The effect of enrofloxacin on blood values of chickens in experimental salmonellosis

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Abstract. The aim of the present study is to research the impact of enrofloxacin on blood values of chickens experimentally infected with Salmonella. In the course of two experiments (experiment “a” and experiment “b”) a number of chickens were experimentally infected with Salmonella typhimurium and Salmonella enteritidis. One of the groups of the infected chickens in either experiment received 200 mg enrofloxacin per l in drinking water 24 hours before the challenge with Salmonella, then each of the following four days after the challenge with Salmonella. The administration of enrofloxacin did not suppress red blood cells in the chickens significantly; the registered reliable changes reflected the development of experimental salmonellosis. Inflammatory response was observed, and reliable tests showed increase in pseudoeosinophils, monocytes and basophils in treatment groups as compared to control group. The impact of developing infection on the leukogram was less marked in the groups of chickens receiving enrofloxacin. The analysis of the leukogram showed that S. enteritidis has a greater effect on the immune system of the chickens as it suppresses the action of polymorphonuclear leukocytes. S. typhimurium has a lesser effect on the immune reactions while retaining its activity and toxicity for a long time.

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. One of the most persistent or recurring diseases in poultry farms is salmonellosis, which accounts for high economic losses relating to high mortality of chickens even at a very young age, low productivity and high cost of diagnosis, treatment and prevention routines [1]. Salmonellosis is caused by different serovars of Salmonella, in poultry the most prevalent serovars are Salmonella typhimurium and Salmonella enteritidis [2]. Very often the prompt detection of infected birds is unlikely due to the fact that infecting with certain serovars such as S. enteritidis is not necessarily accompanied by any clinical symptoms [3]. So some birds may act as asymptomatic carriers, their droppings will spread the disease fast and their meat products will be contaminated [4, 5].

There are quite many different groups of antimicrobial drugs on the market, to be used to treat chickens in poultry farms for salmonellosis. One kind of them, namely fluoroquinolones, are very effective in veterinary medicine against microbial infections and the resultant pathologies [6, 7].
Enrofloxacin, a fluoroquinolone, was originally invented as a means of therapy and prevention in poultry farming. Enrofloxacin is very effective as it has a long-lasting bactericidal action, inhibits the bacterial DNA gyrase and enables phagocytosis [8]. Besides enrofloxacin is known for its ability to penetrate phagocytes and accumulate in them. It is very important as Salmonella is able to persist in macrophages and avoid the action of the immune system [9].

But in spite of its popularity in treatment and its highly valued pharmacodynamics properties we lack knowledge of its impact on blood values. The blood values of chickens give us the immediate evidence of their physiological state following the treatment of bacterial infections. Based on the lack of such knowledge we strove to research the impact of enrofloxacin on blood values of chickens in treatment of experimentally salmonellosis.

2. Object and methods
To achieve the goals mentioned we conducted two experiments (experiment “a” and experiment “b”) on one-day-old male chicks of the Highsex brown cross. Group Ia and Group Ib (control groups) in the course of the experiment received the basic diet and pure drinking water. Group IIa and Group IIb received 200 mg enrofloxacin per l in drinking water 24 hours before the challenge with Salmonella, then each of the following four days after the challenge with Salmonella. On Day 2 of the experiment the chickens of Group Ia and Group Ib were challenged with Salmonella typhimurium, the chickens of Group IIa and Group IIb were challenged with Salmonella enteritidis, by means of intraperitoneal injection of the above-stated culture (3×10³ CFU per 0.5 ml). The blood samples from every chicken were collected with cardiac punctures on Day 1, Day 3, Day 5, Day 7 and Day 9 after the challenge.

The following blood tests were conducted: erythrocyte sedimentation rate (ESR) by Panchenkov’s method; red blood cell count and white blood cell count (computation in Goryaev’s chamber); the colorimetric hematinic method (i.e. Sahli’s acid hematinic method); hematocrit and calculation of color index; eosinophil count, basophil count, pseudoeosinophil count, lymphocyte count, monocyte count in blood smear, stained by Romanovskiy-Giemza stain; and then the content of each kind of leukocytes in the total volume was calculated and the white blood cell differential (i.e. leukogram) was performed.

The statistical analysis of the digital data was performed using SPSS Statistic 17.0 software, reliability of the results was checked with the help of Mann–Whitney nonparametric test.

