The Residue Levels of Enrofloxacin Antibiotics in Catfish (Clarias batrachus)

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Abstract: Enrofloxacin (EXF) in fish comes from feed, water, and the environment. Through these three sources enrofloxacin will accumulate in the body of the fish. Enrofloxacin enters the metabolic process and enters the tissue so that fish meat containing enrofloxacin will be produced, and can threaten human health such as the occurrence of carcinogenic potential. Tests have been carried out to test the time of decay of enrofloxacin in catfish after various doses, namely group A (control), B (25 ppb) and C (50 ppb). The fish samples analyzed were catfish which were tested by Enrofloxacin using the ELISA (Linked Immunosorbent Assay) method. The results showed that the last residual content of enrofloxacin was added to feed every day during the treatment of concentration for group B (17.89 ppb) and group C (17.47 ppb). However, the time for all enrofloxacin residues in catfish to be the same as the control or normal conditions turned out to take 80 days with enrofloxacin residual concentrations for group B (2.12 ppb) and group C (5.13 ppb).

1. Introduction

Quality assurance and food security that have become important issues in global trade competition, the use of antibiotics in fish farming needs serious attention. This is evidenced by various cases of rejection of fishery products containing prohibited substances such as antibiotics chloramphenicol and nitro furan which have harmed aquaculture and economic activities in Indonesia, related to the stipulation of several types of antibiotics as prohibited substances both at home and abroad. Therefore various regulations have been issued that use of all antibiotics is prohibited in the cultivation of fish. EU Regulations (Commission Regulation (EU) No 37/2010 concerning On Pharmacologically active substances and their classification regarding the maximum residual limits of animal origin) and other importing countries (Japan and America) still allow certain types of antibiotics to be resided to a certain degree or Maximum Residues Limits = MRLs in fishery products such as: tetracycline and enrofloxacin. MRL values for enrofloxacin and tetracyclines in the European Union amount to 100 ppb (Wang S, 2006).

The source of the presence of enrofloxacin in fish can be through feed, water, and the environment. Through these three sources enrofloxacin will accumulate in the body of the fish. Enrofloxacin enters into the metabolic process and enters the tissue so that fish meat containing enrofloxacin will be produced.
The European Union provides rules regarding food safety to test the time of decay of enrofloxacin in fish products. Decrease time is the time (specified) from the time the drug is stopped until all drugs and metabolites in the fish body are removed from the body of the fish (24 hours). With data on the time of decay, drug residues are still contained in the body of the fish after the treatment. Enrofloxacin residues can be formed due to excessive use or not paying attention to stopping time, the presence of enrofloxacin residues in fish can be through feed, water or the environment. Enrofloxacin residues when found in humans with higher concentrations will cause harmful toxic effects, such as DNA damage, inflammation of epidermal tissue and so on (Quintero and Miranda, 2000). A method is needed to determine the downtime (decay time) of the enrofloxacin antibacterial. In this study aimed to determine the time of decay of enrofloxacin in catfish after dosing 25 ppb of fish and 50 ppb of fish for 7 days of treatment.

2. The object and the methods of research
The object of research is enrofloxacin in catfish. The ingredients used were catfish meat, 70% methanol, sample diluent buffer, enrofloxacin standard solution, conjugate enzyme, antibody solution, wash buffer, substrate or chromogenic and stop solution. The tools used are analytic balance, tube, vortex, centrifuge, micro pipette, spatula, micro titer plate, ELISA reader R-Bio pharm brand that is connected to a computer.

Testing of the time of decay of enrofloxacin was the evaluation period needed after the fish were treated until the concentration of residual enrofloxacin was below the MRL value. The duration of testing time for all enrofloxacin was 80 days with observation times 10 times, ie 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, 21 days, 24 days, 27 days, 30 days, 55 days, 60 days and 80 days.

In general, in accordance with Council Directive 91/414 / EEC, which states that testing on test animals is carried out with 2 doses of drug administration and one control, so that the total group becomes 3 groups, with details of group A: control, group B: dose treatment treatment, and group C: drug treatment 2 times the treatment dose. Each group was carried out triple and 200 test animals were used each. Thus the number of test fish needed is 1800 animals.

Stages of activity for testing the time of decay of enrofloxacin:
1. Prepare a container of catfish with a size of 183x132x100 cm, amounting to 9 pieces with enough supporting installations. Allotment of containers is 3 pieces for control, and 6 pieces for 2 variations of treatment doses.
2. Add catfish ± 20-30 g with 200 containers each
3. Acclimatization of fish for approximately 7 days by feeding as much as 3% BB (heavy weight)
4. Perform fish treatment by mixing enrofloxacin antibiotics in each fish feed with treatment: 1 treatment dose, and 2 times the treatment dose. Each treatment consisted of three replications.
5. Give feed as much as 3% BB (heavy weight) during the treatment for 7 days by mixing enrofloxacin antibiotics each day differently for each dose, to mix antibiotics with feed using eggs. Can be seen in the table below for mixing antibiotics every day on feed Table 1.
6. Perform 6 fish samples taken from each treatment at the time of treatment each day and after treatment taken again is 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, 21 days, 24 days, 27 days, 30 days, 55 days, 60 days and 80 days.
7. The fish sample is directly stored at the freezer at -20oC.
8. Perform fish preparation for analysis of enrofloxacin residues using ELISA on meat and fish skin.
9. Perform water analysis for parameters of temperature, pH, DO every once a week.
Table 1 The mixture of enrofloxacin antibiotics for each dose for 7 days dose of Enrofloxacin antibiotics

| No. day | fish weight (Kg) | drug weight (g) dose of 25 ppb | drug weight (g) dose of 25 ppb |
|---------|-----------------|--------------------------------|--------------------------------|
| 1 H-1   | 5.00            | 0.6250                         | 1.2500                         |
| 2 H-2   | 4.85            | 0.6063                         | 1.2126                         |
| 3 H-3   | 4.70            | 0.5875                         | 1.1750                         |
| 4 H-4   | 4.55            | 0.5688                         | 1.1376                         |
| 5 H-5   | 4.40            | 0.5500                         | 1.1000                         |
| 6 H-6   | 4.25            | 0.5313                         | 1.0626                         |
| 7 H-7   | 4.10            | 0.5125                         | 1.0250                         |

