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Presence of antibiotic residue and residual effect of tylosin tartrate in broiler

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ABSTRACT

This study was designed with the aim to determine the impact of residual antibiotics on poultry muscle (breast and thigh), kidney, spleen, fat and liver and effects of discriminate and indiscriminate use of antibiotic on haematological constituents in broiler chickens. The chickens were properly reared and on the day of 16, chicks were randomly divided into three groups namely control group (group A), discriminate antibiotic group (group B) and indiscriminate antibiotic group (group C). In case of discriminate group, the chicks were treated with antibiotics followed by withdrawal period of one week before the sacrifice of the birds. On the other hand, indiscriminate group of the chicks was continued without giving any withdrawal period and birds were sacrificed for sampling. Thin Layer Chromatography method was used for screening and detection of tylosin tartrate residues in poultry tissues. The samples were extracted with trichloroacetic acid (30%), diethyl ether, followed by detection inpre-coated thin layer chromatography paper on ultraviolet detector. In case of group B, the residue of tylosin was 100% in livers, 83.33% in kidneys, 0% in thigh muscles, and 16.67% in breast muscles, 33.33% in fat and 16.67% in spleen. In case of group C, the residues of tylosin were 100% in livers, 100% in kidneys, 0% in thigh muscles, and 33.33%in breast muscles, 50% in fat and 33.34% in spleen. Among the poultry tissues, liver and kidney had the highest level of antibiotic residues in comparison to other samples. Tylosin antibiotic showed some changes in hematological parameters of broiler. The Hb and TEC content were significantly decreased at 31stday of tylosin treatment at recommended dose compared to untreated control. But percent PCV was slightly decreased. There was significantly increased live weight gain of broiler from day 16th to day 28th compare to untreated control. However, there was no significant difference among the groups in gaining live weight at 31st day. The study concluded that once antibiotics are administered to broiler, antibiotic residues are present in high or low concentrations in their products which mainly depend on the duration of the administration of antibiotics. After the administration of antibiotics, concentration of their residues gradually reduces in meat & other meat products and mostly after 4-5 days of administration excretion of residues is negligible.

INTRODUCTION

Antibiotic use in commercial poultry can be divided into two categories- therapeutic antibiotics and growth-promotant antibiotics (Anonymous, 2003). Therapeutic dose of antibiotics is used for treatment of bacterial infections. Antibiotics used in feed for the promotion of growth and prevention of disease are administered at levels that are lower than those given for the treatment of disease, and therefore, these uses are termed as sub-therapeutic. The antibiotics in the growth-promotant category are, in some cases, the same antibiotics used in the therapeutic category. The growth-promotant antibiotics are administered only in the birds' feed (Jones and Ricke, 2003). The decision to use growth-promotant antibiotics in commercial poultry is primarily based on economic factors, i.e., whether the improvements in body weight, feed efficiency, and/or growth rates are worth the cost of the antibiotic (Stutz and Lawton, 1984; Sirdar et al., 2012).

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The successful use of antibiotics in veterinary medicine not withstanding their use has become particularly worrisome, especially for the fact of the potential to extend such drug into the human food chain. Also, the possibility of reduced efficacy of such drugs which has been observed in some reports to be administered by non- qualified personnel (Boonmar et al., 1998; Thakur and Bajaj, 2006). In developed countries, stringent control of antibiotic use coupled with effective surveillance of antibiotic resistance patterns in the population have successfully reduced the prevalence of antibiotic resistance to these agents (Collignon, 2003).

Tylosin is a macrolide antibiotic produced by *Streptomyces fradiae* that still considered as one of the most effective antimicrobial agents against different mycoplasmas species and has more activity against mycoplasma than bacteria (Burrows, 1980; Atef et al., 1991; Kowalski et al., 2002). It is extensively and exclusively used in veterinary medicine and is classified as medium-spectrum because it has a high activity against Gram-positive bacteria and mycoplasma but only a limited activity against Gram negative bacteria. Because of the risk that residues of tylosin in edible tissue could lead to the development of resistant strains of bacteria in humans, the European Union decided to ban the use of tylosin together with three other antibiotics (zinc bacitracin, spiramycin, and virginiamycin) as a growth promoter. As a consequence, these community methods of analysis were no longer useful because they lack the specificity required to identify an unknown compound. A few chemical methods for the detection of tylosin in animal feed have been developed (Van Pouckeetal., 2003).

