(RESEARCH ARTICLE)

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Discriminate and indiscriminate use of ciprofloxacin antibiotic and detection of ciprofloxacin residues in edible poultry tissues

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Abstract

Abuse of antibiotics is more common in developing countries. This study was undertaken to investigate the presence of ciprofloxacin residue in broiler tissues and effects of antibiotics misuse on hematological parameters in broilers. Day old chicks (DOC) were collected and reared up to 31 days. The treatment was started from 16th day until sacrifice. On day 14th, the chicks were randomly divided into three groups namely control group (group A), discriminate antibiotic group (group B), and indiscriminate antibiotic group (group C). The control group was left untreated whereas, the discriminate group was treated with antibiotic, ciprofloxacin, followed by withdrawal period of one week. On the other hand antibiotic treatment was continued in the indiscriminate antibiotic group until the day of sacrifice, without maintaining withdrawal period. The mean body weight gain in treatment period (14 days) was the highest in discriminate group (1312± 26.1gm) followed by indiscriminate group (1089± 222.8gm) and control group (823.3±90.2gm). The Thin Layer Chromatography revealed that all the samples were positive in indiscriminate group with an exception of fat tissue (66.7%). The 50% liver and 33.3% kidney was ciprofloxacin positive in discriminate group and all others were negative. There was no positive sample in control group. . The hematological parameters such as Hb, PCV and TEC values of treatment groups showed significantly (p<0.05) lower, while PCV did not show any significant difference compared to the control group. Hence this experiment identifies the potential effects of ciprofloxacin misuse in broilers and their subsequent impacts on hemolytic system.

Keywords: Antibiotic residues; Broiler; Thin layer chromatography; Ciprofloxacin

1. Introduction

Antibiotic was one of the most important inventions on the development of human and veterinary medicine [1]. Antimicrobials were first used with sulfonamides for human therapeutics in the 1930s. However, during the following years, due to the emergence of resistant microorganisms, a constant search for more effective therapeutic alternatives in terms of spectrum of action and toxicity started [2]. In addition to the use of antimicrobials in Veterinary Medicine to prevent and treat diseases, antimicrobials are also used as growth promoters or performance enhancers for livestock. These antimicrobials are continuously added in poultry feeds in sub-therapeutic doses.

The indiscriminate use of antimicrobials leads to several life threatening implications. Among them, the most prevalent effects are antimicrobial resistance, antibiotics residues in food animal products which cause hypersensitivity reaction, alteration of gut micro flora and residual toxicity. There are four microbiological endpoints have been identified that could be of public health concern; modification of the metabolic activity of micro biota, changes in bacterial populations, selection of resistant bacteria, and perturbation of the barrier effect [3, 4, 5, 6]. Antibiotic drug residues in animal tissues

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may cause hypersensitivity reactions in humans. Certain macrolides may in exceptional cases be responsible for liver injuries, caused by a specific allergic response to macrolide metabolite-modified hepatic cells [7]. Aplastic anemia can occur in susceptible individuals exposed to concentrations of chloramphenicol that might remain as residues in edible tissues of chloramphenicol-treated animals [8].

The earliest screening methods used for detecting antibiotic residues in foods, including milk, were based on the detection of growth inhibition of various bacterial strains. Such methods were based on microbial agar diffusion tests or on the inhibition of acid production by starter organisms [9]. From the 1950s, assays were developed for the testing of tissues, primarily from the existing milk testing procedures [10]. But chromatographic analysis like thin layer chromatography, liquid chromatography, gas chromatography, and more advanced mass spectrometry have been replacing the conventional microbiological and immunological detection and quantification of antibiotic residue from food and environmental samples as these methods provided more recovery rate even several-fold higher [11]. Hence, chromatographic techniques are preferred to other analytical techniques even though they are quite expensive and sophisticated.

There have been many studies regarding antibiotics residue, pharmacokinetics of antibiotics, potential ecological risk, and drug toxicity study of antibiotics in poultry. Though these astute analyses of valuable data clearly indicate different phenomena, no studies have performed an indoor trial with the farmer-level perspective of Bangladesh. Hence, we investigated whether the injudicious use of antibiotics pose any threat that is detectable by simple laboratory testing. We selected the most commonly prescribed and used drug in poultry, Ciprofloxacin, for the indoor trial for residual status investigation. The administration of antibiotics was maintained similar way a root level farmer does. The three groups of poultry would clearly provide the different aspects of discriminate and indiscriminate use in farm level.

