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Article

Discriminate vs. indiscriminate use of tylosin in broiler chickens: impacts on antibiotic residues in edible tissues, growth performance, and hematological parameters

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Abstract: Unregulated antimicrobial application in poultry farming represents a critical public health vulnerability in developing nations like Bangladesh, where improper drug usage promotes chemical residue accumulation in edible tissues and accelerates antimicrobial resistance (AMR). This investigation evaluated the comparative impacts of discriminate (incorporating a withdrawal period) and indiscriminate (omitting a withdrawal period) tylosin administration on tissue residue occurrence, growth traits, and blood parameters in Cobb-500 broiler chickens from January to May 2020. Eighteen day-old chicks were raised for 31 days and randomized on day 16 into three groups (n = 6): Group A (control; no antibiotic), Group B (discriminate; tylosin at 2.5 g/L in drinking water from days 16-22 followed by a withdrawal period), and Group C (indiscriminate; tylosin at 2.5 g/L from days 16-31 without withdrawal). On day 31, blood and tissue samples (liver, kidney, spleen, breast muscle, and thigh muscle) were harvested to screen for tylosin residues via thin-layer chromatography (TLC) and evaluate hematological indices (hemoglobin, total erythrocyte count, and packed cell volume). Broilers in the indiscriminate cohort attained the highest final numerical body weight (1805 ± 2.91 g), followed by the discriminate (1718 ± 3.05 g) and control (1352 ± 3.07 g) groups; though inter-group variations were statistically non-significant ($P > 0.05$). Active tylosin residues occurred across all target tissues in both medicated groups-with no traces detected in controls-showing markedly higher prevalence in the indiscriminate cohort than the discriminate cohort: liver (100% vs. 83.33%), kidney (100% vs. 50.00%), spleen (83.33% vs. 33.34%), breast muscle (66.66% vs. 16.67%), and thigh muscle (50.00% vs. 16.67%). Hematological parameters displayed a non-significant declining trend as tylosin exposure increased; for example, hemoglobin levels dropped from 7.93 ± 0.16 g/dL (control) to 7.52 ± 0.07 g/dL (discriminate) and 7.12 ± 0.11 g/dL (indiscriminate) ($P > 0.05$). Overall, continuous tylosin treatment generated widespread residue persistence across all edible tissues, predominantly in hepatic and renal organs, whereas observing the withdrawal window lowered, but did not completely eliminate, detectable residues. Disregarding withdrawal guidelines risks elevating dietary drug exposure for consumers and driving AMR, underscoring the urgent necessity for strict enforcement of withdrawal standards, routine residue monitoring, and responsible pharmaceutical stewardship in commercial poultry operations to safeguard public health.

Keywords: drug residue detection; antimicrobial resistance; therapeutic withdrawal; food safety; veterinary drug monitoring; thin layer chromatography

1. Introduction

The poultry industry is a major contributor to food security and economic development in Bangladesh, providing an affordable and high-quality source of animal protein for a rapidly growing population (Rahman *et al*., 2024). Over the past two decades, the sector has transformed from predominantly backyard production into a commercially intensive industry, driven by urbanization, increasing household income, changing dietary preferences, and rising demand for poultry meat and eggs (Rahman *et al*., 2024; Islam *et al*., 2025a). However, intensified poultry production has also increased disease pressure, resulting in greater dependence on veterinary pharmaceuticals, particularly antibiotics, to maintain flock health and productivity.

Antibiotics have long been used in poultry production for the therapeutic treatment of bacterial infections, prophylactic and metaphylactic disease prevention, and, historically, as growth promoters (Olatoye and Ehinmowo, 2009; Islam *et al*., 2025a). Their use has contributed to improved animal health, lower mortality, better feed conversion efficiency, and increased production efficiency (Das *et al*., 2023; Uddin *et al*., 2025). Nevertheless, the extensive and often indiscriminate use of antibiotics in food-producing animals has become a major global concern because it promotes antimicrobial resistance (AMR), contaminates animal-derived foods with drug residues, and threatens both animal and human health (Beyene, 2016; Mesfin *et al*., 2024; Islam, 2026).

The World Health Organization (WHO) identifies antimicrobial resistance (AMR) as a primary global public health hazard, with estimates indicating that fatal cases could reach 10 million each year by 2050 in the absence of robust control measures (Nazir *et al*., 2025). Agricultural antibiotic administration plays a major role in driving this crisis, as resistant bacterial strains and genetic resistance determinants can spread to human populations via contaminated livestock products, direct contact with animals, or environmental transmission vectors (Collignon *et al*., 2009; Apata, 2009; Beyene, 2016). Furthermore, the ingestion of lingering veterinary drug residues in animal meat can trigger hypersensitivity reactions, causes toxicological side effects, alter gut microbiome balance, and encourage the colonization of drug-resistant pathogens within the human digestive system (Nasr *et al*., 2014; Ahmed *et al*., 2021).