However, sell, use and prescription pattern of veterinary antibiotics in poultry production in Bangladesh are not well documented. People of Bangladesh are not concerned about use and residual effect of antibiotics. Therefore, the study was designed to screening of tylosin tartrate residue in poultry meat after discriminate and indiscriminate administration. The growth promoting effect of tylosin tartrate and the effect of tylosin tartrate antibiotic in haemopoietic system on PCV, TEC and Hb examination were also investigated.

MATERIALS AND METHODS

Preparation of bird house and rearing

The birds were reared in an isolated poultry shed, belongs to the Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. Firstly, the shed was thoroughly washed by sweeping and washing with tapwater. The room was disinfected with a phenolic disinfectant (phenyl) and allowed to dry leaving the roof, unused with the electric fan and the bulb switched on. Eighteen apparently healthy day-old broiler chicks were purchased from CP Hatchery Ltd. The chicks were already vaccinated with Marek's disease vaccine in the hatchery.

All chicks were brooded in the same brooder up to 7 days of age under strict management. Vitamin (Rena-C, Reneta Pharmaceuticals Ltd.) and electrolyte (Glucose) were administered to the birds daily mixing with water. BCRDV (Newcastle L-36) and Gumburo vaccine was given to all chicks in eye (one drop) at 7 and 12 days, respectively.

Broiler starter mash and broiler Grower pellet were obtained from Quality Feeds Ltd. The birds were given adlibitum feed for 7 days. Then feed was given twice daily in the morning & evening. Clean & cold water was provided adlibitum. Antibiotic tylosin 20% was obtained as powder from Eskayef Bangladesh Limited.

Experimental group and medication

Chicks were randomly divided on the first day of age into three groups each having six birds. Group A was kept as untreated control received non medicated water. Groups B was administered with tylosin at recommended therapeutic dose @2.5 g /1L drinking water for seven consecutive days from 16^{th} to 22^{nd} day of age and considered as discriminate antibiotic group. Group C was administered with tylosin at recommended therapeutic dose @2.5g/1L drinking water for fourteen consecutive days from 16^{th} to 31^{st} day of age and considered as indiscriminate antibiotic group. In case of discriminate group, the chicks were treated with antibiotics followed by

withdrawal period of one week before the sacrifice of the birds. On the other hand, indiscriminate group of the chicks was continued without giving any withdrawal period and birds were sacrificed. Birds were received their freshly prepared daily medication in the morning hour of each day. The concentration of tylosin in the water provided per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication. Drinking water was free from pesticides, heavy metals and coliform count. Drinking water medication was used in this study since this method is usually practice in field condition in Bangladesh. The weights of every bird were taken individually in the morning and the results were recorded. The antibiotic (Tylosin tartrate 200) was prepared by dissolving 0.1 g of powder per 0.1 ml of solution in 4 ml of methanol.

Sampling of blood and tissue

Blood samples were collected from wing vein into sterile heparinized vials and test tubes from all groups (Control, discriminate & indiscriminate). Blood smears were prepared simultaneously at the time of blood collection and used for differential leukocyte count.

For collection of edible tissue samples at the end of the experiment, all birds from corresponding treatment and control group were sacrificed ethically. Liver, kidney, breast muscle, thigh muscle, fat and spleen samples were collected and stored at -20^{o}C until analysis.

Sample preparation and antibiotics extraction

Thin layer chromatography was used to separate and recognized component in a mixture according to the protocol described by Poppelka et al. (2005). Briefly, 4 gm of sample (Liver/ Kidney/ Thigh muscle/ Breast muscle/fat/Spleen) was cut into small pieces and grinded by using mortar & pestle. 10 ml of PBS was added & mixed homogenously by vortex machine. Precipitation was performed by 2 ml of 30% TCA. The mixture was then centrifuged @ 60000 rpm for 20 minutes. Supernatant was collected in another falcon tube. Same amount of Diethyl ether was added and left for 10 mins in room temperature. The bottom layer was collected into screw cap vial with proper care and kept into refrigerator for further advanced analysis.

Pointing on TLC plate

Running of TLC for continuous development of

Chromatogram was done as per method of Thangadu et al. (2002). The chromatogram was examined under the ultraviolet lamp at 256 nm for spots that fluorescence. The outlines of the spots were marked with a series of dots using sharp pencil for calculation of retention factor (Rf).

Calculation of Rf values

To define the relative migration rate of substances under various conditions retardation factor is determined. It is the ratio of distance moved by thesubstance and distance moved by solvent. For this, the distance that each spothad traveled from the start line was measured in cm, taken from the center ofthe spot. Then calculation of Rf values was doneusing the following equation

$$\text{Rf} = \frac{Distance\ \ travelled\ \ by\ sample}{Distance\ \ travelled\ \ by\ solvent}$$

Hematological parameters

Hemoglobin concentrations (Hb), total erythrocyte count (TEC) and Packed Cell Volume (PCV) were determined according to the methods described by Lamberg and Rothstein (1977).

