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Article

Investigation of discriminate and indiscriminate use of doxycycline in broiler: an indoor research on antibiotic doxycycline residue study in edible poultry tissue

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Abstract: Abuse of antibiotics is more common in developing countries including Bangladesh. The aim of this study was to detect doxycycline residue after discriminate and indiscriminate administration in broiler poultry. Eighteen broiler chicks, DOC (Cobb-500) was collected & reared up to 31 days. On day 16, they were randomly divided into 3 groups, namely group–A (Control group), group-B (Discriminate group) and group-C (Indiscriminate group). Each group contains 6 birds. The discriminate and indiscriminate groups were treated with antibiotic, doxycycline. In group-B withdrawal period was followed by treatment for 7 days. On the other hand, withdrawal period was not maintained in group-C, i.e. the antibiotic treatment was continued until the day of sacrifice. TLC analysis revealed that intensity of doxycycline in discriminate antibiotic group for liver, kidney, thigh muscle and breast muscle samples were found 63.33%, 65.33%, 22.67% and 26.00% respectively. And in indiscriminate antibiotic group intensity of doxycycline for liver, kidney, spleen, thigh muscle and breast muscle samples were found 50.67%, 50.33%, 39.50% and 48.17% respectively. All the samples of control group were found 0% intensity. The different among intensity were statistically significant. Overall, the present study documented the widespread abuse of doxycycline and failure to implement the recommended withdrawal period will undeniably leads to deposition of residues in broiler tissues.

Keywords: doxycycline; antibiotic residue; broilers

1. Introduction

Antibiotics are used for three different purposes in poultry industry; therapeutic, prophylactic and growth promoter (Oluwasile *et al*., 2014). So antibiotics are directly related to poultry production (Mund *et al*., 2017). But, irrational and indiscriminate use of antibiotics causes deposition of antibiotic in the edible tissue of poultry known as antibiotic residue (Chowdhury *et al*., 2015). In the present world antibiotic residue is a burning question. Antibiotic residues are one of the major sources of public health concerns associated with hypersensitivity reactions, antibiotic resistance, toxicity, teratogenicity, and carcinogenicity (Darwish *et al*., 2013). Doxycycline (DOX) is a semi-synthetic bacteriostatic tetracycline and a broad-spectrum antibiotic effective against Gram-negative and Gram-positive aerobic and anaerobic bacteria, Rickettsiae, Chlamydiae, Mycoplasmas and some protozoa (Anadón *et al*., 1994; Prats *et al.*, 2005). So, Doxycycline is seen to be a valuable antibiotic in terms of therapeutic effect for treating infections in poultry industry. Despite the important therapeutic use of doxycycline in veterinary practices and public health issue, relatively little has been known about residual status of this drug in broiler (Vandenberge *et al*., 2012). Therefore, the present study was undertaken to determine the presence of doxycycline in broiler following discriminate and indiscriminate use.

2. Materials and Methods

2.1. Experimental design

18 DOCs were collected as laboratory animal. Chicks were reared for 14 days without using any antibiotic; only feed and water. Then chicks were grouped in 3 experimental groups (A, B and C); each group having 6 chicks. Group A was kept as untreated control and received no antibiotic medicated water, group B is discriminate group and group C is indiscriminate group. Antibiotic treatment was started from 16th day. Group B was administered with doxycycline at recommended therapeutic dose @ 10 mg/kg through drinking water as described in (Laczay *et al*., 2001). In group C the dose of doxycycline was indiscriminate and more than the normal dose (10mg/kg). After 7 days, at the age of day 23; antibiotic supply was stopped in the group-B and withdrawal period was maintained as drug nomenclature (7 days) whereas, in group C withdrawal period was not maintained and antibiotic continued till 30th day. Samples were collected from every bird at 31th day for thin layer chromatography (TLC) analysis.

2.2. Sample collection

Liver, kidney, breast muscle, thigh muscle and spleen samples were collected. Immediately after collection of sample, these were washed individually several times in physiological saline to remove clotted blood and debris. All samples were marked separately and preserved at -20º C in polythene zipper bags for their extraction and analysis.

