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Assessment of the Influence of Cooking Methods on Antibiotics Residues in Chicken Meat

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Abstract

Unacceptable levels of antibiotics residues in chicken meat have dire implications for food safety and public health. This study assessed the influence of thermal processing methods on gentamicin and doxycycline residues in some tissues (liver, gizzard and skin) of broiler chicken exposed to a cocktail of both agents. Nine (9) one-day-old broiler chickens were reared under controlled conditions and divided into three experimental groups: a control group, an antibiotic-treated group that observed a withdrawal period, and an antibiotic-treated group without withdrawal period. Samples were subjected to boiling, roasting, and deep frying before residue analysis using gas chromatography. Considerable residue levels reduction of both agents were achieved by boiling (72.9 – 81.5%), deep-frying (68.1 – 92.4%) and roasting (61.1 – 91.1%) in the tissues. Kruskal-Wallis analysis indicated that these reductions were significant for deep-frying in the gizzard and skin ($p = 0.014$) and by boiling in the liver ($p = 0.038$) for gentamicin residues. Doxycycline residues were significantly reduced by roasting in the gizzard ($p = 0.028$); boiling in the liver ($p = 0.01$) and deep-frying in the skin ($p = 0.027$). Generally, liver antibiotic residues could be significantly reduced by boiling while gizzard and skin residues by deep-frying and roasting. Notably, thermal processing did not completely eliminate residues, especially in samples from the non-withdrawal group, emphasizing that cooking alone cannot substitute for proper withdrawal compliance prior to slaughter. The observation of withdrawal period of greater than 15 days is recommended as this could well complement the effort to reduce untoward antibiotic accumulation in consumers.

Key words: *doxycycline, gentamicin, broiler, food safety, thermal processing*

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1. Introduction

Broiler chicken has emerged as a readily available and affordable source of meat in most developing countries, Nigeria inclusive (Adenuga and Montowska, 2023; Umair *et al.*, 2021). However, comparable to Nigerian Indigenous Ecotype Chickens (NIEC), rearing broiler chickens for meat production can be challenging because of their high vulnerability to microbial infections (Pewan *et al.*, 2025). Poultry farmers therefore frequently utilize antimicrobials to prevent microbial infestations as well as to promote animal growth (Kamouh *et al.*, 2024; Mohammadzadeh *et al.*, 2022). A considerable amount of chicken meat produced in the Nigerian market comes from unregulated farms where the misuse of antibiotics appears to be the norm. Indiscriminate use of antibiotics in poultry farming is of grave concern since it usually results in residual in tissues (Maharjan *et al.*, 2020; Selaledi *et al.*, 2020). The prevalence of antibiotics residues in chicken meat is increasing (Lee *et al.*, 2018; Shrestha *et al.*, 2026), and has evolved as a major contributor to the global scourge of antimicrobial resistance (Onyinye *et al.*, 2020; Ali *et al.*, 2025).

Drug residues tend to accumulate more in certain organs than others and are impacted by factors such as formulation, the route of administration, the target species, age, physiology, pathology and diet (Gray *et al.*, 2021). In the African farming space, residues of antibiotics such as aminoglycoside, macrolides, β-lactams, fluoroquinolones, tetracyclines, sulfonamides, and phenicols are found to be most prevalent in animal products (Oladeji *et al.*, 2025). Most of these antibiotic residues were found in levels higher than the regulatory permissible limit. Noncompliance with withdrawal periods and potentially high antibiotics contents in animal feeds are possible contributors. Withdrawal periods, defined as the time required for treated animals to reduce drug residues below established maximum residue limits (MRLs). This period is recommended for veterinary drugs for wash out through pharmacokinetic processes (Gray *et al.*, 2021).

