J. Exp. Zool. India Vol. 23, No. 2, pp. 1783-1785, 2020 | www.connectjournals.com/jez | *ISSN 0972-0030*

EVALUATION OF EFFICACY OF OXOLINIC ACID AGAINST *VIBRIO PARAHAEMOLYTICUS* IN PACIFIC WHITE SHRIMP, *LITOPENAEUS VANNAMEI*

K. Rakesh*, M. Ganapathi Naik, Nevil Pinto, K. M. Shankar, B. T. Naveen Kumar, Satish Rama Poojary and P. B. Abhiman

Aquatic Health Management Laboratory, Department of Aquaculture, Karnataka Veterinary, Animal and Fisheries Science University, College of Fisheries, Mangalore-575002, India.
*e-mail : rockysachin10@gmail.com

(Received 14 April 2020, Accepted 21 June 2020)

ABSTRACT : Efficacy of oxolinic acid against *Vibrio parahaemolyticus* was evaluated in *Litopenaeus vannamei*. Among the three dosages feed containing 2, 4 and 6.65g oxolinic acid/kg feed, a dosage of 2g oxolinic acid/kg feed for 3 days was found to be effective against *V. parahaemolyticus* in *L. vannamei*. Further elimination of the oxolinic acid was studied by feeding healthy shrimps with 2 g oxolinic acid/kg of feed at the rate of 3% of body weight. Muscle and hepatopancreas samples were collected at 1, 2, 3, 5, 7, 9, 11, 13 and 15th day after 3 days of medication period. Antibiotic residue was analysed by High Performance Liquid Chromatography (HPLC). The maximum concentration (C_{max}) of oxolinic acid was found in hepatopancreas (1.99µg/g) compared to muscle (1.04µg/g) at 16 h post feeding. On the 15th day, concentration of oxolinic acid in muscle tissue and hepatopancreas found to be 0.15 µg/g and 0.12µg/g, respectively. In the present study, concentration of oxolinic acid did not exceed the maximum residue level limit of 0.3 ppm in both muscle and hepatopancreas. It was found that the withdrawal time of oxolinic acid is 15 days after the cessation of medicated feeding when the residual concentrations in both tissues become less than maximum residual limit and thus safe for human consumption.

Key words : Oxolinic acid, *Vibrio parahaemolyticus*, *Litopenaeus vannamei*, hepatopancreas, antibiotic.

INTRODUCTION

Presently, the biggest problem faced by the aquaculture industry worldwide is disease caused due to various biological and non-biological agents. Many groups of microorganisms affect the shrimp culture among that the best known are bacteria because of the devastating economic effects they have on affected farms. Vibriosis is considered as one of the fatal causes for the mass mortality in the penaeid shrimp hatcheries. Vibriosis is caused by a number of Vibrio species of bacteria including: *Vibrio parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. vulnificus*, *V. penaeicida* (Ishimaru *et al*, 1995). *Vibrio parahaemolyticus*, a gram negative bacterium is one of the important etiologic agents of mass mortalities of shrimp in larval rearing systems. A large number of shrimp hatcheries involved in shrimp seed production often suffer setbacks due to *Vibrio parahaemolyticus* and suffer economic losses.

Antibiotics are frequently used in aquaculture during the production cycle both in the larval and growth phases to control bacterial diseases. The antibiotics most frequently used in aquaculture to control bacterial diseases include florfenicol, oxytetracycline, sarafloxacin and enrofloxacin (Roque *et al*, 2001; Soto-Rodriguez *et al*, 2006), ciprofloxacin, chlortetracycline, quinolones, norfloxacin, oxolinic acid, perfloxacin, sulfamethazine, tiamulin and gentamicin (Holmstrom *et al*, 2003). But the use of pharmaceutical drugs in fisheries sector is still limited. Many of the drugs have been banned by the Food and Drug Administration (FDA), USA due to the persistence of the drug in the tissues of the fishes. Oxolinic acid is a synthetic quinolone antibiotic, which is used in veterinary medicine for the treatment of cattle, pigs, poultry and fin fish. . In several European Union member countries, Oxolinic acid (OA) is a licensed antibiotic that displays a broad spectrum of antibacterial activity, which especially against gram negative bacterial fish pathogens (Ledo *et al*, 1987). Studies related to efficacy of OA in feed against vibriosis in shrimps are very limited. Hence, studies regarding the drug efficacy and its pharmacokinetics are still a challenging aspect in fisheries. Against this background, the present study was aimed to develop an effective dose of oxolinic acid to control the *Vibrio parahaemolyticus* in shrimp production.

MATERIALS AND METHODS

Vibrio parahaemolyticus isolate

The *V. parahaemolyticus* isolate collected from the Cochin University of Science and Technology, Cochin, Kerala, India was confirmed by polymerase chain reaction (PCR) using species specific primer (toxR) (Kim *et al*, 1999).