Tylosin, a 16-membered ring macrolide restricted solely to animal healthcare, is routinely used to combat Gram-positive bacteria and Mycoplasma species in poultry flocks (Giguere *et al*., 2022; Khan *et al*., 2022; Hossain *et al*., 2024). The agent disrupts bacterial translation by targeting and binding the 50S ribosomal subunit, typically delivered to birds through mass medication in feed or drinking water (Ahmed *et al*., 2021). To protect consumers against tissue residue exposure, regulatory authorities mandate explicit pre-slaughter clearance windows. Disregarding these obligatory withdrawal periods allows chemical residues to persist within marketed meat, heightening the selective pressure that favors macrolide-resistant bacterial strains (Nasr *et al*., 2014; Islam *et al*., 2025b).

Concerns regarding tylosin use have led to significant regulatory changes worldwide. Macrolide resistance in pathogenic bacteria has developed as a substantial clinical concern, as it limits therapeutic options for treating infections in both animals and humans (Kumar *et al*., 2023; Sarker *et al*., 2023). The European Union prohibited its consumption as a growth promoter in 1999 because of concerns over AMR and food safety (Van Poucke *et al*., 2003). However, in many developing countries, including Bangladesh, tylosin remains readily available and is frequently used without veterinary supervision or strict adherence to recommended withdrawal periods (Al Sattar *et al*., 2023; Hasan, 2025). Limited regulatory oversight, unrestricted access to veterinary antibiotics, inadequate farmer awareness, and weak enforcement of existing regulations have contributed to inappropriate antibiotic use in commercial poultry production (Trisha *et al*., 2023; Uddin *et al*., 2025). Consequently, several studies have reported the occurrence of antibiotic remains in poultry meat and eggs marketed in Bangladesh, highlighting an important food safety concern (Trisha *et al*., 2021; Rahman *et al*., 2024).

Although national guidelines specify withdrawal periods for veterinary antibiotics, compliance among poultry producers remains inconsistent. Consequently, there is a considerable gap between regulatory recommendations and field practices. Moreover, limited information is available regarding the consequences of discriminate use (withdrawal period observed) compared with indiscriminate use (no withdrawal period) of tylosin under conditions representative of commercial poultry production in developing countries. Most previous studies have focused primarily on residue depletion under controlled experimental conditions or have evaluated residue occurrence without simultaneously assessing production performance and physiological responses (Vandenberge *et al*., 2012; Soliman and Sedeik, 2016; Trisha *et al*., 2021; Islam *et al*., 2024). As a result, evidence integrating tissue residue prevalence, growth performance, and hematological responses under field-relevant conditions remain limited. It was hypothesized that indiscriminate administration of tylosin without observing the recommended withdrawal period would result in a higher prevalence of tissue residues than discriminate administration while also influencing growth performance and hematological characteristics in broiler chickens.

Consequently, this investigation was designed to evaluate how discriminate versus indiscriminate tylosin dosing regimens influence tissue residue frequency, broiler growth outcomes, and blood profiles within a setup modeling commercial poultry farming practices. The insights gained from this work offer valuable empirical data to enhance pharmaceutical stewardship and strengthen food safety protocols across Bangladesh and similar developing markets. By demonstrating the impact of withdrawal-period compliance on tissue residue occurrence, this research contributes to evidence-based policymaking, supports the implementation of routine residue surveillance programs, and reinforces the need for farmer education and stricter enforcement of responsible antibiotic-use practices to reduce consumer exposure to veterinary drug residues and mitigate the spread of AMR.

2. Materials and Methods

2.1. Ethical approval

All protocols involving live birds were executed in strict accordance with institutional regulations governing the care and management of laboratory animals. The trial blueprint underwent formal review and received ethical authorization from the Animal Welfare and Experimentation Ethics Committee (AWEEC) at Bangladesh Agricultural University, Bangladesh (Approval Ref. No. AWEEC/BAU/2021/07). Bird maintenance, handling, and specimen collection protocols were carried out following these sanctioned ethical guidelines to guarantee high standards of animal welfare throughout the study.

2.2. Study site and duration

The experiment was conducted between January and May 2020 at the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, Bangladesh (Figure 1). Before the commencement of the experiment, an isolated poultry shed was thoroughly cleaned, disinfected using a 1% (v/v) phenolic disinfectant, and allowed to dry completely before the introduction of the experimental birds.

