Water replacement to reduce tetracycline residue level in the liver of tilapia (*Oreochromis* sp.)

W Pawestri¹, N Hakimah²,⁴ and M J Pangestika³

¹Departement of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia
²Polytechnic of Marine and Fishery, Sidoarjo, Indonesia
³PDHB drh. Cucu K. Sajuthi, Jakarta, Indonesia
⁴Corresponding author: wari.pawestri@staff.uns.ac.id

Abstract. Tetracycline is an antibiotic that frequently used to treat various bacterial infections. However, the residue of the antibiotic are often found in food of animal origin, including fish and can be hazardous if consumed by human. This study aimed to determine the effect of water replacement on the residual levels of tetracycline in the liver of tilapia after therapy. Fifty four tilapia (*Oreochromis* sp.) were divided into 3 groups, namely group A, B, and C. Group A as a negative control was injected with 0.25 ml NaCl 0.9% without water replacement, group B as a positive control was injected with 0.25 ml tetracycline (50 mg/kg) without water replacement, and group C was injected with 0.25 ml tetracycline (50 mg/kg) with 25% water replacement twice a day for 6 days. Subsequently, 3 tilapias were euthanized from each group daily with cranial concussion. Samples of the liver were taken. Liver were analyzed by HPLC for tetracycline residual level and analyzed with Two Way ANOVA. The results showed that there were significant differences in residual level between groups and day. It can be concluded that 25% water replacement can decrease residual level in the tilapian liver.

1. Introduction

Fish is a major source of food and income globally. Tilapia is favored among aquaculturists due to its ability to tolerate a wide range of environmental conditions and fast growth [1]. Unfortunately, intensive fish farming is associated with a number of challenges including diseases. Bacterial pathogens are a great threat to fish production worldwide due to the high economic importance of diseases they cause. Bacteria of particular importance in fresh water fish include *Streptococcus* spp., *Aeromonas* spp., *Flavobacterium* spp., *Edwardsiella* spp., *Pseudomonas* spp., *Vibrio* spp., and *Mycobacterium* spp. [2].

Tetracycline is an antibiotic that is widely used for treatment in animals including fish. Tetracyclines are often used in fish cultivation to treat bacterial diseases [3]. Tetracyclines are widely used because of their antibacterial activity as broad spectrum. Indiscriminate use of antibiotic could lead to undesirable deposition of their residues in edible tissues which could hamper public health to some extents. Antibiotic residue transferred to humans through food can also alter the intestinal ecology thereby favoring the emergence of resistant microflora [4]. [5] reported the presence of tetracycline residues in *Clarias gariepinus*. As much as 69% of fish contained tetracycline residues.

The maximum residue limit (MRL) of tetracycline in liver of fish is 600 µg/kg (0.6 µg/g) [6]. MRL is defined as the maximum concentration of residue resulting from use of a veterinary drug to be legally permitted or recognized as acceptable in or on food. With the increasing human consumption of aquaculture products, the probability of exposure to potentially ocuring antimicrobial resistant bacteria,
resistance genes, or antimicrobial residues is increasing. It is necessary to increase fish farmers and veterinarians consciousness level to improve fish health reducing the residue antibiotics [7].

Water is a critical factor in the life of all aquatic species. Water quality determines to a great extent the success or failure of fish cultural operation, thus, the quality of water as a media for fish to live must be maintained. One of the methods to maintain water quality is by water replacement. Water replacement is expected to reduce residual levels in the liver of tilapia. Liver in fish has an important role for drug elimination [8]. The analysis was carried out to determine the effect of water change treatment and days on tetracycline residue levels in fish liver.

2. Materials and Method

The experiment was carried out in Balai Pengembangan Teknologi Perikanan Budidaya (BPTPB) Argomulyo, Cangkringan. Tetracycline residue analysis was carried out at the Department of Pharmacology, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

2.1. Materials

The tools used in the research are 3 fiber pools with volume 1500 liters (2 m x 1.5 m x 1 m). The instrument used for the analysis of the residual level of tetracycline is High Performance Liquid Chromatography (HPLC) Shimadzu 6.1. The HPLC consists of several components, namely the SCL-10A VP control system, SPD 10-AV VP UV-Vis detector, LC-10AD VP pump, DGU-14A degasser, CTO-10AC VP oven column and C18 Cliepeus column.

The experiment used fifty four tilapia (Oreochromis sp.) aged six months with body weight ranging from 350-450 grams. The feed was given in pellet (Fish Grower PA Super, JAPFA) with 32% protein content. In addition, the use of tetracycline 50 mg/kg (PT. Actavis Indonesia), NaCl 0.9%, iodine povidone, cotton, ice cubes, alcohol 70%, and distilled water.