Minimum inhibitory concentration (MIC) test

The lowest concentration of oxolinic acid at which no bacterial growth observed (MIC) was determined by broth microdilution method (Banerjee *et al*, 2012). Briefly, an inoculum of *V. parahaemolyticus* was prepared and suspension was approximately adjusted to 5×10^8 CFU/ml. Test tubes containing 5 ml of Muller Hinton broth was taken and different concentrations of antibiotic solutions between 0.1-0.5 µg/ml of oxolinic acid and sarafloxacin and 2-10 µg/ml gentamicin was added separately. The lowest concentration of antibiotics at which no bacterial growth observed was determined to be MIC of antibiotic. The determined effective antibiotic dose was used for efficacy study.

Collection and acclimatization of shrimps, *L. vannamei*

Healthy *L. vannamei* (21-23g) were collected from a shrimp farm at Kundapura, Karnataka, India and acclimatized in 3 tubs for a week providing commercial feed at the rate of 3% body weight. The shrimp samples at random were tested for presence of *V. parahaemolyticus* by PCR using species-specific primer (toxR). The acclimatized and PCR negative shrimps were used for the experiments.

Determination of *Vibrio parahaemolyticus* challenge dose (LD_{50})

Ten shrimps were maintained in each 20 L capacity aquarium in triplicate with continuous aeration. *V. parahaemolyticus* (2×10^9 CFU/ml) culture was serial diluted with sterile PBS (Sigma-Aldrich) and shrimps were injected at 10^2,10^3,10^4, 10^5, 10^6, 10^7 and 10^8 CFU/shrimp. Shrimps injected with only sterile PBS served as negative control. The experiment was conducted for 15 days and shrimp survival and mortality was recorded twice daily. During the period, hepatopancreas of the dead shrimps were used for re-isolation of the bacterium using selective TCBS agar and confirmation by PCR.

Preparation of feed with oxolinic acid

Loss of antibiotic due to leaching (50%) and reduced feed intake (25%) during diseased condition of shrimp (Selvin *et al*, 2009) was considered to determine dose of the Oxolinic acid. Oxolinic acid at 2, 4 and 6.65g per kg of feed was mixed with egg albumin, so that the shrimp may consume the antibiotic @ 15, 30 and 50mg/kg body weight respectively. Oxolinic acid and albumin mixture was mixed with commercial shrimp feed (Grobest) followed by coating with sunflower oil, air dried and stored at 4^0C.

Efficacy of oxolinic acid against the *V. parahaemolyticus* in *L. vannamei*

The shrimps were acclimatized for 7 days in 20 L capacity glass aquarium and fed with normal feed. On the 8th day, *V. parahaemolyticus* at 10^4 CFU/shrimp (0.1 ml) was injected to each shrimp. Infected shrimps were divided into 3 treatment groups T1, T2 and T3 with ten animals each in triplicate, were fed with Oxolinic acid incorporated feed at 2, 4 and 6.65g/kg @ 3% of b.w., respectively. *V. parahaemolyticus* injected shrimps without medicated feed was served as the positive control and healthy shrimps which are not injected with *V. parahaemolyticus*, but fed with medicated feed was served as negative control. Shrimps in all the treatment groups were fed with medicated experimental feed for 3 d followed by normal feed later in the remaining period. Mortality was recorded for 15 d. Dead shrimps were examined for clinical signs of *V. parahaemolyticus* infection and were screened by PCR. The relative percent survival (RPS) was calculated by using following formula.

RPS = 1- (Mortality (%) in treatment group / Mortality (%) in control group) × 100

Analysis of oxolinic acid residue in muscle and hepatopancreas of *L. vannamei*

Acclimatized 360 healthy shrimps (21-23g) were divided into two experimental groups (treatment and control) with triplicate and maintained in fibreglass tank of 1000 L capacity with continuous aeration. Shrimps in treatment groups were fed with oxolinic acid at 2g/kg feed for 3 days @ 3% of b.w. After treatment, 50% of sea water was exchanged and shrimps were switched to normal feed for another 15 days to evaluate the elimination of oxolinic acid. While in control groups, shrimps were fed with control feed without the antibiotic. After 3 days of feeding with medicated feed, three shrimps from each treatment tank were randomly collected on 1, 2, 3, 5, 7, 9, 11, 13 and 15th day. Tail muscle and hepatopancreas of the shrimps were taken aseptically and stored at -40 ^{0}C until they were analyzed. In control groups, tail muscle and hepatopancreas were collected at the beginning and at the end of the experiment.

