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Leaching and Residual Kinetics of Chloramphenicol Incorporated Medicated Feed Treated to Juvenile Black Tiger Shrimp *Penaeus monodon*

Joseph Selvin and A.P. Lipton*
Department of Biotechnology,
Malankara Catholic College
Mariagiri, Kaliakavilai - 629 153, India

The leaching rate of chloramphenicol (CAP) incorporated medicated feed after immersion in water was high during the first 10 min. About 30% loss of CAP from the medicated feed was observed after 2 h of immersion. The leaching rate was not uniform in the period of 10 h experiments. Juvenile black tiger shrimp *Penaeus monodon* fed for 7 days with recommended dose (50-100 mg/kg shrimp) of CAP were examined for residual accumulation and depletion. A higher residue of 7.10 mg/g shrimp tissue was found after 1 day of post-dosing. This was estimated as 2.15% of the total CAP intake in the7 days of experimental period. By the 20th day following cessation of medicated feed treatment the CAP concentration was found below detectable limit.

Key words : Chloramphenicol; leaching; medicated feed; Tiger shrimp *Penaeus monodon*, antibiotic residue, kinetics

Antibiotics are considered as the best choice for reactive management of bacterial diseases in fish and shellfish farming. Their use in global aquaculture is common and mostly unrestricted. However, the number of drugs approved by the United States Food and Drug Administration (USFDA) for use in aquaculture remained at five (Griffin, 1988). The regular use of low concentration of antibiotics, especially in shrimp hatcheries, resulted in development of drug resistant bacterial strains. The genus *Vibrio*, one of the targeted species of bacteria, includes some potent human pathogens as well (Brown, 1989). Chloramphenicol (CAP) was one of the frequently used antibiotics to treat vibriosis and other bacterial infections in Indian shrimp farming (Subasinghe, 1992, Baticados & Paclibare, 1992)

Invariably, antibiotics are incorporated as surface-coatings in feed for oral treatment of bacterial diseases in the grow out phase of shrimp aquaculture (Park *et al.*, 1995). The level of loss during the initial period of shrimp feeding schedule has to be considered as an important factor in order to get an effective therapy. Therefore in the present study, it was intended to evaluate the level of uptake and kinetics of CAP residue in shrimp tissue and leaching rate of CAP following medicated feed treatment for recommended period.

Materials and Methods

The top coated medicated feed was prepared by spraying recommended dose of CAP @ 50-55 mg/kg of shrimp on the surface of the commercial pellet diet (shrimp grower feed No.1, C.P. feeds, Cochin). The shrimps were fed at a rate of 3.2% of the body weight daily. Assuming 50% loss due to leaching and reduced feed intake during diseased condition (about 25%), the medicated feed was prepared with 4 g CAP per kg of shrimp feed so that the shrimp will consume 50 mg/kg of body weight. To

* Marine Biotechnology Laboratory, Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram, Kerala, India

prepare the medicated feed, 4 g of CAP (potency = 980 mg) was dissolved in 50 ml of 4% gelatin water and the mixture was sprayed on 1 kg of pellet feed using a TLC sprayer. The sprayed medicated feed was dried in a hot air oven at 40°C.

Tests to determine the rate of leaching of antibiotics from the medicated feed were conducted in two one litre glass beakers, each containing 500 ml deionised water. The medicated feed, enclosed in a nylon bag with 1 mm mesh, was placed in each beaker. To ensure undisturbed release of antibiotic, the beaker was placed on a magnetic stirrer. The feedbag was carefully lowered without any shaking into the water for 10, 20, 30, 60, 120, 240, 360 and 600 min and then raised above the water column. Then the water was stirred and three water samples (1 ml each) were collected. The level of antibiotic leaching from the medicated feed was determined by using turbidimetric assay method for CAP (USP, 1995 and IP, 1985).