2.3. Chemicals and standard drugs

Purity of all standard chemicals and reagents was at least 99%. HPLC grade methanol (Merck-Germany), trichloracetic acid (TCA), diethyl ether and acetone were used. Doxycycline (DOX) was obtained from Sigma-Aldrich via Renata Limited, Bangladesh. The standard for the TLC analysis was prepared by dissolving 0.1 gm of doxycycline 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

Sample extraction was performed according to Poppelka *et al*. (2005). Four gram of each sample was cut into small pieces, grinded and blended. 10 mL Phosphate Buffer Saline (pH-6.5) was added and mixed by vortexing (Vortex- XHC, Wincom, China). Centrifuged (Hettich D-78532, Germany) @ 60000 rpm for 20 min was done after mixing with 2 mL 30% TCA. Supernatant was collected and filtered by Whatman filter paper and funnel. Filtrated fluid was collected in another falcon tube and same amount of diethyl ether was added and left for 10 min in room temperature. The bottom layer was collected and supernatant extraction was repeated twice using diethyl ether. Final volume of the extracts were pooled carefully into screw cap vial and kept into refrigerator for future analysis.

2.5. Thin layer chromatography (TLC)

2.5.1. TLC apparatus

TLC plate (MN-Germany), TLC tank and UV detection box (UV light: F18W-Germany) were used. TLC was performed according to Tajick and Shohreh (2006) with some required adjustments. TLC plate was cut into appropriate size (4x5 cm) from 20x20 cm. A straight line was drawn across the plate approximately 2 cm from the bottom by a pencil. Another straight line was drawn across the plate below 1 cm from the upper edge of the plate. Desired spots marking were marked on the bottom line where analytes were dropped. Spots were applied to the plate using thin capillary glass pipettes. A volume of 50 µl was used for spotting. Plate was placed in TLC tank (contained mobile phase; Acetone and Methanol: 1:1) and covered by lid and it was left until the mobile phase reached the upper line. Spots were visualized in UV detection box at 256 nm. Spots marking were done by pencil for calculation of retention factor (Rf). Calculation of Rf values: These measurements are the distance travelled by the solvent, and the distance travelled by individual sample spots. Same Rf value of standard and sample considered similar compound.

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

3.1. Detection of doxycycline antibiotic by TLC

3.1.1. Liver sample

Table 1 represents doxycycline residue in liver sample of three different groups. In control group, we could not find any positive liver samples however in both discriminate and indiscriminate groups positive samples were found by TLC analysis.

Figure 1 represents doxycycline residue in liver sample of three different groups. The multiple comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.01$).

3.1.2. Kidney sample

Doxycycline residue in kidney sample of three different groups is presented in Table 2. In control group, we could not find any positive liver samples however in both discriminate and indiscriminate groups positive samples were found by TLC analysis.

Figure 2 represents doxycycline residue in kidney sample of three different groups. The multiple comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.01$).

3.1.3. Thigh muscle

Table 3 represents doxycycline residue in thigh muscle of three different groups. In control group, we could not find any positive liver samples however in both discriminate and indiscriminate groups positive samples were found by TLC analysis.

In Figure 3, the multiple comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.05$).

3.1.4. Breast muscle

Doxycycline residue in breast muscle of three different groups is presented in **Table 4**. In control group, we could not find any positive liver samples however in both discriminate and indiscriminate groups positive samples were found by TLC analysis.

In Figure 4, the multiple comparisons during one way ANOVA (Bonferroni) showed that there was significant difference between discriminate and indiscriminate groups ($P<0.05$).

Table 1. Doxycycline residue in liver sample of three different groups.

Name of Group	Doxycycline intensity in liver by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B(Discriminate group)	50.67± 2.404	**<0.001**	***
Group-C(Indiscriminate group)	63.33± 3.602		

Table 2. Doxycycline residue in kidney sample of three different groups.

Name of Group	Doxycycline intensity in kidney by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B(Discriminate group)	50.33± 2.765	**<0.001**	***
Group-C(Indiscriminate group)	65.33± 2.996		

Table 3. Doxycycline residue in thigh muscle sample of three different groups.

Name of group	Doxycycline intensity in thigh muscle by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B(Discriminate group)	22.67± 7.200	**<0.001**	***
Group-C(Indiscriminate group)	39.50± 1.746		

Table 4. Doxycycline residue in breast muscle sample of three different groups.