2. Material and methods

2.1. Experimental design

Experimental design was prepared as described by Islam et al. and Ali et al.[12, 13]. 18 DOC were collected as laboratory animal and chicks were reared for 14 days without using any antibiotic. Normal feed and drinking water were supplied. Then chicks were divided into 3 experimental groups (A, B and C); each group consisting of 6 chicks. Group A was kept untreated as control group and received no antibiotic medicated water, group B is discriminate group and group C is indiscriminate group. Antibiotic treatment was started from day 16th. Group B was administered with ciprofloxacin at recommended therapeutic dose @1gm/1L drinking water. In group C the dose of ciprofloxacin was indiscriminate and more than the normal dose (1gm/1L drinking water). After 7 days, at the age of day 23; antibiotic supply was stopped in the group-B and withdrawal period of 7 days was maintained. Whereas, in group C withdrawal period was not maintained and antibiotic continued till day 30th. Samples were collected from every bird at 31st day for thin layer chromatography (TLC) analysis.

2.2. Sample collection

On day 31st, before sacrifice; all the birds of three groups (Group- A, B and C) were weighed individually and the results were recorded. After slaughtering of the birds liver, kidney, breast muscle, thigh muscle and spleen were collected for the estimation of antibiotic residue. All samples were marked separately and preserved at -20º C in polythene bags for their extraction and analysis. Blood samples were also collected and preserved for hematological analysis.

2.3. Chemicals and standard drugs

All standard chemicals and reagents were at least 99% pure. HPLC grade methanol 5 (Merck-Germany), trichloracetic acid (TCA), diethyl ether and acetone were used. Ciprofloxacin was obtained from Popular Pharmaceuticals Ltd, Bangladesh. The standard for the TLC analysis was prepared by dissolving 0.1gm of Ciprofloxacin powder in 4 mL solution of methanol. Standard solution was stored in - 4°C and every month fresh solution was prepared.

2.4. Sample preparation

Samples were prepared as described by Ali et al., Das et al., Shumiya et al., and Islam et al. [13, 14, 15, 16]. Samples (Muscle tissue, liver and kidney) were blended with a mortar and pestle properly for three to five minutes. This technique was repeated until tissues were blended properly. These mashed/blended samples were taken into properly cleaned and sterilized petridishes with proper care as well as covering. From this 4gm of aliquot sample was taken into beaker with the help of electric balance and spatula. Then homogenization was done with addition of 10 ml phosphate buffer (pH 7.2). After proper mixing, protein contents of these samples were precipitated with the addition of 2 ml

tricholoracetic acid (30%) maintaining sufficient care and attention. Then the mixed samples were taken into properly cleaned and sterilized test tubes for centrifugation. Then centrifugation was done at 7000 rpm for 15 minutes with the help of automatically time regulated centrifuge machine. The supernatant was extracted with equal volume of diethyl ether and mixing was done properly in order to perform defatation. Then the mixture was kept for 23 minutes to become a separate layer, an upper oily layer and bottom layer. Then these mixtures were separated from each other and upper oily layer was discarded and only bottom layer was collected. Then the extracts were evaporated until dryness. The dried sample was reconstituted within 2ml of mobile phase methanol acetone. Then, extracts were collected into screw capped vial with proper care and kept into refrigerator for 4 further advanced analysis.

2.5. Thin layer chromatography (TLC)

2.5.1. TLC apparatus

TLC plate (MN-Germany), TLC tank, UV detection box (UV light: F18W-Germany), were used. TLC was performed as described by Islam et al. [12] with some modifications. In this experiment, standard antibiotics were used to identify the residues of antibiotics. Spots were visualized in UV detection box at 256 nm and the outlines of the spots were marked with a series of dots using sharp pencil for calculation of retention factor (Rf). Same R_f value of standard and sample considered similar compound.

2.5.2 Preparation of TLC-Silica Plate

TLC Silica plates with 0.25 nm thickness (Merck, Germany), were activated in 120^0C for 2 hour before use [17].

