Effects of ciprofloxacin on chicken blood parameters after an experimental infection

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Abstract. The objective of this paper is to study the dynamics of blood parameters in chicken resulting from use of Ciprofloxacin. Ciprofloxacin is more active in affecting aerobic Gram-negative bacteria and Staphylococci. To study the dynamics, formed three groups of chickens (1st group – control; 2nd and 3rd groups – experimental groups), which included crossbred Hisex Brown roosters. The experimental infection of chickens in the 2nd and 3rd group with Staphylococcus aureus culture performed. One day before and during four days after infection chickens in the 3rd group received 200 mg/L of Ciprofloxacin dissolved in water. Blood samples were drawn from all subjects on the day of infection, and then at the 2nd, 4th and 6th day after infection. The following blood parameters: red blood cell count, hemoglobin level, white blood cell count, and leucograms were studied. Analysis of the data we obtained showed significant changes in some parameters of white blood cells in the 2nd and third groups in comparison to the control group. We found inflammatory response which was characterized by significant increase in the absolute count and percentage of pseudo-eosinophils, monocytes and basophils in the 2nd and 3rd groups in comparison to the control group.

1. Introduction

Keeping plenty of birds in confined small production facilities of contemporary chicken farms results in quick emergence and spreading of infectious diseases. Infections develop swiftly and cause great production losses. Bacterial diseases entail process turndown and increase in death rate among chicken, which, in turn, causes significant damage to poultry farming [1]. Staphylococcus invasion in chickens is the most common infectious diseases prevailing at chicken farms. Despite availability of a broad spectrum of therapeutic and disinfecting drugs, Staphylococcus infections in chickens require long-term and expensive treatment by highly qualified specialists. That is why issues of prevention, diagnosis and treatment of this disease in poultry are invariably topical. High resistance and adaptability of the microorganisms to newly developed drugs is one of the causes of growth in prevalence of Staphylococcus infections.

In this context the role of veterinary activities aimed at reduction of in-process losses is ever growing. At the present moment antimicrobial chemotherapeutic drugs are being widely used in poultry farming. Fluoroquinolones play the major role in this pharmaceutical group, which have proved to be highly active systemic drugs with a broad range of indications to their use in control of infectious diseases [2].
Fluoroquinolones are a swiftly developing group of synthetic antimicrobial drugs, which has a common mode of action in suppression of the key enzyme of the microbial cell, i.e. DNA gyrase [3]. Fluoroquinolones have optimal pharmacokinetics which ensures high bioavailability of the drugs [4]. It should also be noted that the drugs have a good therapeutic effect in monotherapy and, thus, no combined therapy is required, provided that the infectious agent is susceptible to fluoroquinolones [5]. These drugs are characterized by prolonged bactericide action and potentiation of phagocytosis and intracellular destruction of bacteria by white blood cells [6]. The said drugs can penetrate into phagocytizing cells and accumulate there [7].

The growing clinical use of fluoroquinolones increases interest to the effects some drugs of this group have on white blood cells in the process of treatment of infectious diseases. Ciprofloxacin is a drug used in poultry farming. This drug is a broad-spectrum antibiotic which is most active to affect aerobic Gram-negative bacteria. Furthermore, the drug is effective in treatment of Staphylococcus infections [8, 9]. Evaluation of physiological parameters of an organism affected by infectious agents and antimicrobial drugs is a topical trend in research. Hence, for the purpose of this study we counted blood cells in young chicken after effect of Ciprofloxacin as a therapy for experimental Staphylococcus infection.

2. Object and methods

In order to meet the objective of our trial, we formed three peer-based groups of chickens (1st group – control; 2nd and 3rd groups – experimental groups), which included daily-aged crossbred Hisex Brown roosters. The well-balanced ration of all chickens included basic nutrients and bioactive substances. For the purpose of our trial, chickens in the 2nd and 3rd groups were infected with Staphylococcus aureus culture which was administered by an intraperitoneal injection in concentrations 2 McFarland. One day before and during four days after infection chickens in the 3rd group received 200 mg/L of Ciprofloxacin with water. Blood samples were drawn from all subjects on the day of infection, and then at the 2nd, 4th and 6th day after infection. The blood samples were then stabilized with 3.8% sodium citrate.