The quantification of CAP was carried out by using *Escherichia coli* (ATCC No.10536) as the test organism. One ml each of standard and test solutions were added in triplicate 18 x 150 mm test tubes and then 9 ml each of inoculum prepared in the antibiotic assay medium No.2 (HiMedia, 1998) was added. Three control tubes were also prepared: the first one contained the inoculated culture medium (culture control), the second one contained the same medium but was treated immediately with 0.5 ml of dilute formaldehyde solution (blank) and the third one contained uninoculated culture medium. All the tubes were placed, in a Latin square arrangement, in a water bath at 37°C for 4 h. After the incubation period, the growth was stopped by adding 0.5 ml dilute formaldehyde solution and then determined by measuring the light transmittance at 530 nm by spectrophotometry (Spectronic 20, USA). Concentrations of the test solutions were determined in comparison to the growth obtained by using the reference standard solutions.

The 45 days old farm-reared tiger shrimps (*Peneaus monodon*) collected from an extensive pond at Kanyakumari coast (Southwest coast of India) were acclimated in a 1000 l capacity FRP tank. After fifteen days of acclimatization, they were maintained in glass aquaria at a rate of 10 shrimps per tank. Prior to administering the drug, 100 healthy shrimp (w = 4 to 6 g) were kept fasting for 24 h. The shrimp were treated with CAP incorporated medicated feed (3.2% of the shrimp bodyweight per day as three equal instalments for 7 days). Before applying the feed, the waste and unfed materials were collected separately and dried at 40°C for evaluation of the daily feed intake. Based on the daily feed intake, a daily antibiotic intake was calculated.

The extraction solvent was determined on the basis of solubility. CAP was sparingly soluble in water at a rate of 1 g in 400 ml and completely dissolved in dehydrated ethyl alcohol at a rate of 1 g in 2.5 ml (Clarke's 1986, USP 1995). On the day 1, 5, 10, 15 and 20 of post treatment, 4 sets of 5 shrimp each were randomly harvested, immediately killed under ice and peeled off the shell and head. The pooled tissues were weighed and minced in a tissue homogenizer. The tissue was then homogenized thoroughly and the homogenate was centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was collected and two other extractions were made by using 10 ml each of solvent. The combined extract was concentrated under reduced pressure in a rotary vacuum evaporator (Buchi type, JSGW). The concentrated extract was diluted in phosphate buffered saline (pH 6.4) for the microbiological assay. The elimination rate constant (b) was calculated from the slope of the line using the equation b = slope/2.303. The elimination half-life ($t_{1/2}$) of CAP for the tissue was calculated by $t_{1/2} = 0.693/b$ (Gibaldi & Perrier, 1975). The results are presented as mean ± SD of four samples.

Results and Discussion

Microbiological methods were reported to be an effective and inexpensive one for detecting antibiotic residues (Giles *et al.*, 1994; Mohney *et al.*, 1997). Many of these methods were modified from the earlier AOAC method for antibiotic concentration in feeds (MacSwain *et al.*, 1992; Giles *et al.*, 1994). However, in the present study, the assay was developed from the standard pharmacopoeias (USP, 1995; IP, 1985). The efficacy of antibiotic assays is mainly depending on the strain of sensitive bacteria used. Literature indicated that all the existing bioassays were based on the plate diffusion technique. Therefore the present one can be regarded as a maiden attempt to evaluate the concentration of CAP by using turbidimetric assay.

In the present study, 4.125 g of CAP/kg of feed was chosen as the successful treatment level. This level of CAP incorporated medicated feed will provide about 132 mg/kg of shrimp. As about 30% leaching of CAP from the medicated feed was observed in the initial 2 h period of feeding time, the estimated mean treatment level in the present study was about 90 mg/kg of shrimp body weight.

Losses of CAP from medicated feed were continuous throughout the immersion period of 600 min in water (Fig. 1). It was found that 20.46% of CAP were leached in the first 10 min and 25.04% in the first 30 min. 5.26% and 7.27% of CAP were leached between 30 and 60 min and 60 and 120 min respectively. Only 2.18% and 1.72% of CAP were leached between 120 and 240 min and 240 and 360 min respectively. During the final stage, between 360 and 600 min, only 4.85% of CAP was leached. So the total leaching was 46.32% of CAP for 600 min. This indicates that CAP leaching was not uniform and that relatively greater losses occurred during the first few minutes of exposure in water than during the subsequent period of immersion (Rigos *et al.*, 1999). According to Tendenzia & de la Pena (2001), the incidence of *Vibrio* resistance in the shrimp farms were least against CAP (0.66% of the total numbers of isolates) compared to OTC (4.3%). Several studies have stressed the role of feed-derived antibiotics as a source of bacterial resistance (Austin 1985; Samuelsen *et al.*, 1992; Coyne *et al.*, 1994; Smith *et al.*, 1994; Kerry *et al.*, 1995). According to Twiddy & Reilly (1994), antibiotic-resistant human pathogenic bacteria are developed in aquaculture ponds where the antibiotics are routinely incorporated into feeds. This problem can be minimized significantly by using antibiotic mixed with feed. According to Rigos *et al.* (1999), the question is whether the feed manufactures respond promptly to the occasional demand of the farmers, as continual availability of medicated feeds are not practical or cost-effective.

