Iranian Journal of Veterinary Science and Technology

Received: 2024- Jul-08
Accepted after revision: 2025- Feb-15
Published online: 2025- Mar-01

RESEARCH ARTICLE

DOI: 10.22067/ijvst.2025.88457.1389

Tylosin Residue in Chicken: Detection with ELISA, Four Plate Test, HPLC, Effect of Heat Treatment and implications for Human Health

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ABSTRACT

Tylosin residues (TR) in chicken meat pose potential health risks to consumers. This study aimed to detect and quantify TR in chicken tissues from Ikpa slaughterhouse, Nsukka and evaluate the effect of heat treatment on TR concentrations. Sixty randomly sampled chicken were processed, and their muscle, liver, and kidney tissues were collected and tested for TR at raw and after ten, fifteen , and twenty minutes of cooking and microwaving using enzyme-linked immunosorbent assay (ELISA), four plate test (FPT), and high-performance liquid chromatography (HPLC). Of the 180 tissues, 93 (51.7%) were positive for TR. The prevalence of TR was 40% in muscles, 55% in liver, and 60% were in kidney samples with ELISA. Six liver samples exceeded the maximum residue level (MRL) of100 µg/kg. Cooking and microwaving reduced TR concentrations by 97-100% in muscle and liver tissues using HPLC. The mean inhibition zones decreased by 87-100% after cooking and microwaving using FPT. Chicken at Ikpa slaughterhouse, Nsukka have TR even in concentrations above the MRL but were significantly eliminated ($p < 0.05$) after 20 minutes of heat treatments (cooking or microwaving). Hence, mitigating the health risks associated with TR in meat requires regular screening and quantification, public awareness campaigns targeting consumers of raw or improperly cooked chicken, strict policies on antibiotics use in poultry, and enhanced meat handling and processing practices in food industry.

Keywords

Tylosin Residue Analysis, Chicken, Cooking, Microwaving, ELISA, Four Plate Test, HPLC, Food Safety

Number of Figures: 5
Number of Tables: 2
Number of References:: 39
Number of Pages: 9

Abbreviations

ELISA : enzyme-linked immunosorbent assay
FPT : four-plate test
HPLC : high-performance liquid chromatography
MRL : maximum residue level
CRD : chronic respiratory disease

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Introduction

Chicken is the second most widely produced, exported, and consumed meat worldwide [1, 2]. It has contributed significantly to the supply of about 40% of protein needed as demanded by the increasing human population globally [3]. However, the modern-day integrated and intensive production system has been associated with the unwarranted use and misuse of antimicrobials in preventing disease occurrences and as growth promoters for poultry in compounded animal feed [4]. Tylosin is a 16-membered macrolide approved for therapy for a variety of infectious disease agents, including Mycoplasma gallisepticum and M. synoviae, which causes chronic respiratory disease (CRD) in poultry. They are metabolized in the liver, where the highest tissue concentrations are found, especially in chickens and turkeys [5]. TR should not be detected in the edible tissue of treated birds and other products of animal origin in concentrations exceeding the recommended MRL of 100µg/kg [6]. Hence, the recommendation that chickens should not be slaughtered for human consumption 6 days after the last oral tylosin administration [7]. The excess of the residues in meat above MRL could pose high toxicological, microbiologically, or immuno-pathological damage to the consumers of contaminated meat hence the need to effectively prevent and reduce the TR occurrence in meat for human consumption.

Chicken is usually cooked or roasted ("suya") with or without food additives to increase taste, shell life, digestibility, and other sensory properties thereby making them appetizing to the consumers [8]. Nevertheless, the knowledge of the reductive impact of cooking or any other thermal processing methods on TR in heat-and-serve meat is still very limited [9]. There has been a report of an overall reduction rate of TR in meat by 35.3% and 79.9% after 2 and 30 minutes of microwaving and boiling respectively [10]. Meanwhile, other factors, including the concentration of TR in raw meat before processing have been reported to influence the rate of TR reduction in meat [11].

The use of ELISA kit has been reported to have good performance in the analysis of antimicrobial residues like Tylosin in meat as it has the advantages of specificity and sensitivity [12]. It allows the analysis of a large number of samples per kit in a few hrs without the requirement of sophisticated instrumentation unlike the Four Plate Test (FPT) [13]. HPLC on the other hand, quantifies the concentration of the residue in meat unlike FPT [14]. It has more prospects of repeatability, selectivity, resolution, high recovery, and ease of application compared to others [15, 16]. FPT, a microbiological assay, offers a simple and cost effective approach, but may be less sensitive and specific compared to ELISA and HPLC. However, Enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), and other chromatography-mass spectrometry types are generally used in diverse analytical techniques for the detection of antimicrobial residues in foods of animal origin [12, 17, 18]. The respective strength of the three methods was leveraged in this study to ensure a comprehensive and accurate assessment of TR in chicken. Tylosin is indiscriminately used by farmers in the study area while data on the contamination rates in the processed chicken are unavailable in the literature to the best of our knowledge. Hence it has become necessary to constantly monitor and periodically assess the risks of exposure to TR associated with misuse or abuse in poultry production and take apt action to ensure meat safety [19]. Moreover, the detection rates with different tests and effects of thermal meat processing could be validated to guarantee consumers' confidence and meat safety. The study therefore evaluates the concentration of TR in chicken sold to consumers in Nsukka, Nigeria and the impact of heat treatment using ELISA, Four Plate test and HPLC for informed evidence-based strategies for ensuring food safety and mitigating the risk associated with TR and antimicrobial resistance in southeast, Nigeria.