The use of antimicrobials in poultry production is widespread in Nigeria, often without adequate veterinarian prescription or strict adherence to withdrawal periods. Studies have reported extensive use of doxycycline and gentamicin in poultry farms, with detectable residues frequently exceeding international safety limits in poultry tissues and eggs (Olatoye *et al*., 2019; Unukevwere *et al*., 2025). Doxycycline, a tetracycline of second generation, is usually indicated in the infections of the respiratory and alimentary tract in poultry caused by micro-organism susceptible to the agent. Gentamicin, on the other hand, is a broad-spectrum aminoglycoside which find utility in the control bacterial infections like *E. coli* and *Salmonella* in broiler chickens (Agunos *et al*., 2012). The liver, gizzard, and skin of broiler chickens are touted to accumulate higher concentrations of antibiotic residues (FAO/WHO Expert Committee on Food Additives [JECFA], 2019; Kabir *et al*., 2004; Rose *et al*., 1999). The liver serves as the primary organ for drug metabolism; the gizzard is directly exposed to medicated feed and water while the skin accumulate drug residues; making them veritable indicators of residue persistence and potential consumer exposure.

Antibiotic treatment in broiler chickens is allowed for a duration of 5 - 7 days (Gray *et al*., 2021), followed by a recommended withdrawal period of at least 9 days (Mestorino *et al*., 2018; Mostafa *et al*., 2025). In Nigeria, most poultry farmers procure formulations that come as combined drugs (and/or other excipients), a factor which is capable of affecting drug pharmacokinetics. The interrogation of the residual dynamics of administering such typical mix-antibiotics formulation with/without withdrawal period is one of our cardinal objectives. Secondly, the stability of antibiotic residues varies with processing conditions and drug chemical properties, implying that cooking may alter residue levels and consumer exposure risk (Pame *et al*, 2024; Wu *et al*., 2022).Thermal processing methods such as boiling, roasting, and deep frying can degrade/eliminate drug residues making them potential intervention measure prior to meat consumption (Abou-Raya *et al*., 2013; Kamouh *et al*., 2024). Degradation of veterinary drug residues following boiling and frying have previously been reported, with high post processing residue levels observed for aminoglycosides and tetracyclines (Rose *et al*., 1999; Abou-Raya *et al*. 2013). Despite these findings, we intend to evaluate the impact of common Nigerian cooking methods on gentamicin and doxycycline residues in chicken meat based on a cocktail of a 2-drug formulated regimen antibiotics mix commonly used in Nigerian poultry production. The aim of this study therefore is to evaluate the effect of common thermal processing methods on antibiotic residues and modifying impacts of observing withdrawal periods. The findings will contribute to food safety knowledge, enhance public health risk assessment, support regulatory enforcement efforts in Nigeria, and provide evidence-based information to guide consumers on the limitations of domestic processing in eliminating antibiotic residues.

2. Materials and Method

2.1 Materials

Analytical balance, rotary vacuum evaporator, electric oven, aluminum foil deep frying pan, Lasota and Gumboro vaccines, sunflower cooking oil, chloroform, methanol, ethanol, ammonium acetate buffer, ammonium hydroxide, G.DX WSP (Doxycycline hyclate 200 mg + Gentamicin sulphate 200 mg per gram). All chemicals are of analytical grade.

2.2 Experimental Design

2.2.1 Animal experiment

A total of nine (9) healthy one-day old broiler chicks were procured from Asaba, Delta state Nigeria and reared following the American Dairy Science Association, American Society of Animal Science, and Poultry Science Association's Guide for the Care and Use of Agricultural Animals in Research and Teaching (2020), for the first six (6) weeks. This pre-treatment rearing period was intended to minimize biological variability and to establish a homogeneous study population prior to treatment allocation as previously suggested (National Chicken Council, 2024). Thereafter, the birds were randomly assigned into three experimental groups of 3 birds each for the subsequent two-week experimental period as indicated below:

Group A (Control Group): birds received commercial feed and clean drinking water only throughout the study period and were not exposed to any antibiotic treatment.

Group B (Withdrawal Group): birds received commercial feed and drinking water containing G.DX WSP at a concentration of 1 g per 3 L water for five (5) consecutive days. Following exposure, drug administration was discontinued and a nine (9)-day withdrawal period was strictly observed prior to slaughter as previously suggested (Mestorino *et al*., 2018; Mostafa *et al*., 2025).

