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Antibiotic residue analysis of tetracycline and chloramphenicol in banana shrimp (*Fenneropenaeus merguiensis*) from a traditional shrimp farming in Brondong, Lamongan

Alma Ika Fatmawati[1], Nuning Vita Hidayati[2], Mirna Fitrani[3], Boedi Setya Rahardja[4], Sapto Andriyono[5,*]

[1]Bachelor of Aquaculture Program, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto, Indonesia.
[3]Aquaculture, Department of Fisheries, Faculty of Agriculture, Universitas Sriwijaya, South Sumatera, Indonesia.
[4]Department of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia.
[5]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia.

*Corresponding Author: sapto.andriyono@fpk.unair.ac.id

ABSTRACT

Banana shrimp is one of the potential export shrimp cultivated in Brondong, Lamongan. Antibiotic residues, including pollutants, can come from cultivation pond areas and environmental pollution. The antibiotics in shrimp are *tetracycline* groups (*oxytetracycline, chlortetracycline,* and *tetracycline*) and *chloramphenicol.* This study aims to determine the presence of antibiotic residues in banana shrimp and whether the quality of the shrimp has met health safety standards based on antibiotic residue analysis using estimated daily intake (EDI) and hazard quotient (HQ) calculations. Examination of antibiotic residues is carried out at the UPT. Quality Testing and Development of Marine and Fisheries Products in Surabaya. This study used an observation method by taking shrimp samples, measuring pond water quality parameters, and testing the content of antibiotic residues in the laboratory. The examination results showed that residues of *the tetracycline* and *chloramphenicol* groups were not detected in banana shrimp. Calculations of EDI and HQ cannot be performed because antibiotic residues are not detected. The HQ value is below one, which indicates a low-risk level category. Banana shrimp from a farming pond in Brondong, Lamongan is safe for human consumption.

Keywords: *antibiotic, shrimp, bioaccumulation, pollution*

Introduction

Banana shrimp (*Fenneropenaeus merguiensis*) is a type of shrimp originating from Indonesian waters, has high economic value, is resistant to various diseases, has a high dilution rate (80%), and includes potential export products (Kusna et al. 2019, Umam et al. 2021). Shrimp export activities have experienced some resistance in the American, European, and Asian markets in the past decade due to the detection of contaminants such as antibiotic residues. The types of antibiotics found in shrimp are tetracycline group antibiotics (oxytetracycline, chlortetracycline, and tetracycline) (Luu et al. 2021) and chloramphenicol (Chi et al. 2017). However, the Indonesian government continues to monitor and limit the number of types of antibiotics as outlined in the Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 37/Permen-KP/2019. This is the government's commitment to guarantee fishery products

(shrimp) from Indonesia that are free from harmful antibiotic residues.

The antibiotic chloramphenicol has been banned because it has a high level of toxicity in the human body (Hanekamp and Bast 2015). Tetracycline antibiotics are also found in many fishery products in Indonesia because they can still be used in fisheries. The tetracycline group was chosen because it has a wide spectrum, was relatively cheap in price, has low toxicity, and can be applied orally (Borghi and Palma 2014). Antibiotic residues will accumulate in the human body if consumed continuously. Antibiotic residues can come from drug residues in feed, liquid waste from the pharmaceutical industry and hospitals, household waste, to intensive ponds around it (Hidayati et al. 2021).

The banana shrimp pond area in Brondong, Lamongan is located in the north coast of East Java Province which is traversed by the Bengawan Solo River that spreads through small rivers and groundwater flows, then passes through residential areas, hospitals or health centers, and intensive ponds around it (Hidayati et al. 2021). Antibiotic residues can be carried into the pond through the water flow. Examination of antibiotic residues in banana shrimp is necessary to determine the quality of shrimp production and to determine the level of risk caused by antibiotic residues in humans.

