

Article

Effects of colistin sulfate on hematological parameters in broiler

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Abstract: Hematological indication is an important finding for pathophysiological analysis of biological science. This study was designed with the aim to investigate the effects of residual antibiotics on hematological parameters of broiler following discriminate and indiscriminate use. The day old broiler chicks were collected and reared up to 31 days. The treatment was started from the day 16th until sacrifice. The chicks were randomly divided into three groups namely control group (Group A), discriminate antibiotic group (Group B) and indiscriminate antibiotic group (Group C) on the 14th day. The discriminate group was treated with antibiotic, colistin sulfate maintaining the withdrawal period of one week. In case of indiscriminate group the withdrawal period was not maintained and antibiotic treatment was continued till the day of sacrifice. The Hb (%) of control, discriminate and indiscriminate groups were 8.34 ± 0.15 , 6.89 ± 0.27 , and 7.14 ± 0.23 respectively. The differences among means were statistically significant ($P < 0.05$). The Packed Cell Volume (PCV) of control, discriminate, and indiscriminate groups were 23.51 ± 0.76 , 21.17 ± 0.94 , and 22.83 ± 1.19 respectively. The differences among means were statistically significant. The multiple pair wise comparison of means of blood parameters revealed that there was no significant difference among discriminate and indiscriminate groups. From the above findings, this research could be considered a need based research in Bangladesh to ascertain the influential effect of antibiotic on hematological parameters in broiler.

Keywords: colistin sulfate; hematological parameters; PCV; Hb; TEC

1. Introduction

Antibiotics used for chemotherapeutic and prophylactic purposes and also used as feed additives to promote growth and improve feed efficiency (Swatantra *et al.*, 2014). However, the antibiotic residues from milk, meat and egg may persist for longer period after treatment if withdrawal period was not properly maintained. Various antibiotics take different time periods to be excreted from the body. It becomes a potential hazard to human health. In poultry, Antibiotics are widely used as therapeutic, prophylactic, growth promoting agents and reproductive purposes in poultry production (Donoghue, 2003, Jinap *et al.*, 2010). This wide spread use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which include; carcinogenicity, mutagenicity, bone marrow toxicity and allergy (Nisha, 2008). Now a days, colistin sulfate is one of the most widely used antibiotic in poultry sector.

Besides, antibiotic therapy is associated with toxic effect on hematopoiesis process causing a change in blood parameter of poultry (Stolker and Brinkman, 2005). Certain antibiotics show diverse effect on different elements of the blood like thrombocytopenia, anemia, leucopenia etc. (Al-Mayah and Al-Ahmed, 2005). Hematological investigations in monitoring the health status of birds has grown in extent, becoming an indispensable component of the protocols used for testing bioequivalence, safety and tolerance of active substances on the target species (Ognean *et al.*, 2012). As, hematological profile an important physiological indicator of the body associated with animal production (Aboubakr and Elbadawy, 2016) the present study was undertaken to

investigate the effect of colistin sulfate antibiotic on hematological parameter of broiler following discriminate and indiscriminate use of colistin sulfate.

2. Materials and Methods

2.1. Ethical approval of laboratory animals

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)].

2.2. Experimental design

18 apparently healthy day-old "Cobb-500" broiler chicks were purchased from CP Hatchery Ltd, Valuka, Mymensingh. On the 16th days of age chicks were randomly divided into three groups (Group A, B & C). Each group contains 6 birds. The birds of Group-A, B and C were kept in different cages. Group A was kept as untreated control & received non-medicated water. Groups B & C were administered with colistin sulphate at @2gm/1L drinking water. After 7 days, at the age of day 23; antibiotic supply was stopped in the group-B and withdrawal period was maintained. In group-C the antibiotic supply was continued until the day of sacrifice. Birds received their freshly prepared daily medication in the morning hour of each day. The concentration of colistin sulphate in the water to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication.

2.3. Collection of blood samples

Blood samples from all groups (Control, discriminate & indiscriminate) were collected into sterile heparinized and non-heparinized vials during sacrifice and were immediately stored into refrigerator for further use.

2.4. Hematological Parameters

Total erythrocyte count (TEC), Hemoglobin content (Hb %) and Packed cell volume (PCV) were studied for hematological investigation. For determination of hematological parameter, blood samples were collected at the end of experiment (31st day) from all groups. Immediately after collection of blood, blood was transferred to sterile test tube containing anticoagulant at a ratio of 1:10.

2.4.1. Determination of Hemoglobin Concentrations (Hb)

With the help of a dropper the N/10 hydrochloric acid (HCL) was taken in a graduated tube up to 2 marks. Then Citrated well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid solution. The pipette was rinsed 2-3 times by sucking fluid into tube. This blood and acid were thoroughly mixed by stirring with a glass stirrer into the diluting tube. There was a formation of acid hematin mixture in the tube by hemolysed blood RBC & HCL. The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. Then distilled water was added drop by drop, solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus & expressed in gm percent.

2.4.2. Determination of Total Erythrocyte Count (TEC)

The tip of a dry clean red pipette was dipped into the blood sample and blood was sucked up to 0.5 mark of the pipette. Outside of the tip of the pipette was wiped with cotton. Then the pipette was immediately filled with the red cell diluting fluid (Hayem's solution) up to 101 marks. The rubber tube was stretched at the other end of pipette and both ends were held with thumb and finger. The contents of the pipette were mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. The counting chamber was placed with cover glass under microscope using low power (10x) objectives. After discarding 2 or 3 drops of fluid from the pipette, a small drop was placed to the edge of the cover glass on the counting chamber as the entire area under the cover glass was filled by the fluid. One-minute time was spared to allow the cells to settle over the chamber uniformly. The cells were counted from the recognized 80 small squares (16 x 5) under high power objectives (40x). After completion of counting, the total number of RBC was calculated as number of cells counted x 10,000 and the result was expressed in million/ μ l of blood.