3. Results
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]. Throughout the experiment we noted reliable changes in blood values of the Highsex brown chickens, which indirectly pointed to developing anaemic process, evidently caused by experimental infection. In the experiment “a” we observed a change in erythrocyte number. On Day 1 there occurred a 20% reliable increase of erythrocytes in blood of Group IIIa as compared to control group, on Day 3 erythrocyte number in the blood of Group IIa increased by 33%. Then erythrocyte number in the blood of both groups decreased reliably: in Group IIa erythrocyte number decreased by 10%, in Group IIIa it decreased by 24%, though on the final day of the experiment there was no reliable difference (table 1).

The increase of erythrocyte number may be caused by S. typhimurium as it is known that erythrocyte number is likely to increase at the onset of a number of microbial infections. Besides birds’ erythrocytes are assumed to perform an immune-like function [10].

In the experiment “b”, Group IIb, erythrocyte number decreased reliably by 20% on the average on Day 3, Day 5, Day 7, in Group IIb erythrocyte number decreased by 16% on Day 5 and by 23% on Day 7. Since the changes in blood of the chickens receiving enrofloxacin in both experiments were less marked generally as compared to control group, we assume that experimental infection was the cause of erythrocytosis.

Erythrocyte sedimentation rate (ESR) was not informative as we noted no reliable changes in our experiment.
The haemoglobin values of Group IIb as compared to control group fell by 20%, 36%, 10%, 22% on days 1, 2, 2, 2 respectively. In Group IIIb haemoglobin decreased by 30%, 53%, 17%, 14% on the same days. Very similar results were obtained in experiment "a", but reliable changes in blood values occur here immediately after the challenge. It is possibly caused by unique characteristics.

The decrease of haemoglobin, taking place in both experiments, revealed anemia. In experiment “b” we noted reliable changes every time except for Day 1 after the challenge (table 2).

**Table 1.** Dynamics of red blood in chickens infected with *Salmonella typhimurium* (*N*=6, *M±m*).

| days | groups | ESR, mm/h | haemoglobin, g/l | erythrocytes, 10¹²/l | color index, c.u. | hematocrit, % |
|------|--------|-----------|------------------|----------------------|------------------|---------------|
| 1    | Ia     | 2.8±0.31  | 107.0±6.46       | 1.44±0.08            | 2.31±0.27        | 32.2±1.95     |
|      | IIa    | 3.0±0.36  | 82.7±2.76        | 1.52±0.09            | 1.65±0.08        | 24.9±0.83     |
|      | IIIa   | 3.3±0.61  | 85.3±2.67        | 1.73±0.04            | 1.48±0.07        | 25.7±0.81     |
|      | IVa    | 2.6±0.21  | 99.0±2.91        | 1.59±0.11            | 1.89±0.09        | 29.8±0.88     |
| 3    | IIa    | 2.3±0.33  | 98.0±4.71        | 2.12±0.12            | 1.41±0.09        | 29.5±1.42     |
|      | IIIa   | 3.1±0.79  | 80.3±3.16        | 1.78±0.09            | 1.37±0.05        | 24.2±0.95     |
|      | IVa    | 3.0±0.26  | 97.3±3.29        | 1.79±0.04            | 1.64±0.07        | 29.3±0.99     |
| 5    | IIa    | 3.0±0.45  | 79.0±3.57        | 1.49±0.06            | 1.61±0.11        | 23.8±1.07     |
|      | IIIa   | 3.0±0.36  | 80.3±2.27        | 1.74±0.07            | 1.39±0.03        | 24.2±0.68     |
|      | IVa    | 2.5±0.34  | 110.0±2.68       | 1.83±0.02            | 1.81±0.04        | 33.1±0.81     |
| 7    | IIa    | 2.5±0.22  | 100.3±3.56       | 1.66±0.02            | 1.82±0.06        | 30.2±1.07     |
|      | IIIa   | 2.5±0.34  | 89.3±6.19        | 1.47±0.03            | 1.82±0.09        | 26.9±1.86     |
|      | IVa    | 2.8±0.31  | 122.3±4.24       | 1.75±0.03            | 2.09±0.08        | 36.8±1.28     |
| 9    | IIa    | 2.8±0.31  | 108.3±2.71       | 1.79±0.06            | 1.82±0.06        | 32.6±0.81     |
|      | IIIa   | 2.0±0.00  | 105.7±2.98       | 1.77±0.08            | 1.80±0.08        | 31.8±0.89     |

*ap<0.01 (Mann–Whitney U-test).  
bp<0.05 (Mann–Whitney U-test).

