International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (11), pp. 001-009, November, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Adsorption, metabolism and degradation of erythromycin in giant freshwater prawn and tilapia aquaculture in Mekong River Delta

N. P. Minh*, T. B. Lam and T. T. D. Trang

Department of Food Technology, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam.

Accepted 27 August, 2018

Adsorption, metabolism and degradation of erythromycin in freshwater prawn and tilapia aquaculture in Mekong River Delta were monitored and evaluated. They were fed practical diets medicated with erythromycin (50 and 100 mg. kg^{-1} body weight for 7 days). Erythromycin residues in their muscle were determined by the liquid chromatography - mass spectrometry/ mass spectrometry (LC-MS/MS) method. Our study provided preliminary data for a more prudent use of erythromycin in giant freshwater prawn and tilapia, suggesting a possible withdrawal time after treatment as well as clearing away the awareness of forming and accumulating a harmful over-threshold level of derived products from parental drug during veterinary usage in aqua culture.

Key words: Giant freshwater prawn, tilapia, erythromycin, metabolism, degradation, LC-MS/MS.

INTRODUCTION

Erythromycin is a macrolide antibiotic that is produced by the actinomycete species, *Streptomyces erythreus*. The chemical structures of erythromycin A (EA), which is the major component of erythromycin base, and its related substances, are depicted in Figure 1. Erythromycin is a polyhydroxylactone that contains two sugars. The aglycone portion of the molecule, erythranolide, is a 14-membered lactone ring. An amino sugar, desosamine, is attached through a -glycosidic linkage to the C-5 position of the lactone ring. The tertiary amine of desosamine confers a basic character to erythromycin (pK_a 8.8). Through this group, a number of acid salts of
the antibiotic have been prepared. A second sugar, cladi-nose, which is unique to erythromycin is attached via a -glycosidic linkage to the C-3 position of the lactone ring.

The fermentation process that produces commercial grade erythromycin is not entirely selective. It results in the production of small quantities of erythromycin B (EB), C (EC), D(ED), E(EE) and F(EF), in addition to EA, which is the major component. EB, EC and EE are the most important impurities found in commercial samples of erythromycin (Table 1).

In addition to the related substances, the metabolite, demethylerythromycin (dMeE), and acidic and basic degradation products are also present in small quantities in commercial samples of erythromycin. These include erythromycin enol ether (EEE), anhydroerythromycin (AE), erythrolosamine (ESM), pseudoerythromycin A hemiketal (psEAHK), pseudoerythromycin A enol ether (psEAEE) and dehydroerythromycin (DE). Other related substances such as erythromycin A *N*-oxide (EANO), erythromycin oxime (EOXM) and erythromycylamine also exist and are structurally very similar, differing by only hydrogen, hydroxyl and/or methoxy groups.

Moreover, erythromycin also exists in forms of erythromy-cin stearate (ES), erythromycin ethylsuccinate (EESC), propionyl erythromycin (PE), erythromycin estolate (EES), erythromycin lactobionate (EL), erythromycin glucoheptonate (EG), erythromycin ethyl carbonate (EEC) and erythromycin acistrate.

Giant freshwater prawn (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus)* have been considered two of the most important species of freshwater aquaculture in Viet Nam, especially in the Mekong

*Corresponding author. E-mail: stapimex@gmail.com.