Results

Detection of TR in muscles, kidney, and liver tissues of slaughtered chicken using using ELISA

Out of 180 tissues of sampled birds, a total of 93 (51.7%) was positive for TR, with 24 (40%), 33 (55%), and 36 (60%) of the detected proportion in muscles, liver, and kidney respectively while 6 out of 33 (18%) positive liver samples were above the MRL of 100 µg/kg. However, there was no association between the type of tissue and the occurrence of TR ($x^2 = 5.206$; $P = 0.0741$) (Table 1).

Effect of cooking and microwaving on TR in muscles and liver tissues of chicken using FPT

The effect of cooking at different times (10, 15, and 20) minutes revealed reduction rates of TR concentration at 28.2%, 64.1%, and 100% with the decreasing mean inhibition zone from 9.75mm to (7, 3.5, and 0 mm) respectively in muscle tissues, and 22.2%, 53.3%, and 100% with the decreasing mean inhibition zone from 11.25mm to (8.75, 5.25, and 0 mm) in the same order of cooking in the liver tissue (Table 2).

On the other hand, the impact of microwaving revealed a 29.5%, 46.3%, and 100% reduction rate after

Table 1.
Detection of Tylosin residue in raw chicken tissues from Ikpa slaughter, Nsukka, using ELISA test

Tissue (60 each)	Frequency (concentration (µg/kg)		Proportion detected (%)	Above MRL 100 µg/kg (%)
	Undetected (≤ 2.0)	Detected (> 2.0)		
Muscle	36	24	40	0 (0)
Liver	27	33	55	6 (18.0)
Kidney	24	36	60	0 (0)
Total	87	93	51.7	6 (6.5)

($x^2 = 5.206$; P = 0.0741). MRL (Maximum Residue Level) WHO (2004)

Table 2.
Effect of cooking versus microwaving on TR concentrations in chicken tissues using FPT and HPLC

Tissues	Mean inhibition zone raw chicken (mm)	Mean inhibition zone (mm) and reduction rate (%) after 10-20 (mins) cooking			Mean inhibition zone (mm) and reduction rate (%) after 10- 20 (mins) of microwaving		
		10	15	20	10	15	20
FPT	Muscle	7.0	3.5	0	6.7	5.1	0
		(28.2)	(64.1)	(100)	(29.5)	(46.3)	(100)
FPT	Liver	8.75	5.25	0	7.75	5.1	1.5
		(22.2)	(53.3)	(100)	(32.6)	(55. 7)	(87)
HPLC	Muscle	16.4	9.6	0.4	22.2	5	0
		(48.1)	(69.6)	(98.7)	(29.7)	(84.2)	(100)
HPLC	Liver	31.8	15.8	0.6	36.6	16.8	1.4
		(33.2)	(66.8)	(99)	(23.1)	(64.7)	(97.1)

10, 15, and 20 mins with the decreasing mean inhibition zones from 9.5mm to (7.75, 5.1, and 1.5 mm), respectively, in the muscle tissue and 32.62%, 55.7% and 87% with decreasing mean inhibition zones of (6.7, 5.1, and 0 mm) in the same order in the liver tissue (Table 2).

Effect of cooking and microwaving on TR in muscles and liver tissues of chicken using HPLC

The use of HPLC revealed a reduction rate of 48.1%, 69.6%, and 98.7% in the TR concentration from 31.6 µg/kg to (16.4, 9.6, and 0 µg/kg) respectively after 10, 15, and 20 minutes of cooking in the muscle tissue in the same order, while 33.2%, 66.8% and 99% reduction in concentration from the initial 47.6 µg/kg to (31.8, 15.8, and 0.6 µg/kg) in the same order of cooking were revealed for the liver tissue. Significant differences exist between contamination rates in raw and cooking of both muscle and liver tissue at 20 mins ($p < 0.05$) (Table 2) (Figs. 1 and 2).

Moreover, the effect of microwaving on TR con-

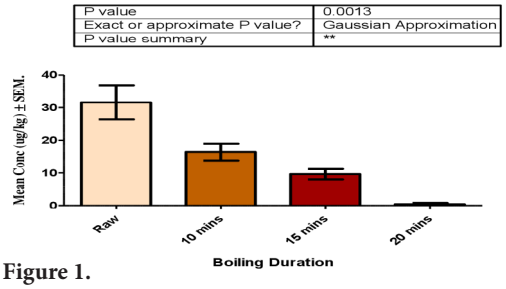

Figure 1.
Mean concentration of Tylosin residue in raw muscle tissue and after different time of cooking.