The haemoglobin values of Group IIb as compared to control group fell by 20%, 36%, 10%, 22% on Day 3, Day 5, Day 7, Day 9 respectively. In Group IIIb haemoglobin decreased by 30%, 53%, 17%, 14% on the same days. Very similar results were obtained in experiment “a”, but reliable changes in blood values occur here immediately after the challenge. It is possibly caused by unique characteristics.

**Table 2.** Dynamics of red blood in chickens infected with *Salmonella enteritidis* (*N*=6, *M±m*).

| days | groups | ESR, mm/h | haemoglobin, g/l | erythrocytes, 10¹²/l | color index, c.u. | hematocrit, % |
|------|--------|-----------|------------------|----------------------|------------------|---------------|
| 1    | Ia     | 2.5±0.34  | 96.7±4.05        | 1.72±0.09            | 1.72±0.14        | 29.1±1.22     |
|      | IIa    | 4.8±2.06  | 102.3±6.54       | 1.81±0.16            | 1.77±0.19        | 30.8±1.97     |
|      | IIIa   | 3.0±0.26  | 88.7±4.15        | 1.68±0.05            | 1.59±0.12        | 26.7±1.25     |
|      | IVa    | 1.8±0.31  | 106.3±6.56       | 1.72±0.07            | 1.86±0.11        | 32.1±1.98     |
| 3    | IIa    | 2.5±0.43  | 88.3±2.61        | 1.64±0.04            | 1.62±0.06        | 26.6±0.78     |
|      | IIIa   | 2.6±0.21  | 81.3±2.29        | 1.49±0.03            | 1.63±0.06        | 24.5±0.69     |
|      | IVa    | 2.3±0.21  | 124.0±4.53       | 1.76±0.04            | 2.12±0.08        | 37.3±1.36     |
| 5    | IIa    | 2.5±0.22  | 90.7±3.68        | 1.51±0.11            | 1.85±0.15        | 27.3±1.11     |
|      | IIIa   | 1.8±0.31  | 81.0±2.91        | 1.55±0.08            | 1.59±0.11        | 24.4±0.88     |
|      | IVa    | 2.6±0.49  | 104.0±1.26       | 1.88±0.07            | 1.67±0.06        | 31.3±0.38     |
| 7    | IIa    | 2.3±0.21  | 94.0±2.31        | 1.52±0.02            | 1.85±0.05        | 28.3±0.69     |
|      | IIIa   | 1.6±0.21  | 88.7±3.68        | 1.54±0.04            | 1.74±0.11        | 26.7±1.11     |
|      | IVa    | 2.5±0.34  | 124.3±3.56       | 1.66±0.03            | 2.25±0.05        | 37.4±1.07     |
| 9    | IIa    | 2.8±0.17  | 101.7±2.75       | 1.75±0.05            | 1.75±0.08        | 30.6±0.83     |
|      | IIIa   | 2.3±0.33  | 108.7±1.43       | 1.82±0.09            | 1.82±0.11        | 32.7±0.43     |

*ap<0.01 (Mann–Whitney U-test).  
bp<0.05 (Mann–Whitney U-test).
of S. typhimurium, which has a more harmful effect on organisms. That can be proved by the fact that hemoglobin decreased reliably by 25%, 23%, 21%, 23%, 21% in Group IIIa throughout the whole experiment. We noted more changes in this group than in Group Ia. Decrease of color index suggests the presence of erythrocytes with low hemoglobin. Nevertheless the changes in Group Ib were noted only on Day 7 and Day 9, and in Group Ia – on Day 1, Day 3, Day 9, that is a less marked reliable change as compared to the data obtained in Groups IIIa and IIIb. In the course of our research we noted decrease of hematocrit in every given treatment group, implying that respiratory function of blood is failing. Remarkably we didn’t get reliable changes in Groups Ib and IIIb only within a very short period of time after the challenge. The change was most pronounced in Group IIIa, where hematocrit decreased throughout the experiment. In Group Ia reliable decreases by 29%, 23%, 12% as compared to control group were noted in blood tests on Day 1, Day 5, Day 9 respectively.

Leukocytes are cells forming an important part of the immune system, tackling foreign bacterial invaders, which in their turn very often cause an increase in leukocyte number. In the two experiments some similar changes occurred in leukocyte number. So in experiment “a” leukocyte number increased reliably by 19% and by 15% as compared to control group on Day 3 in Group IIIa and Group Ia respectively. In experiment “b”, in Group IIIb, leukocyte number increased by 20% as compared to control group only on Day 3 after the challenge. Such transitory increase in leukocytes could be caused by Salmonella activity as Salmonella is capable of penetrating macrophages and bypassing the immune system in this way, that explains why the primary response of the organism was not quite pronounced.