2.5.3 Preparation of Standard solution

Standard was prepared with routinely used antibiotic (Ciprofloxacin) by dissolving of 0.1 ml of liquid in 4 ml of methanol.

2.6. Statistical analysis

Statistical analysis was performed by one way ANOVA using Graphpad Prism; version 6. The results were expressed as mean ± standard error mean (S.E.M).

3. Results and discussion

3.1. Effects of ciprofloxacin on body weight gain

From 16th day onwards, everyday bodyweight was recorded for each bird up to 30th day. The body weight gain in 14 days period was calculated by subtracting initial body weight (16th day) from final body weight (30th day).

Table 1 Body weight gain (gm) in 14 days period (16th day-30th day)

Sl. No.	Discriminate Antibiotic Group (B)	Indiscriminate Antibiotic Group(C)	Control Group (A)
1	1214	1137	798
2	1484	1435	854
3	1345	775	826
4	1273	1287	826
5	1328	942	798
6	1230	961	868
Mean±SD	1312± 90.2a	1089± 222.8a	823.3± 26.1

Values with similar superscript didn't differ significantly

The Table 1 represents that the average body weight gain was highest in discriminate antibiotic group which was 1312± 90.2 whereas the lowest was 823.3± 26.1 in control group. Indiscriminate antibiotic group showed moderate weight gain that was 1089± 222.8. The differences among means of three groups were statistically significant ($P<0.05$). The

multiple comparisons during one way ANOVA revealed that there was no significant difference in means between group B and C. On the contrary, between group A & C, and group A & B, significant differences were observed.
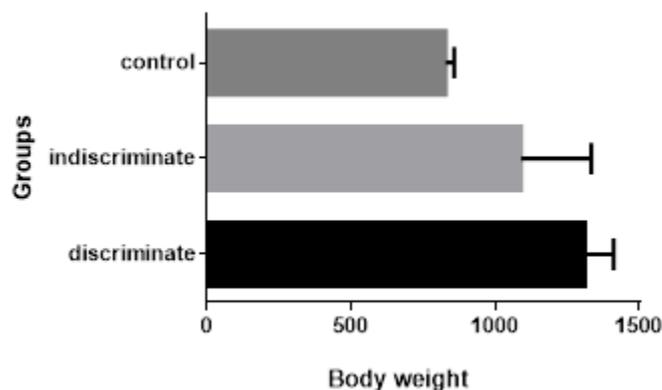
Figure 1 Body weight gain of three individual groups in 14 days period (16th -30th)

Figure 1 represents body weight gain of broilers of control, discriminate and indiscriminate antibiotic groups in 14 days period (16th -30th). It also shows standard deviations of three individual groups.

Table 2 Proportion of ciprofloxacin positive and negative samples in indiscriminate group.

Sample (Frequency)	Positive % (Frequency)	Negative % (Frequency)
Liver (6)	100 (6)	0 (0)
Kidney (6)	100 (6)	0 (0)
Thigh Muscle (6)	100 (6)	0 (0)
Breast Muscle (6)	100 (6)	0 (0)
Fat Tissue (6)	66.7 (4)	33.3 (2)
Spleen (6)	100 (6)	0 (0)

In the indiscriminate antibiotic group, all of the samples were positive (100%) except fat tissue which showed 66.7% positive samples.

Table 3 Proportion of ciprofloxacin positive and negative samples in discriminate antibiotic group.

Sample (Frequency)	Positive % (Frequency)	Negative % (Frequency)
Liver (6)	50 (3)	50 (3)
Kidney (6)	33.3 (2)	66.7 (4)
Thigh Muscle (6)	0 (0)	100 (6)
Breast Muscle (6)	0 (0)	100 (6)
Fat Tissue (6)	0 (0)	100 (6)
Spleen (6)	0 (0)	100 (6)

Of the samples in discriminate antibiotic group, only 50% liver and 33.3% kidney samples showed positivity. All the other samples were negative in TLC analysis.

Table 4 Proportion of ciprofloxacin positive and negative samples in control group.

Sample (Frequency)	Positive % (Frequency)	Negative % (Frequency)
Liver (6)	0 (0)	100 (6)
Kidney (6)	0 (0)	100 (6)
Thigh Muscle (6)	0 (0)	100 (6)
Breast Muscle (6)	0 (0)	100 (6)
Fat Tissue (6)	0 (0)	100 (6)
Spleen (6)	0 (0)	100 (6)

All the samples of control group showed negative result in TLC analysis.

