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Article

Indiscriminate use of ciprofloxacin antibiotic in broiler reveals high antibiotic residues in broiler meat

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Abstract: In Bangladesh, excessive and haphazard antibiotic use is frequently observed in the poultry industry, which is a global issue in terms of antibiotic residue and resistance. This study aimed to detect ciprofloxacin residues in edible tissues and assess their impact on broilers' hematological and major enzymatic parameters. Thirty-day-old Cobb-500 broiler chicks were used to examine the effects of controlled and uncontrolled ciprofloxacin usage during the period from June to December 2021 at Bangladesh Agricultural University (BAU). On the 14th day, the chicks were randomly assigned to control, discriminate, and indiscriminate antibiotic groups. Treatment commenced from day 14, with the discriminate group receiving ciprofloxacin at 100 mg/L in drinking water for 7 days followed by a 7-day withdrawal period. Conversely, the indiscriminate group received ciprofloxacin at the same concentration for 14 days without any withdrawal period. Daily body weights of the broiler were recorded. On day 14, the body weights across all groups showed no significant differences, but by day 28, the indiscriminate group exhibited a significantly higher mean body weight (1601.43±15.50 g). Thin layer chromatography (TLC) analysis revealed the presence of ciprofloxacin residues in all samples from the indiscriminate group, while no residues were detected in the control group. In the discriminate group, residues were found in 2 liver samples, 3 kidney samples, 1 spleen sample, and 1 breast muscle sample, but not in thigh muscle samples. Hematological analysis indicated significant differences in total erythrocyte count (TEC), hemoglobin (Hb), and erythrocyte sedimentation rate (ESR) among the groups, with no significant differences in packed cell volume (PCV). Enzymatic analysis of blood serum for ALT and AST showed no substantial changes between treated and control birds. Therefore, the controlled use of ciprofloxacin in the poultry sector is crucial to safeguard human health.

Keywords: antimicrobial resistance; withdrawal period; hematological analysis; residue detection; food safety

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1. Introduction

Antibiotics are now frequently used in poultry production to prevent diseases and treat infections caused by bacteria, and also to stimulate growth. Ciprofloxacin is one of the most commonly used broad-spectrum antibiotics of the fluoroquinolone group that is widely known for its efficacy against both Gram-negative and Gram-positive bacteria (Islam *et al*., 2016; Islam *et al*., 2024a). However, the misuse of ciprofloxacin has given rise to serious concerns regarding antibiotic resistance and the presence of antibiotic residue, which have detrimental effects on both animal and human health. Antibiotic resistance has become a global threat and the indiscriminate use of antibiotics without proper guideline in animal production is a major contributing factor (Islam *et al*., 2023).

Ciprofloxacin resistance might be developed resulting in resistant bacteria infecting humans by transmitting through the food chain leading to increased morbidity (Van Boeckel *et al*., 2015; Islam *et al*., 2024a). Moreover, it is essential to perform residual analysis of the ciprofloxacin antibiotic in poultry products to ensure safe food for human consumption and safeguard human health. Even after the withdrawal period, antibiotic residues can be found in meat, eggs, and other poultry products, causing a risk of toxicity, allergic reactions, and disturbance of normal human gut flora (Fletouris, 2000; Islam *et al*., 2019; Sarker *et al*., 2018). Though maximum residue limits (MRLs) for ciprofloxacin in poultry products have been established by regulatory bodies, there are still difficulties in compliance and monitoring (Csikó, 2023).

The widespread and often indiscriminate use of antibiotics such as ciprofloxacin in poultry farming raises concerns about antibiotic residues in edible tissues, potential public health risks, and the development of antimicrobial resistance. This study hypothesizes that indiscriminate use of ciprofloxacin in broiler chickens leads to detectable drug residues in tissues, alters hematological and enzymatic parameters, and influences growth performance. To investigate this, the research addresses the following questions: Does ciprofloxacin use result in residue accumulation in different tissues of broilers? How does its administration—discriminate versus indiscriminate—affect body weight gain and blood parameters? And what are the implications of these findings for food safety and public health?