Name of group	Doxycycline intensity in breast muscle by TLC (Mean ± SEM)	P Value	Level of Significance
Group-A (Control group)	0.00± 0.00		
Group-B(Discriminate group)	26.00± 8.591	**<0.001**	***
Group-C(Indiscriminate group)	48.17±2.676		

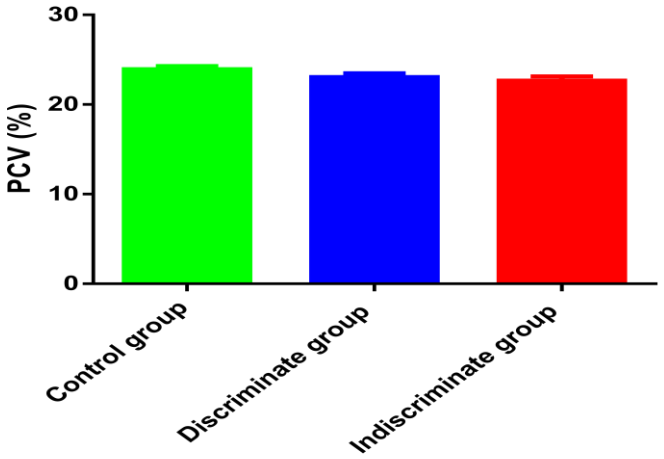

Figure 1. Doxycycline antibiotic in liver.

***, Significantly difference between Control & Discriminate groups and Control & Indiscriminate groups (P<0.001).
##, significantly different between Discriminate and Indiscriminate groups (P<0.01).

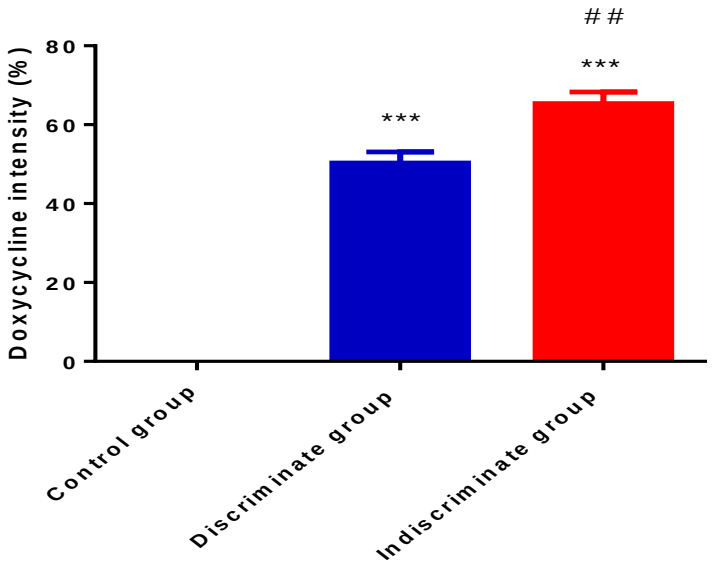

Figure 2. Doxycycline antibiotic in kidney.

***, Significantly difference between Control & Discriminate groups and Control & Indiscriminate groups (P<0.001).
##, significantly different between Discriminate and Indiscriminate groups (P<0.01).

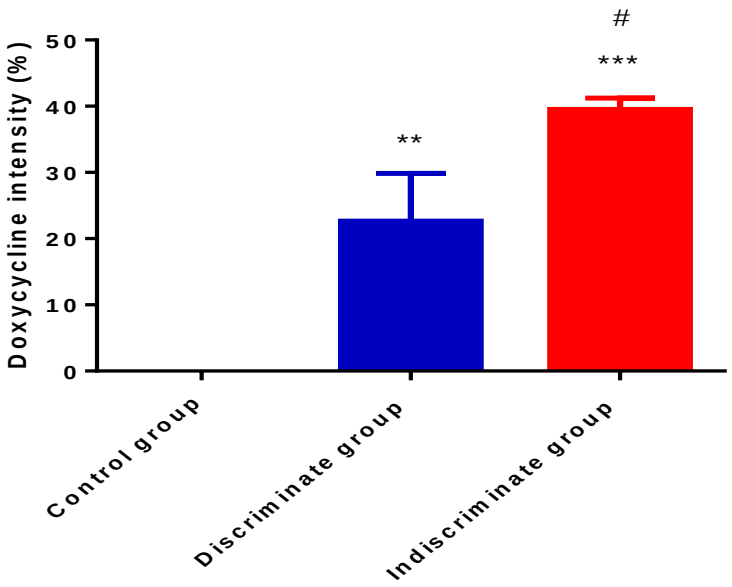

Figure 3. Doxycycline antibiotic in thigh muscle.

