Concentration of Enrofloxacin Residue from Tilapia (Oreochromis niloticus) Muscular That Infected by Aeromonas salmonicida

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Abstract: Aim of this research was to find out the concentration of enrofloxacin residue in tilapia meat for several weeks after antibiotic treatment. Twenty seven tilapia fishes were divided into three groups. The first group was not infected and treated, the second group was infected with A. salmonicida subsp. smithia and the third group was infected with A. salmonicida subsp. achromogenes intramuscularly. Six days after infection, treatment was carried out using Baytril® administered orally for the second group and intramuscularly for the third group during five days. At the 1st, 4th and 8th week after the treatment, Three fish were taken from each group to be analyzed for its concentration of enrofloxacin residue by diffusion on Mueller Hinton Agar (MHA) method and quantitatively using high performance liquid chromatography (HPLC) method. The MHA test showed the formation of inhibition zone, at the 1st week and 4th week after the treatment, while at 8th week after treatment did not show inhibition zone. The HPLC test on enrofloxacin residual concentration in tilapia infected with A. salmonicida subsp. smithia (second group) at the 1st, 4th and 8th week after treatment showed the average of 33.0, 6.10 and 0.0021 µg/g of enrofloxacin residue level. While in tilapia infected with A. salmonicida subsp. achromogenes and treated with enrofloxacin intramuscularly (third group) showed the average of residue level 35.79, 2.18 and 0.00065 µg/g. In conclusion, the residue of enrofloxacin was still high concentration until the fourth week after treatment in the second and third groups. Based on Indonesian National Standards and Rules, the maximum limit of enrofloxacin residue is 0.01 µg/g. The concentration of enrofloxacin residue was very low and the concentration of enrofloxacin residue collected from tilapia using orally and intramuscularly method of treatment was not different.

Key words: Residue, enrofloxacin, Aeromonas salmonicida subsp. smithia, subsp. achromogenes.

1. Introduction

Aeromonas salmonicida is a pathogenic bacterium in fish industry that cause furunculosis disease or ulcerative furunculosis in salmonid aquaculture intensification. It is indicated that non-salmonid fish which live in freshwater, brackish water or sea are highly vulnerable to the infection of these bacteria [1]. Fish have lower heart rate and the cardiac output is smaller, so the tissue gets less blood supply than mammals [2].

One of ways to treat bacterial infection in aquaculture is antibiotics. Infected fish treated with antibiotics have different pharmacokinetics and withdrawal time compared to healthy fish [3]. The result from treatment using enrofloxacin in healthy tilapia which were mixed with fisheries feed with excessive dose of antibiotic (50 mg/kg body weight) for 7 d showed 3.61 µg/g as maximum enrofloxacin residue level in fish muscle and the estimated withdrawal time was 22 d [4]. The usage of enrofloxacin intensively in aquaculture treatment resulted in emerging issue of enrofloxacin in food and

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bacterial resistance to enrofloxacin in animal. Residual analysis in animal origin food are very important because it gives critical information about residue level in tissue after treatment and also gives information about the right and safe time to consume animal origin food.

This research’s goal is to determine enrofloxacin residue level after treatment orally and intramuscularly in tilapia meat infected with *A. salmonicida*.

2. Materials and Methods

This research was done experimentally. About 27 tilapias with approximately 50 gram of body weight were adapted for 7 d and were divided into three different groups. The first group, used as control group, consisted of nine fish without treatment. The second group consisted of nine fishes infected with 0.1 mL of concentration of $10^6$ cell/mL *A. salmonicida* subsp. smithia. The third group was infected intramuscularly with *A. salmonicida* subsp. *achromogenes*. Both subspecies of *Aeromonas salmonicida* were already identified for its morphological and biochemical natures in Department of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University.

Six days after infection, the second and third group was treated with Baytril® given orally for group two and intramuscularly for group three during five days with 10 mg/kg body weight of therapeutic dosage. In the 1st, 4th and 8th week after the treatment, three fishes were analysed for enrofloxacin residue using diffusion method on Mueller Hinton Agar (MHA) and quantitative test by high performance liquid chromatography (HPLC) method.