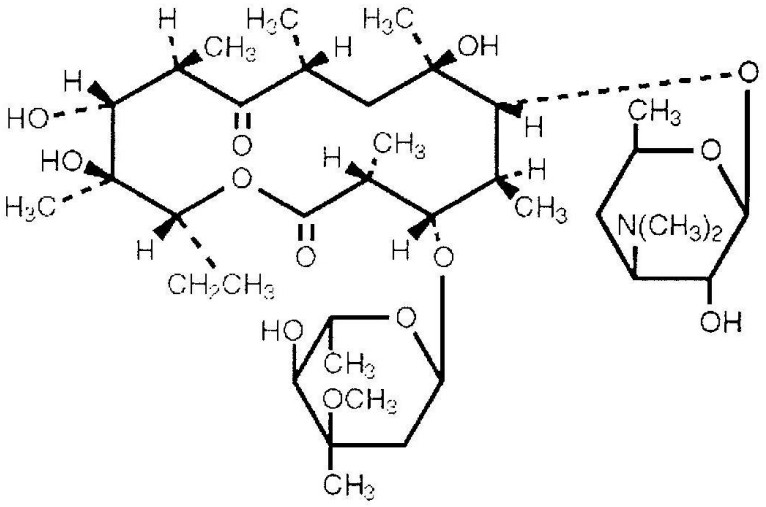

Figure 1. Chemical structures of erythromycin A.

Table 1. Formula of erythromycin A and related substances.

Erythromycin	Formula	Molecular mass	R_1	R_2	R_3	R_4	R_5
A	$C_{37}H_{57}NO_{13}$	734	OH	H	H	OCH_3	CH_3
B	$C_{37}H_{57}NO_{12}$	718	H	H	H	OCH_3	CH_3
C	$C_{38}H_{55}NO_{13}$	720	OH	H	H	OH	CH_3
D	$C_{36}H_{65}NO_{12}$	704	H	H	H	OH	CH_3
E	$C_{37}H_{67}NO_{13}$	748	OH	-O-	OCH_3		CH_3
F	$C_{37}H_{67}NO_{14}$	750	OH	OH	H	CH_3	CH_3

River Delta. Bacterial necrosis is a common disease observed in adult prawns (Winton Cheng and Jiam-Chu Chen, 1998). Bacterial necrosis has variously been termed as 'black spot', 'brown spot', 'shell disease' or chitinolytic bacterial disease. It is caused by the invasion of chitinolytic bacteria, which break down the chitin of the exoskeleton. *Aeromonas hydrophila, Aeromonas caviea, A. sorbia* and *Aeromonas sp.* were bacterial flora isolated from necrosis prawns (Dat N. T., 2002). *Pseudomonas fluorescens, Aeromonas* sp.*, Lactococcus garvieae* and *Edwardsiella tarda* were bacteria flora isolated from adult prawns (Ahmed, 2003; Be, 2002; Lalitha and Surendran, 2006; Shih-Chu et al., 2001; Tran et al., 2002). Meanwhile, the most significant diseases in Nile tilapia (*Oreochromis niloticus)* culture are caused by *Streptococcus iniae, Aeromonas hydrophila*, *Trichodina sp., Flexibacter, Edwardssiella* spp. (Nagla et al., 2005).

The macrolide antibiotic erythromycin has long been the chemotherapeutant of choice to prevent and tackle these pathogenic bacteria. However, there are limited studies being published relating to adsorption, metabolism and degradation of erythromycin in aquatic species. Purpose of this study was to survey the adsorption, metabolism and degradation of erythromycin in giant freshwater prawn muscle (*Macrobrachium rosenbergii*) and Nile tilapia (*Oreochromis niloticus)* after oral administration of the drug given by medicated feed. From that, we can interpolate appropriately tentative withdrawal times. In addition, an evidence of bio-transformative forms of erythromycin, not only in fermentation process but in endo-enzymatic aquaculture pathway as well can be obviously seen.

MATERIALS AND METHODS

Materials

Erythromycin base in white powder and purity 96.5% was purchased from DHG Pharma (Can Tho, Vietnam). Feed and coating agent (squid liver oil) were supplied from Grobest Ltd.

Animals and diet

750 adult giant freshwater prawns (*Macrobrachium rosenbergii*), with an average weight 40 ± 2 g and 120 adult Nile tilapias (*Oreochromis niloticus)* with an average weight 500 ± 5 g were used

for the investigation.

