Leukocytes differential under effect of ciprofloxacin in experimental colibacillosis in chickens

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Abstract. This study was aimed at evaluation of white blood cells of domestic birds over time using a broad-spectrum chemotherapeutic drug Ciprofloxacin. Ciprofloxacin more actively inhibits gram-negative bacteria. In order to evaluate the dynamics, three groups of chickens were formed (1st group – control; 2nd and 3rd groups – experimental ones), which consisted of Hisex Brown cross-bred cockerels. Within the framework of this experiment chickens in the 2nd and 3rd groups were contaminated with Escherichia coli. A day before and for four days after the contamination chickens in the 3rd group received 200 mg/L of ciprofloxacin with water. Blood samples were drawn from all experimental chickens at Day 1, 5, 10, 15 and 20 after the contamination. White blood cell differential count was performed. Analysis of the data obtained showed significant changes in some blood cell parameters in the 2nd and 3rd groups in comparison to the control group. Inflammatory response was detected, which was characterized by significant increase in absolute count and percentage of pseudoeosinophils, monocytes and basophils in the 2nd and 3rd group in comparison to the control group. Nevertheless, white blood cells differential count in the 3rd group was relatively closer to analogous count in the control group.

1. Introduction

Intensification of production translates into the development of the continuous technological process in poultry farming. But the eventual cost-effectiveness may sometimes cause the deterioration in physical conditions of poultry. Possible deviations in poultry maintenance or veterinary and sanitary control add to the stress which decreases resistance of poultry and facilitates bacterial outbreaks.

Analysis of treatment protocols for infectious diseases at poultry farms shows a noteworthy trend in use of fluoroquinolone medicines. Such situation can be explained by the fact that by now medicines of this pharmacological group have solidly proven to be effective chemotherapeutic drugs which have systemic action [1-3] and are highly effective in management of severe infectious and inflammatory diseases [4], which share a common mechanism of action inhibiting the key enzyme (DNA-gyrase) of bacterial cells [5]. Fluoroquinolones affect two targets in a microbial cell – enzymes responsible for transformation of spatial configuration of DNA [6-9]. These medicines are well-tolerated and highly efficient against many gram-positive and gram-negative bacteria, and multidrug-resistant microorganisms. Pharmacokinetics of fluoroquinolones is characterized by high volume of distribution and biotransformation in the organism, prolonged half-life, and active penetration into cells and tissues of macroorganisms [10-13].
The high efficiency of fluoroquinolone medicines against severe bacterial infections caused by agents resistant to medicines of other classes of chemical substances is an important property of fluoroquinolones, which can penetrate phagocytizing cells and accumulate in them while staying in active form. Intracellular accumulation of antibacterial medicines in active form facilitates transformation of phagocytic activity of macrophages and neutrophils and affects viability of phagocytized bacteria [14-17]. Specificity of fluoroquinolone effect on bacterial cells lies in the fact that these medicines are prominently active in sub-bacteriostatic concentrations, which have post-antibiotic effect [18].

The studies of fluoroquinolone application show that this kind of drugs has a slightly negative effect on circulatory system, but it only concerns mammals and little is known about the fluoroquinolones’ impact on circulatory system of birds [8].

There is rather controversial information about effects of fluoroquinolones antibacterial medicines on lymphocytes. Ofloxacin, Norfloxacin and Pefloxacin have antiproliferative effect on cells. Fluoroquinolones may affect production of interleukin-2, also known as lymphokine, which activates lymphocyte proliferation. Fluoroquinolones also stimulate “respiratory explosion” in neutrophils. Some authors noted that use of such medicines resulted in mild changes in the hemic system in the form of anaemia, thrombocytopenia, eosinophilia, and increase in erythrocyte sedimentation rate, leukopenia, and leucocytosis [19-20]. Such side effects raise the question about effects of fluoroquinolones on the hemic system.

This paper is the first to provide percentage and absolute count of different white blood cells of chickens treated with ciprofloxacin. This study was aimed at evaluation of white blood cells of chickens treated for experimental colibacillosis with ciprofloxacin.