The aim of this research is to investigate the effects of the ciprofloxacin antibiotic in poultry, emphasizing its residual analysis in poultry products. By comprehending these effects, we can develop strategies to ensure the appropriate use of ciprofloxacin in poultry, addressing the public health concerns associated with antibiotic use in poultry farming.

2. Materials and Methods

2.1. Ethical approval

The experiment was conducted in accordance with the ethical and welfare guidelines established by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University (Approval No: AWEEC/BAU/2021[07]).

2.2. Experimental design

Thirty-day-old Cobb-500 chicks from CP Company Ltd. were purchased, and initial body weights were recorded upon arrival. Chicks were fed fresh starter feed and supplied with vitamin C and 5% glucose solution in drinking water to alleviate the stress of transport. The experiment was conducted between June 2021 and December 2021 in a controlled shed located within the premises of Bangladesh Agricultural University. On day 14, the chicks were randomly divided into three groups: Group A (control), which did not receive any antibiotic treatment; Group B (discriminate use), which was given ciprofloxacin at 100 mg/L in drinking water for 7 days, followed by a 7-day withdrawal period; and Group C (indiscriminate use), which was administered the same ciprofloxacin dosage continuously for 14 days without a withdrawal period (Table 1).

Table 1. The experimental design for the current experiment.

Group name	Specification	Description
Group A	Control	No antibiotic treatment
Group B	Discriminate group	Treated with ciprofloxacin at 100 mg/L in drinking water for 7 days, followed by a withdrawal period of 7 days
Group C	Indiscriminate group	Received ciprofloxacin at 100 mg/L in drinking water for 14 days with no withdrawal period

2.3. Data and sample collection
Birds were weighed each day to monitor body weight progression. On day 28, they were humanely sacrificed, and tissue samples were collected for analysis. Samples from the liver, kidney, spleen, breast, and thigh muscles were obtained to assess ciprofloxacin residue levels.

2.4. Hematological and biochemical analysis
Blood samples were analyzed to evaluate hematological parameters such as total erythrocyte count (TEC), hemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), and packed cell volume (PCV), following established protocols (Islam *et al*., 2019). Additionally, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed through standard enzymatic procedures (Senanayake *et al*., 2015).

2.5. Thin layer chromatographic analysis
Thin-layer chromatography (TLC) is an analytical technique used to distinguish and identify the constituents of a mixture. TLC offers a quick, easy, and affordable way to analyze small amounts of material, which is why it is widely used in both academic and industrial settings. This method is useful for preliminary testing prior to utilizing more sophisticated chromatographic techniques (Sherma and Fried, 2003; Cieśla *et al*., 2019).

2.6. Sample preparation and antibiotic extraction
Prior to analysis, the samples were stored at -20 °C. At first the samples were ground with a mortar and pestle and 4 g of the sample was transferred into a beaker. Then they were homogenized with 10 ml of phosphate buffer (pH 7.2), and 2 ml of tricholoroacetic acid (TCA) (30%) was added. Then the mixture was centrifuged for 20 minutes at 6000 rpm to precipitate proteins. An equal amount of diethyl ether was used to defeat the supernatant, dividing the mixture into layers that were watery and oily. The bottom aqueous layer was collected for additional examination. The approach is in accordance with the (Popelka *et al*., 2005).

2.7. TLC plate and standard solution preparation
For the preparation of the standard solution, 0.1 ml of ciprofloxacin solution was added to 4 ml of methanol solution and stored in -4 °C (Billah *et al*., 2014). The mobile phase was prepared with acetone–methanol (1:1) (Sarker *et al*., 2018; Islam *et al*., 2024b).

2.8. Pointing, running, and detection
The standard solution was poured to the plate using a capillary tube, and when the spots dried, the plate was placed in the mobile phase in the TLC tank. Chromatograms were examined under UV light at 256 nm, and the retention factor (R*f*) values were determined by comparing the distance traveled by each compound spot to the distance traveled by the solvent front.