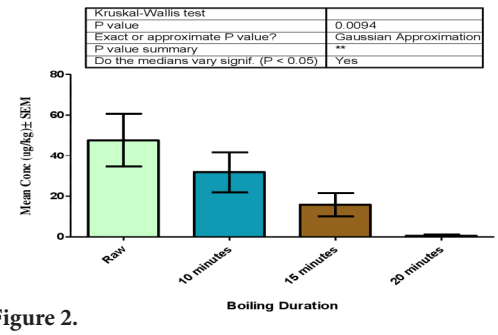

Figure 2.
Mean concentration of Tylosin residue in raw liver tissue and after different time of cooking.

centration reduction using HPLC also revealed 29.7%, 84.2%, and 100% reduction rate with corresponding values from 31.6 µg/kg to (22.2, 5 and 0 µg/kg) after 10, 15, and 20 mins of microwaving respectively in the muscles tissue and 23.1%, 64.7% and 97.1% with corresponding values from 47.6 µg/kg to 36.6, 16.8 and 1.4 µg/kg in the liver tissues in the same order (Table 2). Statistically significant differences exist between the raw and after 20 mins microwaving of both muscle and liver tissues ($p < 0.05$) (Figs. 3 and 4).

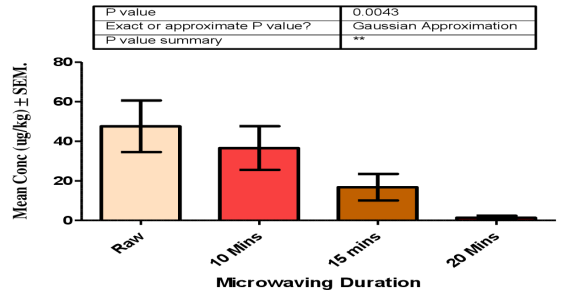

Figure 3.
Mean concentration of Tylosin in raw liver tissue and after different time of microwaving.

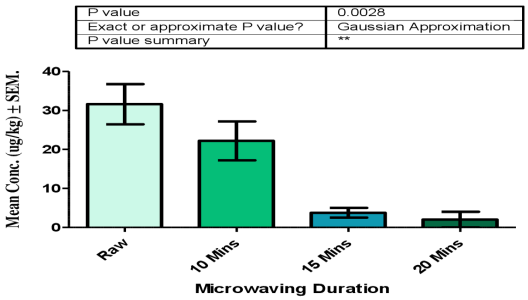

Figure 4.
Mean concentration of Tylosin in raw muscle tissue and after different time of microwaving.

Discussion

The contamination rate of TR in slaughtered chicken at 51.7% is of public health concern, especially with the values above the MRL in the liver tissues. Humans especially the consumers of such muscle parts and liver of contaminated chicken are at the risk of the many chronic health challenges which have been associated with the TR accumulation in the body system [23]. The residue is known to interrupt the colonization barrier of the gastrointestinal tract in humans because of its antibiotic activities against bacterial strains in the human colonic flora [23, 24]. This could lead to antibiotic resistance development and resistant gene transfer especially when such contaminated chicken is not properly processed via cooking or other thermal heat methods before consumption [25]. The effect is equally of economic consequences with regards to resistant or difficult-to-treat infections, high cost, and longer duration of medication or stay at the hospitals [26]. The health implications of the findings in this study could involve a wider range of consumer populations as the meat from Ikpa slaughterhouse, Nsukka, Enugu State, Nigeria are usually transported to the neighboring states of the country including Kogi and Benue. The chicken meat are processed, and consumed in the form of pepper soup by the street meat vendors or as roadside ready-to-eat meat 'suya' joints [27]. The accumulation of TR in chicken as revealed in the study could be a result of constant abuse of the drug in poultry production in Nsukka ranging from wrong dosage, wrong route of administration, non-adherence to the withdrawal minimum period of 3 days before slaughtering[11, 28]. There is enough evidence that the majority of the farmers are ignorant of the consequences of antibiotic misuse or abuse in poultry in the Nsukka area in particular and Nigeria at large [29, 30].