Group C (Non-Withdrawal Group): birds received feed and clean drinking water for nine (9) days only. Thereafter, drinking water was replaced with water containing G.DX WSP at a concentration of 1 g per 3 L water for the final five (5) days preceding slaughter. This treatment arm was specifically designed to simulate the indiscriminate use of antibiotics and failure to observe recommended withdrawal periods.

All the birds were slaughtered using the Halal method. Following slaughter, the liver, gizzard and skin (JECFA, 2019; Kabir *et al*., 2004; Rose *et al*., 1999) were aseptically harvested, packaged in sterile plastic bags and stored on ice for residue analysis for the raw samples and food processing in duplicate as proposed by Shaltout *et al*. 2019; **Boiling**: tissue samples were completely immersed in distilled water and boiled at approximately 100°C for 15 minutes; **Deep frying**: tissue samples were submerged in pre-heated sunflower oil and fried for 15 minutes at an approximately constant temperature of 170–180°C; **Roasting**: tissue samples were individually wrapped in aluminum foil to minimize moisture loss and prevent contamination, and then placed in a preheated electric oven at approximately 180°C for 15 minutes.

2.2.2 Sample Preparation

Samples were prepared according to the method described by Santos *et al*. (2005). Briefly, 1 g of tissue sample was homogenized with phosphate buffer (pH 6.0, 0.1 M) and then centrifuged at 2600 ± 100 × *g* for 5 min. Supernatant was decanted into a new glass tube and defatted with 10 ml of *n*-hexane. Antibiotic residues were subsequently extracted using 1 mL of ethyl acetate. The mixture was vortexed for 2 minutes and centrifuged at 5000 rpm for 10 minutes to separate the organic phase. The supernatant containing the extracted analytes was carefully collected into a clean eppendorf tube. The extraction process was repeated twice to ensure maximum recovery, and the supernatants were combined. The combined extract was evaporated to dryness at 40°C under a gentle stream of nitrogen to prevent thermal degradation of the analytes.

2.2.3 Derivatization Procedure

The presence of hydroxyl and amino groups in the antibiotics makes them good candidates for chemical derivatization. Samples were derivatized to enhance detection efficiency prior to chromatographic analysis as follows: **Gentamicin**: Dry residue was dissolved in 50 ml of anhydrous pyridine. To the pyridine solution, 100 ml of trimethylsilylimidazole (TMSI) were added and the vial was closed and incubated for 15 min at 60 °C. Thereafter, 70 ml of trifluoroacetic anhydride (TFAA) were added and the vial was closed and incubated for 60 min at 60 °C. (Isoherranen and Soback, 2000). **Doxycycline**: Dry residue was dissolved in 500 µL of pyridine solution. 250 µL of acetic anhydride were subsequently added and the vial was closed tightly. The residue was dissolved by swirling and allowed to stand at room temperature overnight (Thangadurai, 2011). Solutions were reconstituted in 1 mL of acetonitrile, followed by centrifugation at 13,000 rpm for 10 minutes to remove any remaining particulate matter. The clarified extract was then transferred into a gas chromatography (GC) vial for analysis.

2.2.4 Sample Analysis

Quantitative determination of gentamicin and doxycycline residues in the processed tissue samples was carried out using an Agilent 6890 Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID) operated under optimized analytical conditions. Aliquots of the purified sample extracts were injected into the GC system using splitless injection to enhance sensitivity. Separation of analytes was achieved using a capillary column under controlled temperature programming, while helium was employed as the carrier gas. Identification of analytes was based on comparison of the retention times of sample peaks with those of corresponding analytical standards. Quantification of antibiotic residues was performed using an internal standard calibration approach, in which the concentrations of analytes were determined based on the ratio of the peak area of each analyte to that of the internal standard. Calibration was achieved using standard solutions analyzed under identical chromatographic conditions. The concentrations of gentamicin and doxycycline residues in the tissue samples were expressed in µg/kg. All analyses were conducted in duplicate, and mean values were recorded to ensure analytical precision and reproducibility.

