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Original research article

Elimination of Chloramphenicol by Tiger Shrimp (*Penaeus monodon*) and White Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Chloramphenicol (CAP) has been illegally used in many shrimp farms in South East Asia, including Indonesia. We performed an experiment of elimination simulation of CAP in tiger shrimp (*Penaeus monodon*) and white shrimp (*Litopenaeus vannamei)*. After 5 days of depuration process, the concentration of CAP in *P. monodon* decreased to 94.85% (muscle), 97.98% (cephalothoraxes), and 90.30% (exoskeleton). The elimination half-life of CAP in *P monodon* was 0.596 day in the muscle, 0.716 day in cephalothorax, and 0.437 day in exoskeleton. On the other hand, concentrations of CAP in *L. vannamei* decreased to 97.74% (muscle), 90.30% (cephalothoraxes), and 97.63% (exoskeleton). The elimination half-life of CAP in *L. vannamei* was 0.6624 day (muscle), 0.859 day (cephalothorax), and 0.796 day (exoskeleton). CAP was retained better by *P. monodon* compared to *L. vannamei*.

1. Introduction

Shrimp production from aquaculture in Indonesia continues to increase: in 2011, it reached 410,000 tons and 415,703 tons in 2011. It is expected to increase to 785,900 tons in 2015. The shrimps are exported mostly to the United States, European Union, and Japan. Extensive, semi-intensive, and intensive techniques have been applied to manage shrimp production in Indonesia (Graslund and Bengtsson 2001). Intensive techniques have high risk of disease outbreaks caused by virus, bacteria, fungi, and other pathogens. The structures of antibiotics are largely composed of structures that are covered by cyclic components, represented by piperazine units, benzene rings, hexahydropyrimidines, and quinolone, morpholine, and sulfonamides groups. Activated metabolites, conjugates, and hydroxylated forms are metastable compounds after treated in humans or animals (Manzetti and Ghisi 2014). Among antibiotics that are commonly used in aquaculture, chloramphenicol (CAP), erythromycin, streptomycin, prefuran, and neomycin are also used in the treatment of bacterial disease in shrimp and ornamental fish (Supriadi *et al.* 2000). Because of its effectiveness against gram-positive and gram-negative bacteria, CAP is well known as a broad spectrum antibiotic. The use of CAP in shrimp farms and hatcheries has been reported (Graslund and Bengtsson 2001; Leston *et al.* 2013). Although the use of antibiotics in aquaculture production can promote growth and improve the production of aquatic products, excessive use of these compounds will lead to concentration of antibiotics in aquatic products that is higher than national and international food safety standards. Because of the well-known risk of anemia and carcinogenic properties of CAP, the presence of CAP in food is considered illegal and unacceptable in the European Community (EC) since 1994. Furthermore, according to the European Commission Decision 2001/699/EC, 2001/705/EC, 2002/249/EC, 2002/250/EC, and 2002/251/EC, certain fishery and aquaculture products, imported from China, Vietnam, Indonesia, Thailand, and Myanmar, intended for human consumption, must be subjected to testing to ensure the absence of CAP residues (Impens *et al.* 2003; Conti *et al.* 2015). CAP is one of nine types of food additives that are banned in Indonesia (Permenkes No. 722/Menkes/Per/IX/88). Nevertheless, the use of CAP in fishery commodities (shrimp and fish) has spread in local, regional, and international levels that it has hampered exports, especially shrimp from Indonesia to various countries in the world. Peak export failure the implementation of zero tolerance occurs when the content of CAP by EU countries for commodities imported shrimp (Islamulhayati *et al.* 2005).

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Indonesia also has national standards for CAP at shrimp product. According to Indonesia National Standard no SNI 01-2705.1-2006, frozen shrimp products have to contain zero CAP.

