Efficacy of Sarafloxacin in Broilers after Experimental Infection with *Escherichia coli*

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**ABSTRACT**

Infections of chickens with *Escherichia coli* serotype O78 can be treated with the antibiotic sarafloxacin. Three experiments were conducted on the administration of this drug to chickens that had been experimentally infected with *E. coli*. The birds were monitored for 10 days after infection for their average daily gain (ADG) and feed conversion ratio (FCR), and the post-mortem pathology was assessed. In the first experiment, sarafloxacin (20 mg/L, equivalent to 5 mg/kg live weight per day), given in the drinking water for 3 days after infection, led to a reduction in the mortality from 75% to 27%, but the ADG of the treated birds was still less than that of the uninfected controls. In the second experiment, when the sarafloxacin was administered at the same dose in the water but over only 2 h, there was also a considerable reduction in mortality, and the ADG and the FCR also improved significantly. In the third experiment, the dose dependence of the drug was tested. The birds were given 5 and 10 mg/kg per day sarafloxacin in each group, starting within 2 h after infection. This rapid administration of the drug completely prevented mortality, while the ADG and FCR were similar to those of the uninfected controls.

**Keywords:** chickens, *Escherichia coli*, fluoroquinolone, live weight, mortality, sarafloxin

**Abbreviations:** FCR, feed conversion ratio; ADG, average daily gain

**INTRODUCTION**

Broiler chickens are frequently infected with *Escherichia coli*, which often results in disease and hence in economic losses. Poultry of all ages are susceptible to infections with *E. coli*, but the most affected are birds of 4 to 5 weeks old (Chansiripornchai et al., 1995). The major clinical signs of *E. coli* infection range from subacute fibrinopurulence in chickens 2–8 weeks of age to an acute septicaemia in younger birds, that can cause sudden death (Leitnes and Heller, 1992). Infections with *E. coli* are usually secondary infections that are triggered by diseases such as Newcastle disease, infectious bronchitis, infectious laryngotracheitis, or mycoplasmosis. Infections of chickens with *E. coli* can be prevented and controlled by antibiotics. However, a serious drawback of the prophylactic use of such chemotherapy is the development of
resistance in avian *E. coli* to most antibiotics (Chansiripornchai et al., 1995). Genes that are located on plasmids often encode resistance to antibiotics. These plasmids easily spread through bacterial populations, which leads to the spread of resistance, so rendering the drugs ineffective. One group of antibiotics, the fluoroquinolones, is less likely to undergo this fate, because they inhibit the synthesis of DNA gyrase A, which is essential for duplication of plasmids (Glisson, 1994; Chansiripornchai et al., 1995). Fluoroquinolones are broad-spectrum drugs with a bactericidal activity and their use for the prevention and control of avian infections with *E. coli* has increased. A recently introduced member of the fluoroquinolone group of antibiotics is sarafloxacin. The chemical structure of this third-generation fluoroquinolone is similar to that of norfloxacin, the most commonly used fluoroquinolone. Joong Kim (1995) reported that sarafloxacin in the drinking water of broilers that were challenged with *E. coli* reduced the mortality and improved the feed conversion ratio (FCR) and average daily weight gain (ADG). The aim of our studies was to define the optimal dosage of sarafloxacin to control *E. coli* infections in experimentally infected broilers.

**MATERIALS AND METHODS**

*Chickens*

Unvaccinated Arbor Acres broiler type chicks of mixed sexes were obtained on the day of hatching from a commercial hatchery. The chickens were fed *ad libitum* before and during the experiments. At the onset of the experiments, there was no statistically significant difference in average weight between the experimental groups. The chickens in experiments 1, 2 and 3 were brought from different batches of the same hatchery, so that there might be differences among the chickens in each experiment.

*Bacterial strain*

The chickens were challenged with an *E. coli* strain of serotype O78 that was originally isolated from the diseased air sacs of a chick with a field case of colisepticaemia. The challenge material was a logarithmic-phase culture produced by 10 h of static incubation of *E. coli* in nutrient broth. Sekizaki and colleagues (1989) showed that this strain of *E. coli* produced high mortality in a very short time. In the first and second experiments, we injected 0.5 ml of the suspension of *E. coli*, containing 10⁷ cfu/ml, into each bird. In the third experiment, 1 ml of *E. coli* (approximately 10⁷ cfu/ml) was injected into each bird. The suspensions of *E. coli* were injected into the left caudal thoracic air sac of the chickens.
Medication

Sarafloxacin (Floxsol, Solvay Duphar, Olst, The Netherlands) was administered to the chickens in their drinking water at the concentrations specified in the experimental designs. The water intake of the birds had been measured before the experiments commenced, so the approximate water intake of the chickens was known and the quantity of the drug to be dissolved in the water to give a known average intake of drug per kg live weight of the chickens could be calculated. In the first experiment, the birds had access to the drug dissolved in the water for 24 h. After 24 h, there was only a small volume of medicated water left (< 100 ml), so the birds had received almost all the drug dissolved in the water. For the second and third experiments, the water was withdrawn for half an hour before giving the medicated water. The withdrawal period resulted in the birds being sufficiently thirsty to consume all the medicated water within 2 or 3 h, in the respective experiments. Unmedicated water was substituted for the medicated water immediately after all the medicated water had been drunk. The water was changed daily.