The concentration of oxolinic acid residue in the shrimp tail muscle and hepatopancreas were analyzed by High Performance Liquid Chromatography (HPLC)

by outsourcing to Sealab, Cochin, Kerala, India. Briefly, muscle and hepatopancreas tissues were homogenized separately using blender. Then, 2 g from each homogenized tissue was added into a 50 ml polypropylene centrifuge tube separately and 2 g anhydrous sodium sulphate was added into each tube. Then, 25 ml of ethyl acetate was added to each tube and vortexed followed by centrifugation at 3000–3200 rpm for 15 min. Extraction was repeated twice with 25 ml ethyl acetate. Then ethyl acetate fraction was taken out and evaporated to dry. The residue from each tissue was dissolved in 2 ml mobile phase and defatted with 3-5 ml hexane and the separated aqueous layer was filtered. Twenty µl of the each filtered sample was injected to HPLC for analysis. The chromatography separation was achieved on a RP-C_{18}, 250 x 4.6 mm id, 5 µm column. The mobile phase was 0.01M oxalic acid solution: Acetonitrile (60:40 v/v), Isocratic elution at a flow rate of 1.2 ml/min and a total run time of 7 min. Oxolinic acid present in the shrimp tissue is detected by Fluorescence detector with excitation wavelength 327 nm and emission wavelength 369 nm.

RESULTS

Minimum inhibitory concentration (MIC) of the PCR confirmed *V. parahaemolyticus*

The Vibrio isolate was confirmed as *V. parahaemolyticus* by PCR (Fig. 1). Minimum inhibitory concentration of Oxolinic acid was determined by using broth microdilution method. The MIC of Oxolinic acid was found to be 0.2µg/ml, which was better than Gentamicin (2µg/ml) and Sarafloxacin (0.5µg/ml). Thus Oxolinic acid was selected for the study.

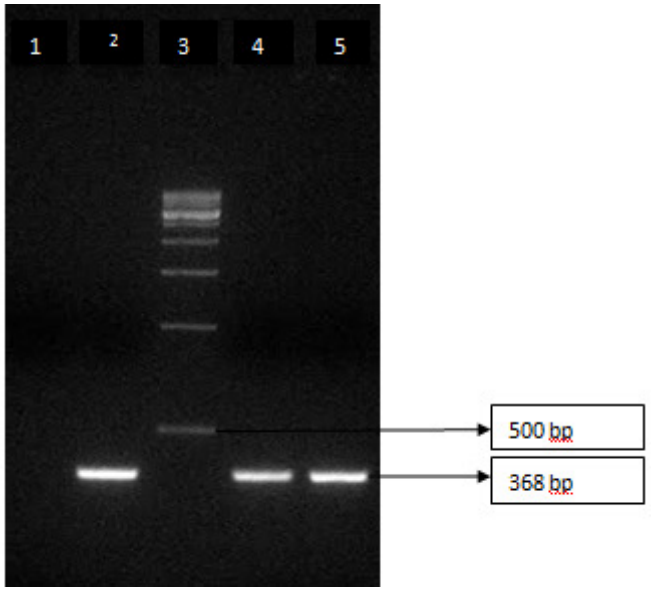

Fig. 1 : Confirmation of *V. parahaemolyticus* isolate by polymerase chain reaction (PCR). LANE 1: *V. parahaemolyticus* negative control; LANE 2: *V. parahaemolyticus* positive control; LANE 3: 500 bp molecular weight marker; LANE 4 & 5: Tested samples.

Determination of LD_{50} of *V. parahaemolyticus*

LD_{50} of *V. parahaemolyticus* in *Litopenaeus vannamei* was found to be 2×10^4 CFU/shrimp having the probits value 4.82 (Fig. 2). At this dose the percentage dead shrimp was 43.33. Shrimp mortality was observed 1d post injection at 10^4, 10^7 and 10^8 CFU/shrimp. No mortality was observed in shrimps injected with sterile PBS. The dose 2×10^4 CFU/shrimp was considered for the challenge studies. The water temperature, salinity and pH during the experiment were 29 ÚC – 30 ^{0}C, 21‰ and 7.8–7.9, respectively.