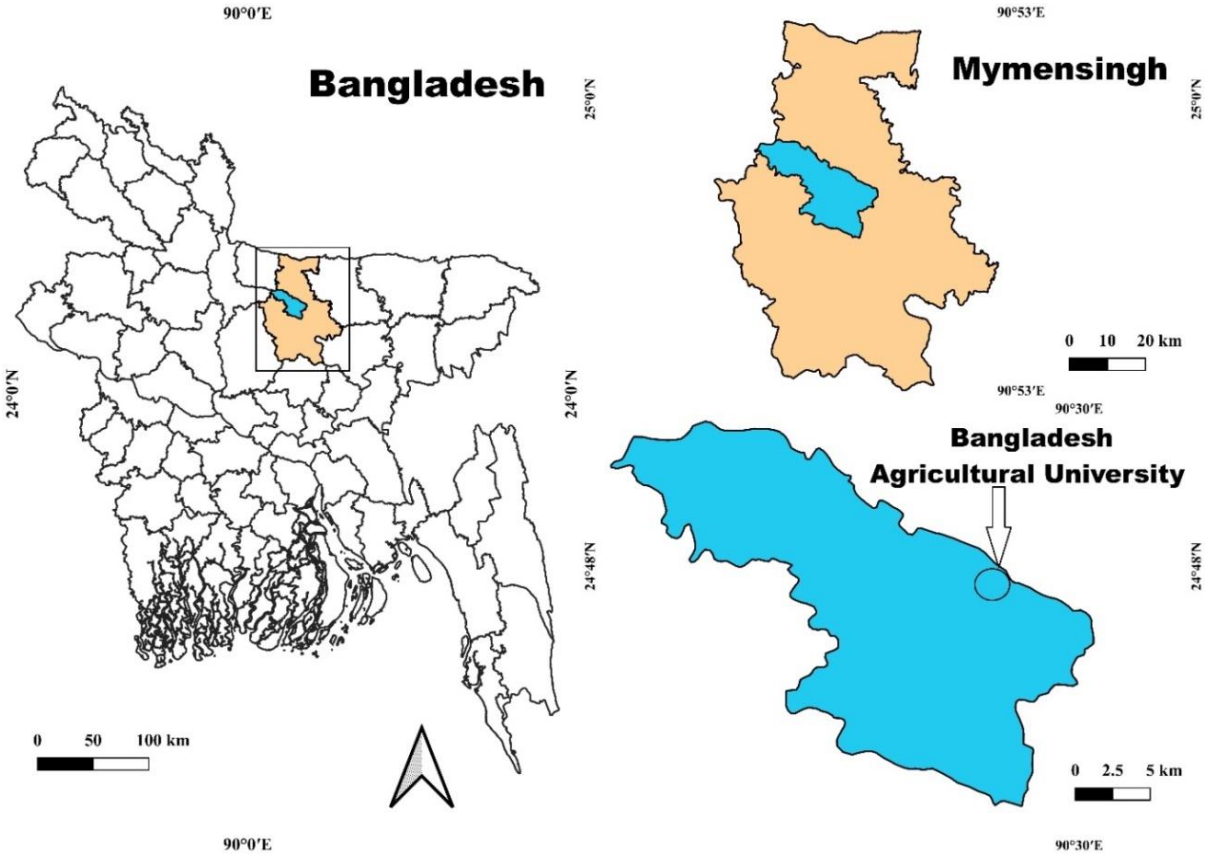

Figure 1. Geographic location of the experimental site.

2.3. Experimental design

A completely randomized experimental setup comprising three distinct cohorts was utilized for this investigation. Following an initial 15-day conditioning phase, a total of 18 day-old Cobb-500 broiler chicks were randomized on day 16 into three equal subgroups (n = 6 birds per group) (Table 1).

Table 1. Experimental design for discriminate and indiscriminate tylosin administration in broiler chickens.

Group	Treatment description	Tylosin administration	Withdrawal period	Sacrifice day
A (control)	No antibiotic throughout	None	N/A	Day 31
B (discriminate)	Tylosin 2.5 g/L drinking water	Days 16-22 (7 days)	Days 23-31 (9 days)	Day 31
C (indiscriminate)	Tylosin 2.5 g/L drinking water	Days 16-31 (14 days)	None	Day 31

N/A=not applicable.

2.4. Experimental birds and management

Overall 18 apparently healthy day-old Cobb-500 broiler chicks, previously vaccinated against Marek's disease at the hatchery, were purchased from CP Hatchery Ltd., Bangladesh. The birds were reared under standard commercial management practices and brooded collectively during the first week. During the initial week, the ambient brooding temperature was set at roughly 33°C, after which it was systematically lowered until reaching 25°C by week four. Throughout the first week, a vitamin-mineral premix (Rena-C, Renata Pharmaceuticals Ltd., Bangladesh) and 5% glucose solution were supplied daily through the drinking water. Routine vaccination was carried out according to the commercial broiler vaccination schedule, with BCRDV (Newcastle disease vaccine, L-36 strain) administered on day 7 and Gumboro vaccine on day 11 as a single ocular drop per bird.

2.5. Feed and water

The birds were fed a commercial broiler diet (Quality Feeds Ltd., Gazipur, Bangladesh) formulated according to their growth stage, comprising starter mash (days 1-14), grower pellets (days 15-24), and finisher feed (days 25-30) (Table 2). Rations were supplied two times per day at 08:00 and 16:00 h using sanitized trough feeders, with unconsumed feed weighed and cleared every morning prior to the initial feeding session. Potable, fresh drinking water was made available *ad libitum* across the entire trial using clean drinkers.