2.1 Residue Test with Diffusion Kier by Bauer Method

Fish were killed in the 1st, 4th and 8th week after treatment. Fresh tilapia meat about ± 1 g were cut and mashed with mortar, then added 1 mL of phosfat buffer saline (PBS) and put into centrifuge tube and centrifuged for 10 min. 50 µL of supernatant were taken for Kierby Bauer disk diffusion test. Subcultures of *A. salmonicida* subsp. *smithia* and *A. salmonicida* subsp. *achromogenes* were streaked onto MHA plates using a sterile cotton swab.

The disc containing extracted meat which had been treated with enrofloxacin was put on the surface of MHA plate, and then incubated in 30 °C for 20 h. The bacterial growth zone was measured for its inhibition zone diameter.

2.2 Quantitative Test Using HPLC

The results from linearity test, the curve of relationship between pure enrofloxacin level and chromatogram square area resulted in linear regression equation $y = 147855x + 521724$ with correlation coefficient variable was 0.99, while the linear curve between enrofloxacin residue level in tilapia meat and chromatogram square area resulted in linear regression equation $y = 31014x + 95173$ with correlation coefficient was 0.99. Axis line or $x$ was a chromatogram square area.

Based on calculation, the limit of detection (LOD) was 0.00001 µg/mL, and the limit of quantification (LOQ) was 0.001 µg/mL. The antibiotic residue level was performed by modification method according to Ref. [5].

Fish meat were put into centrifuge tube, added with 12.5 mL of 1% anhydrate acetate acid in acetonitrile, then were agitated for 5 min. Samples were separated with its supernatant and evaporated. After it had been dried, then 1.5 mL of phosphate buffer with pH 7.4 and 1 mL of hexane were added to the tube. The mixtures then were reagitated and centrifuged in 3,000 rpm for 10 min. The supernatant was thrown away. Suspension containing residue was analyzed with HPLC. Citrate acid liquid (0.126%): asetonitrile: metanol were used as the mobile phase with ratio 6:3:1; flow rate: 1 mL/min, detector: UV, 270 nm, and oven degree: 30 °C.

The observation results of inhibition zone diameter
Concentration of Enrofloxacin Residue from Tilapia (*Oreochromis niloticus*) Muscular That Infected by *Aeromonas salmonicida*

formed on MHA and residue level detected by HPLC were analyzed descriptively, while residue resulted from different treatment route were analyzed with Mann Whitney test.

3. Results and Discussion

3.1 Diffusion Test on Mueller Hinton Agar

Residue test by MHA at the 1st week and 4th week after the treatment showed the formation of inhibition zone. However, samples on the 8th week after treatment showed no inhibition zone. The result of inhibition zone from fish meat samples on the 1st, 4th and 8th week could be seen in Fig. 1.

The results of measurement of inhibition zone could be seen in Table 1.

The diameter of inhibition zone on 1st week compared to 4th and 8th weeks after treatment showed a decreased in inhibition zone diameter which resulted in the decreased of residue level in fish meat. In 8th week after treatment, no inhibition zone was formed. It means that the residual concentration of enrofloxacin in tilapia meat was already under the minimum inhibitory concentration (MIC) against *A. salmonicida*.

3.2 Quantitative Test of Tilapia Meat Treated with Enrofloxacin Orally and Intramuscularly against *Aeromonas salmonicida* at the 1st, 4th and 8th Week

Enrofloxacin residue test results with HPLC on tilapia meat infected with *A. salmonicida* could be seen in Table 2.

The average of HPLC test of enrofloxacin residues in tilapia meat infected with *A. salmonicida* subsp. *smithia* and treated orally at the 1st, 4th and 8th week post treatment were 33.0 mg/g, 6.10 µg/g and 0.0007 µg/g, while enrofloxacin residue level in tilapia meat was below the detection limit.

### Table 1 Measurement results of inhibition zone diameter of enrofloxacin residue on MHA from tilapia meat infected with *A. salmonicida* on the 1st, 4th and 8th week after treatment.