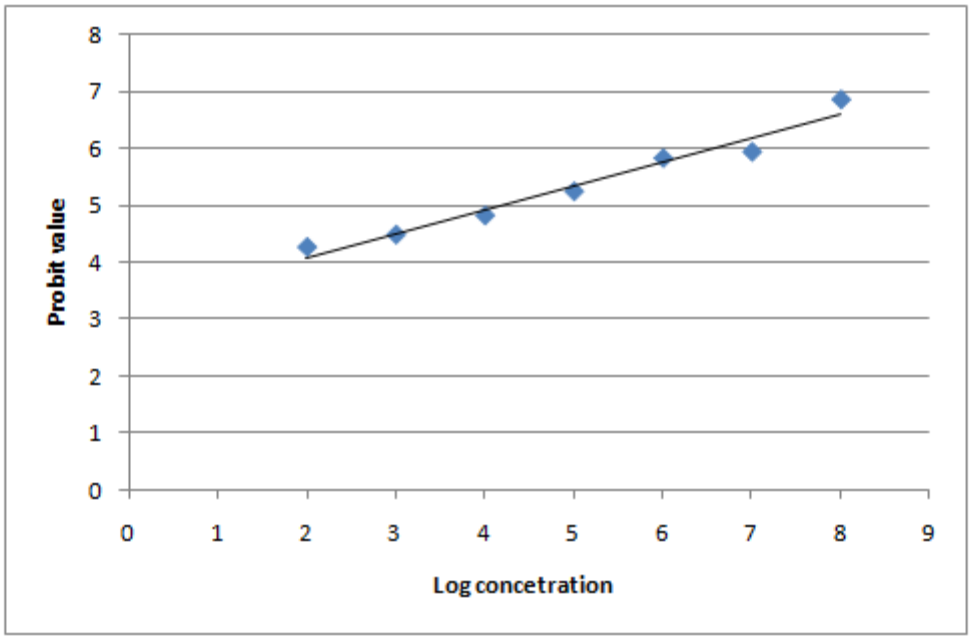

Fig. 2 : Log concentration versus probits for calculation of LD_{50} of *V. parahaemolyticus* in *L. vannamei*.

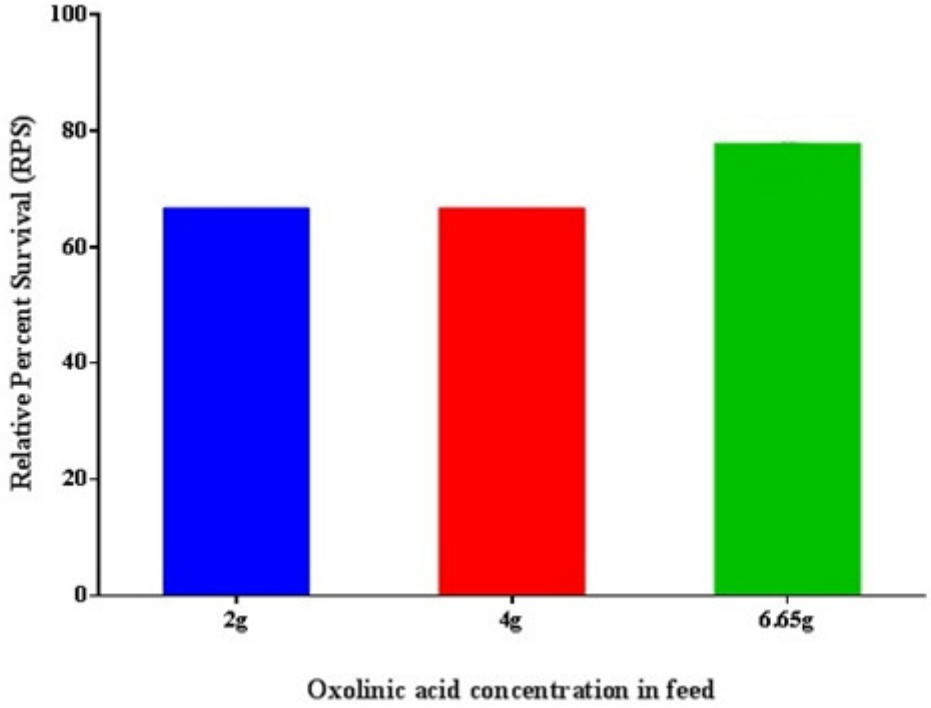

Fig. 3 : Details of Relative Percentage survival (RPS) of efficacy of oxolinic acid against the *V. parahaemolyticus* infected *L. vannamei*.

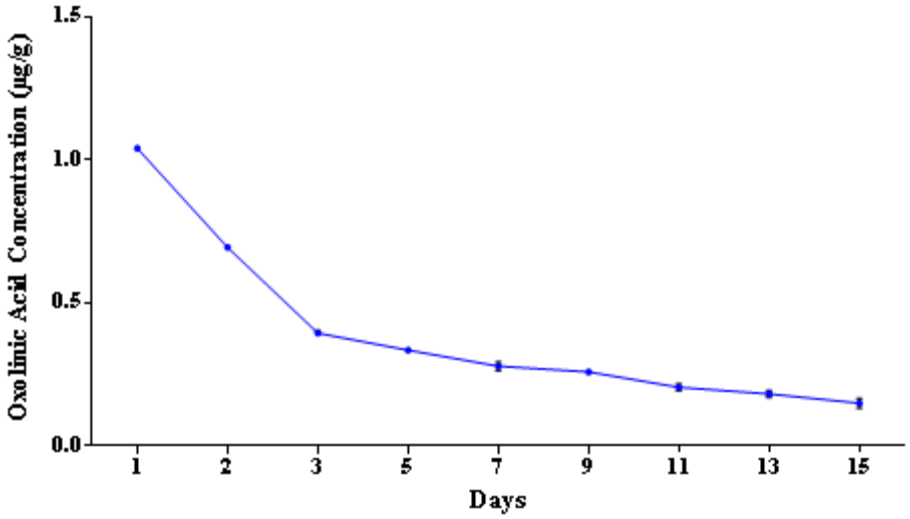

Fig. 4 : Concentration of oxolinic acid in muscle of *L. vannamei* after oral administration (2 g OA/ kg feed).