750 adult giant freshwater prawns (*Macrobrachium rosenbergii*) were separated into two groups: Group A (375 prawns) and Group B (375 prawns). Two different diets were prepared for the experimental trial. Group A was treated with 50 mg kg^{-1} prawn body weight day^{-1} for 7 days through medicated feed (water temperature, 28°C). Group B was treated with 100 mg kg^{-1} prawn body weight day^{-1} for 7 days through medicated feed (water temperature, 28°C). 120 adult Nile tilapias (*Oreochromis niloticus*) were divided into Group A (60 tilapias) and Group B (60 tilapia). Two different diets were prepared for the experimental trial. Group A was treated with 50 $mg.kg^{-1}$ tilapia body $weight.day^{-1}$ for 7 days through medicated feed (water temperature, 28°C) while Group B was treated with 100 $mg.kg^{-1}$ tilapia body $weight.day^{-1}$ for 7 days through medicated feed (water temperature, 28°C).

Two groups of medicated feed were conditioned by weighing and mixing feed with erythromycin base at appropriate dosages. Combination between drug and feed was adhesively guaranteed by a coating agent (squid liver oil).

Temperature monitoring

Freshwater prawn and tilapia were poikilothermic species. The optimum metabolic temperature range for them is between 26 and 32°C. Temperature could strongly affect to their survival and enzymatic metabolism, including drug biotransformation. So influence of temperature fluctuation at sampling time was recorded and mentioned in drug metabolism calculation.

Sample collection

Erythromycin base material had been estimated previously to screen and confirm whether other derivatives of erythromycin A, such as erythromycin B, C, D, E and F have been available or not. Sampling times for the prawn and fish in Group A and B were 1, 3, 6, 9 and 23 days after 7 days of the pharmacological treatment. At each sampling time, individuals in each group were sacrificed to confirm erythromycin A residue. Meanwhile, bio-transformation of erythromycin in prawn and tilapia was monitored by screening and confirming derivative forms of erythromycin at the beginning and the end of sampling stage.

Muscle samples in natural proportion were collected, and placed into polyethylene bags, coded and transferred to the laboratory on dry ice, stored at −40°C before analysis.

Analytical procedures

The methodology used for the determination of erythromycin A as well as derivatives of erythromycin in erythromycin base material, in prawn and fish muscle was based on LC- MS/MS. Parameters of measurements: methanol as the extraction solvent; a temperature of 80°C; a pressure of 1500 psi; an extraction time of 15 min; 2 cycles; a flush volume of 150% and a purge time of 300 s.

RESULTS

Adsorption and depletion of erythromycin A

The influence of water temperature on fish metabolism and, consequently, on the drug pharmacokinetics, the time parameter was also expressed as °C-day. Degree-days were calculated by multiplying the mean daily water temperature by the total number of days at which the temperature was measured to that point.

Prawn

Results of erythromycin depletion at different times in prawn muscle samples treated with 50 mg kg^{-1} (Group A) and 100 mg kg^{-1} (Group B) prawn body weight day^{-1} for 7 days were shown in Table 2 and Figure 2.

Tilapia

Results of erythromycin A depletion at different times in tilapia samples treated with 50 and 100 mg kg^{-1} fish body weight day^{-1} for 7 days were shown in Table 3 and Figure 3.

Determination of withdrawal time and metabolism and degradation of parental drug

The MRL value for erythromycin was set at 30 $\mu g.kg^{-1}$, as reported by CFIA (*Canadian Food Inspection Agency*), date 17/11/2009. The regression line and the upper, one-sided tolerance limit (95%) regression line with a confidence of 95% were also traced. This graph had been obtained using the statistical program recommend-ded by the European Agency for the Evaluation of Medicinal Products (EMEA) and was downloadable from the same EMEA web site (2009).

Prawn

A withdrawal time of 976°C days was interpolated for giant freshwater prawn treatment - Group B (Figure 4). The results for metabolism and degradation of parental drug are shown in Table 4.

Tilapia

A withdrawal time interpolated for tilapia treatment was 908°C-days (Group A) and 1150 °C-days (Group B) (Figures 5 and 6). The results for metabolism and degradation of parental drug are shown in Table 5.

DISCUSSION

Our research was designed in conditions that were quite close to actual aquaculture. The minimum inhibited concentrated of erythromycins A, B, C and D and some of

Table 2. Erythromycin depletion at different times in giant prawn muscles treated with 50 mg kg^{-1} prawn body weight day^{-1} for 7 days.