Table 2. Stated nutritional profiles of the commercial broiler rations utilized during the trial (expressed in g/100 g, excluding metabolizable energy value).

Nutrients	Broiler starter	Broiler grower	Broiler finisher
Moisture (max)	11.00	11.00	11.00
Protein (min)	25.00	24.00	24.00
Fat (min)	5.00	5.00	5.00
Fiber (max)	4.00	4.00	4.00
Calcium (min)	1.20	1.20	1.20
Phosphorus (min)	0.75	0.75	0.75
Methionine (min)	0.65	0.60	0.60
Lysine (min)	1.40	1.40	1.30
ME (Kcal/kg, min)	3150	3175	3175

*ME=metabolizable energy; source=Quality Feeds Limited, Gazipur, Bangladesh.

2.6. Experimental grouping and treatment administration

On day 16, the chicks were randomly divided into three experimental cohorts (n = 6 birds per group). Birds in Group A (control) were supplied with plain, non-medicated drinking water across the entire trial duration. Group B (discriminate use) was treated with water-soluble 20% tylosin tartrate powder (Eskayef Bangladesh Ltd., Bangladesh) administered at 2.5 g/L in drinking water for 7 consecutive days (days 16-22). Following this medication phase, Group B birds were transitioned back to non-medicated drinking water from days 23 to 31, providing a 9-day withdrawal window prior to harvest. Group C (indiscriminate use) received tylosin tartrate at the same concentration (2.5 g/L) in drinking water for 14 consecutive days (days 16-31), with no withdrawal period before sacrifice. Fresh medicated drinking water was prepared daily, and any remaining solution from the previous day was discarded before replenishment. The tylosin concentration was determined according to the manufacturer's recommended dosage and adjusted based on the birds' estimated daily water consumption and body weight to maintain consistent drug administration throughout the treatment period.

2.7. Growth performance assessment

Individual body weights of all birds were measured on days 16, 21, 26, and 31 using an electronic balance (OHAUS Corporation, Model PA214, USA). All birds were weighed in the morning before feeding to minimize

variations associated with gut fill and feed intake. Net body weight gain for every treatment cohort was determined by deducting baseline body mass (day 16) from terminal body mass (day 31). Additionally, feed conversion ratio (FCR) was derived to assess feed utilization efficiency.

2.8. Sample collection

At day 31, 2 mL blood samples were harvested from each bird's brachial (wing) vein using sterile needles and syringes, then transferred right away into sterile heparinized tubes for hematological profiling. Following blood sampling, birds were humanely euthanized via cervical dislocation following the approved ethical guidelines. Tissue samples, including the liver, kidney, spleen, breast muscle, and thigh muscle, were collected aseptically, placed in sterile labeled plastic bags, assigned unique identification codes, and stored at −20°C (LG Electronics, Model GR-B247, South Korea) until tylosin residue analysis.

2.9. Detection of tylosin residues by thin-layer chromatography (TLC)

Tylosin residues in tissue samples were detected using a validated thin-layer chromatography (TLC) method with appropriate quality control procedures, following previously published protocols with minor modifications (Chowdhury *et al*., 2023; Rahman *et al*., 2025; Islam *et al*., 2026).

2.9.1. Reagents and preparation of solutions

Analytical-grade diethyl ether, ethanol, methanol, and phosphate buffer powder (pH 7.2) were obtained from Merck KGaA (Darmstadt, Germany). Trichloroacetic acid (TCA, 30%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was prepared in-house. TLC silica gel 60 F_{254} plates (Catalog No. 1.05554.0001; Merck KGaA, Germany) were used for chromatographic separation. Chromatograms were visualized under a UV lamp (256 nm; VILBER LOURMAT, Model VL-6.LC, France).

To formulate the phosphate buffer (pH 7.2), 5.4 g of phosphate buffer powder was dissolved in 500 mL of distilled water, with final pH accuracy confirmed using a digital pH meter (HANNA Instruments, Model HI2211, Romania). The 30% (w/v) trichloroacetic acid (TCA) reagent was made by dissolving 30 g of TCA crystals into 100 mL of distilled water. A primary tylosin stock solution (25 mg/mL) was produced by dissolving 0.1 g of tylosin tartrate powder into 4 mL of pure methanol, which was then serially diluted to yield the working standard concentrations. The mobile phase was freshly mixed prior to each run using ethyl acetate, acetone, and methanol in a 10:6:4 (v/v/v) ratio. This solvent mixture was left to saturate the thin-layer chromatography (TLC) development chamber for 30 min to reach vapor equilibrium before running the plates.

2.9.2. Sample extraction

For each tissue specimen, roughly 4 g was blended with 10 mL of phosphate buffer using a mechanical tissue homogenizer. Protein precipitation was induced by treating the homogenate with 2 mL of 30% TCA, after which the mixture was spun at 6,000 rpm for 20 min in a refrigerated centrifuge (Eppendorf, Model 5804, Germany). The clean supernatant layer was harvested and washed twice using equal parts diethyl ether to strip out lipid components. The remaining aqueous layer was concentrated to complete dryness under nitrogen gas flow, then re-suspended in 200 µL of methanol to prepare for TLC loading.