Efficacy of oxolinic acid against the *V. parahaemolyticus* in *L. vannamei*

Efficacy of oxolinic acid medicated feed was assessed through homologous challenge with *V. parahaemolyticus* (2×10^4 CFU/shrimp). Three feed were prepared with oxolinic acid at a dose of 2, 4 and 6.65g/kg feed. The survival rate of the challenged shrimps at 2 and 4g oxolinic acid/kg feed was 80% (Table 1). A survival rate of 86.66% was observed in challenged shrimps fed with 6.65g oxolinic acid/kg feed (Table 1). Relative percent survival (RPS) was found to be 66.66 for challenged shrimps fed with 2g oxolinic acid and 4g oxolinic acid/kg feed and 77.77 for shrimps fed with 6.65g oxolinic acid/kg feed (Table 1 & Fig. 3). No mortalities were observed in shrimps fed with 2, 4 and 6.65g oxolinic acid/kg feed without challenge. The water temperature, salinity and pH during the experiment were 29^0C – 30^0C, 21‰ and 7.8–7.9, respectively.

Analysis of oxolinic acid residue in muscle and hepatopancreas of *L. vannamei*

The maximum concentration (C_{max}) of oxolinic acid found in muscle tissue was 1.04µg/g at 16 h post feeding. The C_{max} of oxolinic acid in the hepatopancreas found to be 1.99µg/g at 16 h. On the 15th day post feeding, concentration of oxolinic acid in muscle tissue and hepatopancreas found to be 0.15µg/g and 0.12µg/g, respectively (Figs. 4 & 5). Oxolinic acid was not detected

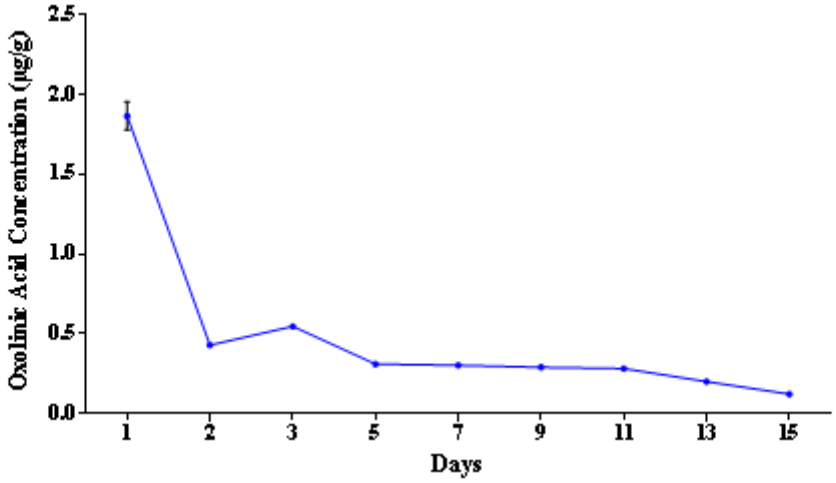

Fig. 5 : Concentration of oxolinic acid in hepatopancreas of *L. vannamei* after oral administration (2 g OA/kg feed).

Table 1 : Details of protection upon challenge in shrimps fed with different doses of oxolinic acid.

Treatments	No. of shrimps challenged	No. of shrimps survived	No. of shrimps dead	% survival	RPS (%)
2g OA/kg feed	30	24	6	80	66.66
4g OA/kg feed	30	24	6	80	66.66
6.65g OA/kg feed	30	26	4	86.66	77.77
Control	30	12	18	40	

in the control shrimps held under the same experimental conditions. The water temperature, salinity and pH during the experiment were 29^{0}C – 30^{0}C, 21‰ and 7.8 - 7.9, respectively.

DISCUSSION

The *V. parahaemolyticus* isolate was confirmed by PCR. Minimum inhibitory concentrations are considered as the gold standards for determining the susceptibility of organisms to antimicrobials (Andrews, 2001). In the present study, MIC of oxolinic acid against *V. parahaemolyticus* was 0.2µg/ml. Barnes *et al* (1990) reported the sarafloxacin MIC of 0.20-4.00µg/ml for oxolinic acid resistant *Aeromonas salmonicida* and 0.0075-1.00µg/ml for oxolinic acid susceptible *Aeromonas salmonicida*. Interestingly, *V. parahaemolyticus* strains isolated from shrimps were resistant to ampicillin, amikacin, tetracycline and chloramphenicol and sensitive to gentamicin and streptomycin (MIC value of <4µg/ml) (Marcus *et al*, 2012). Al-Othrubi *et al* (2011) reported a clear development of *V. parahaemolyticus* resistance towards ampicilin in Malaysian shrimp farms. The MIC of ampicilin was 24µg/ml in 2004 and 64µg/ml in 2007. It is clearly understood that *V.parahaemolyticus* strain used in this study did not develop resistant towards oxolinc acid as its MIC value was lower.