2.3 Data Analysis

Data of residue levels obtained from the sample analysis were analyzed using descriptive and inferential statistical methods. Residue concentrations in the different tissues were expressed as mean ± standard deviation (SD). The Kruskal–Wallis test, a non-parametric statistical test, was used to determine whether significant differences existed among the various thermal processing methods and treatment groups. All analyses were conducted at a 95% confidence level, and differences were considered statistically significant at $p < 0.05$. The results were presented in tables and figures to facilitate comparison of residue levels across the experimental groups and processing methods.

3. Results and Discussion

3.1 Impacts of withdrawal period on residue levels

Table 1 presents the mean ± SD of antibiotic residue levels of samples, established MRL and the fold change (FG) between residue levels of window period compliant and non-compliant groups. This table indicates that antibiotic residue levels in all raw (unprocessed) samples from the control group was below the detection limit of the instruments. In contrast, considerable levels of both antibiotics were detected in groups B (withdrawal period observed) and C (withdrawal period not observed). Non-detectable levels of antibiotics residues in group A apparently confirms the absence of background contamination and the adequacy of the analytical method used. Similar findings in untreated control samples have also been reported in residue-monitoring studies involving poultry tissues (Olatoye *et al.*, 2013).

Table 1: Mean ± S.D of Antibiotic Residues

Tissue	Drug	MRL (ppb)	Control	Withdrawal period observed		Fold Change
			(A)	Yes (B)	No (C)	
Gizzard	**G**	100[a,b]	ND	450.27±146.55	274.83 ± 49.33	0.61
	D	100[b]	ND	39.07 ± 13.05[*]	130. 22 ± 73.50	3.33
Liver	**G**	200[a,b]	ND	626.58 ±274.59	2114.91±1009	3.37
	D	300[b]	ND	8.33 ± 1.29[*]	65.34 ± 13.09[*]	7.84
Skin	**G**	100[a,b]	ND	1532.85±509.10	7879.53±884.08	5.14
	D	100[b]	ND	2.63 ± 1.23[*]	175.47 ± 53.21	66.72

D = Doxycycline; G = Gentamicin; ND = not detected; S.D = standard deviation

[a]JECFA (1995); [b]Codex (2024);[*]Within the MRL

Mean doxycycline levels in group B were found to be well below regulatory values but were generally higher than the recommended MRLs in group C. In contrast, mean gentamicin residue levels in both test groups were considerably higher than the recommended MRLs in all the tissues assayed ranging from skin (1532.85 ± 509.10 ppb), the liver (626.58 ± 294.59 ppb) and the gizzard (450.27 ± 146.55 ppb) tissues. In order to estimate how impactful not observing the withdrawal window could be, we employed the use of fold change (mean values from group C divided by mean values from group B). We observed that non-compliance to withdrawal period could increase drug persistence in the tissues to as much as 3 - 66 times. Doxycycline consistently showed a comparative higher fold change across board positing that it may apparently be more persistent than gentamicin in the event of non-compliance to the washout period when such cocktail of antimicrobial agent is administers to chickens. However, we observed, oddly so that the gentamicin FG in the gizzard was less than unity. Indeed, antibiotic residue occurrence in poultry tissues is governed by a combination of pharmacokinetic properties such as dosage, drug-drug interactions, redistribution kinetics and tissue type. The increased residue in withdrawal compliant group could be as a consequence of gentamicin redistribution from high affinity tissues such as the kidneys during the wash out period. The MRLs are intended to protect consumers from adverse health effects and to limit the development of antimicrobial resistance associated with prolonged exposure to antibiotic residues in food (Baynes *et al*., 2016). Our findings indicate that compliance with the withdrawal period was effective in reducing doxycycline residues to levels considered safe for human consumption. This as had been reported in previous studies (Donoghue, 2003; Landoni and Albarellos, 2015). On the contrary, gentamicin residues persisted in tissues at levels above regulatory thresholds, an observation that could be attributable to apparently short withdrawal period adopted in this study comparatively to greater than 14 days previously recommended for aminoglycosides in food-producing animals (Khatun *et al*., 2018). Finally, abdication from observing the withdrawal period could result to critical residues fold changes, as much as is observed in the present study, can induce protein carbonylation, leading up to oxidative stress (Marquez *et al*., 2021) in consumers.