2. Object and methods
In this study performed on the basis of comparison of analogous parameters three groups of chickens were formed (1st group – control; 2nd and 3rd groups – experimental ones), which consisted of Hisex Brown cross-bred cockerels. All experimental chickens were fed with a feed with a well-balanced composition of nutrients and biologically active substances. Chickens in the 2nd and 3rd groups were contaminated with Escherichia coli, which were administered intra-abdominally in concentration 2 McFarland. A day before and for four days after the contamination chickens in the 3rd group received 200 mg/L of Ciprofloxacin with water. Blood samples were drawn from all experimental chickens at Day 1, 5, 10, 15 and 20 after the contamination.

The following blood parameters were studied: white blood cell (WBC) differential count. White blood cells were counted directly in a Goryaev chamber. Eosinophils, basophils, pseudoeosinophils, lymphocytes and monocytes were counted in blood smears stained using Romanowsky-Giemsa method.

Statistical processing of digital data included computation of the mean (M) and standard error (m) using software. Significance of differences was assessed using the Mann-Whitney U-test; differences were considered significant at p < 0.05.

3. Results
Our studies showed that contamination of chickens with colibacillosis is accompanied by certain changes in white blood cells. In the experiments reported in this paper WBC count remained within normal in all experimental chickens. In spite of the fact that changes in blood of the chickens taking Ciprofloxacin were less pronounced (which is caused by effect of the drug on infection) we note reliable changes in blood values as compared to control group to the final day of the experiment.

Significant increase of eosinophils was observed in the 2nd group at Day 10 and in the 3rd group at Day 15 after the contamination (table 1). Increase in absolute basophil (table 2) count observed in the 2nd group at Day 5, 10 and 15, and in the 3rd group at Day 1, 10 and 15 after the contamination suggests that the organisms responds to introduction of the antigen. It is assumed that basophils after the parenteral penetration of alien proteins are involved in phagocytic protection. It is possible that increase in basophil number occurs in connection with this function after the impact of E. coli.
phagocytic activity of the cells as they are the first to attack microbes and start the chain of immune response of the organism. Pseudoeosinophils are among the first cells to contact infectious agent and, thus, they trigger a cascade of immune reactions. Such pseudoeosinophilia was probably caused by phagocytic activity of the cells as they are the first to attack microbes and start the chain of immune response reactions.

Table 1. Dynamics of chickens blood leukograms of the groups under study, %.

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| 1 2  | 2.1±0.25 | 41.5±1.72 | 8.7±0.66   | 45.1±1.52   | 2.6±0.32          |
| 3   | 2.5±0.21 | 40.7±1.35 | 9.6±0.62   | 42.1±1.36   | 5.1±0.57          |
| 1   | 1.5±0.33 | 44.7±1.01 | 8.3±0.75   | 43.1±1.52   | 2.4±0.21          |
| 5   | 7.7±3.51b| 23.1±2.62b| 9.2±2.14   | 54.8±2.43b  | 5.2±0.62          |
| 3   | 2.5±0.21 | 32.4±1.72b| 11.8±0.62b | 49.8±1.62b  | 3.5±0.42          |
| 1   | 1.8±0.32 | 41.8±1.25 | 9.1±0.76   | 44.8±1.34   | 2.5±0.21          |
| 10  | 3.1±0.35 | 29.8±1.53b| 14.2±1.07b | 49.1±1.02   | 3.8±0.32          |
| 3   | 2.7±0.32 | 32.5±1.62b| 10.8±1.32  | 50.7±1.62b  | 3.3±0.22          |
| 1   | 1.3±0.22 | 42.7±1.53 | 9.2±0.72   | 44.3±1.53   | 2.5±0.21          |
| 15  | 3.1±1.17 | 28.4±1.93b| 10.7±1.07  | 54.1±1.17b  | 3.7±0.86          |
| 3   | 1.5±0.21 | 26.8±0.86b | 14.3±0.75b | 54.3±0.98b  | 3.1±0.25          |

p<0,01 (Mann–Whitney U-test).  
* p<0,05 (Mann–Whitney U-test).

Significant increase in monocyte percentage detected at the first and fifth day gives evidence to active phagocytosis in acute inflammation. In the 2nd group the increase in monocyte number was transitory; the values did not leave the normal range. Such one-time change is probably associated with transient pseudoeosinophilia in accordance with the fact that increase in monocyte number can be caused by increase in the number of polymorphonuclear leukocytes.