2.9. Statistical analysis
The data from the experimental study were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Numerical values were presented as mean ± standard error of the mean (SEM). To assess the differences among the three experimental groups (control, discriminate, and indiscriminate) for parameters like body weight, hematological values (TEC, Hb, PCV, ESR), and serum enzyme levels (ALT, AST), a one-way analysis of variance (ANOVA) was performed. When ANOVA revealed significant differences ($P < 0.05$), post-hoc comparisons were carried out using Tukey's HSD (honestly significant difference) test to identify specific group differences. The presence of ciprofloxacin residues in tissues (liver, kidney, breast muscle, thigh muscle, spleen, and feather) was recorded as categorical data and analyzed using frequency distribution and percentages. A significance threshold of $P < 0.05$ was set for all tests.

3. Results
3.1. Body weight
Body weight measurements were taken daily for each bird from day 14 through day 31. At the beginning of the treatment period, there were no notable differences in weight among the three groups. By day 21, the highest average weight was observed in the Group C, while the group A had the lowest; however, these differences were not statistically significant ($P>0.05$). By day 28, the group C maintained the highest weight gain, followed by the group B, with the group A still showing the lowest weight. Statistical analysis using one-way ANOVA and Bonferroni post-hoc testing indicated that these differences were significant ($P<0.05$) (Table 2).

Table 2. Body weight of broilers at 14th, 21st and 26th day.

Age	Groups	Weight (g) (mean ± SEM)	*P* value	Level of significance
	Group A	431.86 ± 7.00		
14th day	Group B	435.71 ± 6.41	0.69	ns
	Group C	439.14 ± 6.76	0.47	ns
	Group A	833.86 ± 16.91		
21st day	Group B	875.14 ± 14.21	0.09	
	Group C	888.71 ± 21.45	0.07	ns
28th day	Group A	1435.14 ± 18.03		ns
	Group B	1525.86 ± 17.13	0.003	**
	Group C	1601.43 ± 15.50	0.00001	**, ##

*SEM=Standard error of mean; compare between control and dis, $P<0.01$, **; compare between control and Indis, $P<0.01$, **; compare between Dis and Indis, $P<0.01$, ##; ns=non-significant.

3.2. Thin layer chromatographic analysis of poultry tissues

The indoor trial showed no ciprofloxacin residues in the group A. In the group B, residues were detected in 20% of liver, 30% of kidney, 10% of spleen, and 10% of breast muscle samples, with no residues in thigh muscle. Overall, 14% (7 out of 50) samples tested positive in this group. In contrast, all samples (100%) from the group C were positive. Out of 150 total samples, 67 (38%) showed ciprofloxacin residues under UV detection using TLC (Table 3).

Table 3. Ciprofloxacin antibiotic residue in control and treated samples of broiler.

Name of sample		Positive samples	
	Control (n=10)	Discriminate (n=10)	Indiscriminate (n=10)
Liver	0 (0%)	2 (20%)	10 (100%)
Kidney	0 (0%)	3 (30%)	10 (100%)
Spleen	0 (0%)	1 (10%)	10 (100%)
Thigh muscle	0 (0%)	0 (0%)	10 (100%)
Breast muscle	0 (0%)	1 (10%)	10 (100%)

3.3. Blood parameters

3.3.1. Hemoglobin (Hb) status

The highest mean hemoglobin concentration was observed in the group C (9.01 ± 0.16%), while the group B recorded a moderate level (8.14 ± 0.13%), and the lowest value was noted in the group A (7.73 ± 0.09%) (Table 4). Statistical evaluation using one-way ANOVA followed by Bonferroni post-hoc comparisons demonstrated significant differences between the group A and group B ($P < 0.05$), as well as highly significant differences between the group A and group C ($P < 0.001$) and between the group B and Group c ($P < 0.001$).

