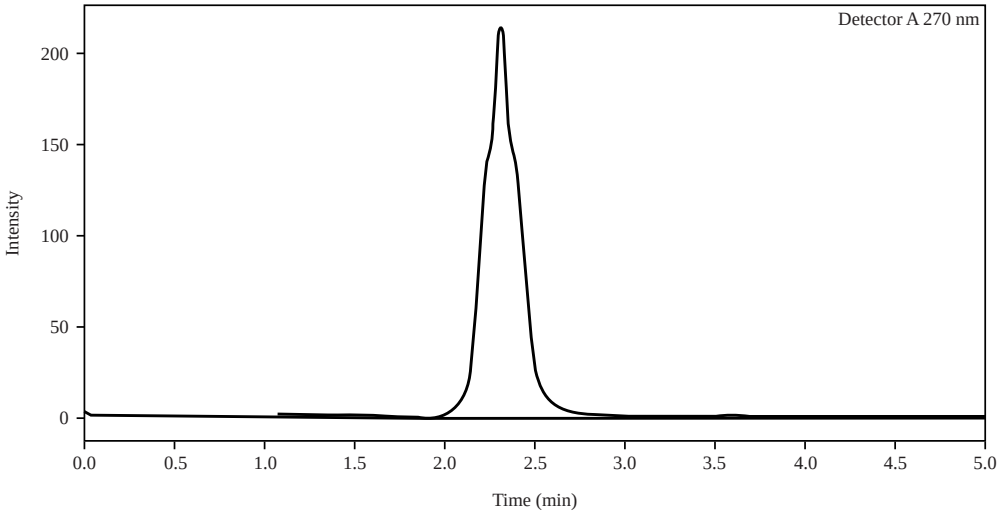

Fig. 4: HPLC chromatogram of (60 mg kg^{-1}) liver

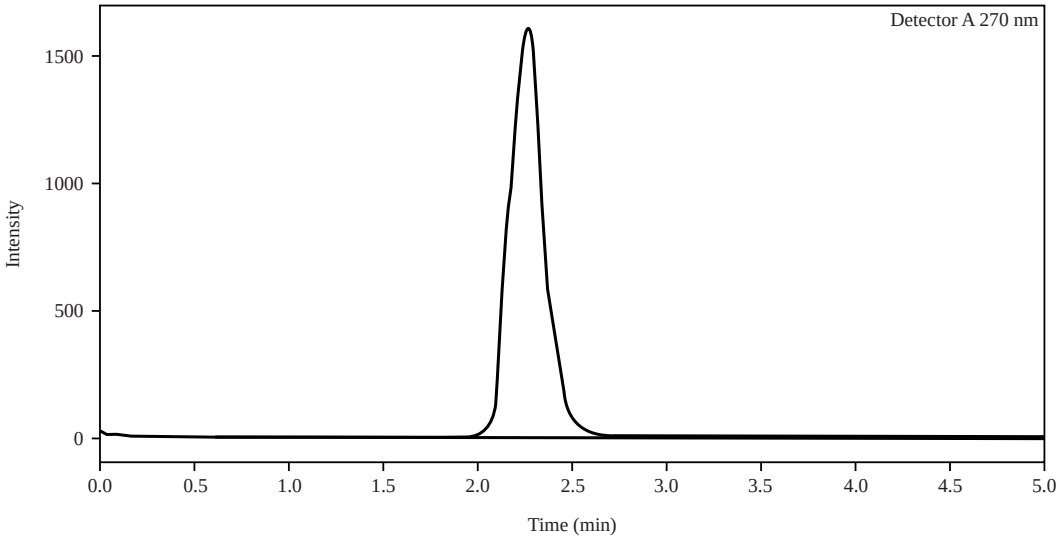

Fig. 5: HPLC chromatogram of (60 mg kg^{-1}) muscle

DISCUSSION

Oxytetracycline residue quantification results using HPLC analysis shows no residue in all the sampled organs (heart, liver and kidney) and muscle collected for all the observations. T_1 (20 mg kg^{-1}), T_2 (40 mg kg^{-1}), T_3 (60 mg kg^{-1}) and T_4 (control), across all the standard set that was 0.63, 1.25, 2.5, 5.0 and 10.0 mg mL^{-1}, respectively. This may be due to the complete metabolism of OTC, as a result of 7 days withdrawal period of the drugs. This result contradicted the findings of Cetinkaya *et al.*[8], who reported, that residue of OTC was detected (at zero withdrawal period) in his study as a result of non-adherence to the recommended days of withdrawal of the drugs. Corroborating this finding, OTC levels were also below Maximum Residual Limits (MRL) in bones and muscle of broiler[9] eggs[10], chicken meat[11] and broiler chicken's claws[12]. However, it was reported that samples were having concentrations above the MRL for OTC in fat and organs[13] and before 4 days of withdrawal in meat[14].

Our results show that OTC is completely metabolized from treated birds and is undetected in organs up to 7 days post-treatment. In this study, a low probability of detection was observed. This may be mainly due to the adherence to the withdrawal period that gives a window to complete

metabolism of the drug since up to 7 days was observed. Therefore, the factor of detection of the antimicrobial through other methods than HPLC was not determined in this study. The carry-over and persistence of this antibiotic may be a risk for the development of antibiotic-resistant pathogens Harada and Asai[15]. For this reason, it is indispensable to observe OTC residue transfer from animals to help reduce the risk of antibiotic resistance for both public and animal health.

While the obtained results are interesting and raise some concerns that can be addressed in further studies, some limitations should be recognized. The administration of the drug did recreate industry conditions since the objective was to ensure the administration of the exact, above and below the recommended dose. Likewise, only one type of antibiotic was studied. Therefore, it would be interesting to take these aspects into account in future research to compare the results obtained and thus determine the factors that may play a role in the persistence and transfer of OTC residues into broiler products.

CONCLUSION

Based on the findings of this research, at 7 days withdrawal period, traces of oxytetracycline in muscles and organs (heart, kidney and liver) of broiler birds sampled for HPLC analysis were within the acceptable limit. It is recommended that, a withdrawal period of at least 7 days should be adhered to (depending on the drug) before slaughtering the birds.

SIGNIFICANCE STATEMENT

This work discovers the aspect of antimicrobial residue limits in broiler meat to minimum days of withdrawal vis-a-vis the threshold of antimicrobial drug administration. This study will help researchers and policy makers in addressing issues of pharmacokinetics, therapeutic drug monitoring, pharmacovigilance and pharmacoeconomics of antibiotic usage in the Sokoto metropolis which is the bedrock in controlling antibacterial resistance in the food chain.

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