RESULT AND DISCUSSION

The present study investigated tylosin antibiotic in broiler both as discriminate and indiscriminate measure. Results showed in case of group A (Control group) no positive sample found. In case of group B (discriminate group), the residue of tylosin was 100% in livers, 83.33% in kidneys, 0% in thigh muscles, and 16.67% in breast muscles, 33.33% in fat and 16.67% in spleen. In case of group C (indiscriminate group), the residues of tylosin were 100% in livers, 100% in kidneys, 0% in thigh muscles, and 33.33% in breast muscles, 50% in fat and 33.34% in spleen.

Detection of Tylosin tartrate in different samples

Tylosin was detected in all organs investigated in discriminate group except thigh muscle. Hundred percent liver samples were positive for tylosin followed by kidney (83.33%), fat (33.33%), spleen and breast muscle (16.67%). Tylosin residue was absent in thigh muscle (Table 1).

In indiscriminate group, tylosin was present in all samples of liver and kidney (100%) followed by fat (50%), spleen and breast muscle (33.33%) whereas, thigh muscle was free from Tylosin residue. Based on the results it indicated that thigh muscle is free from tylosin residue compared to other organs.

Following repeated oral administration of 50 mg tylosin base/kg.b.wt once daily for 5 consecutive days, the blood (µg/ml) and tissue (µg/g) residues of tylosin showed that liver, kidney and lung contained the highest tylosin residues and completely disappeared from those tissues at 6 days after the last oral dose. Chickens should not be slaughtered for human consumption within the treatment and 6 days after the last oral administrations of tylosin (Soliman and Sedeik, 2016).

Hematological parameters of broiler

Tylosin antibiotic showed some changes in hematological parameters of broiler. The Hb and TEC content was significantly decreased at 31st day of tylosin treatment at recommended dose compared to untreated control. But percent PCV was slightly decreased (Table 3). This result is similar to the result of Solimon and Sedeik (2016).

Body weight and weight gain

There was significantly increased live weight gain of broiler from day 16th to day 28th compared to untreated control, whereas, there was no significant difference among the groups in gaining live weight at 31st day (Table 4).

Table 1: Detection of tylosin antibiotic in different samples of group B (Discriminate group) by TLC

Name of Sample	Total No. of Sample	Number of sample		% of sample	
		Positive	Negative	Positive (%)	Negative (%)
Liver	6	6	0	100	0.00
Kidney	6	5	1	83.33	16.67
Thigh muscle	6	0	6	0.00	100
Breast muscle	6	1	5	16.67	83.33
Fat	6	2	4	33.33	66.67
Spleen	6	1	5	16.67	83.33

Table 2: Detection of tylosin antibiotic in different samples of group-C (Indiscriminate group) by TLC

Name of Sample	Total No. of Sample	Number of sample		% of sample	
		Positive	Negative	Positive (%)	Negative (%)
Liver	6	6	0	100	0.00
Kidney	6	6	0	100	0.00
Thigh muscle	6	0	6	0.00	100
Breast muscle	6	2	4	33.33	66.67
Fat	6	3	3	50	50
Spleen	6	2	4	33.34	66.66

Table 3: Different hematological parameters of Broiler

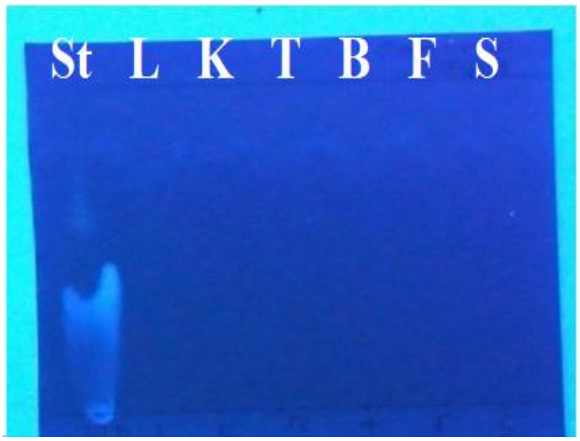
Day	Blood Parameters	Name of group	Average blood parameters value (Mean± SEM)	P Value	Level of Significance
31st Day	Hb (g/ml)	Group-A	8.19 ± 0.063		
		Group-B	7.31 ± 0.05	<0.01	**
		Group-C	7.19 ± 0.06		
	PCV (%)	Group-A	24.17 ± 0.48		
		Group-B	24.23 ± 0.068	0.023	*
		Group-C	23.13 ± 0.039		
	TEC (million/mm3)	Group-A	3.167 ± 0.066		
		Group-B	2.77 ± 0.029	<0.01	**
		Group-C	2.61 ± 0.041		