We performed the white blood cell differential (leukogram) and the reliable data obtained in it were more informative. So in studying the chickens, challenged with S. enteritidis, in Group IIIb, we noted significant changes in the relative and absolute numbers of basophils. On Day 1 after the challenge we noted a decrease in basophil number by 65% and then an increase on Day 7 and Day 9 by 67% and by 48% respectively. This ambiguous change – decrease followed by increase – in basophil number was evidently the result of antigen inflow into the organism of the chickens. The reliable changes in basophil number in groups challenged with S. typhimurium were not so pronounced but they are different from the changes in the second experiment. The relative and absolute numbers of basophils increased, but in Group Ia such increase was noted only on Day 1 after the challenge, by 55% on average, unlike Group IIIa where the values as compared to control group rose on Day 1 and on Day 3 by 55% and 57% respectively (table 3).

In the conducted experiments we noted eosinopenia which was rather pronounced in male chicks challenged with S. enteritidis, practically on each day of the experiment (table 4). So the chicks in Group IIIb had the most protracted reliable changes: the absolute and relative numbers of eosinophils decreased almost immediately on the day following the challenge, the values read 53% and 61% respectively, and they continued to decrease every day afterwards. Such a change directly reflected the emerging infectious process in the organism, caused by S. enteritidis.

Pronounced reliable decrease in lymphocyte number was noted in both experiments, and a significant difference in the relative and absolute numbers of lymphocytes was noted in chickens, which had been challenged with Salmonella but were not fed enrofloxacin. Lymphopenia might be caused by the developing immune deficiency and blocked lymphopoiesis, resulting from Salmonella infection.

The reliable decrease of lymphocyte number in Group Ia occurred only on Day 1 after the challenge; the absolute and relative numbers of lymphocytes fell by 20% and 32%, in Group Ib lymphocyte number reliably decreased by 29% as compared to control group on Day 5. It is a well-known fact that in birds’ lymphopenia is relative, and usually it is associated with increasing number of polymorphonuclear leukocytes.

The reliable changes in the absolute number of pseudoeosinophils in both experiments (in chickens receiving enrofloxacin) are less pronounced. In Group Ia the increase by 15% as compared to control group was observed on the final day of the experiment, in Group Ib the increase occurred only on Day 5. In Group IIIa the increase in the absolute number of pseudoeosinophils was observed throughout the experiment.
Table 3. Dynamics of absolute values blood leukogram of chickens infected with *Salmonella typhimurium* (*N*=6, *M*=m), 10^7·1−1.

| days | groups | monocytes | lymphocytes | eosinophils | pseudoeosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| Ia   | 0.46±0.08 | 13.1±1.13 | 2.95±0.57 | 16.1±1.57 | 0.39±0.05 |
| Ila  | 3.97±1.36a | 10.43±0.74b | 2.79±0.54 | 20.5±0.99 | 1.01±0.13a |
| IIIa | 1.57±0.41a | 7.91±0.97a | 3.17±0.48 | 18.8±1.64 | 0.86±0.12a |
| Ia   | 0.81±0.29 | 12.0±1.41 | 2.09±0.21 | 14.5±0.71 | 1.29±0.36 |
| Ila  | 0.54±0.14 | 14.0±0.91 | 1.23±0.23b | 17.8±1.16 | 1.03±0.18 |
| IIIa | 5.57±0.43b | 7.97±0.61b | 0.78±0.21a | 21.9±0.99a | 1.48±0.31 |
| Ia   | 0.31±0.01 | 11.9±0.52 | 1.23±0.23b | 16.8±1.34 | 0.46±0.06 |
| Ila  | 0.61±0.12a | 14.6±1.24 | 1.46±0.42 | 19.3±1.19 | 0.36±0.01 |
| IIIa | 2.46±0.52a | 6.14±0.63a | 1.08±0.29 | 23.9±1.48b | 1.12±0.21a |
| Ia   | 0.32±0.06 | 14.7±1.02 | 0.61±0.14 | 13.1±0.47 | 0.27±0.01 |
| Ila  | 0.43±0.06 | 16.4±0.85 | 0.64±0.16 | 14.2±0.63 | 0.33±0.01 |
| IIIa | 3.31±0.71a | 8.35±0.74a | 0.37±0.05 | 19.1±1.09a | 0.54±0.17 |
| Ia   | 0.78±0.29 | 13.4±1.08 | 0.93±0.11 | 16.5±1.01 | 0.39±0.07 |
| Ila  | 0.33±0.01 | 12.1±0.54 | 1.74±0.41 | 19.5±0.97b | 0.33±0.01 |
| IIIa | 2.54±0.99 | 10.3±1.35 | 0.59±0.13 | 20.9±1.02a | 0.67±0.21 |

*a*p<0.01 (Mann–Whitney U-test).