The detected concentration (51.7%) of the TR using the ELISA test, serves as a true reflection of the contamination rate in the study area since it screened tylosin, specifically not macrolids. The observed rate is higher than 6.3% in meat samples in Croatia using the same ELISA test [31]. Furthermore, the detection rate of TR at 40% in the muscle in this study was slightly lower than 50.6 % in chicken breast meats as reported in Oman using the ELISA method [12]. The differences may be a reflection of differences in the level of exposure of poultry to tylosin or abuse by farmers in developing countries, where farmers easily assess drugs over the counter without prescription, compared to the developed countries where restrictions on drug use are fully implemented. The maximum Residue Limit (MRL) is the maximum concentration of drug residue like tylosin permitted in food products including chicken. It was established in accordance with international standards to guarantee food products safety for human consumption and to protect public and environmental health. The higher distribution of the residue in the liver tissues with values above the MRL was in agreement with the report of Pavlov et al. (2008) that recorded higher residues in kidney and liver tissues, and this could be attributed to the biotransformation and detoxification actions of the liver with slower depletion rate when compared with the muscle tissues [32]. This further agrees with the JECFA (2006) report of positive meat samples with higher TR in the liver and kidney than in muscle tissues [33]. However, the estimated daily intake of muscle tissues is more than that of liver and kidney, thereby making muscle tissues a more important risk assessment point for TR effect in humans. The 18% rate of TR exceeding MRL as found in the study, was lower than 47.83% in

chicken meat with the use of high-performance liquid chromatography method [34]. Meanwhile, the range of tylosin concentration in the study (31.6-47.7) µg/kg was within the average amount of tylosin (38.8) µg/kg reported in China even though it was in swine and bovine tissue samples (muscle, liver, kidney) using LC-MS/MS [35]. On the other hand, very high values (105.4-109.2) µg/kg in 2 (0.6%) of 300 chicken meats have been reported using the HPLC method [36].

The effect of the heat treatments on the TR, as detected using FPT, has revealed total elimination in both the muscle and liver tissues after boiling for 20 minutes compared to 87% and 100% in muscle and liver tissues after 20 minutes of microwaving. This was in agreement with the effect of the cooking process that significantly reduced the TR in both thermal processing procedures with negative correlations between the length of cooking time and the reduction percentage of tylosin using HPLC [10]. However, it disagrees with the overall effect of cooking time on TR reduction in meat, which was reported to significantly decreased after cooking but not microwaving [37]. This may be because of the longer microwaving time of 20 minutes in this study, as opposed to the two minutes in the reported study. A lower reduction rate of 35.3% has also been reported for TR after two minutes of meat microwaving [11]. Limited education and training, easy access to antibiotics in the country, absence of regulations and enforcement of antibiotic use by poultry farmers and lack of oversight by veterinarian may have contributed to the misuse or overuse of tylosin.

Limited monitoring and surveillance of antibiotic residues in poultry products may also have played a role in addition to economic pressure to maximize production and make profit. The reductive effects of cooking and microwaving were further confirmed with the detection of the TR concentration in the tissues using HPLC, which also recorded similar reduction rates with significant impact after microwaving at 20 minutes [38]. The use of both tests has shown that raw muscles and liver with a high concentration of TR have a time-dependent reduction rate when cooked or microwaved. The 100% significant reductions in the concentration of TR for both muscle and liver tissues over time between the raw, cooked, and microwaved chicken were in agreement with work done by Salaramoli et al. (2015), who also recorded a significant reduction in TR in chicken meatballs subjected to microwaving and boiling treatments [10]. Furthermore, other similar studies have reported a 90-100% reduction of antibiotic residues including that of ciprofloxacin, oxytetracycline, and sulfamethazine in meat tissues using HPLC [11, 39]. It has been reported that the residue levels in meat tissues following heat treatment of different cooking methods and time reduced in the tissues but increased in the juiced water, however, the level in the juiced water was not checked in this study and can be a limitation.

As a result of abuse or misuse of Tylosin, and not complying with the withdrawal period in treated poultry, TR was found in slaughtered chicken tissues from Ikpa slaughterhouse even in concentrations above the MRL, hence it constitutes a health risk to the consumers. Awareness campaigns on the health implications of TR in ready-to-eat meat, judicious use of antibiotics, adherence to withdrawal time before slaughter, and the use of probiotics as alternatives in poultry production have become inevitable. It has been shown that 20 minutes of cooking or microwaving significantly eliminates TR in meat. Therefore, adequate heat application on meat, either by cooking or microwaving, should be encouraged, especially for ready-to-eat meat products. Further studies to check the concentration of the TR in the juice (broth) of the cooked chicken should be encouraged since consumers also drink the juice of cooked meat in different forms.

Materials & Methods

Ethical approval

The protocol for the research project was approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, with reference no: FVM-UNN-IACUC-2024-03/147.

Study area and sample collection

The study area is Nsukka, while the sample collection site was Ikpa slaughterhouse (Fig. 5) [20]. The Ikpa slaughterhouse was visited twice a week, and on each visit, a 1 in 4 systematic random sampling technique was used to select three out of 12 to 15 birds from poultry retailers that bring birds for slaughter from different towns within Nsukka environs, including the university community. Two birds were selected using a simple random sampling technique from each selected retailer. In each visit, six birds were purchased, i.e., 12 birds per week for 5 weeks. A total of 60 birds were purchased and slaughtered; the breast muscles, kidney, and liver tissues were harvested. A total of 180 tissue specimens were collected using sterile universal bottles and transported in cold conditions, which were maintained with ice blocks to the Department of Veterinary Public Health and Preventive Medicine, University of Nigeria, Nsukka laboratory for analysis.