<table>
<thead>
<tr>
<th>Infection enrofloxacin application</th>
<th>Code</th>
<th>Inhibition zone diameters (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st week</td>
</tr>
<tr>
<td><em>A. salmonicida</em> Per oral</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td><em>A. salmonicida</em> Intramuscular</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22</td>
</tr>
</tbody>
</table>

### Table 2 Enrofloxacin residue test results with HPLC.

<table>
<thead>
<tr>
<th>Enrofloxacin residues</th>
<th>Group</th>
<th>1st week (µg/g)</th>
<th>4th week (µg/g)</th>
<th>8th week (µg/g)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.01</td>
<td>2.49</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>32.27</td>
<td>2.3</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66.72</td>
<td>13.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>33.00</td>
<td>6.10</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>2.56</td>
<td>0.00065</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.73</td>
<td>2.00</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>93.4</td>
<td>1.99</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>35.79</td>
<td>2.18</td>
<td>0.0003</td>
<td></td>
</tr>
</tbody>
</table>

ND: not detected.
Concentration of Enrofloxacin Residue from Tilapia (Oreochromis niloticus) Muscular That Infected by Aeromonas salmonicida

infected with *A. salmonicida* subsp. *achromogenes* and treated intramuscularly showed residues level of 35.79, 2.18 and 0.00065 µg/g. The overall residues of enrofloxacin found in tilapia’s meat after one week and four weeks post treatment exceed the enrofloxacin maximum residue limit according to the provisions of the Indonesian national standards at 0.01 µg/g.

Enrofloxacin as one of the antibiotics used in aquaculture is widely distributed in tissues and body fluids. The end of the elimination phase of the drug depends on the formulation, dosage, route of administration and duration of administration [3]. Liver, kidney and gill are organs that metabolize and excrete the drug in the fish body [6, 7]. Any bacterial infection that causes disturbance in those organs will affect the drug levels in tissue and cell.

Enrofloxacin in fish had longer half-life compared to mammals. This happens because of the differences in physiological blood flow, membrane permeability and muscle composition. Fish have lower heart rate and the cardiac output is smaller so the tissue gets less blood supply than mammals. Besides, freshwater fish also have low glomerular filtration rate [2]. Administration of enrofloxacin mixing with feed at excessive dose of 50 mg/kg of body weight for 7 d to tilapia could cause withdrawal time on 22 d [8]. The results of this study indicated that fish which were infected with *A. salmonicida* and then treated with enrofloxacin with a therapeutic dose of 10 mg/kg of body weight for 5 d given orally or intramuscularly resulted in high residue level in fish meat, so it is best to extend the withdrawal time of enrofloxacin treatment, and it is also important to look for another alternative therapeutic dose that does not result in high residues level in fish meat.

There was no significant difference of enrofloxacin residues resultant 1st week and 4th week post treatment, from different administration route, orally and intramuscularly, although they were infected with different bacteria, *A. salmonicida* subspecies *smithia* and *achromogenes*.

In overall, the average enrofloxacin residue found in tilapia meat with oral administration route was higher than intramuscular administration route, especially at the 4th week and 8th week post treatment. Antibiotics which were administered orally, some of them will be metabolized by enzymes in the intestine wall and liver in the first route through these organs. This metabolism is called metabolism or first-pass elimination or parasytemic elimination. The bioavailability of the drug will be significantly reduced (usually called first-pass effect). The decrease in bioavailability is influenced by anatomical factor, physiological and pathological factor. The first elimination of this route can be avoided or reduced by parenteral administration [8]. Pharmacokinetics of intramuscular or oral administration of enrofloxacin in *Cyprinus carpio* at 10 mg/kg dosage of body weight for five days, showed a difference in half-life and volume of enrofloxacin distribution for intramuscular and oral administration. The difference in half life for intramuscular administration and oral administration were 17.9 h, 16.6 h and the volume distribution were 3.1l/kg and 1.5l/kg. This indicates that *A. salmonicida* infection could change the pharmacokinetic profile of enrofloxacin administration.

The results of this study indicated that tilapia meat which had been infected with *A. salmonicida* and then treated with enrofloxacin given orally or intramuscularly could be safely consumed after eight weeks post treatment, because it contained residue level below the provisions according to Indonesian national standards.

4. Conclusions

Enrofloxacin residue test with diffusion on MHA at the 1st week, 4th week post treatment showed inhibition zone, while at the 8th week after treatment showed no inhibition zone.

Quantitative results of enrofloxacin residue concentration in tilapia meat infected with *A.
Concentration of Enrofloxacin Residue from Tilapia (*Oreochromis niloticus*) Muscular That Infected by *Aeromonas salmonicida*

Enrofloxacin treatment orally had higher average residue level than that of the intramuscularly in the 1st and 4th week post treatment.

It was safe for consumption after eight weeks post treatment.

References