3.2 Effect of thermal processing on residue levels

The influence of thermal processing on antibiotic residue concentrations in broiler tissues where the recommended withdrawal period was observed is presented in Table 2. The results indicate that boiling, frying, and roasting generally reduced both gentamicin and doxycycline residues across the examined tissues. In the liver tissue, boiling has the highest percentage residue reduction; 81.5% for doxycycline and 72.9% for gentamicin. Deep-frying showed highest percentage residue reductive effect on the skin tissues; 68.1 and 92.4 % for doxycycline and gentamicin respectively. Roasting indicated a 91.1% reductive impact on gentamicin residue in the skin and 61.1 % reduction of doxycycline on the liver tissue. The effect of thermal processing on antibiotic residues in broilers slaughtered without observing the recommended withdrawal period is shown in Table 3. In contrast to the withdrawal-compliant group, raw tissues from the non-withdrawal group exhibited substantially higher residue concentrations, reflecting the presence of recently administered antibiotics in the birds' tissues. However, all the processing methods followed the same pattern in the reduction of doxycycline residues; skin (62.0 – 68.7%) > gizzard (46.3 – 59.4%) > liver (26.7 – 39.5%). All the processing methods also followed similar patterns for the reduction of gentamicin residues in the order of skin (77.0 – 84.6 %) > liver (29.8 – 48.2 %) > gizzard (18.5 – 38.1 %). Cooking methods (boiling, frying, roasting and grilling) have previously been used as innocuous methods of reducing antibiotic residues in meat (Adegbeye *et al*., 2024). The magnitude of residue reduction observed in this study is in agreement with related studies (Elbayoumi *et al*., 2018; Kamouh *et al*., 2024). However, reported reductions vary widely depending on the drug, tissue type, and cooking methods (Rose *et al*., 1999; Abou-Raya *et al.*, 2013; Adesokan *et al.*, 2015) as also clearly indicated in the present study.

Table 2: Mean ± SD (ppb) of antibiotic residue from withdrawal period-compliant Broiler

Antibiotic	**Tissue**	**Residue levels (ppb)**				**% reduction**		
		Raw	**BD**	**DF**	**RD**	**BD**	**DF**	**RD**
D	**Skin**	2.63 ± 1.23	2.25±1.42	0.84 ± 0.27	2.17 ± 1.2	14.4	68.1	17.5
	Gizzard	39.07 ± 13.05	34.63±8.54	34.81 ± 12.3	19.26 ± 6.3	11.4	10.9	50.7
	Liver	8.33 ± 1.29	1.54 ± 0.55	6.48 ± 0.7	3.24 ± 1.10	81.5	22.2	61.1
G	**Skin**	1532.85 ±509.1	720.76±107.1	116.31 ± 16.3	124.24 ± 20.4	53.0	92.4	91.9
	Gizzard	450.27 ± 146.5	207.60± 27.7	139.39 ± 41.9	185.84 ± 34.9	53.9	69.0	58.7
	Liver	626.58 ± 294.5	169.50± 82.8	402.36 ± 94.4	268.12 ± 121.1	72.9	35.8	57.2

D = Doxycycline; G = Gentamicin
BD = Boiling; DF = Deep Frying; RD = Roasting

Table 3: Mean ± SD (ppb) of antibiotic residues in withdrawal period non-compliant broilers