The following blood parameters: red blood cell (RBC) count, hemoglobin level, white blood cell (WBC) count, leucograms were studied. RBCs and WBCs were counted directly using a Goryaev chamber. Eosinophils, basophils, pseudo-eosinophils, lymphocytes, and monocytes were counted in stained blood smears. The differential count of white blood cell types count by percentage of total WBC was performed. Haemoglobin levels were measured in a colorimetric assay. Statistical processing of digital data was performed using SPSS Statistic 17.0 software, while significance of the findings was assessed using the non-parametric Mann–Whitney U-test.

3. Results

During the first day after infection haemoglobin level in blood of chickens in the experimental groups were significantly (by 16%) lower than control levels, while at the 2nd, 4th and 6th days the haemoglobin level was lower than the control levels by 9, 10 and 16%, respectively. Within the period from the first to the sixth day, a tendency to compensatory growth in RBC count was observed in chickens of the experimental groups. On the background of low haemoglobin concentrations such compensatory growth resulted in decrease in colour index. Low colour index suggests that there are RBCs with reduced haemoglobin concentration.

Despite some decrease in RBC count in chickens after administration of Ciprofloxacin, all blood parameters remained within the age norm. It is a well-known fact that use of fluoroquinolones may cause some changes in blood parameters, development of anemia, and increase in erythrocyte sedimentation rate. Furthermore, the drug may have a short-term and reversible effect on haematopoiesis. For instance, it has been described that Temafloxacin triggers haemolytic reactions in the hematopoietic system [10].

It is common knowledge that infectious agents affect the organism triggering development of leucocytosis [11]. Our findings showed that Staphylococcus infection administered to chicken
organisms was accompanied by certain changes in white blood cell count. In our trial we noted increase in WBC concentration by 31% (moderate leucocytosis) at the 2nd day in the 2nd group only. This observation suggests that St. aureus caused development of inflammatory processes. However, no significant changes were subsequently observed in this parameter which remained within the normal range in all chickens.

Infection triggered significant decrease in eosinophil percentage in the 2nd and 3rd groups (table 1). Over the following days this parameter varied significantly. However, at the sixth day no significant changes were detectable in the 3rd group.

**Table 1. Dynamics of chickens’ blood leukograms of the groups under study (N=6, M±m), %.**

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| 1    | 2      | 2.1±0.26  | 38.3±2.39   | 9.1±0.82    | 46.4±1.96         | 4.1±1.03  |
| 2    | 1      | 4.7±0.81a | 15.8±1.08a  | 5.2±0.79a   | 68.2±2.52a        | 6.1±0.86  |
| 3    | 4.5±1.18b| 24.4±0.67a | 5.8±0.61a   | 60.8±1.67a  | 4.5±0.62          |
| 2    | 1      | 4.7±0.81a | 15.8±1.08a  | 5.2±0.79a   | 68.2±2.52a        | 6.1±0.86  |
| 3    | 4.5±1.18b| 24.4±0.67a | 5.8±0.61a   | 60.8±1.67a  | 4.5±0.62          |

| 2    | 1      | 21.1±2.39a| 10.3±0.42a  | 2.1±0.52a   | 53.4±1.81a        | 13.1±1.32a|
| 3    | 17.8±1.72a | 15.7±1.76a | 2.8±0.61a   | 51.5±1.91a    | 12.2±0.98a       |
| 4    | 2      | 7.1±0.52a | 15.8±1.45a  | 5.1±0.68b   | 61.3±1.71a        | 10.7±1.08a|
| 3    | 11.1±3.04a | 15.3±2.52a | 3.4±1.38b   | 59.1±1.62a    | 11.1±1.92a       |
| 1    | 1.6±0.22 | 48.7±2.14  | 10.3±1.14   | 37.1±1.29    | 2.3±0.33          |
| 6    | 2      | 4.5±1.91a | 19.3±2.17a  | 4.8±0.48a   | 63.7±1.47a        | 7.7±0.56a |
| 3    | 3.2±0.31a | 22.2±1.68a | 7.1±1.18    | 60.1±1.63a   | 7.4±1.08a         |

*p<0.01 (Mann–Whitney U-test).