Sample preparation for TR detection

Two grams of each of the harvested tissues was weighed, macerated, and emulsified with an equal volume of distilled water in a 1:1 ratio and introduced into centrifuge tubes. The tubes were centrifuged at 5000 rpm for 10 mins, and the supernatant was decanted while the required quantity of the solution was used for Tylosin detection.

Detection of TR with ELISA test

The microtiter plates of the ELISA test kit and the reagents were sourced from Shenzhen Lvshiyuan Biotechnology Company Lim-

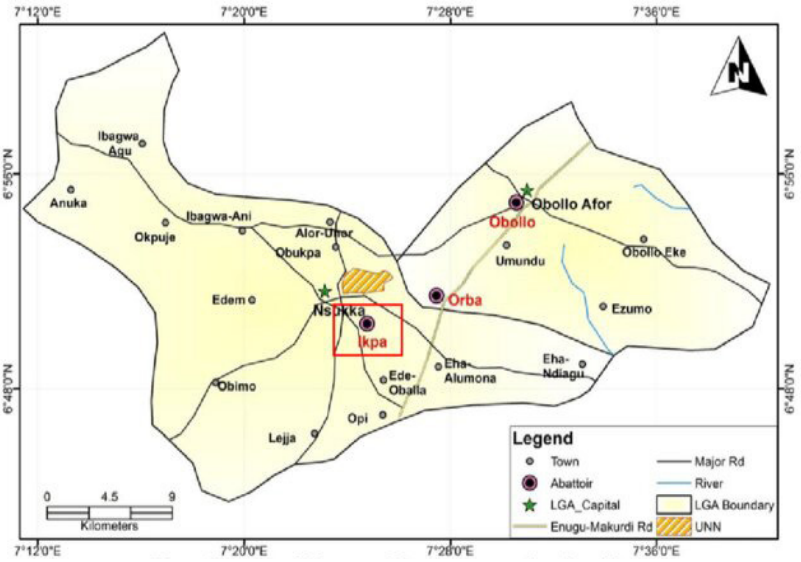

Figure 5
Map of Nsukka urban with blue rectangle showing the study site (Ikpa slaughter). Source: Nwankwo *et al.* (2021).

ited, Shenzhen, China. The microtiter plates, and the reagents were adjusted to room temperature before use. The lyophilized conjugate was reconstituted first with 1ml of conjugate diluents, vortexed, and diluted with the same conjugate diluents at a 1:10 ratio. The standards and the control were reconstituted with 1 ml of deionized water. The wash buffer (5X) concentrate was diluted at a ratio of 1:5. For each plate, a working scheme was prepared, and the standards and samples were run in duplicates as previously reported [21] and briefly described. Twelve strips, each containing 8 wells, were fixed on the plate. Each of the six provided 50 µl standard solutions (0, 2, 6, 18, 54, and 162 ng/kg) was added in duplicate wells according to the working scheme. 50 µl of each tissue extract sample was added in duplicate wells following the standards according to the working scheme. The antibody conjugate (50 µl) was added to each of the wells. The plate (wells) was covered with paraffin tape, and the content was mixed by circular motion on the bench for several seconds and then incubated at 30oC for 30 seconds. It was tapped from time to time to remove bubbles. The microtiter wells were further washed with a wash solution five times and tapped to remove bubbles completely. Solution A (50 µl) color was added, followed by solution B color immediately and mixed thoroughly by shaking. The microtitre plate was incubated at 37oC for 10 minutes. Stop solution (50 µl) was then added. The absorbance was read at 450 nm wavelength within 5 min of adding the Stop solution.

Thermal treatments of ELISA-positive samples

Each positive sample with a high concentration of tylosin after the ELISA test was divided into two parts by weight, and then subjected to different processing methods (boiling and microwaving). Twenty-gram sample each was placed into a strainer, immersed in a 10 ml water bath preheated to 100oC and cooked for 10, 15, and 20 min, and allowed to cool before extraction while the same quantity of sample was placed in a glass tray and microwaved at 450 W for 10, 15, and 20 min and allowed to cool before extraction.

Analysis of raw and heat-treated meat samples using FPT and HPLC

All the raw and heat-treated samples were subjected to FPT and HPLC analysis to determine the residue level using modified methods as previously reported [21, 22].