Efficacy of oxoilinic against *V. parahaemolyticus*

The oxolinic acid is increasingly used in shrimp culture due to its high antimicrobial potency and to its shorter withdrawal period (Hustvedt *et al*, 1991b). Studies related to efficacy of oxolinic acid in feed against vibriosis are very limited. Limsuwan *et al* (1993) found higher survival rate of 73.33% in *P. monodon* fed with oxolinic acid medicated feed @ 2.5g/ kg feed. Frelier *et al* (1993) recommended the application of 3 g oxytetracyclin(OTC)/ kg feed in *L. vannamei* NHP (Necrotising hepatopancreatitits) infection treatment. Satisfactory survival rates, healthy behaviour and a weight increase in OTC fed animals with no gross NHP infection signs were observed in case of *L. vannamei* (Nogueira-Lima *et al*, 2006).

It is well established that higher dose of antibiotic medication can result in accumulation of residual antibiotic in shrimp tissue and contribute to emergence of resistant bacteria (Selvin *et al*, 2009; Flores-Miranda *et al*, 2012). In the present study, 6.66% higher survival rate of shrimp was observed when the oxolinic acid dose was 6.65g/kg feed. The survival rate of shrimp was just 6.66% less when the lower oxolinic acid dose of 2g/kg feed. This study indicates that to achieve 6.66% higher survival rate, oxolinic acid dosage must be increased by 4.65g/kg feed. This higher dosage may cause complications in shrimp such as down-regulation of immune genes and as a result shrimp may be susceptible to infection and survival rate also decreases. However, higher dose of oxolinic acid will result in down- regulation of genes like penaeidin,

proPO, clotting protein, profilin and whey acidic protein which is involved in the immune response in shrimp and fish (Fagutao *et al*, 2009). An earlier study on pharmacokinetics of oxolinic acid in *Penaeus japonicus* showed that it was rapidly and widely distributed to tissues outside of the haemolymph and found to be eliminated more slowly (Uno, 2004). The higher dosage of oxolinic acid may lead to bioaccumulation in tissue and also residual accumulation in culture environment. Interestingly, in the present study, percentage survival and RPS of *L. vannamei* were found to be 80% and 66.66% for both medicated feed with oxolinic acid @ 2g/kg feed and 4 g/kg feed respectively. Similar results were observed in *Ulva* medicated feed where higher doses 1000 and 1500 mg/kg shrimp elicited more or less similar protection. The exact mechanism was not known why the higher dose (1500 mg/kg) did not produce high responses compared to that of 1000 mg/kg (Selvin *et al*, 2009). In the case of antibiotics, it is well established that over-dosing of antibiotic medication lead to residual accumulation in the shrimp tissue which otherwise have produced higher responses than the actual dose (Selvin *et al*, 2009). To avoid all these complications, in the present study, a lower dose of 2g oxolinic acid/kg feed was considered as the better dosage against *V. parahaemolyticus* in *L. vannamei*.

Oxolinic acid residue level in *L. vannamei*

The concentrations of oxolinic acid in the muscle tissue and hepatopancreas of shrimps after oral administration of 2g /kg feed @ 3% of body weight/day for 3 days were analysed by HPLC. The maximum concentration (C_{max}) of oxolinic acid found in the muscle tissue and hepatopancreas was 1.04µg/g and 1.99µg/g at 16 h after the cessation of medicated feeding, respectively. The time for attaining maximum concentration (T_{max}) of oxolinic acid both in the muscle and hepatopancreas was estimated at 16h after the cessation of medicated feeding. On the 15th day after cessation of medicated feeding, concentration of oxolinic acid in the muscle tissue and hepatopancreas found to be 0.15µg/g and 0.12µg/g respectively which were less than maximum residual level(MRL) limit of oxolinic acid (0.3 ppm) in shrimp for human consumption (Expert Group Organised by Aquaculture Authority, Government of India, 2002). This clearly indicates that the withdrawal time of oxolinic acid is 15 days in *L. vannamei* when the residual concentrations in tissues become less than MRL and thus safe for human consumption. The oxolinic acid concentration in the muscle and hepatopancreas showed gradual decrease after 3 days of medicated feeding till the termination of the experiment. The reduction in oxolinic acid concentration may be due to the drug distribution to the different site such as the shell (Uno *et al*, 2004).