Table 2. Dynamics of absolute values of chickens blood leukogram of the studied groups, 10⁶.L⁻¹.

| days | groups | monocytes | lymphocytes | eosinophils | pseudo-eosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| 1 2  | 0.11±0.01 | 2.06±0.16 | 0.44±0.07   | 2.25±0.21   | 0.16±0.03          |
| 3   | 0.17±0.02a | 2.60±0.26 | 0.65±0.08   | 2.59±0.26   | 0.35±0.06a         |
| 1   | 0.12±0.03 | 3.14±0.24 | 0.59±0.05   | 3.01±0.18   | 0.18±0.02          |
| 5   | 0.59±0.29 | 1.49±0.16b| 0.61±0.15   | 3.64±0.36b  | 0.37±0.07b         |
| 3   | 0.16±0.02 | 1.95±0.18b| 0.71±0.06   | 2.98±0.26   | 0.23±0.04          |
| 1   | 0.18±0.03 | 3.76±0.22 | 0.82±0.08   | 4.05±0.23   | 0.23±0.02          |
| 10  | 0.27±0.04 | 2.58±0.28b| 1.25±0.14   | 4.26±0.44   | 0.33±0.02a         |
| 3   | 0.26±0.03 | 3.15±0.24 | 1.03±0.09   | 4.93±0.41   | 0.33±0.02a         |
| 1   | 0.14±0.02 | 4.12±0.26 | 0.88±0.09   | 4.29±0.35   | 0.25±0.01          |
| 15  | 0.35±0.16 | 3.11±0.31 | 1.16±0.09   | 5.97±0.62a  | 0.45±0.14a         |
| 3   | 0.17±0.02 | 2.87±0.16b| 1.54±0.11b  | 5.79±0.22a  | 0.33±0.02a         |

p<0,01 (Mann–Whitney U-test).  
* p<0,05 (Mann–Whitney U-test).

Significant increase observed in pseudoeosinophils concentrations in the 2nd and 3rd group during the entire period of this study in comparison to the control group gives evidence to inflammatory response of the organism. Pseudoeosinophils are among the first cells to contact infectious agent and, thus, they trigger a cascade of immune reactions. Such pseudoeosinophilia was probably caused by phagocytic activity of the cells as they are the first to attack microbes and start the chain of immune response reactions.
Significant decrease was observed in WBC count in chickens of the 2nd and 3rd groups. At Day 5, 10 and 15 significant lymphocytopenia was detected in the 2nd and 3rd groups, which was caused by massive lymphocyte recruitment aimed at inhibition of infection agents.

A less significant difference was found between lymphocyte count in the control and 3rd groups at Day 5 and 10 after the contamination, which is likely to be associated with ability of fluoroquinolones to intensify production of interleukin-2, which activates lymphocyte proliferation.

At Day 20 of the experiment no significant differences in WBC differential count were detectable in any of the groups.

In our experiments we found some changes in WBC differential count in chicken treated with fluoroquinolones. In should be notes that just a few studies have been performed to investigate effects of antimicrobial drugs on WBC differential count in chickens, while information about effects of biologically active substances on blood parameters is much more comprehensive.

Published information about pathophysiological changes in the hemic system under effect of ciprofloxacin confirms our conclusion about mild effects this fluoroquinolone has on WBC differential count [21].

This study showed absence of any significant differences in WBC differential count after administration of ciprofloxacin in different doses in comparison to that in the control group. At the same time, earlier we noted significant changes in count of different types of white blood cells in blood of chickens under effect of ciprofloxacin in the context of experimental Staphylococcus infection and colibacillosis. Therefore, a conclusion can be drawn that leukocytic responses to fluoroquinolones in healthy chickens and chickens in the context of experimental infection are different.

Thus, the detected specific features of effects of fluoroquinolones on ratios of different types of white blood cells in blood of chickens broadens the general notion of physiological responses of domestic birds to antimicrobial drugs.

The results we obtained make it possible to determine the white blood cells of domestic birds which are most susceptible to medications. This information contributes to knowledge about WBC differential count in chickens at an early age. It should be noted the, on the whole, just a few studies investigating changes in blood cells of birds under effect of antimicrobial drugs have been conducted [22-25], and one can hardly find any information about age-related aspects of such effect.

4. Conclusion

Analysis of the data obtained showed significant changes in white blood cell count in the 2nd and 3rd groups in comparison to the control group. Increase was observes in absolute count and percentage of pseudoeosinophils, monocytes and basophils in the 2nd and 3rd groups in comparison to that in the control group. At the same time, WBC differential count in the 3rd group was closer to that in the control group. 

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