Experimental designs

The age of the chickens in the first and second experiments and the dosage of *E. coli* they received differed from those of the chickens in the third experiment. Consequently, there were positive control groups in all the experiments in order to allow comparison of the data within each experiment. The ADG, FCR, and mortality were recorded for 10 days following infection of the chickens with *E. coli*. The pathological lesions in the dead chickens were investigated at necropsy. After 10 days, the surviving chickens were killed and *E. coli* was isolated from their livers and identified by standard culture media and biochemical tests. The unpaired Student’s *t*-test was used for statistical comparison of the groups. The FCR and ADG were calculated from the formulae FCR = feed intake (g)/average weight gain (g) and ADG = weight gain (g)/days.

Experiment 1

One hundred and eighty, 18-day-old broilers were divided into three groups of 60 birds, with each group subdivided into three replicates of 20 birds. The broilers were killed at 28 days of age. Group 1 was challenged with *E. coli* and received drinking water containing 20 mg/L of sarafloxacin (equivalent to an average of 5 mg/kg live weight per day) for the first 3 days after infection. Group 2 was challenged with *E. coli* but did not receive sarafloxacin. Group 3 was not challenged with *E. coli* and did not receive sarafloxacin.
Experiment 2

The aim was to study the efficacy of administering saraflloxacin in the drinking water for a limited time to chickens that were challenged with *E. coli*.

Seventy-eight, 18-day-old broilers were divided into groups 1 and 2 of 42 and 36 birds, respectively. The groups were subdivided into three replicates of 14 and 12 birds, respectively. The broilers were killed at 28 days of age. Group 1 was challenged with *E. coli* and received saraflloxacin at 20 mg/L per day for 3 days, starting within 2 h of challenge and available for 2 h. Group 2 was challenged with *E. coli* but did not receive saraflloxacin.

Experiment 3

The aim was to study the dose dependence of saraflloxacin administration in chickens that were challenged with *E. coli*.

Sixty-four broilers of 40 days of age were divided into four groups of 16 birds each. They were killed at 48 days of age. Group 1 was challenged with *E. coli* and received saraflloxacin at 20 mg/L per day for 3 days, starting within 2 h of challenge and available for 3 h. Group 2 was challenged with *E. coli* and received saraflloxacin at 40 mg/L, equivalent to 10 mg/kg live weight per day for 3 days, starting within 2 h of challenge and available for 3 h. Group 3 was challenged with *E. coli* but did not receive saraflloxacin. Group 4 was neither challenged with *E. coli* nor received saraflloxacin.

RESULTS

Experiment 1

The mortality in the chickens that were treated with sarafloxin after challenge with *E. coli* was significantly lower than that in the group that was challenged but not treated with the drug (Table 1). The greatest mortality occurred on the first day after challenge. The chickens that died within one day after challenge showed only a mild degree of air sacculitis, but typical lesions of *E. coli* infection – air sacculitis, fibrinopurulent pericarditis and peritonitis – were clearly present in the chickens that died after the first day after challenge. These typical clinical lesions were similar in the sarafloxacin-treated group and the untreated group, but fibrinopurulent perihepatitis was less evident in the treated group. Also, the number of bacteria in the livers of the treated group was less than that for the untreated birds (*p* < 0.05) (Table 1).

Although treatment of the challenged chickens with saraflloxacin reduced the mortality, the ADG of the treated chickens was significantly lower and their FCR was significantly higher than those of the birds in the negative control group. The ADG and FCR of the chickens in the positive control group could not be calculated because too many of them died during the experiment.
TABLE I