There is a lack of study on biokinetic approach for bioaccumulation and depuration of CAP in shrimp. Most studies focus on environmental monitoring regarding the CAP content at fish product (Impens *et al.* 2003; Conti *et al.* 2015). Zhao *et al.* (2015) have studied bioaccumulation and sulfonamides in common carp (*Cyprinus carpio*) under experimental controlled conditions. The uptake and depuration of a range of pharmaceuticals in the freshwater shrimp (*Gammarus pulex*) and the water boatman (*Notonecta glauca*) have been studied by Meredith-Williams *et al.* (2012). Moreover, pharmacokinetics of antibiotic have been studied in shrimp by Reed *et al.* (2004) and in abalone by Rosenblum *et al.* (2008). Furthermore, Weifen *et al.* (2004) reported depletion of antibiotic residue (CAP, sulfamethoxazole, and oxytetracycline [OTC]) in shrimp muscle after oral administration in shrimp *Penaeus chinensis* under field conditions. There are uptake and depuration of some pharmaceutical products (5-fluorouracil, carvedilol, diazepam, and moclobemide) by freshwater shrimp (*G. pulex*) and water boatman (*N. glauca*). Furthermore, Kim *et al.* (2014) investigated the bioaccumulation of tetracycline through dietary and aqueous routes in *Daphnia magna*. The objectives of our study were to characterize depuration kinetic of CAP from some part (head, muscle, and exoskeleton) of tiger shrimp and white shrimp. The aim of this study was to find information on the residue of CAP in some part of shrimp after depuration procedure carried out by farmers in field.

2. Methods

2.1. Shrimp feeding

The experiment was carried out at the Laboratory for Coastal Development, Diponegoro University. The experiment followed Weifen *et al.* (2004) with some modifications. Briefly, shrimps *Penaeus monodon* and *Litopenaeus vannamei* were caught from the pond at Jepara aquaculture areas. The size of shrimps was 11.2–12.5 cm. About 50 shrimps were cultured in concrete tanks. Temperature was ambient, and the salinity was 29 ppt. Bubbling air to produce oxygen content was kept close to saturation. Acclimatization of shrimp was carried out for 2 weeks. CAP for veterinary use was evenly mixed with the normal shrimp ration at the theoretical concentration of 2000 mg drug/kg. Shrimps were fed twice once in the morning and once in the afternoon in accordance with the procedures of local farmers. Shrimp samples were collected on 0, 1, 2, 3, 4, and 5 days after feeding. Six shrimps were collected for each sampling time, and they were separated into three parts: muscle, head, and exoskeleton. Each part was mixed and homogenized. Control sample was collected. All samples were held frozen until analyzed.

2.2. Sample analysis for CAP

Analysis of CAP was performed at the laboratory of Institute for brackish Aquaculture (BBPAP). The laboratory is accredited by National Accreditation Committee of Indonesia (KAN). The method of CAP analysis was referred to BIOO Scientific Corp (2007). Briefly, samples were homogenized with a mixer. Three grams of the homogenized sample was weighed and mixed with 6 mL of ethyl acetate, and then, the sample was vortexed for 3 minutes at maximum speed. Next, the sample was centrifuged for 5 minutes at 4000× *g* at room temperature (20°C–25°C). Four milliliters of the ethyl acetate supernatant was transferred into a new vial and a rotary evaporator to dry the sample in a 60°C–70°C water bath under reduced pressure. The dried residues were dissolved in 2 mL of n-hexane. Sample extraction buffer was added and was mixed by vortexing at maximum speed for 2 minutes. Centrifugation was carried out for 10 minutes at ×4000 *g* at room temp. The upper hexane layer was discarded. Fifty microliters of lower aqueous layer per well was used for the assay. In case emulsion happened, the upper n-hexane layer was removed, and the lower aqueous layer was incubated in water bath for 3 minutes at 80°C–95°C. Optical density was measured at 450 nm with a R. Bioparm Well Reader Multiskan microplate (Thermo Fisher Scientific, USA) for all antimicrobials tested. Standard curves were constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ppb on a logarithmic curve. The mean relative absorbance values for each sample were used to determine the corresponding concentration of the tested drug.

3. Result and Discussion

We used enzyme-linked immunosorbent assay method for CAP analysis because it is quite well-known for its speed and accuracy. Nevertheless, this method has disadvantages, both of which are derived from the color noise and disruption caused by the presence of metal ion samples (Yang *et al.* 2012). After feeding, the CAP residues in muscle, cephalothorax, and exoskeleton of *P. monodon* were 0.661, 2.23, and 1.65 μg/kg, respectively (Figures 1 – 3) (Suseno *et al.* 2015). On the other hand, CAP contents in *L. vannamei* were 1.33, 3.00, and 2.4 μg/kg. The cephalothoraxes contained the highest concentration of CAP than muscle and exoskeleton. The digestive track of shrimp is located in cephalothoraxes and responsible for nutritional function such as ingestion, nutriments transit, chemical and mechanical digestions, cellular absorption, and transfer of excreta (Ceccaldi 1989). Within elimination treatment, the concentrations of CAP in shrimp muscle, exoskeletons, and cephalothorax dropped rapidly.