Antibiotic	Tissue	Residue levels (ppb)				% reduction		
		Raw	BD	DF	RD	BD	DF	RD
D	Skin	175.47±53.21	66.67±6.16	60.47 ± 4.62	54.89±25.6	62.0	65.5	68.7
	Gizzard	130.22±73.50	52.87±40.23	67.70±43.64	69.98±9.42	59.4	48.0	46.3
	Liver	65.34±13.09	39.52±17.40	47.92 ± 30.46	46.41±41.9	39.5	26.7	29.0
G	Skin	7879.53±884.08	1209.93±369.91	1236.07±759.41	1809.95±131.6	84.6	84.3	77.0
	Gizzard	274.83±49.33	174.17±35.28	169.99 ± 50.81	223.85±32.20	36.6	38.1	18.5
	Liver	2114.91±1007.6	1096.25±269.47	1478.95±537.73	1484.87±573.4	48.2	30.1	29.8

D = Doxycycline; G = Gentamicin
BD = Boiling; DF = Deep Frying; RD = Roasting

3.3 Impact of processing methods on residue levels

In order to determine the relevance of these reductions given the small sample size, we employed the Kruskal–Wallis test, with Dunn's post hoc analysis as presented in Figure 1 (Gentamicin) and Figure 2 (Doxycycline). In Figure 1, the mean rank of the gentamicin residues levels shows a significant reduction from the unprocessed tissues by all the processing methods as indicated in 1A (withdrawal period observed); gizzard (H = 8.13; p = 0.015), liver (H = 7.51; p = 0.028), and skin (H = 9.65; p = 0.006). Figure 2A also shows the same effect for doxycycline residues in gizzard (H = 7.31; p = 0.033), liver (H = 9.97; p = 0.0003), and skin (H = 7.21; p = 0.038). However, post hoc analyses found that these significant reductions in gentamicin residues were by deep-frying in the gizzard and skin (p = 0.014) while by boiling in the liver (p = 0.038). On the other hand, doxycycline residues were significantly reduced by roasting in the gizzard (p = 0.028); boiling in the liver (p = 0.01) and deep-frying in the skin (p = 0.027). Antibiotic residues in tissues from group C (withdrawal period not observed), despite meaningful numerical reductions (as indicated in the section above), were not significantly reduced (p > 0.05) by any of the processing methods (Fig 1B and 2B).

Generally, our observation in this analysis indicates that liver antibiotic residues could apparently be significantly reduced by boiling while gizzard and skin residues by deep-frying and roasting. Boiling, a moist-heat method conducted at approximately 100 °C, promotes both thermal degradation and leaching of water-soluble residues into the cooking liquid. This makes it particularly effective for reducing hydrophilic antibiotics, although residues may persist in the broth (Rose *et al.*, 1999). Boiling, though effective, its impact is generally lower than deep frying probably due to slower heat penetration and less direct interaction with cooking media (Rose *et al.*, 1999). Previous studies have shown that frying often achieves greater residue reduction compared to boiling, largely due to higher thermal intensity (Abou-Raya *et al.*, 2013; Adesokan *et al.*, 2015). Roasting, a dry-heat method conducted at 160–220 °C, reduces residues primarily through thermal degradation, with limited contribution from leaching. We also observed that when initial residue concentrations are relatively low due to compliance with withdrawal periods, thermal processing can further contribute to the reduction of antibiotic residues in edible tissues. However, complete elimination is rarely achieved, emphasizing that cooking cannot substitute for proper withdrawal compliance prior to slaughter.