In the present study, muscle tissue and hepatopancreas were collected for oxolinic acid residue analysis because the muscle is used to test the MRL for antibiotic residues in many countries and hepatopancreas is the target site of vibriosis. The C_{max} of oxolinic acid found to be high in hepatopancreas compared to the muscle at 16h after cessation of medicated feeding. This may be due the medicated feed remaining in the hepatopancreas acts as a time released dosage form gradually releasing oxolinic acid *in vivo* into the shrimp circulation as shrimp has a ruminating digestive system. In addition, oxolinic acid in hepatopancreas of shrimp had higher concentration than in haemolymph and muscle and it also persisted in hepatopancreas longer than muscle (Chantaraprateep *et al*, 1998). Metabolism and elimination of drugs in vertebrates take place in separate organs such as the liver and kidney, but the shrimp only possess a hepatopancreas, which plays several roles such as collection, metabolism, absorption and elimination of antibiotics (Verri *et al*, 2001; Reed *et al*, 2006; Faroongsarng *et al*, 2007). The higher Cmax of antibiotics in hepatopancreas is also due the drug delivered from the shrimp stomach to the hepatopancreas after oral administration (Tu *et al*, 2010). Tipmongkolsilp *et al* (2006) found higher concentration of florfenicol in hepatopancreas (0.7µg/g) than in muscle (0.05µg/g) in *P. monodon*. Somjetlertcharoen *et al* (1993) studied the residues of oxolinic acid in *P. monodon* after oral administration at a dose 2.5g/kg in feed for 7 days. The result found that oxolinic acid persisted in hepatopancreas longer than muscle of shrimp. Payooha *et al* (1993) studied the residue of oxolinic acid in *P. monodon* at a dose of 10, 20 and 30 mg/kg b.w. The maximum level of oxolinic acid residue was detected in the hepatopancreas and the concentration in hepatopancreas was higher than in muscle and haemolymph. The water temperature, salinity and pH maintained during the experiment were 29^{0}C–30^{0}C, 21‰ and 7.8–7.9, respectively. Furthermore, in the present study, from the residual analysis, it was found that on the 15th day after cessation of medication, the concentration of oxolinic acid in the muscle tissue and hepatopancreas found to be 0.15µg/g and 0.12µg/g respectively, which were less than maximum residual level (MRL) limit of oxolinic acid (0.3ppm) in shrimp for human consumption. This indicates that oxolinic acid in the feed 2 g/kg feed is the most appropriate dosage for controlling *V. parahaemolyticus* infection in *L. vannamei* as well as to avoid health risk problems. With these results, the use of oxolinic acid is of more use for treating infected

shrimp, since low concentrations have to be used to eliminate the potential pathogens *in situ* and those low concentrations are not toxic to culture environment.

CONCLUSION

In the present study, concentration of oxolinic acid did not exceed the maximum residue level (MRL) limit of 0.3 ppm in both muscle and hepatopancreas of *L. vannamei*. It was found that the withdrawal time of oxolinic acid is 15 days after the cessation of medicated feeding when the residual concentrations in tissues become less than maximum residual limit and thus safe for human consumption. Therefore, shrimp farmers may use oxolinic acid at a dose level of 2g/kg feed (15mg/kg b.w/day) for effective control of *V. Parahaemolyticus* in *L. vannamei*.

ACKNOWLEDGEMENT

The guidance and facilities provided by Dr. K. M. Shankar, Dean (Fisheries), College of Fisheries, Mangalore, India are gratefully acknowledged.

REFERENCES

Banerjee S, Kim L M, Shariff M, Khatoon H and Yusoff F M (2012) Antibacterial activity of Neem (*Azadirachta indica*) leaves on Vibrio spp. isolated from cultured shrimp, *Asian J. Anim. Vet. Adv.* **8**, 355-361.

Ishimaru K, Akarawa-Matsushita M and Muroga K (1995) *Vibrio penaeicida* sp., nov., a pathogen of kuruma shrimps (*Penaeus japonicas), Int. J. Syst. Bacteriol*. **43**, 8-19.

Roque A, Molina A A, Bolan M C and Gomez G B (2001) *In vitro* susceptibility to 15 antibiotics of vibrios isolated from penaeid shrimps in Northwestern Mexico, *Int. J. Antmicrob. Ag*. **17**, 383-387.

Soto-Rodriguez S, Armenta M and Gomez-GIL B (2006) Effects of enrofloxacin and florfenicol on survival and bacterial population in an experimental infection with luminescent *Vibrio campbellii* in shrimp larvae of *Litopenaeus vanname. Aquacul*. **255**, 48-54.

Holmstorm K, Graslund S, Wahlstrom A, Poungshompoo S, Bengtsson, B E and Kautsky N (2003) Antibiotic use in shrimp farming and implications for environmental impacts and human health. *Int. J. Food Sci. Tech.* **346**, 49-59.

Kim Y B, Okuda J, Matsumoto C, Takahashi N, Hashimoto S and Nishibuchi M (1999) Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *tox* R gene. *J. Clin. Microbiol*. **37**, 1173– 1177.

Selvin J, Ninawe A S and Lipton A P (2009) *Shrimp Disease Management: Prospective Approaches*. (ANE Publishers; New Delhi, India).