Residue analysis was performed using a McIlvaine buffer containing di-Sodium hydrogen phosphate (Na2HPO4) (Merck), Citric acid monohydrate (Merck), Titriplex III (Na2EDTA) (Merck), and aquabidestilata sterile pro injection (PT Ikapharmindo Putramas). The mobile phase used in HPLC mix of methanol (Macron), acetonitrile, and 0.126% Oxalic acid dihydrate, crystal (HOCOCOOH.2H2O) (J.T. Baker) dissolved in aqua destilata.

2.2. Method

Fifty-four tilapia were adapted for seven days before the experiment was carried out. These tilapia were divided into 3 groups (A, B, and C) with each group containing 18 fish. Group A as a negative control was injected with 0.25 ml NaCl 0.9% without water replacement, group B as a positive control was injected with 0.25 ml tetracycline (50 mg/kg) without water replacement, and group C was injected with 0.25 ml tetracycline (50 mg/kg) with 25% water replacement twice a day for 6 days. Three fish from each group were euthanized daily for liver samples and analyzed for residual levels by HPLC. Euthanasia is done by cranial concussion [9].

Liver extraction was carried out according to the modified standard for the tetracycline according to the Association of Official Analytical Chemistry (AOAC) [10]. The analysis of tetracycline residues was performed using HPLC based on a validated method [11]. The mobile phase used acetonitrile, oxalic acid, and methanol in a ratio of 15: 80: 5 and the stationary phase used Cliepeus C18. The water speed is 1 ml/minute. The oven temperature is 30ºC, the detector uses UV-Vis, the wavelength is 355 nm and the injection volume is 20 µl. Data of residual levels in the liver were analyzed using Two Way ANOVA SPSS version 16.

3. Result and Discussion

The peak area of tetracycline can be seen between the 4th minute and 5th minute [11]. The results of the analysis of residual levels in liver using High Performance Liquid Chromatography, group A (negative control group) did not show a tetracycline peak area (Figure 1). This indicated that group A did not contain tetracycline residues.
In the positive control group (group B), there was a peak area of tetracycline. The peak area appears between the 4th and 5th minutes (Figure 2).

The analysis results of residual levels of tetracycline in the liver of tilapia can be seen in Table 1. The results of the analysis, in the negative control group did not show any antibiotic residues on the first day to the sixth day. These results indicate that the water used during the research can be assumed to be clean and does not contain tetracycline that can contaminate fish. Based on these data, it can be seen that the residual content analysis method using HPLC is accurate.

| Group | Tetracycline residue levels (µg/g) | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
|-------|-----------------------------------|-------|-------|-------|-------|-------|-------|
| Group A | ND | ND | ND | ND | ND | ND | ND |
|        | ND | ND | ND | ND | ND | ND | ND |
|        | ND | ND | ND | ND | ND | ND | ND |
|        | ND | ND | ND | ND | ND | ND | ND |
| Group B | 22.43 | 21.96 | 25.05 | 14.03 | 14.68 | 7.20 |
|        | 22.60 | 23.54 | 25.15 | 13.68 | 14.23 | 6.59 |
|        | 22.64 | 23.46 | 25.18 | 14.00 | 13.41 | 7.21 |
| Group C | 4.82 | 7.84 | 19.41 | 4.65 | 10.31 | 2.98 |
|        | 4.51 | 7.34 | 18.24 | 4.59 | 10.38 | 2.03 |
|        | 4.31 | 8.54 | 18.15 | 4.46 | 10.79 | 3.13 |

*ND = not detected

Based on Table 1, it can be seen that residual levels in group B and group C were decrease. The decrease in residual levels in group C was greater than that in group B. Figure 3 shows a graph of the decrease in residual levels in the positive control group and group with water replacement twice a day based on the average residue levels (Table 2). The pattern of residue reduction in all groups was the same, but there was a difference in the rate of residue reduction.
Table 2. Average levels of tetracycline residues in fish liver

| Group  | Average tetracycline residue levels (µg/g) |
|--------|------------------------------------------|
|        | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 |
| Group A| ND    | ND    | ND    | ND    | ND    | ND    |
| Group B| 22.55 | 22.98 | 25.13 | 13.90 | 7.00  |
|         | ±0.11 | ±0.89 | ±0.68 | ±0.19 | ±0.65 | ±0.35 |
| Group C| 4.55  | 7.91  | 18.73 | 4.57  | 10.49 | 3.05  |
|         | ±0.25 | ±0.60 | ±0.61 | ±0.09 | ±0.25 | ±0.07 |

*ND = not detected

Figure 3. Graph of tetracycline residue levels in the liver of tilapia

Based on the graph (Figure 3), shows that the level of tetracycline residues in the liver of tilapia after injection increased slightly on the 2nd day of water replacement. On the 3rd day there was an increase in the amount of residue, but it decreased quite dramatically on the 4th day of water replacement. On the 5th day of water replacement, there was an increase in the amount of residue in the fish liver. Then on the last day of the water replacement treatment (6th day), there was a decrease again to below the residual amount on the 4th day.