Table 5 Proportions of positive samples in three different groups.

Sample	Indiscriminate Antibiotic Group	Discriminate Antibiotic Group	Control Group
Liver	100	50	0
Kidney	100	33.3	0
Thigh Muscle	100	0	0
Breast Muscle	100	0	0
Fat Tissue	66.7	0	0
Spleen	100	0	0
Mean ± SD	94.45 ±13.59[a]	13.88 ±22.14	0[a]

Values with similar superscript didn't differ significantly

The highest average was in indiscriminate antibiotic group which was 94.45 ±13.59 and the lowest was in control group (0). In the discriminate antibiotic group, the average proportion was 13.88 ±22.14. The differences among means of three individual groups were statistically significant ($P<0.05$). The multiple pairwise comparison revealed that the difference of means between discriminate and control group was not significant. On the other hand, the other two pairs showed statistically significant difference among means.
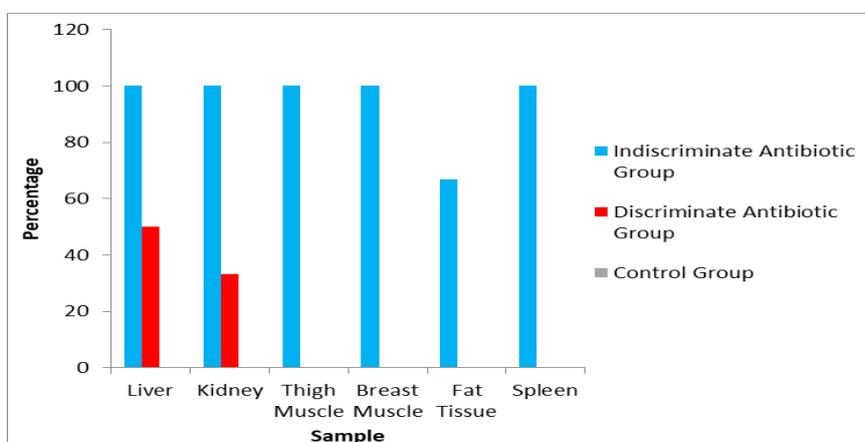
Figure 2 Proportion of positive ciprofloxacin residue samples in three groups

Figure 2 represents proportions of ciprofloxacin positive samples in three individual groups.

Table 6 Total Erythrocyte Count (Million/mm³) of Three Individual Groups.

Sl. No.	Discriminate Antibiotic Group	Indiscriminate Antibiotic Group	Control Group
1	2.81	2.17	3.41
2	2.66	1.86	3.07
3	2.59	2.16	3.23
4	2.78	2.69	3.01
5	2.71	2.44	3.13
6	2.23	2.53	3.33
Mean±SD	2.63± 0.19[a]	2.31± 0.28[a]	3.20±0.14

Values with similar superscript didn't differ significantly

The highest mean TEC was obtained from control group that was 3.20±0.14. The mean TEC of discriminate and indiscriminate groups were 2.63± 0.19 and 2.31± 0.28 respectively. The differences among means of three different groups were statistically significant ($P<0.05$). The multiple pairwise comparison showed that the difference of means between indiscriminate and discriminate was not statistically significant. But other two pairs showed significant differences among means.
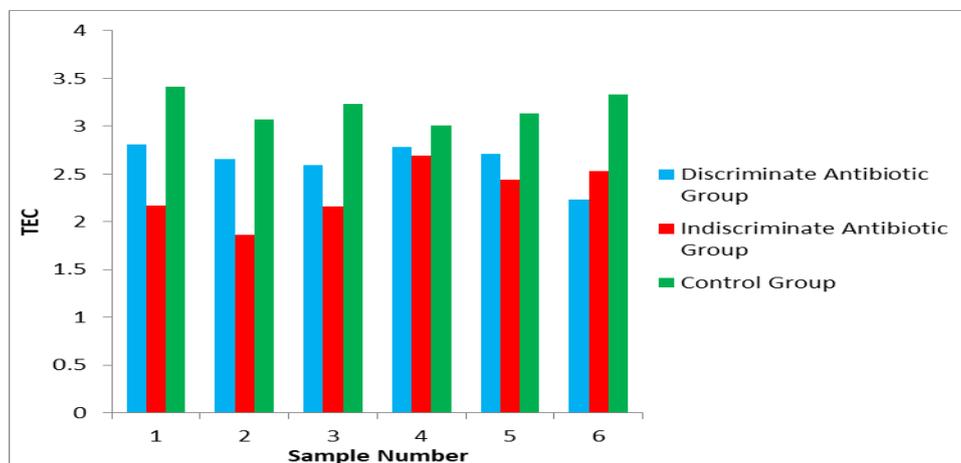
Figure 3 TEC of three individual groups