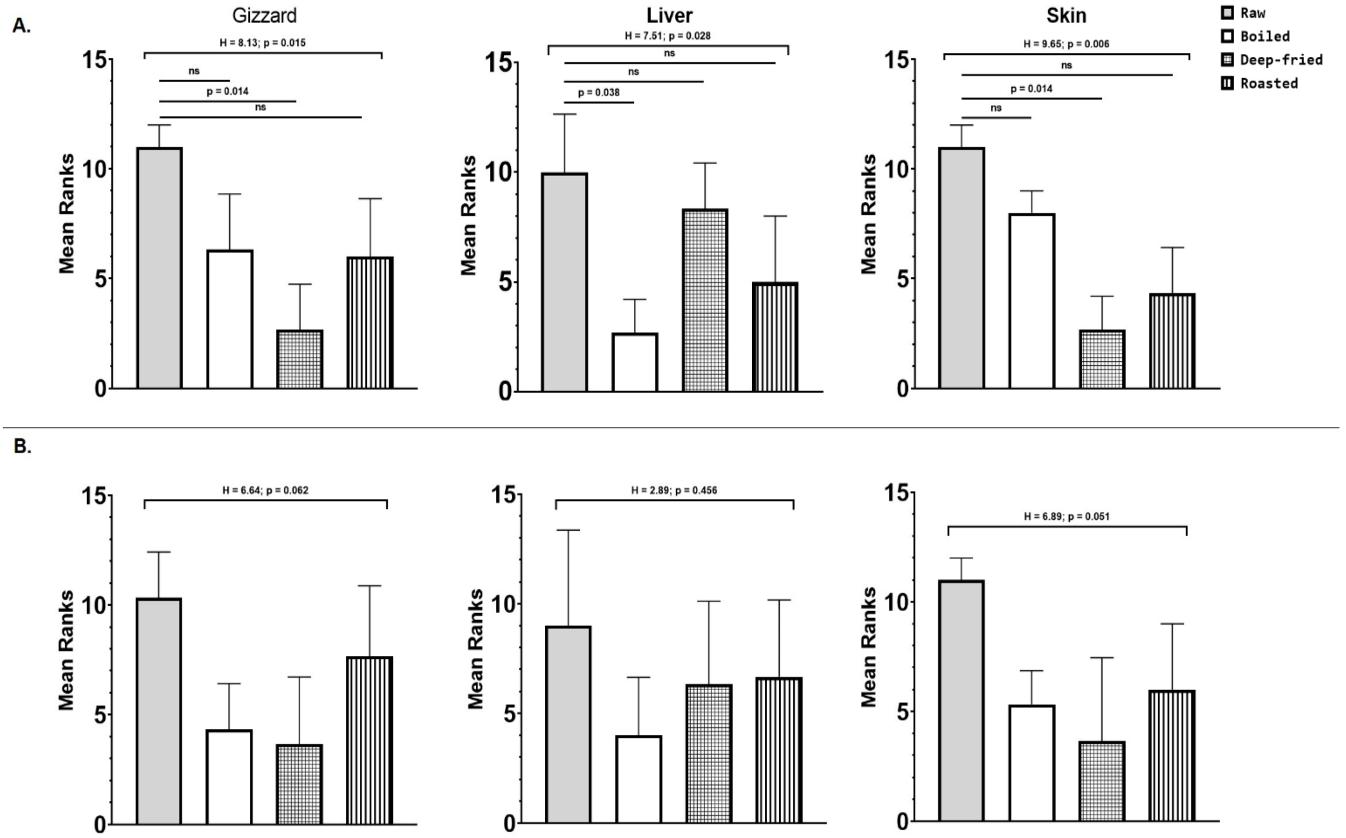

Figure 1: Impact of thermal processing methods on gentamicin residues tissues.
A = Withdrawal period observed; B = Withdrawal period not observed