Table 4. Hemoglobin (g %) of three individual groups.

Name of group	Hemoglobin % (mean ± SEM)	*P* value	Level of significance
Group A	7.73 ± 0.09		
Group B	8.14 ± 0.13	0.02	*
Group C	9.01 ± 0.16	0.00001	***, ##

*SEM=Standard error of mean; compare between control and Dis, $P<0.05$, *; $P<0.001$, ***; compare between control and Indis, $P<0.05$, *; $P<0.001$, ***; compare between Dis and Indis, $P<0.01$, ##; $P<0.001$, ***.

3.3.2. Total erythrocyte count (TEC)

The TEC reached its peak in the group C (3.45 ± 0.09), with the group B showing a moderate value (2.84 ± 0.08) and the group A recording the lowest count (2.62 ± 0.04). These variations were found to be statistically significant ($P < 0.05$) (Table 5). Further analysis using Bonferroni post-hoc testing revealed significant differences between the group A and group B ($P < 0.05$), and highly significant differences between both the group A and group C, as well as the group B and group C ($P < 0.001$). In the case of PCV, the highest average was observed in the group C (27.86 ± 0.63), followed by the group B (27.14 ± 0.55) and group A (26.57 ± 0.37).

Despite this trend, statistical analysis showed no significant differences (*P* > 0.05) among the three groups (Table 6).

Table 5. Total erythrocyte count (million/mm^3) of three individual groups.

Name of group	TEC (Million/mm^3) (mean ± SEM)	*P* Value	Level of significance
Group A	2.62 ± 0.05		
Group B	2.84 ± 0.08	0.04	*
Group C	3.45 ± 0.09	0.000003	***, ###

*SEM=standard error of mean; compare between control and Dis, *P*<0.05, *; *P*<0.001, ***; compare between control and Indis, *P*<0.05, *; *P*<0.001, ***; compare between Dis and Indis, *P*<0.001, ***.

Table 6. Packed cell volume (%) of three individual groups.

Name of group	PCV % (mean ± SEM)	*P* value	Level of significance
Group A	26.57 ± 0.37		
Group B	27.14 ± 0.55	0.41	ns
Group C	27.86 ± 0.63	0.11	ns

*SEM=standard error of mean; ns=non-significant.

3.3.3. Erythrocyte sedimentation rate (ESR)

The mean ESR was highest in the group C (3.42 ± 0.20), with the group B showing a slightly lower value (3.14 ± 0.26), and the group A presenting the lowest mean ESR (2.86 ± 0.34) (Table 7). While overall analysis indicated a statistically significant variation among the groups, subsequent pairwise comparisons using Bonferroni post-hoc testing did not reveal any statistically significant differences between individual groups (*P* > 0.05).

Table 7. Erythrocyte sedimentation rate (%) of three individual groups.

Name of group	ESR (mm/hr) (mean ± SEM)	*P* Value	Level of significance
Group A	2.86 ± 0.34		
Group B	3.14 ± 0.26	0.51	ns
Group C	3.42 ± 0.20	0.17	ns

*SEM=standard error of mean; ns= non-significant

3.4. Enzymatic analysis SGOT (AST) and SGPT (ALT)

3.4.1. Alanine aminotransferase (ALT)

The group C had the highest mean serum ALT enzyme level (16.63 ± 1.01), followed by the group B (15.83 ± 1.05) and the group A (13.08 ± 0.71) (Table 8). Statistical analysis revealed significant differences among the groups. One-way ANOVA followed by Bonferroni post-hoc analysis revealed no statistically significant difference between the group A and group B (*P* > 0.05), nor between the group B and group C (*P* > 0.05). In contrast, a significant difference was observed between the group A and group C (*P* < 0.001).

Table 8. Serum ALT enzyme levels of three individual groups.