* P<0.05; ** P<0.01

Table 4: Body weight of broilers at different age

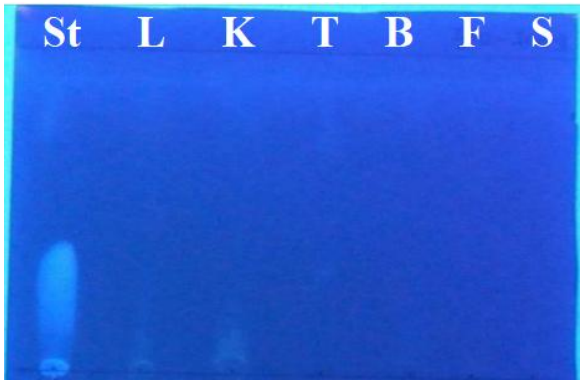
Variables	Group of Broilers	Average weight (g) (Mean ± SEM)	P Value	Level of significance
Live weight(g) on 16th day	Group-A	613.7 ± 38.77		
	Group-B	716.4 ± 17.92	0.0469	*
	Group-C	704.8 ± 26.35		
Live weight(g) on 22th day	Group-A	987.5 ± 17.31		
	Group-B	1081 ± 26.26	0.0180	*
	Group-C	1100 ± 32.54		
Live weight(g) on 28th day	Group-A	1611 ± 28.99		
	Group-B	1714 ± 18.27	.004	**
	Group-C	1768 ± 14.18		
Live weight(g) on 31st day	Group-A	2082 ± 28.66		
	Group-B	2176 ± 81.95	0.1709	Ns
	Group-C	2277 ± 82.38		

NS= Non significant; *= P<0.05; **= P<0.01



Group A

Group B



Group C
St=standard, L=liver, K=kidney, T=thigh muscle, B=breast muscle, F=fat, S=spleen.

Figure 1: TLC Plate showing the tylosin in group A, B & C

Based on limited data on tylosin residues in poultry reviewed by JECFA in 1991, it was concluded that no tylosin residues were found in chicken tissues 24 hours after treatment withdrawal. When tylosin was administered in drinking water (500 mg/l) for eight days or in feed (1000 g/ton) for seven days, the highest residues of tylosin observed at zero withdrawal time were 1030 µg/kg in the liver and 432 µg/kg in the kidney. In turkeys that received tylosin in drinking water (5 g/gallon ≈ 1.319 g/l) for seven days, tylosin residues at zero withdrawal time were similar to those in chickens, 385 µg/kg in the liver and 240 µg/kg in fat (JECFA, 1991).

Little data has been published about depletion of tylosin residues in edible tissues, especially on therapeutic dose regimes. In one study, it was analyzed that several poultry matrices to determine tylosin's transfer from feed at concentration levels representative of cross-contamination events (Vandenberge et al., 2012). They found no residue concentration levels above the limit of quantification in muscle matrix, therefore this molecule did not exceed maximum residue limits established at 100 µg kg-1 by EU legislation (EMEA, 2002). Another two studies concluded that the administration route determines the concentration of tylosin in different organs. Specifically, orally administered tylosin resulted in lower concentration levels than those from tylosin injections, and it could be a consequence of low oral bioavailability (Kowalski et al., 2002; Lewicki 2006).

CONCLUSION

The study concluded that once antibiotics are administered to animal body, antibiotic residues are present in high or low concentrations in their products. However, it mainly depends on the duration of the administration of antibiotics. After the administration of antibiotic, concentration of their residues gradually reduces in meat & other meat products and mostly after 4-5 days of administration excretion of residues are negligible. Residues of antibiotics have been detected in chicken meat by various investigators in several countries. The antibiotics were identified on the basis of their retention times (RTs) as compared to the standards and by adding known quantities of the standards to the sample and re-chromatography which showed complete overlap of analyte peaks. Hence, the withdrawal time of different drugs should be strictly followed and during this period meat and other animal products like liver, spleen, fat and kidney should not be used for human consumption. Use of antibiotic as growth promoter should be strictly prohibited and whenever they are used for therapeutic purpose must be used in proper doses and for proper time. Thus, by observing proper scientific guidelines and precautions we can minimize the harmful effects of antibiotic residues.

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