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sarafloxacin</th>
<th>E. coli</th>
<th>ADG ± SD (g)</th>
<th>FCR ± SD</th>
<th>Mortality</th>
<th>Air sacculitis (%)</th>
<th>Pericarditis (%)</th>
<th>Peritonitis (%)</th>
<th>Perihepatitis (%)</th>
<th>Culture from the liver ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>119 ± 64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 ± 12&lt;sup&gt;e&lt;/sup&gt;</td>
<td>27</td>
<td>36/44</td>
<td>22/44</td>
<td>24/44</td>
<td>11/44</td>
<td>6/44</td>
</tr>
<tr>
<td></td>
<td>-  +</td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>75</td>
<td>13/15</td>
<td>10/15</td>
<td>9/15</td>
<td>8/15</td>
<td>9/15</td>
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<td></td>
<td>-  -</td>
<td></td>
<td>537 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>8/30</td>
<td>0/30</td>
<td>0/30</td>
<td>0/30</td>
<td>0/30</td>
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<tr>
<td>2</td>
<td>+  +</td>
<td></td>
<td>409 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.1 ± 0.2&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5</td>
<td>11/12</td>
<td>2/12</td>
<td>1/12</td>
<td>5/12</td>
<td>7/12</td>
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<td></td>
<td>-  +</td>
<td></td>
<td>57 ± 9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37.9 ± 25.4&lt;sup&gt;h&lt;/sup&gt;</td>
<td>33</td>
<td>12/12</td>
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<td>7/12</td>
<td>10/12</td>
<td>10/12</td>
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<td>3</td>
<td>+  + (5 mg)</td>
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<td>250.6</td>
<td>2.9</td>
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<tr>
<td></td>
<td>+  10 mg</td>
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<td>263.8</td>
<td>2.8</td>
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<td>56</td>
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<td></td>
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<td></td>
<td>291.8</td>
<td>2.8</td>
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</table>

NR, not relevant in view of the high mortality

<sup>a,b</sup>Different superscripts indicate statistically significant differences (p < 0.05) in each experiment
Experiment 2

The mortality in the chickens that were treated with sarafloxacin after challenge with *E. coli* was significantly lower than that in the group that was challenged but not treated with the drug (Table I). The pathology observed post mortem was similar to that in experiment 1. The numbers of bacteria cultured from the livers of the chickens in the sarafloxacin-treated group was lower than from the untreated group ($p<0.05$). The ADG of the sarafloxacin-treated group was higher and the FCR was lower than those of the untreated group ($p<0.05$).

Experiment 3

No mortality had occurred by 8 days after challenge in the two groups that were treated with 5 or 10 mg/kg per day of sarafloxacin, while the group that was not treated with sarafloxacin had a mortality of 56%. The pathology seen post mortem was similar to that in experiments 1 and 2, except that there was less haemorrhage at the duodenal loop in the chickens that were treated with sarafloxacin. The ADG of the sarafloxacin-treated chickens was only slightly lower than that of the unchallenged birds and the FCRs in the two groups were similar. The ADG and FCR of the challenged but untreated group could not be calculated because of the high mortality.

DISCUSSION

The ADG and FCR both improved significantly as a result of the sarafloxacin treatment in all three experiments, although those of the treated birds did not attain the levels in the unchallenged controls (Table I). The ADG and FCR in the infected and treated birds in the first experiment were not as good as those in experiments 2 and 3. An unlimited time for consuming a drug can reduce its efficacy. Thus, Prabhavathi and colleagues (1986) gave sarafloxacin at 4 times the minimum inhibitory concentration to experimental mice and found that the highest efficacy against *E. coli* (99.9%) occurred within 2 h of giving the drug. The efficacy of the drug appeared to be reduced when the time was over which it was available was prolonged. The chickens in experiment 1 had access to the drug in solution for more than 2 h each day for 3 days and a lower average daily gain and higher mortality were found than in the experiments in which the birds had limited time in which to drink the solution of the drug. In the third experiment, there were no statistically significant differences in the mortality rate or FCR of the chickens in the groups that had received 5 or 10 mg/kg per day of the drug.

However, administration of sarafloxacin significantly reduced the mortality of chickens that were challenged with *E. coli* serotype O78 ($p<0.05$) in all the experiments. This finding is in agreement with the data published by McCabe and Rippel (1993) and by Joong Kim (1995) on sarafloxacin treatment of *E. coli*-infected chickens. Piercy and West (1976) reported that injection of *E. coli* into the air sacs did not differ
from other routes of challenge apart from tending to give a shorter incubation period for the colibacillosis (Harry, 1964). Gross (1991) found air sacculitis within 1 h after challenge, which was supported by the necropsy findings in the present study. There were differences in the mortality of chickens in the positive control groups in all the experiments. This might have come about because commercial birds were used in these studies, so that their microbiological and/or immunological status was uncertain, and may have affected to the infections. The other lesions seen, including fibrinopurulent pericarditis, fibrinopurulent perihepatitis and fibrinopurulent peritonitis, were similar to those described by Sasipreeyajan and Pakpinyo (1992). A low level of air sacculitis was found in the unchallenged chickens, but no other lesions and no E. coli were found. The air sacculitis in these birds may be attributed to a high ammonia level in the experimental room without E. coli infection. A high ammonia level causes irritation of the respiratory tract (Chansiripornchai et al., 1995), which may induce bacterial infections from the environment (Whiteman and Bickford, 1983).

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