Table 7 Hemoglobin (%) of Three Individual Groups.

Sl. No.	Discriminate Group	Indiscriminate Group	Control Group
1	7.4	6.0	8.4
2	7.0	5.6	8.0
3	7.0	6.2	8.2
4	7.4	7.0	8.0
5	7.6	7.0	8.1
6	6.4	7.2	8.3
Mean±SD	7.13± 0.39[a]	6.5± 0.65[a]	8.17±0.15

Values with similar superscript didn't differ significantly

The highest mean Hb (%) was obtained from control group that was 8.17±0.15. The mean Hb (%) of discriminate and indiscriminate groups were 7.13± 0.39 and 6.5±0.65 respectively. The differences among means of three different groups were statistically significant ($P<0.05$). The multiple pairwise comparison showed that the difference of means

between indiscriminate and discriminate was not statistically significant. But other two pairs showed significant differences among mean.
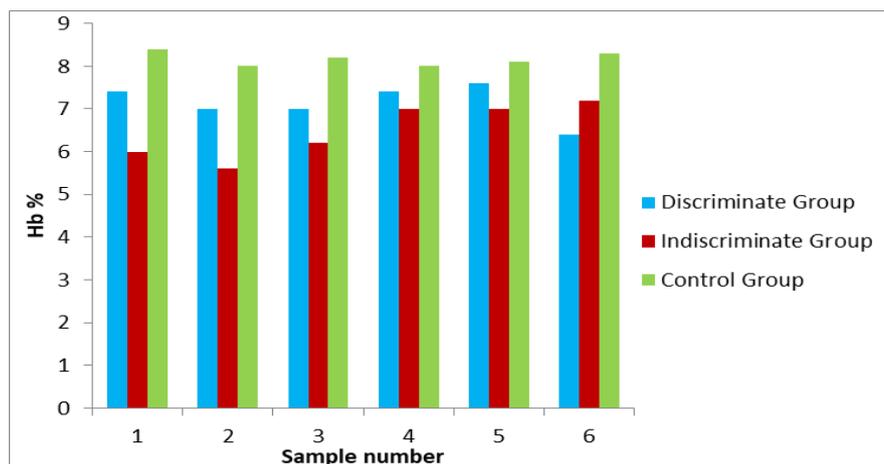
Figure 4 Hb (%) of three individual groups

Figure 4 Represents Hb (%) of discriminate antibiotic, indiscriminate antibiotic and control group**.**

Table 8 Packed Cell Volume (%) of Three Individual Groups.

Sl. No.	Discriminate Group	Indiscriminate Group	Control Group
1	24	19	26
2	21	17	23
3	21	21	24
4	24	23	23
5	23	21	24
6	19	22	25
Mean±SD	22± 1.83[ab]	20.5± 1.98[a]	24.17± 1.07[b]