2.4.3. Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette) and pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. The rubber bulb of the pipette was pressed continuously to expel the blood out of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming. The tube was exactly filled to the 10 cm mark and then placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. After 30 minutes the tubes were taken out of centrifuge machine and PCV was read directly from the calibration on the right side of the tube.

The result was expressed in percentage.

$$\text{PCV}\% = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \times 100$$

3. Results

3.1. Total Erythrocyte Count (Million/mm³)

Table 1. Total Erythrocyte count of three different groups.

SL No.	Antibiotic group (Discriminate)	Antibiotic group (Indiscriminate)	Control group
1	2.89	2.25	3.55
2	2.71	2.01	3.15
3	2.63	2.29	3.37
4	2.76	2.41	3.09
5	2.61	2.28	3.26
6	2.19	2.05	3.33
Mean±SEM	2.63±0.09	2.21±0.06	3.29±0.07

The highest mean TEC was obtained from control group that was 3.29±0.07 (Table 1). The mean TEC of discriminate and indiscriminate group were 2.63± 0.09 and 2.21±0.06 respectively (Table 1). 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 were not statistically significant. But other two pairs showed significant differences among means.

3.2. Hemoglobin (gm%) count of three different groups

Table 2. Hemoglobin percentage (%) of three different groups.

SL No.	Antibiotic group (Discriminate)	Antibiotic group (Indiscriminate)	Control group
1	6.88	7.04	8.18
2	7.11	7.09	8.02
3	7.56	6.17	8.59
4	6.22	7.33	8.15
5	6.04	7.29	8.13
6	7.49	7.97	9.02
Mean±SEM	6.89±0.27	7.14±0.23	8.34±0.15

The highest mean Hb (%) was obtained from control group that was 8.34±0.15 (Table 2). The mean TEC of discriminate and indiscriminate group were 2.63±0.09 and 2.21±0.06 respectively (Table 2). 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 and discriminate was not statistically significant. But other two pairs showed significant differences among means.

3.3. Packed cell volume (%) count of three different groups

Table 3. Packed Cell Volume of the three different groups.

SL No.	Antibiotic group (Discriminate)	Antibiotic group (Indiscriminate)	Control group
1	22	24	22
2	19	26	25
3	21	23	26
4	24	21	21
5	23	25	24
6	18	18	23
Mean±SEM	21.17±0.94	22.83±1.19	23.51±0.76

The highest mean PCV was obtained from control group that was 23.51±0.76 (Table 3). The mean TEC of discriminate and indiscriminate group were 2.63±0.09 and 2.21±0.06 respectively (Table 3). 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 and discriminate group and discriminate and control group were not statistically significant. But discriminate and control group showed significant differences among means ($P<0.05$).

4. Discussion

Antimicrobial residues in food of animal origin have received much attention in developed countries to ensure food safety. Many countries have monitoring programs to avoid antimicrobial residue in food of animal origin (Ellis, 2008). In Bangladesh, there are regulations regarding the use of antimicrobials or the maximum allowable antimicrobial concentrations in food. Additionally, there are no systems to monitor the presence of antimicrobial residue in animal products in Bangladesh. Therefore, screening of food products from animal origin intended for human consumption for the presence of antimicrobial residue is essential to ensure food safety. In this study, antimicrobial residue in poultry meat was evaluated. The most commonly sold antimicrobial classes in the major livestock especially in poultry production in 15 countries from Europe, Asia and Australia were penicillins, tetracyclines, macrolides and aminoglycosides, especially since each of these classes has been in use for more than 50 years (Page and Gautier, 2012). These antimicrobials are administered to broilers by injections (intramuscularly or subcutaneously) and orally in food or water (Kirbis, 2007). Screening techniques are the first step in determination the presence of antimicrobials in food of animal origins; these techniques may use biological methods, biochemical methods and physicochemical methods (Chafer-Pericas *et al*, 2010). Microbiological inhibition tests are cheap and permit to analyze a large number of samples in a short time. Microbiological screening relies on a common property of all antibacterials; they inhibit growth of microorganisms (Wasch *et al.*, 1998).

The use of colistin sulfate 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. The effect is large when chronic exposure occurs. Moreover, the leukocytes are the primary cells those are destroyed by the chronic antibiotics use. It is evident that the indiscriminate use of colistin sulfate doesn't bear any fruitful effect on broiler. Rather there is potential harmful effects of antibiotics residue which might enter the human food chain and produce deleterious impact on human health. Moreover, the higher cost is a drawback to the profitability of the farmers. So, they must realize that use of antibiotics at large can't increase their profitability rather decrease the profits. The lower blood parameters are the clear indication of decreased immunity of the live birds. 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. So from the above discussion it is clear that, antibiotic residue already exist in our food chain, especially in broiler. However, a comprehensive study require in Bangladesh to detect and estimate all the antibiotics used in broiler and layer chicken to take potential steps to protect the mankind and environment from antibiotics residue hazards.

5. Conclusions

Discriminate and indiscriminate use of colistin sulfate has some effects on hematological parameters but the effects are not significant in small duration. The result of the hematological monitoring show that within a short period no potential risks associated with indiscriminate dose of colistin sulfate. The result also showed that broiler chickens could tolerate more than the recommended dose of colistin sulfate without any deleterious effect on the hematological parameter.

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Conflict of interest

None to declare.

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