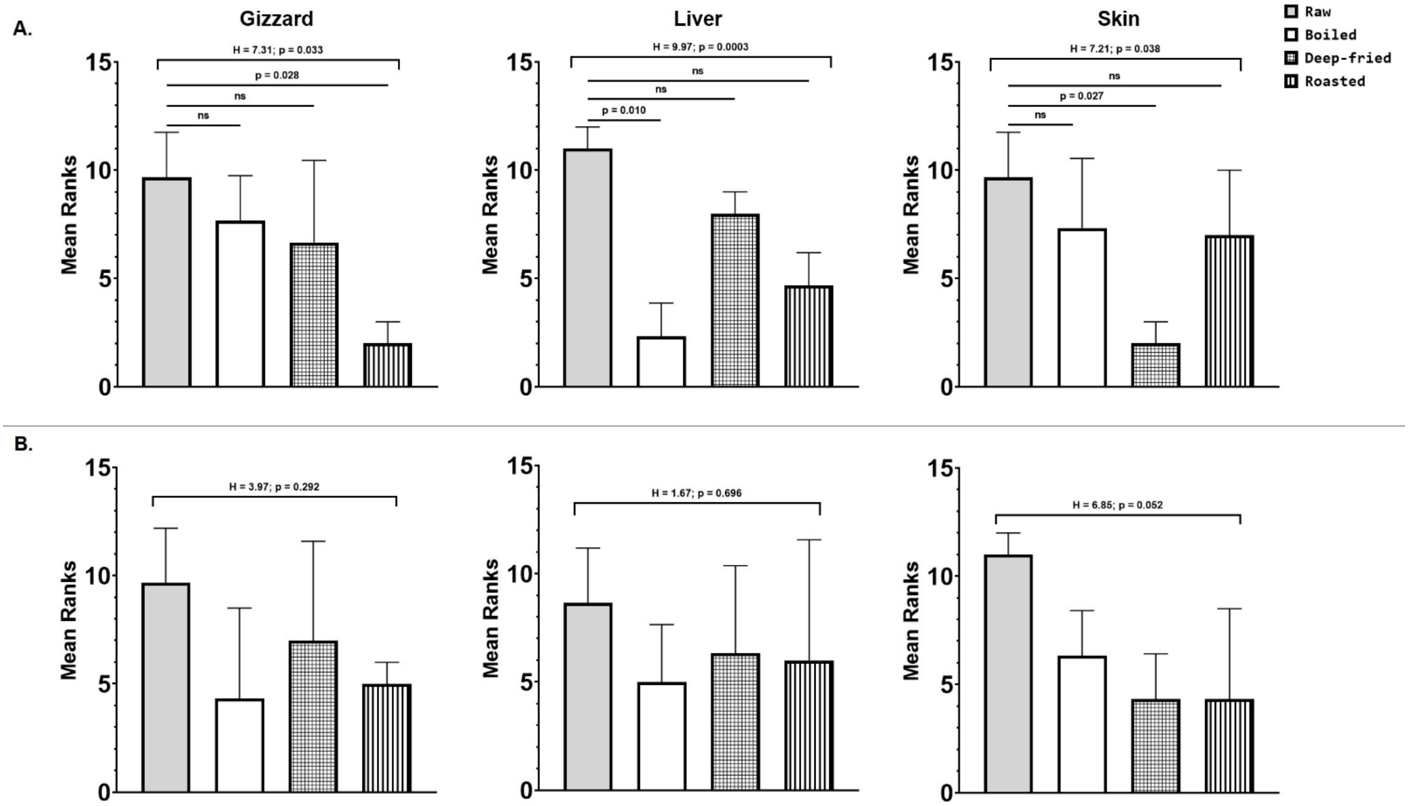

Figure 2: Impact of thermal processing methods on doxycycline residues tissues.
A = Withdrawal period observed; B = Withdrawal period not observed

4. Conclusion

This study has demonstrated that gentamicin and doxycycline residues can be found in in broiler chicken tissues following administration of common 2-drug cocktail antibiotic formulation, with their concentrations significantly influenced by withdrawal compliance, tissue type, and thermal processing methods. Thermal processing methods, including boiling, deep-frying, and roasting, significantly reduced antibiotic residue levels with the extent of reduction depending on the type of antibiotic and tissue involved. Deep-frying and roasting produced significant greater reductions for both drugs in gizzard and skin, while boiling impacted liver tissue more. Notably, thermal processing did not completely eliminate residues, especially in samples from the non-withdrawal group, emphasizing that cooking cannot substitute for proper withdrawal compliance prior to slaughter. Our findings also highlight the variability in elimination profiles among different classes of antibiotics and underscores the limitations of relying solely on withdrawal periods without considering drug-specific characteristics. However, we recommend that withdrawal periods for most antibiotics used for local poultry faming in Nigeria should not be less than 15 days as previously recommended by Khatun *et al*. (2018).

Conflict of Interest

The authors declare that they have no conflict of interest.

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