**, Significantly difference between Control & Discriminate groups (P<0.01).
***, Significantly difference between Control & Indiscriminate groups (*P*<0.001).

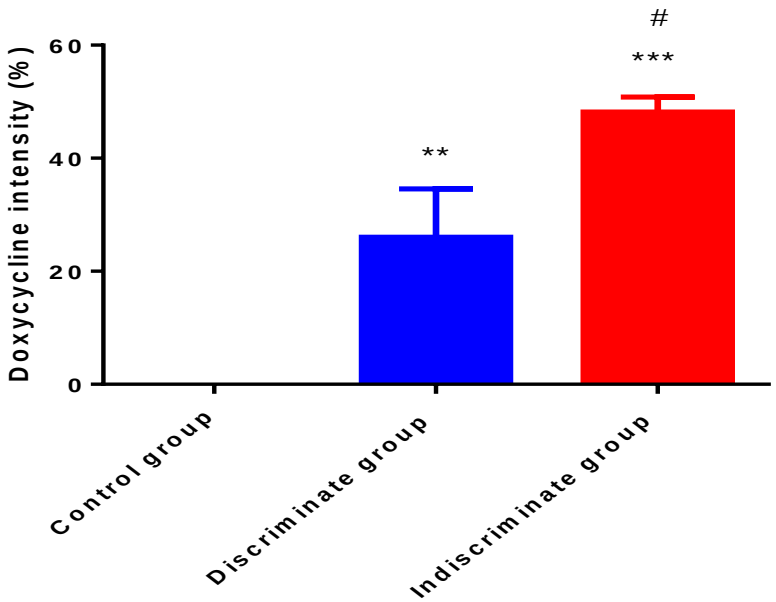

Figure 4. Doxycycline antibiotic in breast muscle.

**, Significantly difference between Control & Discriminate groups (*P*<0.01).
***, Significantly difference between Control & Indiscriminate groups (*P*<0.001).

4. Discussion

The presence of antibiotic residues in poultry meat has received colossal worldwide attention from public health agencies. This is due to the importance and significance of antibiotic residues on public health. Lack of knowledge about the proper withdrawal times of drugs and the overuse or misuse of various drugs lead toward

the formation of drug residues into the animal end products (Seri, 2013). These residues ultimately pose serious health threats to human beings when meat from such animals is consumed (Shareef *et al*., 2009). The doxycycline has a wide spectrum of activity and being widely used as veterinary drug to improve health conditions. It has good absorption and high bioavailability with good penetration into body tissues and found at high concentrations in the excretory organs, especially the liver and kidney with high withdrawal time (Newton *et al*., 2005). In present study, intensity of doxycycline in discriminate antibiotic group for liver samples 63.33± 3.602, kidney samples 65.33± 2.996, thigh muscle samples 22.67± 7.200, breast muscle samples 26.00± 8.591 were found. And in indiscriminate antibiotic group intensity of doxycycline for liver samples 50.67± 2.404, kidney samples 50.33± 2.765, thigh muscle samples 39.50± 1.746, breast muscle samples 48.17±2.676 were found. All the samples of control group were found 0% intensity. The highest concentration of doxycycline was in liver and lowest in muscle. This finding is similar to (Al-Ghamdi *et al*., 2000) who reported that the highest doxycycline residues in poultry liver samples. Our results were also consistent with other studies (Hussain *et al*., 2013 ; Popelka *et al*., 2005; Sarker *et al*., 2018) regarding the highest doxycycline residues in poultry liver samples than meat.

5. Conclusions

Doxycycline is widely used in the treatment of infections in both human and veterinary medicine. A number of these drugs have been licensed to be administered in broiler chickens for the prophylaxis and treatment. But the indiscriminate use of doxycycline in food-producing animals may leave drug residues in foods. These residues represent a risk to public health, including stimulation of bacterial resistance, alterations on intestinal micro flora and hypersensitivity reactions. TLC analysis revealed that intensity of doxycycline in discriminate and indiscriminate antibiotic groups was found. All the samples of control group were found 0% intensity. The different among intensity were statistically significant. The differences among means of blood parameters of three individual groups were statistically non-significant (P>0.05).Thus, there is a need to educate the farmers about the ill effects of residual drugs on human health and withdrawal time in poultry birds. National authorities should also adopt more judicious approaches to ensure prudent use of antibiotics in food animals.

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Conflict of interest

None to declare.

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