Four Plate Test: Briefly, two grams of each organ were macerated with an equal volume of sterile water in porcelain mortar and pestle, centrifuged at 3000 revolutions per minute (rpm) in a test tube for 10 minutes after which the supernatant was decanted and stored for analysis. Three batches of Mueller Hinton agar were prepared according to the manufacturer's recommendations and autoclaved. After cooling to 45 – 50oC, they were adjusted to pH 6, 7.2, and 8 using NaOH (base) and HCL (acid). Ten milliliters of the media was poured on sterile Petri dishes and allowed to solidify. Each plate with pH 6, 7.2, and 8 was seeded with a ready-to-use suspension of Bacillus subtilis (Merck Darmstadt, Germany), and another media at pH 8 was seeded with 24-hour culture of Micrococcus luteus bacterial suspension (ATCCR 10240). Four wells were bored, and 80 µl of each tissue extract was inoculated into each of the wells and the fourth well was inoculated with tissue extract from oxytetracycline treated bird as positive control. After, the plates were incubated for 24 hours at 37°C. They were observed for the clear zone of inhibition with an annular diameter ≥ 2 mm, which indicated a positive test for antimicrobial residues.

HPLC-based test: Briefly, tylosin stock solution was obtained from Sigma (St Louis, MO, USA). One mg/ml tylosin was prepared by dissolving 10 mg tylosin tartrate in 10 ml methanol and stored at -18oC. Working standard solutions for the calibration curve were prepared by appropriate diluting of the stock solution, using a dilution factor. The kidney, liver, and muscle samples that were tested and confirmed to be free of macrolide antibiotic residues (control) were used as blanks for the preparation of matrix-matched calibration curves. For fortification, standard solutions were prepared by dissolving a standard substance in methanol at concentrations 40, 20, 10, 5, and 2.5 mg/ml. Two grams each of the kidney, liver, and muscle samples of birds were weighed and macerated with mortar and pestle. 2 ml of distilled water was added, followed by 10 ml HPLC grade acetonitrile. It was then mixed with a vortex mixer to homogenize for 1 minute. Then, the sample was centrifuged for 15 minutes at 3,000 rpm. The clear extracted solvent layer was then collected using disposable pasture pipettes and diluted to 50 ml with distilled water. The SPE Cartridges Bond Elute C18 500 mg/3ml were activated with 2 ml of methanol and 5 ml of distilled water. The cartridge was washed with 20 ml of distilled water and allowed to dry. The extracted sample solution was loaded and allowed to elute from the cartridge with 3 ml of HPLC-grade methanol. The solution was passed through 0.45 µm membrane filter The samples were manually injected into the HPLC. Chromatographic analysis was performed with isocratic elution on Zorbax Eclipse XDB - C18 (150 x 4.6mm, 5 µm) analytical column at 30oC. The mobile phase composed of HPLC grade acetonitrile and water (90:10), at the flow rate of 1.00 ml/min, 20µl was injected. The chromatogram was monitored at wavelength 292 nm.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism statistical software version 5.02. The Chi-square ($\chi 2$) test was used to evaluate the association between TR concentrations with the tissue types. Kruskal–Wallis test was used to compare differences between the mean concentration of the raw values and each of the different cooking and microwaving times. p-value < 0.05 was considered statistically significant.

Authors' Contributions

S.O.O., and E.V.E. conceived and planned the experiments. S.O.O. carried out the experiments. E.V.E., J.A.N., and A.O.A supervised the work. I.O.N, S.O.O and E.V.E. contributed to sample preparation and the interpretation of the results. I.O.N. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

The authors would like to thank Mrs S.N. Ikechukwu of the Department of Veterinary Public health and Preventive Medicine, Faculty of Veterinary Medicine, UNN, for assistance in performing the sample analysis. This study did not receive any financial support as declared by the authors.

Conflict of interest

The authors declare that there is no conflict of the interest

References

1. EuroStat. Agricultural Production—Livestock and Meat. Accessed December 15, 2022.https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Agricultural production livestock and meat#Poultry.

2. USDA. United States Department of Agriculture Foreign Agricultural Service October 12, 2023 Livestock and Poultry: World Markets and Trade. Accessed November 10, 2023. https://public.govdelivery.com/accounts/USDAFAS/subscriber/new.

3. F.A.O. Gateway to Poultry Production and Products. Accessed March 15, 2023. https://www.fao.org/poultry-production-products/production/en/.

4. Njoga EO, Ogugua AJ, Nwankwo IO, Awoyomi OJ, Okoli CE, Maigawu BD. Oyeleye FA, et al. Antimicrobial drug usage pattern in poultry farms in Nigeria: implications for food safety, public health, and poultry disease management. Vet Italiana. 2021;57(1):5-12. Doi: 10.12834/VetIt.2117.11956.1.

5. Patel T, Marmulak T, Gehring R, Pitesky M, Clapham MO, Tell LA. Drug residues in poultry meat: A literature review of commonly used veterinary antibacterials and anthelmintics used in poultry. Journal of Veterinary Pharmacology and Therapy. 2018; 41(6):761-789. Doi: 10.1111/jvp.12700.