Materials and Method

Location and time of research

This research has been conducted in November 2021-March 2022. Data analysis was carried out in March-April 2022. The sampling location in this study was in traditional ponds that use a polyculture pattern for cultivating banana shrimp, grouper, and seaweed, located in Ngesong, Sedayulawas Village, Brondong District, Lamongan Regency, East Java (6°52'25.3"S 112°14'27.4"E). Testing of antibiotic residue content was carried out at UPT. Quality Testing and Development of Marine and Fisheries Products in Surabaya, which certified by KAN (Komite Akreditasi Nasional) LP-088-IDN and LSPR-056-IDN.

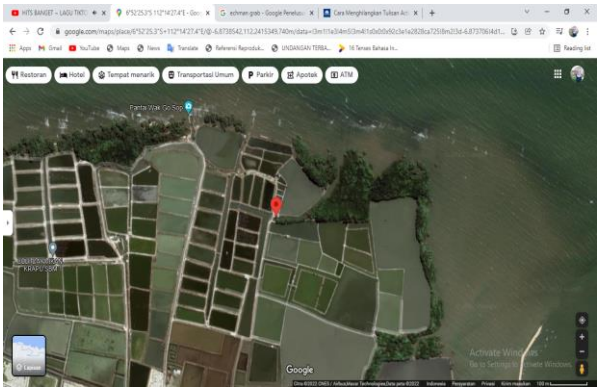

Figure 1. Sampling location of traditional ponds in Brondong, Lamongan

Methods

This study used an observation method by making observations at the pond location, measuring pond water quality parameters, and taking shrimp samples to test the content of antibiotic residues in the laboratory. Water quality parameter are salinity by Refractometer (Atago), DO (DO meter YSI 550A), pH using pH meter (MediaTech), and temperature (GEA Medical S-006). The HPLC (High Performance Liquid Chromatography) method was used for tetracycline group residues test (Shahbazi et al. 2015). This method using based on the provisions of SNI 2354.11: 2009, while testing for chloramphenicol residues uses the ELISA method (Chughtai et al. 2017) based on the provisions of IK. 2.4.15.

Data analysis

The data obtained are then described quantitatively. The data obtained was in the form of antibiotic residual value in banana shrimp, and then a comparison was carried out with the maximum residue limit (BMR) in fishery products based on the Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 37/Permen-KP/2019 (Yasin 2021) and the European Commission in 2003/181/EC (Sallam et al. 2022). Data analysis was also carried out

through the calculation of estimated daily intake (EDI) and hazard quotient (HQ).

1. **Estimated Daily Intake (EDI)**

Estimated Daily Intake (EDI) is an estimate of the daily intake of antibiotic residues in shrimp consumed. EDI calculation based on the following formula (Li et al. 2021).

$$EDI = \frac{C \times M}{W}$$

Information:
EDI : Estimated daily intake (µg/kg bw/day)
C : Antibiotic concentration in shrimp banana (µg/kg)
M : Daily consumption per capita (g/day)
W : Average body weight of Indonesians (kg)

Statistical data from the Ministry of Maritime Affairs and Fisheries states that fish consumption per capita in Indonesia is 56 kg/year in 2020 (Virgantari et al. 2022). The average body weight of Indonesians is 60 kg (Triwinarto et al. 2019).

2. **Hazard Quotients (HQ)**

Hazard Quotients (HQ) is the ratio of the amount of potential exposure to a substance and the level of a substance that does not cause adverse effects. HQ in banana shrimp can be calculated by the ratio of EDI and ADI (Acceptable Daily Intake) (Liu et al. 2018).

$$HQ = \frac{EDI}{ADI}$$

Information:
HQ : Hazard ratio
EDI : Estimated daily intake (µg/kg bw/day)
ADI : Acceptable daily intake (µg/kg bw/day)

The ADI value for tetracycline, oxytetracycline, and chlortetracycline is 30 µg/kg bw/day, while chloramphenicol does not have an ADI value so there is no need to calculate the HQ value (Sallam et al. 2022). The risk of antibiotic residues for health in Liu *et al*. (2018) is classified into two levels. The risk level is low if the HQ value is <1, while the risk level is high if the HQ value is ≥1.