Values with similar superscript didn't differ significantly

The highest mean PCV was obtained from control group that was 24.17± 1.07. The mean PCV of discriminate and indiscriminate groups were 22±1.83 and 20.5± 1.98 respectively. The differences among means of three different groups were statistically significant ($P<0.05$). The multiple pairwise comparisons showed that the difference of means between indiscriminate & discriminate group and discriminate & control groups were not statistically significant. But indiscriminate and control group showed significant differences among means ($P<0.05$).
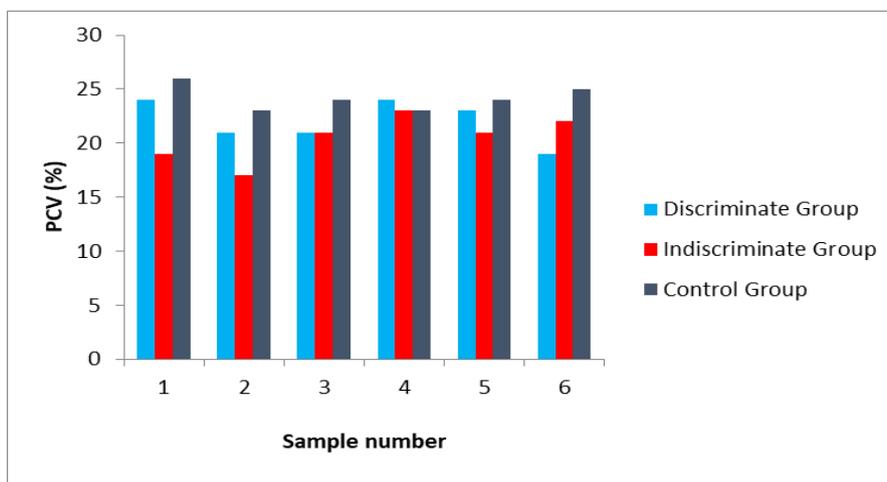
Figure 5 PCV of three individual groups

Figure 5 represents Packed Cell Volume of discriminate, indiscriminate, and control groups.

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4. Discussion

The present study was conducted to investigate the presence of ciprofloxacin residue in broiler tissue by indoor trial. Body weight gain and hematological parameters were also investigated.

The body weight gain was the highest in group B and lowest in control group A whereas moderate in group C. It is not apparent that the antibiotic promotes the weight gain only, it is the genetic characteristics of the broiler that body weight is gained to a certain extent with the advancement of age. The moderate body weight gain in group C justified the genetic characteristics of broiler. As use of antibiotics is expensive and there is no apparent health benefit of using antibiotics until sacrifice, rather potential harmful effects might prevail, there is no use of using antibiotics up to last day of rearing. The body of broilers is adapted to gain weight at a certain extent where it might be incapable of gaining body weight even if more feeding and growth promoter is provided [18]. The lower weight gain in control group was expected but it is not recommended by the study that antibiotics are must be used to promote growth of live broilers. Rather proper management and scientific feeding practices can attain such goal more profitably than feeding of antibiotics. Furthermore, it will be consumer and environment friendly. The results are in accordance with other researchers [12, 13, 19]. Now a day, alternatives to antibiotic growth promoters is an interesting topic. Various researches showed that use of natural feed additive as alternative to antimicrobial growth promoters is highly efficient [20]. Recently medicinal plant extracts are being used to promote the growth and performance [21]. The most beneficial effects of using plant extracts are no side effect, antibiotic resistance doesn't develop, cost-effectiveness and eco-friendliness [20]. So, we should also consider using medicinal plants as growth promoters in broilers rather than antibiotics.

In TLC analysis, samples collected from indiscriminate group showed positive result with an exception of fat tissue. It might happen that the protocol used in this study doesn't allow for vigorous fat tissue dissolve, rather a mixture of protein and fat. So, if the extraction and cleaning steps could be increased, we could find positive results from fat tissues. On the other hand, only liver, and kidney showed positive results in discriminate groups, and other samples were negative in TLC analysis. As the liver and kidney is the main metabolic and excretory organs, all drug are metabolized and excreted via liver and kidney chiefly [17]. So, even in discriminate groups, the presence of antibiotics residue was found in liver and kidney. There are other evidences of similar results where kidney and liver was considered the predilection site of antibiotics residues and analyzed accordingly [13, 16]. Another study found 60% ciprofloxacin positive samples in broiler meat [22]. The most surprising fact is that ciprofloxacin is such residue that is deposited even in chicken feather following oral administration [23]. So, ciprofloxacin residue poses a serious threat and a severe public health consequence if consumed for long term.

Chromatographic analysis is a major tool in identifying and quantifying antibiotic residue in broiler tissues [14]. Among them, TLC is the most inexpensive method and the results are reliable, if proper detecting substances are used. Debora et al. [24] found ciprofloxacin residue above MRL up to two days following administration in broiler.