6. Kim MJ, Kim JY, Choi JD, Moon G. Re-evaluation of a microbiological acceptable daily intake for tylosin based on its impact on human intestinal microflora. Toxicology Research. 2023;1-6. Doi: 10.1007/s43188-023-00179-z.

7. Soliman AM, Sedeik M. Pharmacokinetics and tissue residues of tylosin in broiler chickens. Pharmacology and Pharmacy. 2016;7(1):36-42. Doi: 10.4236/pp.2016.71006.

8. De Wasch K, Okerman L, Croubels S, de Brabander H, van Hoof J, De Backer P. Detection of residues of tetracycline antibiotics in pork and chicken meat: correlation between results of screening and confirmatory test. Analyst. 1998;123(12):2737-41. Doi: 10.1039/a804909b.

9. Javadi A, Mirzaie H, Khatibi SA. Effect of roasting, boiling and microwaving cooking methods on Enrofloxacin residues in edible tissues of broiler. African Journal of Pharmacy and. Pharmacology. 2011;5(2):214-218. Doi: 10.5897/AJPP10.378

10. Salaramoli J, Heshmati A, Kamkar A, Hassan J. Effect of cooking procedures on tylosin residues in chicken meatball. Journal für Verbraucherschutz und Lebensmittelsicherheit 2015;11(1):53-60. Doi: 10.1007/s00003-015-1005-1.

11. Gowtham P, Muthukumar M, Eswara RB, Kalpana S, Keerthika V, Dani N. Impact of cooking methods of meat on antibiotic residues: Review. TPI International Journal of science and technology. 2020; 9(12):303-9.Doi: 10.22271/tpi.2020.v9.i12e.5454.

12. El Tahir Y, Elshafie EI, Asi MN, Al-Kharousi K, Al Toobi AG, Al-Wahaibi Y, Al-Marzooqi W. Detection of Residual Antibiotics and Their Differential Distribution in Broiler Chicken Tissues Using Enzyme-Linked Immunosorbent Assay. Antibiotics (Basel). 2021;10(11):1305. Doi: 10.3390/antibiotics10111305.

13. Debeb D. Review on Effect of Antimicrobial Residual on Human. Journal of Veterinary Science and Medical Diagnostics. 2021;10(7):1-4.

14. Prats C, Francesch R, Arboix M, Pérez B. Determination of tylosin residues in different animal tissues by high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;766(1):57-65. Doi: 10.1016/s0021-9673(01)01325-5.

15. Ghanjaoui ME, Mandil A, Ait Sidi Mou A, Slimani R. High-performance liquid chromatography quality control. International Journal of Advances in Chemistry. 2020;8:160-169. Doi: 10.14419/ijac.v8i1.30723

16. Treiber FM, Beranek-Knauer H. Antimicrobial Residues in Food from Animal Origin-A Review of the Literature Focusing on Products Collected in Stores and Markets Worldwide. Antibiotics (Basel). 2021 May 6;10(5):534. doi: 10.3390/antibiotics10050534.

17. Jayalakshmi K, Paramasivam M, Sasikala M, Tamilam TV, Sumithra A. Review on antibiotic residues in animal products and its impact on environments and human health. Journal of Entomology and Zoological Studies 2017;5(3):1446-1451. Doi:10.47672/ejah.1104

18. Tasci F, Secilmis Canbay H, Doganturk M. Determination of antibiotics and their metabolites in milk by liquid chroma-

tography-tandem mass spectrometry method. Food Control. 2021;127:108-147. Doi: 10.1016/j.foodcont.2021.108147.

19. Khaniki GJ, Aghaee EM, Sadighara P. Chemicals and drugs residue in meat and meat products and human health concerns. Journal of Food Safety and Hygiene 2018;4:1-7.

20. Nwankwo IO, Ezenduka EV, Nwanta JA, Ogugua AJ, Audu BJ. Prevalence of Campylobacter spp. and antibiotics resistant E. coli on poultry carcasses and handlers' hands at Ikpa slaughter, Nsukka, Nigeria. Notulae Sciencia Biologica. 2021;13:(2)10866. Doi:10.15835/nsb13210866.

21. Onwumere-Idolor OS, Nwankwo IO, Ezenduka EV, Nwanta JA and Anaga AO. Comparison of Premi® test, Four Plate Test, ELISA, and HPLC tests in the detection of tylosin residue and its depletion time in chickens treated with tylosin by intramuscular injection and oral routes. Journal of Veterinary and Applied Sciences. 2024;14(1):469–480.

22. Onwumere-Idolor OS, Ogugua A J, Ezenduka EV, Nwanta JA, Anaga A. Prevalence of Antimicrobial Residues in Tissues of Broilers Sold at a Local Market in Enugu State, Nigeria Using the European Four Plate Test. Journal of Food Nutrition and Research. 2022; Doi: 10.26502/jfsnr.2642-110000102.

23. International Programme on Chemical Safety & Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of certain veterinary drug residues in food /prepared by the seventieth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization. 2009:https://apps.who.int/iris/bitstream/handle/10665/44086/9789241660617_eng.pdf?sequence=1&isAllowed=y Accessed on 15 September 2022.