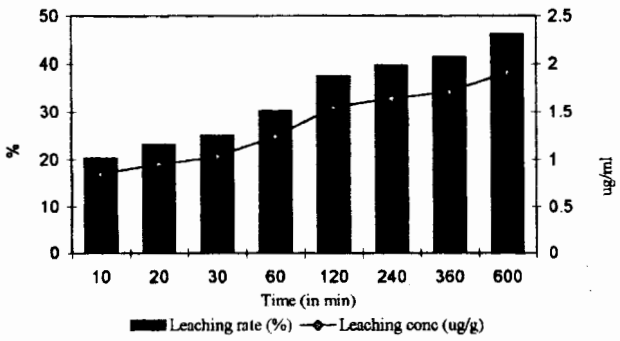

Fig. 1. Leaching rate and concentration of CAP in the environment.

Residual accumulation of CAP in shrimp tissue at 24 h of post dosing was 7.1 µg/g. This higher value may be due to the slower leaching rate and resultant high treatment level (90 mg/kg of shrimp). A substantial quantity of residue was observed 5, 10 and 15 days after the end of the treatment. The quantity of residues was 5.64 µg/g on day 10 and 0.48 µg/g on the day 15 respectively. However no residue was observed 20 days after end of the treatment.

Elimination rate of CAP from shrimp tissue was 1.46 µg/g on the first 5 days after the end of the treatment, 4.28 µg/g between the 5th and the 10th day after the end of the

treatment and only 0.88 μg/g between the 10th and 15th day after the end of the treatment (0.88 μg/g). The trend of bioavailability of CAP in shrimp tissue to estimated antibiotic intake (Table 1) indicated that 2.15% of CAP remained as residue in the shrimp tissue on the day 1 of post-treatment. The level fell below the detection limit on the 20th day through 1.7%, 0.41% and 0.14% CAP on the days 5, 10 and 15 respectively after end of the treatment. The calculated value of terminal elimination constant β is 0.30 and the corresponding elimination half-life $t_{1/2}$ of CAP is 2.26 days.

Table 1. Residual kinetics of CAP in shrimp tissue

Days	Average weight of shrimp tissue (g)	Residual CAP shrimp tissue (μg/g)	% of total CAP absorbed (330 μg/g) remained as residue*
1	5.2	7.10±0.57	2.15
5	5.5	5.65±0.37	1.71
10	4.96	1.36±0.18	0.41
15	4.6	0.48±0.12	0.14
20	5.8	Not detectable	0.0

Values expressed as mean ± SD. N=4
*Rough estimate based on the feeding rate

According to Anastasio *et al.*, 1995, CAP was one of the antibiotic residues detected in 21.0% of marketed shrimp samples. The pharmacokinetic profile of a CAP derivative, 'florfenicol' was reported in Atlantic salmon *Salmo salar* (Horsberg & Hoff, 1994).

Among the commonly used antibiotics, CAP had a bad reputation, since prolonged ingestion of small doses can induce aplasia of bone marrow (Huber, 1986) and growth retardation in fish. Because of its adverse effects in the consumers, it was banned for use in food fishes in many countries (Baticadas & Paclibare, 1992 and Huber, 1986). However it was being heavily used in shrimp farms because of its high positive results obtained during laboratory sensitivity tests (Thonguthai & Chanratchakool, 1992). Based on the present observations, it could be inferred that the CAP treated shrimp should be given at least 25 days of withdrawal period in order to prevent the adverse effect to the consumers.

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