2.9.3. Thin-layer chromatography analysis

Using calibrated micropipettes, 20 µL portions of each extracted specimen alongside 10 µL of the tylosin reference standard were spotted onto TLC plates 2 cm above the bottom edge. Chromatographic separation took place over 30 min at ambient temperature within a saturated TLC chamber holding the mobile solvent. Following plate development, the stationary phase was allowed to air-dry before visual inspection under 256 nm UV radiation, and the retention factor (R_f) for every visible signal spot was subsequently computed. Samples were considered positive for tylosin when the R_f value and fluorescence characteristics corresponded to those of the tylosin standard ($R_f = 0.61$).

2.9.4. Quality control

Quality control procedures included the analysis of spiked tissue samples (positive controls) and extraction blanks (negative controls) with each analytical batch to verify the reliability of the extraction procedure and chromatographic detection.

2.10. Hematological analysis

Hematological parameters, including hemoglobin (Hb) concentration, total erythrocyte count (TEC), and packed cell volume (PCV), were determined using standard hematological methods. Hemoglobin concentration was

measured by the Sahli acid-hematin method using a Sahli hemometer (Hirschmann Laborgeräte, Germany). Total erythrocyte count was determined using a Neubauer hemocytometer (Marienfeld Superior, Germany) with Hayem's solution as the diluent, where erythrocytes were counted in five designated squares of the counting chamber. PCV was assessed via the Wintrobe micro-hematocrit technique using dedicated Wintrobe tubes (Hirschmann Laborgeräte, Germany), which were subsequently spun at 3,000 rpm for 30 min in a micro-hematocrit centrifuge.

2.11. Statistical analysis

Experimental datasets were consolidated, coded, and structured using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Statistical evaluations were executed through IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA). Continuous metrics—such as live body weight and hematological values-are expressed as mean ± standard error of the mean (SEM). Inter-group variations were evaluated via one-way Analysis of Variance (ANOVA), with mean separation performed using Tukey's post-hoc test. Tissue residue occurrences are reported as percentage positive rates for each treatment cohort. Statistical significance was established at $P < 0.05$. Graphical illustrations were created using GraphPad Prism Version 9.5 (GraphPad Software, San Diego, CA, USA). The map of the study site was prepared by QGIS version 3.40.

3. Results

3.1. Growth performance

From day 21 through the end of the trial, the continuous, indiscriminate treatment cohort (Group C) maintained the highest average body mass, followed by the discriminate treatment cohort (Group B), while the non-medicated control cohort (Group A) consistently demonstrated the lowest weight values across all evaluation points. On day 16, the mean body weights were 680 ± 2.09 g, 720 ± 2.12 g, and 725 ± 1.94 g for Groups A, B, and C, respectively. These values increased to 871.67 ± 2.36 g, 985 ± 2.39 g, and 1020 ± 2.13 g on day 21, and further increased to 1138.33 ± 2.48 g, 1451.67 ± 1.25 g, and 1501.67 ± 3.01 g on day 26. By the end of the experimental period (day 31), the final mean body weights reached 1351.67 ± 3.07 g, 1718.33 ± 3.05 g, and 1805 ± 2.91 g for groups A, B, and C, respectively. Despite the consistently greater numerical body weights observed in group C throughout the study, one-way ANOVA showed no statistically significant differences among the treatment groups at any sampling time ($P > 0.05$). The cumulative body weight gain between days 16 and 31 was 671.67 g, 998.33 g, and 1080 g for groups A, B, and C, respectively, corresponding to percentage increases of 98.8%, 138.7%, and 149.0% (Table 3). Although birds receiving continuous tylosin administration (group C) achieved the greatest numerical weight gain, this apparent growth-promoting effect was not statistically significant under the experimental conditions of the present study.

Table 3. Body weight (g) of broiler chickens at different ages (mean ± SEM, n=6).

Age	Group A (control)	Group B (discriminate)	Group C (indiscriminate)	*P* value
16th day	680 ± 2.09	720 ± 2.12	725 ± 1.94	>0.05
21st day	871.67 ± 2.36	985 ± 2.39	1020 ± 2.13	>0.05
26th day	1138.33 ± 2.48	1451.67 ± 1.25	1501.67 ± 3.01	>0.05
31st day	1351.67 ± 3.07	1718.33 ± 3.05	1805 ± 2.91	>0.05

Group A = control (no antibiotic); Group B = discriminate use (withdrawal period observed); Group C = indiscriminate use (no withdrawal period). Values are presented as mean ± SEM (n = 6). P values were obtained using one-way ANOVA.