**Table 2. Dynamics of absolute values of chickens’ blood leukogram of the studied groups (N=6, M±m), 10⁶/l.**

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| 1    | 2      | 0.21±0.03 | 4.05±0.59   | 0.95±0.14   | 4.74±0.25         | 0.39±0.07 |
| 2    | 1      | 0.52±0.09a| 1.76±0.11a  | 0.57±0.05   | 7.81±0.78a        | 0.69±0.12b|
| 3    | 0.49±0.09a | 2.94±0.27  | 0.71±0.11   | 7.41±0.68a      | 0.55±0.11 |
| 1    | 0.23±0.03 | 4.29±0.22  | 0.85±0.11   | 3.74±0.39      | 0.23±0.02 |
| 2    | 2.79±0.34a| 1.38±0.12a | 0.29±0.09a  | 7.11±0.53a     | 1.73±0.23b       |
| 3    | 2.03±0.25a| 1.60±0.18a | 0.31±0.06a  | 5.83±0.41a     | 1.53±0.18a       |
| 1    | 0.28±0.07 | 5.56±0.41  | 1.12±0.05   | 5.02±0.51      | 0.36±0.04 |
| 2    | 2      | 0.89±0.13a | 1.99±0.24a  | 0.65±0.13b    | 7.83±0.93b       | 1.30±0.11b|
| 3    | 1.77±0.56a | 2.28±0.32  | 0.51±0.19b  | 9.06±0.42a     | 1.73±0.32b       |
| 1    | 0.19±0.03 | 6.45±0.85  | 1.29±0.09   | 4.75±0.35      | 0.33±0.05 |
| 6    | 2      | 0.69±0.31a | 2.82±0.36a  | 0.71±0.08a   | 9.31±0.57        | 1.14±0.15b |
| 3    | 0.43±0.04a | 3.07±0.29a | 0.95±0.14   | 8.44±0.82a     | 1.11±0.22a       |

*p<0.01 (Mann–Whitney U-test).

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This finding is an indication of inflammatory response, since pseudo-eosinophils are actively involved in phagocytosis. These cells are among the first to engage with foreign agents and, thus, trigger a cascade of immune responses.

Significant decrease in lymphocyte count in the blood differential was observed in chickens in the 2nd and 3rd group immediately after infection. However, during this day the absolute count in the 3rd group showed no significant changes. Nevertheless, at the second, fourth and sixth days of the trial we identified highly significant lymphocytopenia in the 2nd and 3rd groups, which was caused by mass escape of lymphocytes from blood channels into tissues, which, in turn, facilitated suppression of infectious agents.

Pseudo-eosinophilic leucocytosis was less prominent in the 3rd group, since this was the group which received Ciprofloxacin. The difference between lymphocyte counts in the control group and the 3rd group was less significant, which, most likely, is attributable to the ability of fluoroquinolones to increase production of interleukin-2 also known as lymphokine, which stimulates proliferation of lymphocytes [14].

4. Conclusion

Thus, we found that use of 200 mg/L Ciprofloxacin dissolved in water does not cause any significant changes in RBCs of young chickens. At the same time, it should be noted that the drug triggered short-time reversible anemic presentations which did not have any negative effect on physiological status of the chickens. Our study gives indirect evidence to the fact that anti-Staphylococcus activity of Ciprofloxacin is rather low and, therefore, this drug is barely useful in treatment of Staphylococcus infections.

However, it should be noted that, on the whole, differential white blood cell count in the 3rd group was closer to the control parameters in comparison to differential count in the group which did not receive Ciprofloxacin. Nevertheless, it is widely known that other fluoroquinolones, such as Trovafloxacin and Moxifloxacin, are more effective against St. aureus than Ciprofloxacin [15, 16].

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