Andrews J M (2001) Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemoth*. **48**, 5-16.

Barnes A C, Lewin C S, Hastings T S and Amyes S G (1990) *In vitro* activities of 4-quinolones against the fish pathogen *Aeromonas salmonicida*. *Antimicrob. Agents. Chemother.* **34**(9), 1819-1820.

Marcus H Y W, Ming L, Hoi W and Sheng C (2012) Characterization of Extended-Spectrum-â-Lactamase-Producing *Vibrio parahaemolyticus. Antimicrob. Ag. Chemother*. **56**, 4026–4028.

Al-Othrubi S M, Hanafiah A, Radu S, Neoh H Jamal R (2011) Rapid detection and E-test antimicrobial susceptibility testing of *Vibrio parahaemolyticus* isolated from seafood and environmental sources in Malaysia. *Saudi Med J.* **32**(4), 400-406.

Hustvedt S O, Salte R and Vassvik V (1991) Absorption, distribution and elimination of oxolinic acid in Atlantic salmon (*Salmo salar* L.) after various routes of administration. *Aquacult.* **95**, 193-199.

Flores-Miranda M C, Luna-González A, Campa Córdova A I, FierroCoronado J A, Partida-Arangure B O, Pintado J and González-Ocampo H A (2012) Isolation and characterization of *Vibrio sinaloensis* strains infectious for the Pacific shrimp *Litopenaeus vannamei* (Decapoda: Penaeidae). *Rev. Biol. Trop*. **60**(2), 567-576.

Uno K (2004) Pharmacokinetics of oxolinic acid and oxytetracycline in Kuruma shrimp, *Penaeus japonicas*. *Aquacult.* **230**, 1–11.

Fagutao F, Yasuike M, Santos M D, Lila R L, Sangrunggruang K, Anchalee T A, Takahashi Y, Ueno R, Kondo H, Hirono I and Aoki T (2009) Differential gene expression in black tiger shrimp, *Penaeus monodon* following administration of oxytetracycline and oxolinic acid. *Devel. and Comp. Immunol.* **33**, 1088–1092.

Limsuwan C T, Lamkom P and Chanratchakool Capelo F (1993) Efficacy of oxolinic acid against *Vibrio alginolyticus* infection in black tiger shrimp. *Second Symposium on Diseases in Asian Aquaculture*. Phuket, Thailand,.

Frelier P F, Loy J F and Kruppenbach B (1993) Transmission of Necrotizing Hepatopancreatitis in *Penaeus vannamei*. *J. Invert. Pathol.* **61**, 44-48.

Nogueira-LIMA A C, Gesteira T C V and Mafezoli J (2006) Oxytetracycline residues in cultivated marine shrimp (*Litopenaeus vannamei* Boone, 1931) (Crusta- cea, Decapoda) submitted to antibiotic treatment. *Aquacult.* **254**, 748-757.

Chantaraprateep P, Sinhaseni P, Udomprasertgul V, Rungphitackchai B, Issaravanich S and Boonbundarlchai (1998) Toxicity and residues of Oxolinic acid. *Thai. J. Hlth. Res.* **12**(2):125-139.

Faroongsamg D, Chandumpai A, Chiayvareesajja S and Theapparat Y (2007) Bioavailability and absorption analysis of oxytetracycline orally administered to the standardized moulting farmed Pacific white shrimps (*Penaeus vannamei*). *Aquacult.* **269**, 89–97.

Reed L A, Siewicki T C and Shah A C (2006) The biopharmaceutics and oral bioavailability of two forms of oxytetracycline to the white shrimp, *Litonenaeus setiferus. Aquacult.* **258**, 42-54.

Verri T, Mandal A, Zilli L, Bossa D, Mandal P K, Ingrosso L, Zonno V, Vilella S, Ahearn G A and Storelli C (2001) D-Glucose transport in decapod crustacean hepatopancreas. *Comp. Biochem. and Physio. Part A: Mol. & Intgr. Physiol.* **130**, 585–606.

Tu H T, Silvestre F, Phuong N T and Kestemont P (2010) Effects of pesticides and antibiotics on penaeid shrimp with special emphases on behavioural and biomarker responses. *Environ. Toxicol. Chem.* **29**, 929–938.

Somjetlertcharoen A, Chinabut S and Limsuwan C (1993) Oxolinic acid residues from *Penaeus monodon* Fabricius in intensive culture pond. *The 31st Kasetsart University Annual Conference.*

Payooha K, Limsuwan C, Chinabut S and Chanratchakul P (1993) Study of residues of oxolinic acids in *Penaeus monodon* Fabricius. *The 31st Kasetsart University Annual Conference*.

Tipmongslip N, Yanin L Y, Patamalai P, Lusanandana P and Wongtavatchai J (2006) Oral medication with florfenicol for black tiger shrimp *Penaeus monodon*. *Thai. J. Vet. Med.* **36**, 39-47.