3.2. Tylosin residues in edible tissues

No antibiotic residues were detected in any of the tissue samples collected from the control group (group A), indicating the absence of cross-contamination and confirming the reliability of the analytical procedure. In contrast, tylosin residues were detected in all examined tissue types from both treated groups, with consistently higher residue prevalence in the indiscriminate group (group C) than in the discriminate group (group B) (Table 4). In the discriminate group (group B), tylosin residues were detected in 83.33% (5/6) of liver samples, 50.00% (3/6) of kidney samples, 33.34% (2/6) of spleen samples, and 16.67% (1/6) of both breast and thigh muscle samples. In comparison, the indiscriminate group (group C) exhibited substantially higher residue prevalence, with residues detected in 100% (6/6) of both liver and kidney samples, 83.33% (5/6) of spleen samples, 66.66% (4/6) of breast muscle samples, and 50.00% (3/6) of thigh muscle samples.

Among all tissues examined, the liver and kidney exhibited the highest residue prevalence in both treatment groups, whereas the breast and thigh muscles showed comparatively lower detection rates. Overall, the markedly greater residue prevalence observed in group C demonstrates that failure to observe the recommended

withdrawal period substantially increases the persistence of detectable tylosin residues in edible tissues. Nevertheless, the detection of residues in several tissues of the discriminate group indicates that withdrawal reduced, but did not completely eliminate, detectable tylosin residues under the conditions of the present study.

Table 4. Comparison of tylosin residue positivity in edible tissues of broiler chickens among control (group A), discriminate (group B), and indiscriminate (group C) groups.

Tissue	Control (group A)	Discriminate (group B)	Indiscriminate (group C)
Liver	0/6 (0%)	5/6 (83.33%)	6/6 (100%)
Kidney	0/6 (0%)	3/6 (50.00%)	6/6 (100%)
Spleen	0/6 (0%)	2/6 (33.34%)	5/6 (83.33%)
Breast muscle	0/6 (0%)	1/6 (16.67%)	4/6 (66.66%)
Thigh muscle	0/6 (0%)	1/6 (16.67%)	3/6 (50.00%)

Values indicate the proportion of positive samples out of the total number of samples tested for each group. Group A = control (no antibiotic); Group B = discriminate use (withdrawal period observed); Group C = indiscriminate use (no withdrawal period).

3.3. Hematological parameters

A progressive numerical reduction in hemoglobin (Hb) content was recorded across the treatment cohorts, yielding mean concentrations of 7.93 ± 0.16 g/dL in group A, 7.52 ± 0.07 g/dL in group B, and 7.12 ± 0.11 g/dL in group C. An identical declining pattern emerged for total erythrocyte counts (TEC), decreasing from 2.91 ± 0.07 × 10^6 /mm³ in group A to 2.75 ± 0.06 × 10^6 /mm³ in group B and 2.63 ± 0.08 × 10^6 /mm³ in group C. Likewise, PCV decreased progressively, with mean values of 23.87 ± 0.52%, 22.53 ± 0.33%, and 20.52 ± 0.85% in groups A, B, and C, respectively (Figure 2). Although all hematological parameters exhibited lower numerical values in the tylosin-treated groups, particularly in the indiscriminate group (group C), one-way ANOVA revealed no statistically significant differences among the treatment groups ($P > 0.05$). Overall, the results indicate that tylosin administration at the tested dose and duration did not produce significant alterations in hematological indices, despite the consistent downward trends observed across all measured parameters.