Name of group	ALT (U/L) (mean ± SEM)	*P* Value	Level of significance
Group A	13.08 ± 0.71		
Group B	15.83 ± 1.05	0.05	ns
Group C	16.63 ± 1.01	0.001	***, ns

*SEM=standard error of mean; compare between control and Dis, *P*<0.001, ***; compare between control and Indis, *P*<0.001, ***; compare between Dis and Indis, *P*<0.001,***; ns= non-significant

3.4.2. Aspartate transaminase (AST)

The group C exhibited the highest mean serum AST enzyme level (264.5 ± 6.45), followed by the group B (250.67 ± 8.00) and the group A (246.83 ± 7.81) (Table 9). Statistical evaluation showed that there were no meaningful differences among the three groups ($P > 0.05$). Additionally, Bonferroni post-hoc comparisons from the one-way ANOVA confirmed the absence of significant variation between any pair of groups ($P > 0.05$).

Table 9. Serum AST enzyme levels of three individual groups.

Name of group	ALT (U/L) (mean ± SEM)	*P* Value	Level of significance
Group A	246.83 ± 7.81		
Group B	250.67 ± 8.00	0.74	ns
Group C	264.5 ± 6.45	0.11	ns

*SEM= standard error of mean; ns= non-significant

4. Discussion

Indiscriminate and excessive use of antibiotics in animal industry has been a concern for a long time. The application, and occasionally improper use, of antimicrobials in livestock production has contributed to the rise and spread of resistant pathogens and genes. Developing country like Bangladesh where animals and human suffered from many infectious diseases, warrant special precautions for using antibiotics in poultry industry. Therefore, this study was designed to investigate the presence of ciprofloxacin residues in the edible tissues of broiler chickens through a controlled experimental trial. Additionally, a correlation was observed between ciprofloxacin use and live birds' overall body weight gain. The research also explored the impact of antibiotic misuse on the hematological parameters of the birds, providing a comprehensive insight into its physiological effects. The body weight gain was observed to be lowest in the control group, highest in the indiscriminate group, and moderate in the discriminate group. While antibiotics may play a role in promoting weight gain, it is important to note that broilers are genetically predisposed to gain weight as they mature to a certain extent. The moderate weight gain observed in the discriminate group is consistent with the inherent genetic traits of broilers. Numerous studies have highlighted the growth-enhancing effects of antibiotics (Dahiya *et al*., 2006; Castanon, 2007; Miyakawa *et al*., 2024).

Antibiotics influence several physiological functions in poultry, leading to improved feed efficiency. Their use significantly alters the composition of the microbial population in the gut. Additionally, antibiotics reduce the density of villi in the duodenum while eliminating harmful microorganisms within the digestive tract (Mitchell *et al*., 1998). Antibiotic growth promoters have been commonly used in poultry for many years (Costa *et al*., 2017). However, improper use not only raises costs but also compromises meat safety for consumption. Maintaining an appropriate withdrawal period ensures more profitable weight gain. Since antibiotics are costly and offer no clear health benefits until slaughter, their use up to the final day of rearing is unnecessary and may lead to potential negative effects.

Broilers naturally reach a certain weight gain capacity, beyond which additional feeding or growth promoters may not further enhance weight (Costa *et al*., 2017). The lower weight gain observed in the control group was expected, and this study does not support the use of antibiotics for promoting growth in broilers. Instead, effective management and scientific feeding practices are more profitable alternatives to antibiotic use. These approaches also benefit consumers and the environment. This aligns with findings from other studies (Demir *et al*., 2003). Nowadays, the use of natural feed additives as alternatives to antibiotic growth promoters has gained significant attention, with numerous studies highlighting their efficiency. Chromatographic techniques play a pivotal role in detecting and quantifying antibiotic residues in poultry tissues, with TLC standing out for its simplicity and cost-effectiveness. TLC is a particularly suitable choice for direct residue analysis on poultry farms due to its affordability, speed, and ability to analyze multiple samples simultaneously, making it a practical tool for routine screening (Sattar *et al*., 2014). While high-performance liquid chromatography (HPLC) offers superior accuracy, it is often hindered by higher costs and complex operational requirements (Choma, 2003).