The first order of one-compartment kinetic model was fitted to the depuration measurements for *P. monodon* and *L. vannamei*. The CAP concentration at muscle of *L. vannamei* decreased fast within 1 day, and the curve began to slow down at the later stage of the eliminations. On other hand, CAP concentration in the muscle of *P. monodon* decreased fast within 3 days. Depuration CAP process at exoskeleton and cephalothorax did decrease quickly within 2 days, both in *P. monodon* and in *L. vannamei*. The elimination of CAP was modeled using double-component exponential model

$$C_t = C_0 e^{k_{ef}} + C_{01} e^{k_{es}} \quad (1)$$

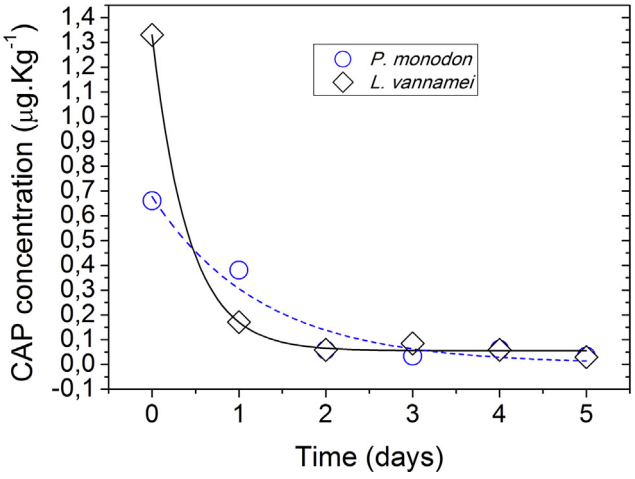

Figure 1. CAP residue in muscle.

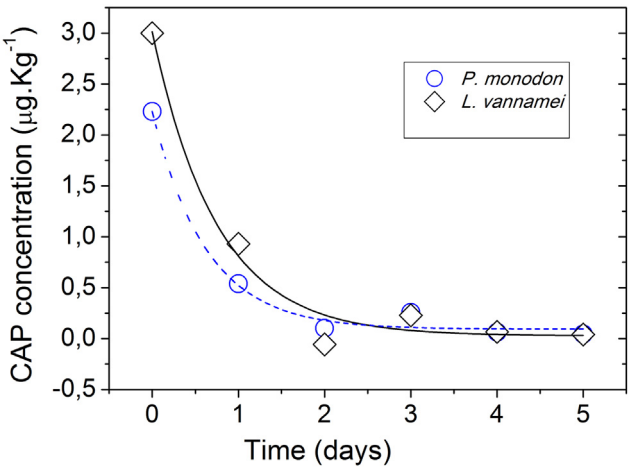

Figure 2. CAP residue in cephalothoraxes.

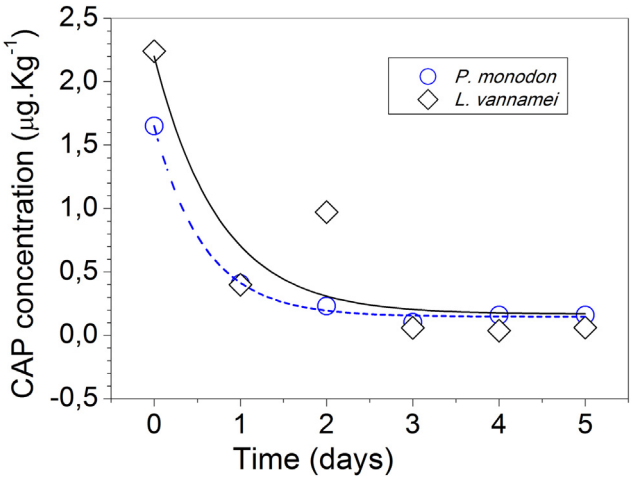

Figure 3. CAP residue in exoskeleton.

where C_0 and C_{01} are CAP concentrations (µg/kg) at time 0 (d) and t, respectively, for short-lived (s) and long-lived (l) components, and k_e is the elimination rate constant (d^{-1}) (Reinardy *et al.* 2011). The retaining concentrations of CAP in the shrimp muscle, head, and exoskeleton were plotted against time. Regression linear was used to determine the elimination of CAP. Elimination half-life ($t_{1/2}$) was then calculated using the equation:

$$t_{1/2} = \frac{\ln 2}{k_e} \quad (2)$$

where k_e is the slope (elimination rate coefficient). The kinetic parameter was provided in Table. The elimination half-life of CAP in *P monodon* was 0.596 day in the muscle, 0.716 day in cephalothorax, and 0.437 day in exoskeleton. On the other hand, in *L. vannamei*, it was 0.6624 day in muscle, 0.859 day in cephalothorax, and 0.796 day in exoskeleton. Thus, CAP was retained better by *P. monodon* than by *L. vannamei*. Weifen *et al.* (2004) reported that elimination half-life ($t_{1/2}$) of CAP was 10.04 hours. Reed *et al.* (2004) reported that OTC did not accumulate in muscle tail. Furthermore, results of analysis of exoskeleton have shown that OTC concentration was below limit detection. Reed *et al.* (2004) also explained that the distribution of antibiotic in tissue of shrimp is very fast. In our experiment, the elimination half-life was short because the distribution rates in muscle, cephalothoraxes, and exoskeleton were very fast. Nogueira-Lima *et al.* (2006) studied the depletion and distribution OTC residue in *L. vannamei* tissue. The levels of OTC residue rapidly dropped after 72-hour termination treatment and then reached a residue level of 0.1 µg/g. In contrast, Leston *et al.* (2013) reported that CAP uptake by sea weed *Ulva lactuca* reached equilibrium within hours. Our experiment results were different from previous works on antibiotic elimination because we studied different organisms and different kinds of antibiotics. Furthermore, antibiotic elimination from shrimp is also influenced by environmental factors such a temperature and salinity (Reed *et al.* 2004; Nogueira-Lima *et al.* 2006). Moreover, the present experiment was performed in open mini-hatchery tanks near the site of shrimp farming.

Table. Biokinetic parameter of CAP elimination from tiger shrimp and white shrimp

Shrimp	Body parts					
	Muscle		Cephalothorax		Exoskeleton	
	k_{ef}	k_{es}	k_{ef}	k_{es}	k_{ef}	k_{es}
Penaeus *monodon*	0.596	0.0068	0.716	0.016	0.437	0.0096
Litopenaeus vannamei	0.6624	0.0085	0.859	0.03	0.796	0.0006

After 5 days of depuration process, the concentrations of CAP in *P. monodon* were 0.034 (muscle), 0.062 (exoskeleton), and 0.045 µg/kg (cephalothorax). On the other hand, CAP concentrations in *L. vannamei* were 0.03, 0.062, and 0.041 µg/kg, as well as *P. monodon*. There have been few studies on CAP elimination from shrimp. Weifen *et al.* (2004) reported that after feeding, CAP concentration in muscle of *P. chinensis* was 0.84 µg/kg. After 4 hours of elimination process, its concentration increased to 3.5 µg/kg. However, the concentration decreased gradually and became undetected after 84 hours. Although in our experiment, the CAP decreased to more than 90%, its residue was still retained. Moreover, according to Council Regulation EEC No. 2377/90 (European Communities), CAP should not be able to be detected in food products at all, regardless of concentrations (Hanekamp and Bast, 2003). Furthermore, according to the Indonesia National Standard No SNI 01-2705.1-2006 about quality and food safety requirements, the concentration of CAP should be 0 ppm. On the other hand, the CAP is still used at a dose of 5 ppm in shrimp aquaculture and ornamental fish (Supriyadi *et al.* 2000). Susanti *et al.* (2009) reported that CAP is still used illegally in shrimp farming to prevent disease. Ardina (2011) reported that CAP was detected at 0.038 ppb–0.054 ppm level in frozen black tiger shrimp. It is difficult to control the use of CAP in shrimp farming in Indonesia because there are two market segmentations. The first market is export oriented, and it requires zero content of CAP. The product for this market should be certified through a legal and accredited laboratory. On the other hand, the second market is for local Indonesian consumers, and the products can be sold without any laboratory testing.

In conclusion, after 5 days of depuration process, the concentration of CAP in *P. monodon* decreased to 94.85% (muscle), 97.98% (cephalothoraxes), and 90.30% (exoskeleton). The elimination half-life of CAP in *P. monodon* was 0.596 day in the muscle, 0.716 day in cephalothorax, and 0.437 day and in exoskeleton. On the other hand, concentration of CAP in *L. vannamei* decreased to 97.74% (in muscle), 90.30% (cephalothoraxes), and 97.63% (exoskeleton). The elimination half-life of CAP in *L. vannamei* was 0.6624 (muscle), 0.859 (cephalothorax), and 0.796 day (exoskeleton). CAP was better retained by *P. monodon* than by *L. vannamei*.

Conflict of interest

The authors report that they have no conflict of interest.

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