*b*p<0.05 (Mann–Whitney U-test).

Pseudoeosinophilia of the chicks in Group IIIb did not last so long. The absolute number of pseudoeosinophils increased only on Day 1, Day 3, Day 5 of the experiment by 38%, 39%, 31% respectively as compared to control group. Such an effect is evidently associated with intensive suppression of phagocytosis by S. enteritidis which inhibited the primary reaction of the immune system, started by pseudoeosinophils and that activated macrophages. This is confirmed by reliable long-lasting monocytosis, observed first on Day 3 and then till the end of the experiment.

Table 4. Dynamics of blood leukograms of chickens infected with *Salmonella enteritidis* (*N*=6, *M*=m), %.

| days | groups | monocytes | lymphocytes | eosinophils | pseudoeosinophils | basophils |
|------|--------|-----------|-------------|-------------|-------------------|-----------|
| Ia   | 1.5±0.22 | 39.2±3.55 | 2.2±0.39 | 13.8±0.95 | 1.7±0.32 |
| Ila  | 6.7±1.73a | 31.7±3.23 | 0.8±0.31b | 18.8±1.93 | 0.9±0.44 |
| IIIa | 2.5±0.62 | 28.2±2.57b | 1.0±0.33b | 22.3±0.95a | 0.6±0.17b |
| Ia   | 1.9±0.31 | 40.8±1.19 | 1.7±0.38 | 49.5±1.84 | 0.8±0.13 |
| Ila  | 2.2±0.61 | 34.3±2.67 | 2.2±0.37 | 55.0±4.06 | 0.8±0.12 |
| IIIa | 11.2±2.82a | 20.5±3.48b | 0.8±0.15b | 64.0±5.75 | 0.9±0.34 |
| Ia   | 2.0±0.52 | 50.2±3.24 | 3.3±0.33 | 43.3±3.59 | 0.3±0.05 |
| Ila  | 2.8±0.61 | 35.2±2.71a | 3.2±0.83 | 57.3±2.69a | 0.5±0.11 |
| IIIa | 12.0±3.32a | 16.5±1.78a | 1.7±0.42b | 68.1±3.58a | 0.5±0.12 |
| Ia   | 2.0±0.82 | 41.0±2.49 | 2.3±0.76 | 53.5±2.21 | 1.2±0.17 |
| Ila  | 2.1±0.41 | 42.0±3.09 | 2.2±0.48 | 52.5±2.75 | 1.2±0.17 |
| IIIa | 6.3±1.49b | 31.7±3.17 | 1.2±0.17 | 57.3±3.76 | 3.5±0.56b |
| Ia   | 1.6±0.49 | 32.7±1.86 | 6.5±0.34 | 58.0±1.75 | 1.2±0.17 |
| Ila  | 1.2±0.17 | 31.3±1.02 | 7.4±0.81 | 58.8±1.05 | 1.3±0.21 |
| IIIa | 9.7±3.13ab | 21.7±2.63a | 1.8±0.31a | 64.5±1.91 | 2.3±0.42b |

*a*p<0.01 (Mann–Whitney U-test).

*b*p<0.05 (Mann–Whitney U-test).
In Group IIIa the monocyte number increased on Day 1, Day 3, Day 5, Day 7 after the challenge as compared to control group, but then it reaches the normal value. Such monocytosis could be considered a “monocytic protective phase”, i.e. the phase of fighting the infection [1].

4. Conclusion
The studies we conducted show that enrofloxacin is effective against the two serotypes of Salmonella, but at the same time it does not have a substantial toxic effect on leukogram (WBC differential) of birds. In the course of both experiments in the chickens, receiving fluoroquinolone, the suppression of bacterial infection was more pronounced. It is probable that enrofloxacin except its immediate action on bacteria interacts with cellular nonspecific immunity system and enhances its efficacy. This hypothesis is supported by the data obtained in both experiments on chickens in Groups III, whose reliable changes in leukogram were significant and long-lasting. Analyzed duration of changes registered in the leukogram of these chickens suggests that S. enteritidis suppresses the action of pseudoeosinophils and has a greater effect on immune system of birds. S. typhimurium in its impact on the organism suppresses the immune reactions in a lesser degree, though it retains its active action for a longer time, at the same time providing a toxic effect.

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