The use of ciprofloxacin for a specific period had some effects on hematological parameters. The TEC, Hb (%), and PCV were the highest in control group. This group had no exposure to antibiotics and the results were normal. But indiscriminate and discriminate groups showed lower mean than the control group. The means of three groups were statistically significant but pairwise comparison revealed that there was no statistical significance in difference of means among discriminate and indiscriminate groups. The antibiotics have effects on hematopoiesis and hemolysis but the effects are not significant in small duration. This might be significant if used for longer duration.

It is evident that the indiscriminate use of ciprofloxacin doesn't bear any fruitful effect on broiler. Rather, there are potential harmful effects of antibiotics residue which might enter the human food chain and produce deleterious impact on human health. Moreover, the higher cost should discourage the use of antibiotic growth promoters. So, they must realize that use of antibiotics at large can't increase their profitability rather decrease the profits. The lower TEC, Hb%, and PCV in treated groups indicated depressive impact on bone marrow. So, if the antibiotics are used prudently and proper withdrawal period is maintained, there is lower risk of antibiotics resistance and other residue related problems and there is increased chance of profitability of broiler farmers. This study also indicated that the indiscriminate use of antibiotics is not beneficial and profitable.

5. Conclusion

Fluoroquinolones, as ciprofloxacin, are the mostly used antibiotics in veterinary and human medicine due to its broad spectrum activity and low toxicity. The indiscriminate use of ciprofloxacin in farm level has caused a serious concern due its residual effect and subsequent public health and environmental consequences. This indoor trial was designed to investigate the presence of ciprofloxacin residue in broiler tissues. Besides, the effect of long term use of ciprofloxacin on hematological parameters was also investigated. The treatment of broiler was started from 16th day until sacrifice. The mean body weight gain in treatment period (14 days) was the highest in discriminate group (1312± 26.1 gm). The differences among mean weight gain among discriminate, indiscriminate, and control group were statistically significant ($P<0.05$). The TLC was performed for identification of antibiotic residues. The analysis revealed that all the samples were positive in indiscriminate group with an exception of fat tissue (66.7%). There was no positive sample in control group. The results were statistically significant ($P<0.05$). The TEC of control, discriminate, and indiscriminate group were 3.20± 0.14, 2.63±0.19, and 2.31± 0.28 respectively. The hemoglobin % of control, discriminate, and indiscriminate group were 8.17± 0.15, 7.13± 0.39, and 6.5±0.65 respectively. And Packed Cell Volume (PCV) of control, discriminate, and indiscriminate group were 24.2± 1.07, 22±1.83, and 20.5± 1.98 respectively. The differences among means of blood parameters of three individual groups were statistically significant ($P<0.05$). The multiple pairwise comparison of means of blood parameters revealed that there was no significant difference among discriminate and indiscriminate groups. The experiment showed that there is a substantial risk of ciprofloxacin residue in consumer level if withdrawal period is not maintained accordingly. Indiscriminate use of ciprofloxacin doesn't result in significant body weight gain. Furthermore, there was significant fluctuation in blood parameters like TEC, HB (%), and PCV among three groups. So this indoor trial of discriminate and indiscriminate ciprofloxacin use in broiler revealed that prudent use of ciprofloxacin would reduce the risk of antibiotic residue in broiler products.

Compliance with ethical standards

Acknowledgments

Disclosure of conflict of interest

The authors state no conflict of interest.

Statement of ethical approval

The experimental broilers were used ethically and at the end of the experiment sacrificed humanely following the ethical and welfare guidelines set by the Animal Welfare and Experimental Ethics Committee of Bangladesh Agricultural University [approval 5 number: AWEEC/ BAU/2021(09)].

References

[1] Finch R. Innovation- drugs and diagnostics. Journal of Antimicrobial Chemotherapy. 2007; 60: 79-82.

[2] Jackson LC, Reyes LAM. Quinolones and Antimicrobial Therapy. Acta Medica. 1998; 8(1): 58-65.

[3] Sundlof SF. Drug and chemical residues in livestock. Veterinary Clinics of North America: Food Animal Practice. 1989; 5(2): 411–49.

[4] Gorbach SL. Perturbation of intestinal microflora. Veterinary and Human Toxicology. 1993; 35 suppl 1: 15-23.

[5] Boisseau J. Basis for the evaluation of the microbiological risks due to veterinary drug residues in food. Veterinary Microbiology. 1993; 35(3-4): 187-192.