Tetracyclines are absorbed by the liver from the blood circulation and transported to the small intestine via the bile. From the small intestine, tetracyclines are reabsorbed into the blood circulation and excreted through the kidneys [12]. Tetracycline is metabolized in the liver as much as 5% to a metabolite in the form of epi-tetracycline. While 95% of other tetracyclines are excreted by the bile and by the kidneys to be excreted in the form of urine [13]. Tetracyclines present in water can be reabsorbed into the fish’s body through the gills. This is demonstrated by an increase in level of tetracycline residues on the 3rd day and 5th day. The water replacement method can reduce the tetracycline in the water that can be reabsorbed by the gills during the period of water replacement treatment which can be observed on the 4th day and 6th day.

The pattern of residual reduction in this study is in accordance with the research of [14] who discussed the pharmacokinetics and accumulation of tetracycline in fish after drug administration. The highest concentration of tetracycline residues was found on day 3 after drug administration. This is due to the slow absorption of tetracycline by the gastrointestinal tract. The peak absorption of tetracycline by liver occurred twice, namely on the 3rd day and on the 5th day. This is in accordance with the results of this study. The distribution of tetracyclines from liver to plasma always increases from day 3rd to day 7th after drug administration and decreases sharply at 2 weeks after drug administration [10].
Analysis of the residual levels of tetracycline for 6 days using the Two Way ANOVA method resulted in a significance value of less than 0.05. This result means that there is a difference in tetracycline residue levels in liver based on water replacement treatment and days. Based on observations on the 6th day of Group C which shows the average levels of tetracycline residues in fish liver is 3.05 ±0.07 µg/g (below MRL) [6]. It can be concluded that after 6th day water replacement treatment, tilapia in this study was safe for consumption.

4. Conclusion
Based on the results of the study, it can be concluded that the 25% water replacement treatment in tilapia after tetracycline therapy was able to reduce residual levels in the liver. The results of this study can be used for fish farmers in reducing residual levels in fish that have been treated with tetracycline.

5. References
[1] Grammer G, William T, Mark S, and Mark A 2012 *Aquatic Invasions*. 7 367-376.
[2] Wamala S, Mugimba, Mutoloki, Eversen, Mdegela, Byarugaba, and Sorum 2018 *Fisheries and Aquatic Sciences*. 21 1-10.
[3] Nurhasnawati H, Jubaidah S, dan Elfia N 2016 *Jurnal Ilmiah Manuntung*. 2 6.
[4] Barman A K A, Hossain, Rahim, Hassan, and Begum 2018 *Bangladesh Journal of Scientific and Industrial Research*. 53 41-46.
[5] Alarape S A and Olanike K A 2017 *African Journals Online*. 35.
[6] Rafati L, Mohamad H E, Mehd M, Aria S, Sahar S, Hossein M, and Seyed M M 2018 *Journal of Health Scope*. 7 1-6.
[7] Monteiro S H, Graziela C, Fabiana G, and Fabiana P 2018 *The Pharmaceutical and Chemical Journal*. 5 127-147.
[8] Xie W 2017 *Drug Metabolism in Diseases* (Oxford: Elsevier) p 182.
[9] Anonim 2013 *The AVMA Guidelines for the Euthanasia of Animals* (Schaumburg: The American Veterinary Medical Association) p 68-76.
[10] Wijayanti A, Lukman H, dan Irkham W 2007 *Jurnal Sain Vet*. 25 68-74.
[11] Hakimah N, Gagak D, Wari P, dan Soedarmanto I 2018 *Jurnal Sain Veteriner*.
[12] Turk E dan Oguz H 2016 *Eurasian Journal of Veterinary Sciences*. 32 74–79.
[13] Agwuh K N dan MacGowan A 2006 *Journal of Antimicrobial Chemotherapy*. 58 256–265.
[14] Rogstad A, Hormazabal V, Ellingsen O F, dan Rasmussen K E 1991 *Aquaculture*. 96 219-226.