Results

Concentration of Antibiotic residues

The results of testing tetracycline, oxytetracycline, chlortetracycline, and chloramphenicol antibiotic residues on the four banana shrimp samples in shrimp farming ponds in Brondong, Lamongan Regency showed that no residue was detected (Table 1).

Table 1. Results of Antibiotic Residue Testing on Banana Shrimp Samples

Parameter	Test Results (µg/kg)				BMR (µg/kg)
	1	2	3	4	
Tetracycline	ND	ND	ND	ND	100[a]
Oxytetracycline	ND	ND	ND	ND	100[a]
Chlortetracycline	ND	ND	ND	ND	100[a]
Chloramphenicol	ND	ND	ND	ND	0.3[b]

ND: Not detected
[a] Maximum Residual Limit based on PerMen KP No. 37 of 2019.
[b] Maximum Residual Limit by European Commission 2003/181/EC.

Water quality parameters

Measurement of water quality parameters was carried out at the banana shrimp farming pond in Brondong, Lamongan. Such water quality parameters include temperature, acidity degree (pH), salinity, oxygen solubility (DO), and brightness (Table 2).

Table 2. Water Quality Parameter Measurement Results

Parameter	Value	Quality standards

Temperature (°C)	32.67	29-33
pH	6.86	6-7
Salinity (ppt)	30	28-32
DO (mg/L)	6.78	4-8
Brightness (cm)	49	>30

Discussion

Analysis of antibiotic residues in banana shrimp

Based on the study's results, the analysis using EDI and HQ calculations could not be carried out because no residual antibiotic value in banana shrimp was detected. These results show that there is no maximum amount to consume banana shrimp in one day because the level of risk indicated by the shrimp is below the low category of <1 (Liu et al. 2018). The non-detection of antibiotic residues in banana shrimp during testing can be caused because farmers do not apply excessive antibiotics (Pawestri et al. 2019). The analysis of tetracycline and chloramphe- nicol group antibiotic residues showed that the production of banana shrimp from aquaculture ponds in Brondong, Lamongan were not at risk and safe for consumer health, so it could be consumed in the long term. Previous research on the analysis of antibiotic residues in tiger shrimp (*Penaeus monodon*) was 0.0024634 ppm, which is still below the SNI SNI 01-6366-2000 standard, which is 0.01 ppm (Saputra and Arfi, 2019). Meanwhile, white shrimp species (*Penaeus vannamei*) were found at concentrations of 0.14 ppb and 0.12 ppb, which were also below the food safety threshold (Virgianti et al. 2022).

Antibiotics have an elimination time where, at a certain period, the residual content is no longer detected in body tissues (Avunje et al. 2021). Each antibiotic has a different elimination time. Tetracycline group antibiotics have an elimination time of 48-96 hours after stopping drug administration (Avunje et al. 2021), while chloramphenicol antibiotics have an elimination time of 3 days (Bilandžić et al. 2012). If sampling or harvesting is carried out after the elimination period, antibiotic residues will not be detected in shrimp.

Analysis of water quality parameters of aquaculture ponds

The water quality parameter values in (**Table 2**) show that the waters of the banana shrimp farming pond follow the reference used. The use of waterwheels can maintain the availability of dissolved oxygen in waters so that the two main biotas cultivated, namely grouper fish and shrimp, do not lack dissolved oxygen (Fuady and Nitisupardjo 2013). The high temperature (32.67^{o}C) in the measurement results is caused by the measurement time, which is daytime. However, this condition is still within the range that shrimp can tolerate (Vance and Rothlisberg 2020). During the day, the process of photosynthesis increases so that the level of dissolved oxygen increases (Supriatna et al. 2020). The salinity of pond waters is in the optimum range, so it is safe for shrimp farming (Ighwerb et al. 2021). The high brightness of water is influenced by the abundance of micro-organisms and organic matter in the water body. This condition causes the brightness to be added to the traditional, which is relatively high, namely 49 cm. This value has fulfilled the life of the shrimp (Lestari et al. 2014). Meanwhile, the parameters of the acidity of the waters indicate that the pond conditions are still safe for shrimp culture activities, with the pH value still in the range of 6-7 values. Temperature, pH, salinity, DO, and brightness of water that does not exceed or less than the tolerance limit of water quality parameters in shrimp farming indicates that the aquaculture waters in Brondong pond, Lamongan are still included in the excellent category.