[6] Perrin-Guyomard A, Cottin S, Corpet DE, Boisseau J, Poul JM. Evaluation of residual and therapeutic doses of tetracycline in the human-flora-associated (HFA) mice model. Regulatory Toxicology and Pharmacology. 2001; 34(2): 125-136.

[7] Dewdney JM, Maes L, Raynaud JP, Blanc F, Scheid JP, Jackson T. Risk assessment of antibiotic residues of beta-lactams and macrolides in food products with regard to their immuno-allergic potential. Food and Chemical Toxicology. 1991; 29(7): 477–83.

[8] Settepani JA. The hazard of using chloramphenicol in food animals. Journal of American Veterinary Medical Association. 1984; 184(8): 930–931.

[9] Mitchell JM, Griffiths MW, McEwen SA, McNab WB, Yee AJ. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. Journal of Food Protection. 1998; 61(6): 742–56.

[10] Huber, WG, Carslon MB, Lepper MH. Penicillin and antimicrobial residues in domestic animals at slaughter. Journal of the American Veterinary Medical Association. 154(12): 1590-1595.

[11] Moats WA. Chromatographic methods for determination of macrolide antibiotic residue ion tissues and milk of food producing animals. Journal Association of Official Analytical Chemists. 1985; 68(5): 980-984.

[12] Islam MS, Hasan MR, Islam MS. Thin layer chromatographic investigation of antibiotics residues in edible poultry tissues in Bangladesh. World Journal of Biology Pharmacy and Health Sciences. 2021; 05(03): 024–032.

[13] Ali MR, Sikder MMH, Islam MS, Islam MS. Investigation of discriminate and indiscriminate use of doxycycline in broiler: an indoor research on antibiotic doxycycline residue study in edible poultry tissue. Asian Journal of Medical and Biological Research. 2020; 6(1): 1-7.

[14] Das D, Islam MS, Sikder MMH, Alom F, Sharmin MA, Ashraf MZF. Presence of antibiotic residue and residual effect of tylosin tartrate in broiler. International Journal of Natural and Social Sciences. 2020; 7(2): 29-35.

[15] Shumiya MK, Das D, Sharmin MA, Ashraf MZF, Das S, Tuhin MRI, Islam MS. Residual effect of amoxicillin in broiler. International Journal of Natural and Social Sciences. 2020; 7(3): 51-58.

[16] 16. Islam MS, Islam MZ, Islam MS. Discriminate and indiscriminate use of amoxicillin and its effects on hematological parameters of broiler. Asian Journal of Medical and Biological Research. 2019; 5(2): 153-157.

[17] Sattar S, Hassan MM, Islam SKMA, Alam M, Faruk MSA, Chowdhury S, Saifuddin AKM. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Veterinary World. 2014; 7(9): 738–743.

[18] Marcio CC, Jose AB, Amauri AA, Alexandre O. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLOS ONE. 2017; 12(2): 1-14.

[19] Donoghue DJ. Antibiotic residues in poultry tissues and eggs: human health concerns? Poultry Science. 2003; 82(4): 618–21.

[20] Demir E, Sarica S, Ozcan MA, Suicmez M. The use of natural feed additives alternative to antibiotic growth promoters in broiler diets. European Poultry Science. 2005; 69(3): 110-116.

[21] Barreto MSR, Menten JFM, Racanicci AMC, Pereira PWZ, Rizzo PV. 2008: Plant extracts used as growth promoters in broilers. Brazilian Journal of Poultry Science. 2008; 10(2): 109-115.

[22] Buket E, Fatma KO, Burak D, Ufuk A. Screening of quinolone antibiotics in chicken meat and beef sold in markets of Ankara, Turkey. Poultry Science. 2013; 92(8): 2212-5.

[23] Griselda H, Gustavo HM, Jorge OE. Quantification of residual ciprofloxacin in feathers of broiler by high performance liquid chromatography- fluorescence after oral drug administration. Journal of Advanced Pharmaceutical Technology and Research. 2016; 7(1): 2-5.

[24] Debora CSA, Isabela PL, Silvana VC. Evaluation of the presence and levels of enrofloxacin, ciprofloxacin, sulfaquinoxaline, and oxytetracycline in broiler chicken after drug administration. PLOS ONE. 2017; 11(11): e0166402.