24. Murray SA, Holbert AC, Norman KN, Lawhon SD, Sawyer JE, Scott HM. Effects of Tylosin, a Direct-Fed Microbial and Feedlot Pen Environment on Phenotypic Resistance among Enterococci Isolated from Beef Cattle Feces. Antibiotics. 2022;11(1):106. Doi: 10.3390/antibiotics11010106.

25. Cazer CL, Eldermire ERB, Lhermie G, Murray SA, Scott HM, Gröhn YT. The effect of tylosin on antimicrobial resistance in beef cattle enteric bacteria: A systematic review and meta-analysis. Preventive Veterinary Medicine. 2020;176:104934. Doi: 10.1016/j.prevetmed.2020.104934.

26. WHO Antibiotics resistance: Accessed October 15, 2022. https://www.who.int/news-room/fact- sheets/detail/antibiotic-resistance.

27. Ali A, Ahmad N, Liaqat A, Farooq MA, Ahsan S, Chughtai MFJ, et al. Safety and quality assessment of street-vended barbecue chicken samples from Faisalabad, Pakistan. Food Science and Nutrition. 2022;11(2):930-939. Doi: 10.1002/fsn3.3127.

28. Codex Alimentarius Commission Maximum. Residue Limits for Veterinary Drugs in Foods Updated as at the 34th Session of the Codex Alimentarius Commission 2011 Accessed March 14, 2023.

29. Badger-Emeka LI, Emeka PM, Okosi M. Evaluation of the extent and reasons for increased non-prescription antibiotics use in a University town, Nsukka Nigeria. International Journal of Health Sciences. (Qassim). 2018;12(4):11-17.

30. Chah JM, Nwankwo SC, Uddin IO, Chah KF. Knowledge and practices regarding antibiotic use among small-scale poultry farmers in Enugu State, Nigeria. Heliyon. 2022: 26;8(4):e09342. Doi: 10.1016/j.heliyon.2022.e09342.

31. Kolanovic BS, Bilandzic N, Varenina I, Bozic D. Tylosin content in meat and honey samples over a two-year perıod in Croatia. Journal of Immunoassay Immunochemistry. 2014; 35: 37-47. Doi: 10.1080/15321819.2013.784198.

32. Pavlov AI, Lashev LI, Vachin, Rusea V. Residues of antimicrobial drugs in chicken meat and offals. Trakia Journal of Sciences 2008;6(1):23-25.

33. JECFA. Evaluation of Certain Veterinary Drug Residues in Animals and Foods (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO 2006: Technical Report Series, No. 939.

34. Gürel Yücel M, Seçilmiş H, Taşçı F. Investigation of tylosin and tilmicosin residues in meat by high-performance liquid chromatography method. Polish Journal of Veterinary Sciences. 2023; 26(1):39–46. Doi: 10.24425/pjvs.2023.145005.

35. Tao Y, Yu G, Chen D, Pan Y, Liu Z, Wei H, Peng D, Huang L, Wang Y, Yuan Z. Determination of 17 macrolide antibiotics and avermectins residues in meat with accelerated solvent extraction by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B Analytical Technology and Biomedical Life Sciences. 2012;897:64-71. Doi: 10.1016/j.jchromb.2012.04.011.

36. Arslanbaş E, Şahin S, Kalın R, Moğulkoç MN, Güngör H. Determination of some antibiotic residues by HPLC method in chicken meats prepared for consumption. Erciyes University Veteriner Fakultasi Dergisi. 2018;15:247-252. Doi: 10.32707/ercivet.477315.

37. Heshmati A, Kamkar A, Salaramoli J, Hassan J, Jahed GH. The effect of two methods cooking of boiling and microwave on tylosin residue in chicken meat. Iranian Journal of Nutritional Science and Food Technology. 2013;8(1):231-240.

38. Pikkemaat MG. Microbial screening methods for detection of antibiotic residues in slaughter animals. Analytical and Bioanalytical Chemistry. 2009;395(4):893–905. Doi: 10.1007/s00216-009-2841-6.

39. Shaltout AF, Mohammed A, El S, Heba MF. Studies on antibiotic residues in beef, and effect of cooking and freezing on antibiotic residues beef samples. Scholarly Journal of food nutrition. 2019; 2(1).Doi: 10.32474/DSJFN.2019.02.000129.

How to cite this article
Nwankwo IO, Onwumere-Idolor SO, Ezenduka EV, Nwanta JA, Anaga AO. Evaluation of ELISA, Four Plate and HPLC Tests in the detection of Tylosin residue in raw and heat treated chicken. Iran J Vet Sci Technol. 2025; 17(1): 55-63.
DOI: https://doi.org/ 10.22067/ijvst.2025.88457.1389
URL: https://ijvst.um.ac.ir/article_46332.html