Enrofloxacin testing in catfish which has been prepared is using the Elisa method. Catfish meat in slices then the catfish meat is milled (blender). After that, weighed 1 gram and added 4 mL of methanol 70%, then homogenized with 10 minutes vortex with maximum speed, then centrifuged at 4000 rpm for 5 minutes. After that, 0.5 ml of the supernatant (filtrate) is transferred and then added 0.5 ml of extracted buffer and vortexes for 1 minute.

A solution containing 50 µl of sample is inserted into the well for testing and a standard solution of enrofloxacin with concentrations of 0 ppb, 0.1 ppb, 0.25 ppb, 0.5 ppb, 1 ppb, and 5 ppb added 50 µl to in the well too, then carefully added 100 µl of the enrofloxacin antibody solution in each well containing samples and standards and incubated for 30 minutes at room temperature. The solution is removed first then a washing solution of 250 µl is added to each well, washing is done three times (each time the washing solution is removed). Conjugate HRP was added as much as 150 µl into an empty well. Mixing was done by manually shaking the plate then incubating for 30 minutes and doing the washing again after the incubation was complete. The stop solution was added as much as 100 µl to each well and re-incubated for 1 minute before reading. The resulting solution is yellow then its absorption is measured at a wavelength of 450 nm using the R-bio pharm well Reader.

3. The Results of Research

The results of the administration of enrofloxacin antibiotics in catfish during 7 days of treatment with 3 dose treatments, namely group A (control), group B (dose 25 ppb) and group C (dose 50 ppb) can be seen in the table below:

Table 2. Concentration of enrofloxacin during treatment

| Time   | Group A (ppb) | Group B (ppb) | Group C(ppb) |
|--------|---------------|---------------|--------------|
| H-1    | 5.72          | 18.24         | 18.03        |
| H-2    | 5.13          | 17.71         | 17.75        |
| H-3    | 5.25          | 18.22         | 18.09        |
| H-4    | 7.89          | 18.16         | 18.06        |
| H-5    | 6.83          | 18.42         | 18.21        |
| H-6    | 5.21          | 17.74         | 17.61        |
| H-7    | 5.46          | 17.89         | 17.47        |

Table 2 Shows that the results of observations during feeding containing enrofloxacin for group A and group B fish showed normal eating ability marked by fish looking for feed on the surface of the water when feed was given. However, the disrupted group C of eating will be characterized by the intake of feed at the mouth of the fish several times then eaten as well this is due to the large amount of enrofloxacin added to the feed affecting the aroma of feed Enrofloxacin concentrations in catfish during treatment between group B and group C did not differ greatly for each day due to the group C
intake of enrofloxacin eaten by catfish only a portion of enrofloxacin was added due to the length of feed in water. This can be seen in the picture Figure 1.

Figure 1. Enrofloxacin residue in catfish after treatment

The enrofloxacin residue in the body of catfish left behind during treatment for group B (17.89 ppb) and group C (17.47 ppb) means administration of enrofloxacin antibiotics orally for catfish in group B (71.56%) and group C (34.98%) that enter the body of catfish.

The results of the observations during feeding which contain enrofloxacin showed that catfish group B whose appetite was undisturbed compared to group C showed a lack of appetite. But after being stopped feeding the enrofloxacin with regular food, appetite began to return to normal, marked by the rapid catfish looking for food on the surface of the water when the feed was given.

Maintenance of catfish by giving normal feed to accelerate catfish metabolism so that the enrofloxacin residue given during treatment decreases or decays with length of maintenance. Decrease time is the time (specified) from the time the drug is stopped until all drugs and metabolites in the fish body are removed from the body of the fish (per 24 hours). The time of all enrofloxacin residues from each treatment can be seen in the picture Figure 2.

Figure 2 Enrofloxacin residues in catfish after treatment

From Figure 2 It shows that the total time of enrofloxacin residues in catfish until the residue is completely equal to the control turns out to take 80 days with enrofloxacin residual concentrations for group B (2.12 ppb) and group C (5.13 ppb). While the results of enrofloxacin residues from control animals were found because in the feed used there were enrofloxacin antibiotics used by feed producers to prevent rotten feed with an enrofloxacin concentration of 2.76 ppb. Most of the time of the decay of enrofloxacin in the body of catfish is caused by metabolism (enzymatic) in the digestive tract and in the excretion through feces.
4. Conclusions
The end result of enrofloxacin was added to feed every day during the treatment of concentration for group B (17.89 ppb) and group C (17.47 ppb). However, the time for all enrofloxacin residues in catfish to be the same as the control or normal conditions turned out to take 80 days with enrofloxacin residual concentrations for group B (2.12 ppb) and group C (5.13 ppb).

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