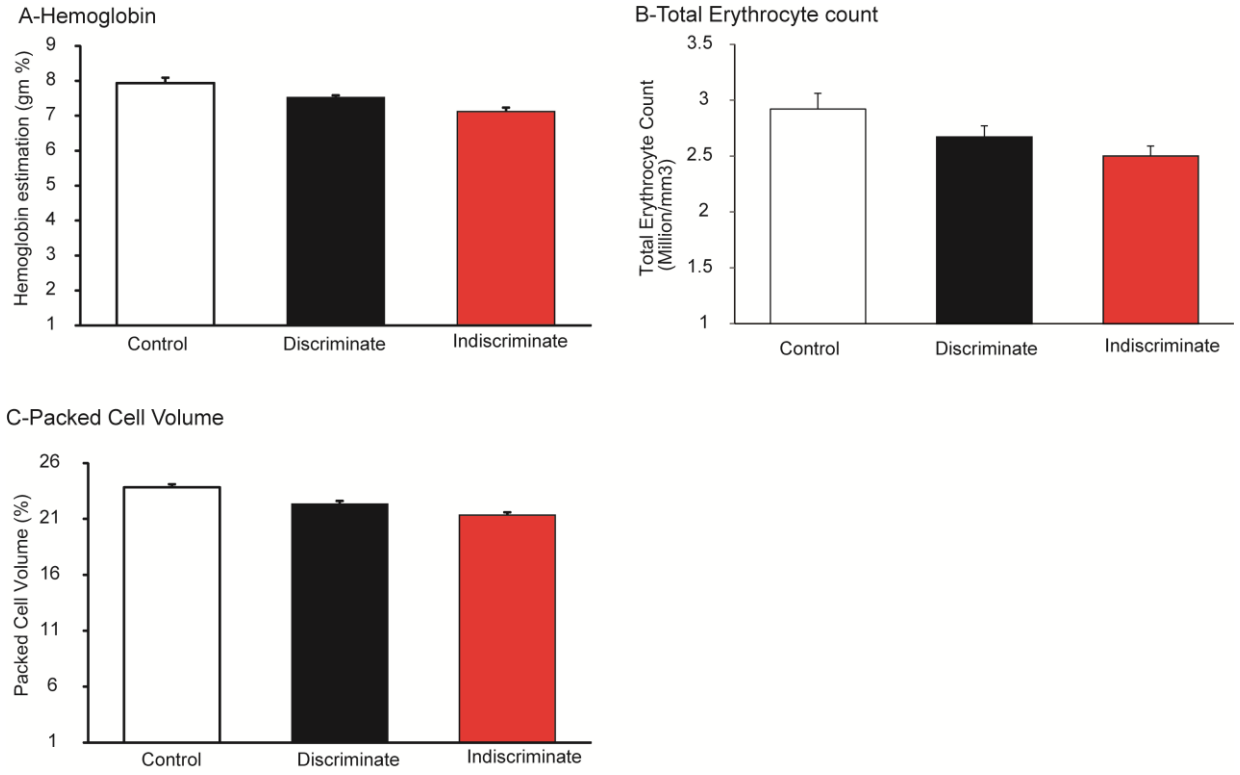

Figure 2. Impact of targeted (discriminate) versus continuous (indiscriminate) tylosin regimens on hematological profiles in broiler chickens (n = 6 per group). (A) Hemoglobin (Hb; g/dL); (B) Total erythrocyte count (TEC; ×10⁶ cells/mm³); (C) Packed cell volume (PCV; %). Bars represent mean ± SEM. No significant differences were observed among treatment groups for any hematological parameter (one-way ANOVA, *P* > 0.05).

4. Discussion

The present study demonstrates that indiscriminate administration of tylosin without observing the withdrawal period leads to a markedly higher prevalence of antibiotic residues in all major edible tissues of broiler chickens, whereas discriminate use with a withdrawal period substantially reduces residue occurrence. The liver and kidney exhibited the highest residue prevalence, particularly in the indiscriminate group where both tissues showed 100% positivity. This distribution is biologically plausible because tylosin, like other macrolide antibiotics, undergoes extensive hepatic metabolism and renal excretion, resulting in preferential accumulation in these organs (Ahmed *et al*., 2021; Khan *et al*., 2022). The high prevalence of residues in the liver is especially important from a food safety perspective because liver is widely consumed in many countries and may represent a significant route of human exposure to veterinary drug residues.

The observed pattern of residue distribution is consistent with previous studies. Soliman and Sedeik (2016) reported that liver and kidney contained the highest tylosin residues following repeated oral administration in broiler chickens, with detectable residues persisting for several days after treatment. Similarly, the Joint FAO/WHO Expert Committee on Food Additives reported that, at zero withdrawal time, tylosin concentrations in liver and kidney reached 1030 µg/kg and 432 µg/kg, respectively (Kim *et al*., 2023). In the present study, residues remained detectable in several tissues of the discriminate group even after a 9-day withdrawal period, indicating that withdrawal substantially reduced, but did not completely eliminate, detectable residues under the experimental conditions. This finding suggests that residue depletion may vary according to management conditions, physiological status of birds, environmental factors, and analytical sensitivity, and it highlights the need for further quantitative studies to determine whether current withdrawal recommendations consistently ensure compliance with food safety standards in commercial production systems.

The detection of tylosin residues in breast and thigh muscles in the indiscriminate group is of particular public health concern because these tissues constitute the most commonly consumed portions of chicken meat in Bangladesh and many other countries. Dietary exposure to antibiotic residues may contribute to allergic reactions in susceptible individuals, alteration of the intestinal microbiota, and selection of macrolide-resistant bacteria within the human gastrointestinal tract (Shareef *et al*., 2009; Beyene, 2016). Macrolide resistance has become increasingly widespread worldwide, and resistance determinants such as erm(B) and mef(A) can confer reduced susceptibility to erythromycin, tylosin, and related macrolides (Kumar *et al*., 2023; Sarker *et al*., 2023). Although the present study did not evaluate AMR directly, the persistence of residues in edible tissues may contribute to selective pressure that favors the emergence and maintenance of resistant bacterial populations.

Such outcomes ought to be considered against the wider backdrop of the global antimicrobial resistance (AMR) emergency. According to the WHO, AMR ranks among the most critical dangers facing worldwide public health, with forecasts indicating that drug-resistant pathogens may claim up to 10 million lives every year by 2050 unless stringent containment strategies are put into action (Salam *et al*., 2023). Inappropriate antibiotic use in livestock production, particularly prolonged or sub-therapeutic exposure, is recognized as an important driver of this problem (Collignon *et al*., 2009; Apata, 2009). The continued availability and use of tylosin without adequate veterinary supervision in countries such as Bangladesh may therefore contribute not only to residue contamination of food but also to the broader dissemination of resistance determinants through animal, human, and environmental pathways.