Sources of pollution of antibiotic residues

Sources of antibiotic pollution can come from internal and external areas of the pond. In the

internal area, antibiotics enter the body by administering drugs mixed in the feed. Antibiotics are absorbed by the stomach and intestines and then distributed to body organs and tissues through blood circulation (Pawestri et al. 2019). Antibiotics undergo an elimination process that occurs in the kidneys or liver, and then antibiotics that are not absorbed by the body will become residues and are wasted in the waters through urine or feces. Antibiotic administration is carried out several days in a row, so the elimination process takes time (Avunje et al. 2021). Sources of antibiotic residues from the external area of the pond, including household waste, medicinal industry waste, liquid waste from hospitals or clinics, livestock liquid waste, agricultural waste using animal manure fertilizer, and intensive pond activities in the vicinity (Hidayati et al. 2021). Until now, there have been no reports of the use of these antibiotics in fishing activities in Lamongan. However, we want to ensure that there is no accumulation of cultivated biota in this area. No report regarding the application of antibiotics due to this chemical compound being prohibited used in aquaculture under Indonesian (Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 37/Permen-KP/2019).

Impact of antibiotic residue pollution

The impact of exposure to tetracycline group antibiotic residues is nausea, vomiting, and diarrhea. Exposure to antibiotic residues in pregnant women and children can cause calcification of bones and teeth during the growth period due to the deposition of residues during pregnancy (Borghi and Palma 2014). Other reactions caused are problems on the skin, such as allergies and the skin becoming sensitive (Jayalakshmi et al. 2017). Chloramphenicol residues can cause aplastic anemia, leukemia, bone marrow suppression, and gray baby syndrome (Hanekamp and Bast 2015). Side effects of chloramphenicol are rare in the health world due to the ban on these antibiotics by PERMENKES No. 1168/Menkes /PER/X/1999. The use of antibiotics in cultivation can lead to the development of antibiotic resistance among pathogens such as bacteria. Resistant bacteria appear as a form of reducing the level of sensitivity of antibiotics in inhibiting bacterial performance. The impact of reducing the level of antibiotic sensitivity can make bacteria resistant to antibiotics so that they can inhibit the treatment process (Sukertiasih et al. 2021).

Efforts to reduce antibiotic residues

Shrimp farmers can reduce antibiotic residues by reducing and controlling the use of antibiotics and then using alternative ingredients derived from plants or probiotics (Bacanlı and Başaran 2019). Antibiotic residues derived from urban waste, hospitals, and the pharmaceutical industry can be treated in wastewater treatment installation, which uses a membrane bioreactor system that can reduce antibiotic residues properly (Le et al. 2018). Antibiotic residues in fishery products can be reduced by paying attention to the time of residue elimination when harvesting so that the amount of residue in shrimp has decreased and is not detected. The elimination time of tetracycline group antibiotics is 48-96 hours, while the antibiotic chloramphenicol is three days (Bilandžić et al. 2012). In addition, fishery products heating and the freezing process can degrade various chemicals (Bacanlı and Başaran 2019).

Conclusion

The results showed that banana shrimp from a cultivated pond in Brondong, Lamongan, did not contain residues of *tetracycline, oxytetracycline, chlortetra-cycline,* and *chloramphenicol* antibiotics. The banana shrimp produced has met safety standards for health and is safe for long-term consumption.

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