In this study, TLC was employed to efficiently separate and identify ciprofloxacin residues in poultry samples, demonstrating its reliable performance for rapid residue detection in broiler tissues. In the TLC analysis, the indiscriminate group showed the highest levels of ciprofloxacin residues, while no positive samples were found in the control group. In both the discriminate and indiscriminate groups, the liver and kidney had the highest concentrations of antibiotic residues. These findings align with earlier research by Sattar *et al*. (2014) and Sarker *et al*. (2018), who reported that antibiotic residues were more concentrated in the liver than in muscles or other tissues. Given that the liver and kidneys play central roles in metabolizing and eliminating drugs, it is logical for these organs to exhibit the highest levels of residue. Similar conclusions were drawn by Metli *et al*. (2015), who also identified the liver and kidneys as primary accumulation sites for antibiotic residues

Residual intensity was found to be lowest in the breast and thigh muscles when compared to the liver and kidney tissues. These findings are generally consistent with those reported by Al-Mashhadany *et al*. (2018), though slight discrepancies were noted. In the indiscriminate group, several spleen and breast muscle samples showed no detectable ciprofloxacin residues, while in the discriminate group, the majority of spleen, breast, and thigh muscle samples also tested negative for residues. In contrast, liver and kidney samples from both the discriminate and indiscriminate groups were consistently positive for residues. A particularly surprising finding was that ciprofloxacin residues were also detected in chicken feathers after oral administration (Haag *et al*., 2016), highlighting the potential public health risks associated with its use. Regarding hematological parameters, the control group, which did not receive antibiotics, had the lowest values for TEC, Hb (%), PCV, and ESR, consistent with normal values. In comparison, both the discriminate and indiscriminate groups exhibited higher mean values. Significant statistical differences were observed in TEC, Hb (%), and ESR between the groups, suggesting that ciprofloxacin impacts hematopoiesis, though changes in PCV were not significant, likely due to the short duration of antibiotic exposure.

According to findings by Kasprzak *et al*. (2006), chicks treated with antibiotics between the ages of 22 and 27 days exhibited reduced levels of hematological indicators like PCV, red blood cell count (RBC), and Hb in comparison to untreated controls. However, these reductions were not statistically significant. This may be due to the stimulation of the bone marrow and immune system following antibiotic treatment. Based on these findings, it is clear that the indiscriminate use of ciprofloxacin offers no benefits and instead creates significant health risks, including the development of antibiotic-resistant microbial strains that can be transmitted to humans through food. The improper use of antibiotics presents two major concerns: the potential for toxic or allergic reactions in consumers and the rise of resistant microorganisms. Additionally, the increased cost of antibiotics reduces the profitability of farmers. Therefore, farmers must understand that excessive antibiotic use does not enhance their profits and may, in fact, diminish them. It is crucial for veterinarians, farmers, and poultry producers to adhere to proper antibiotic usage guidelines, including maintaining the appropriate withdrawal periods.

5. Conclusions

While antibiotics like ciprofloxacin improve poultry growth and feed efficiency, their excessive use poses serious public health risks, including antimicrobial resistance and harmful residues in meat. Significant changes in hematological parameters further highlight its physiological effects. Educating farmers on withdrawal periods and enforcing strict monitoring policies are essential to ensure food safety and align with international standards.

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Data availability

The data presented in this study are contained in this manuscript.

Conflict of interest

None to declare.

Authors’ contribution

Md. Rakibul Hasan: data collection, methodology, data analysis, draft writing; Md. Shakil Islam: conceptualization, methodology, data analysis; Md. Mominul Islam: data collection, data analysis; Jahagir Alam: methodology; Al Wasef: data analysis, draft writing; Md. Tanvir Hasan: reviewing and editing; Kazi Rafiq: conceptualization, methodology; Md. Shafiqul Islam: conceptualization, supervision, funding. All authors have read and approved the final manuscript.

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