Although birds in the indiscriminate group achieved the highest numerical body weight gain, the differences among groups were not statistically significant. The absence of significance may be related to the relatively small sample size and the short experimental duration. Nevertheless, the trend toward greater weight gain in tylosin-treated birds is consistent with the historical use of macrolides as growth-promoting agents (Liu *et al*., 2021). Importantly, any potential production advantage must be weighed against the associated food safety and public health risks. The European Union prohibited the use of tylosin as a growth promoter because the perceived benefits did not outweigh concerns regarding AMR and residue exposure (Van Poucke *et al*., 2003). The persistence of antibiotic use for growth promotion in some developing countries reflects ongoing challenges related to regulatory enforcement, economic pressure, and access to veterinary guidance.

Hematological parameters showed a consistent numerical decline from the control group to the indiscriminate group, but these changes were not statistically significant. This finding agrees with the observations of Trisha *et al*. (2021), who reported no significant alterations in hematological indices following antibiotic administration in broiler chickens. The lack of significant differences suggests that tylosin, at the dose and duration used in this study, did not induce overt hematological toxicity. However, the downward trend observed for Hb, TEC, and PCV may indicate subtle physiological effects associated with prolonged exposure or treatment-related stress. Because hematological indices can serve as sensitive indicators of systemic responses to xenobiotic, future studies involving larger sample sizes, longer exposure periods, and additional biochemical markers would be valuable for clarifying the clinical relevance of these changes.

The analytical approach used in this study also deserves consideration. Thin-layer chromatography is a useful and cost-effective screening method for detecting antibiotic residues, particularly in resource-limited settings; however, it is inherently less sensitive and less quantitative than high-performance liquid chromatography coupled with tandem mass spectrometry. Because TLC provides qualitative or semi-quantitative information, the present study could not determine the exact concentrations of tylosin in tissues or directly compare the results with international maximum residue limits, including the European Union MRL of 100 µg/kg for tylosin in muscle (Sani *et al*., 2023). Future investigations should therefore employ validated quantitative methods such as HPLC-MS/MS to determine residue concentrations and assess regulatory compliance more accurately. In addition, the limited sample size reduces statistical power and may have constrained the ability to detect modest but biologically relevant differences in growth and hematological parameters. Despite these limitations, the contrast between discriminate and indiscriminate use was pronounced. Residue prevalence increased substantially when the withdrawal period was omitted, demonstrating the critical importance of withdrawal compliance for food safety. At the same time, the persistence of detectable residues in the discriminate group indicates that withdrawal recommendations should be evaluated under field-relevant conditions rather than relying solely on controlled laboratory depletion studies.

The findings have important policy implications for Bangladesh and other developing countries. Previous surveys have documented poor compliance with withdrawal recommendations among poultry producers (Al Sattar *et al*., 2023; Hasan, 2025). Contributing factors include limited farmer awareness, economic pressure to minimize disease losses, inadequate veterinary supervision, over-the-counter access to antibiotics, and weak regulatory enforcement (Rahman *et al*., 2024; Uddin *et al*., 2025). To effectively combat these issues, an integrated One Health framework is essential-one that unifies human, livestock, and ecological well-being into a cohesive strategy (Uddin *et al*., 2025). Priority actions should include farmer education on responsible antibiotic use, stronger regulation of veterinary drug sales, routine residue surveillance in slaughterhouses and markets, improved access to diagnostic and advisory services, promotion of non-antibiotic alternatives such as probiotics and enhanced biosecurity, and the implementation of antibiotic stewardship programs within veterinary practice. Together, these measures are essential for reducing consumer exposure to veterinary drug residues and mitigating the spread of AMR.

5. Conclusions

This study demonstrated that indiscriminate use of tylosin without observing the recommended withdrawal period resulted in a substantially higher prevalence of antibiotic residues in edible tissues, particularly the liver and kidney, compared with discriminate use. Although no statistically significant effects on growth performance or hematological parameters were observed, the persistence of residues highlights a potential public health risk through increased consumer exposure and the potential contribution to AMR. These results highlight the imperative for rigorous compliance with withdrawal timelines, systematic residue surveillance, and prudent antimicrobial stewardship across poultry management systems. Subsequent research ought to utilize high-precision quantitative assays-such as HPLC-MS/MS-alongside expanded sample cohorts to accurately quantify tissue residue levels, verify adherence to statutory maximum residue limits (MRLs), and gain a deeper understanding of the food safety and public health risks associated with antibiotic residues in poultry-derived goods.

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Data availability

All relevant data and information are presented in the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Md. Shafiqul Islam: conceptualization, supervision, data analysis, initial drafting and revisions; Md. Al-Amin Haque: sample acquisition, data collection, data analysis, initial drafting of the manuscript, and revisions; Md.

Shakil Islam: data validation, manuscript review and editing. All authors have reviewed and approved the final version of the manuscript.

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