Time		Erythromycin residue in prawn muscle (µg/kg) $^{\alpha}$	
Day	°C – Day	Group A	Group B
1	28	15.4 ± 3.3	632.4± 74.1
3	84	10.6 ± 2.1	199.0± 31.2
6	168	5.9 ± 3.1	141.8 ± 3.1
9	252	5.5 ± 4.1	54.2± 9.0
23	644	2.8 ± 0.8	31.4± 7.5

α Values shown are concentration means ± standard deviations from 5 prawn samples.

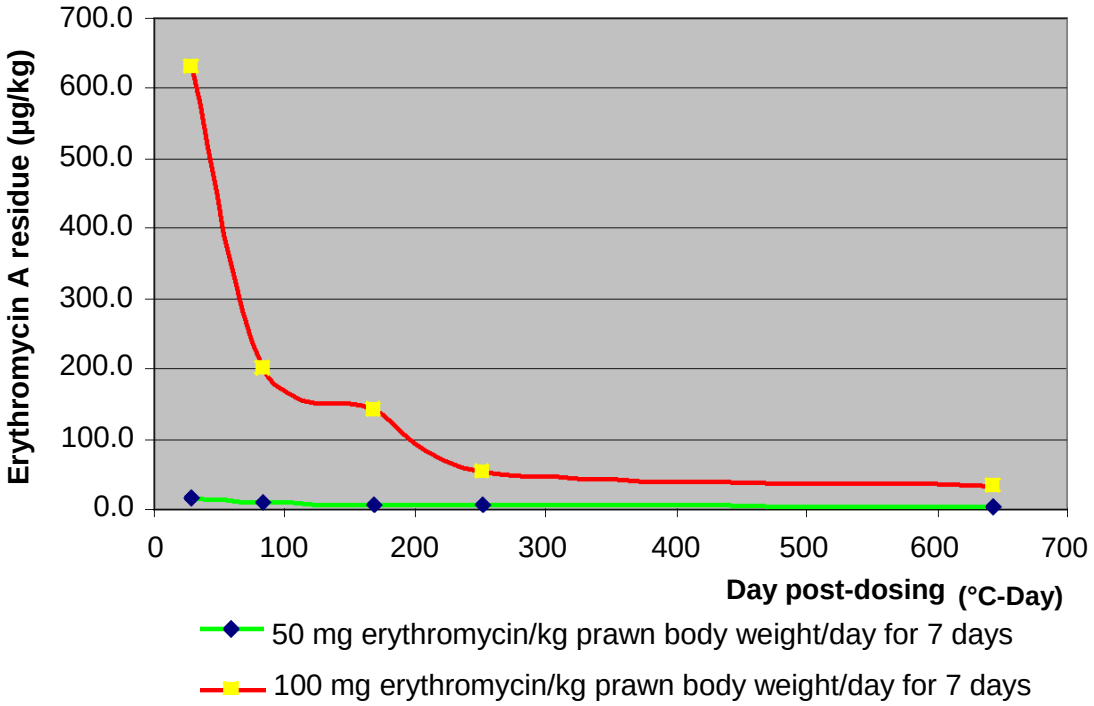

Figure 2. Erythromycin A depletion at different times in giant prawn muscles treated with 50 and 100 $mg.kg^{-1}$ prawn body $weight.day^{-1}$ for 7 day.

Table 3. Erythromycin A depletion at different times in tilapia fillet samples treated with 50 $mg.kg^{-1}$ and 100 mg.kg fish body $weight.day^{-1}$ for 7 days.

Time		Erythromycin A residue in tilapia fillet (µg/kg) $^{\alpha}$	
Day	°C – Day	Group A	Group B
1	28	22,216.0± 22,023.0	46,960.0 ± 9,054.7
3	84	13,590.0± 14,415.9	14,328.0± 18,336.1
6	168	940.8± 460.3	6,382.0± 5,582.5
9	252	131.4 ± 31.9	379.7 ± 99.3
23	644	34.7 ± 9.6	42.9± 17.4

α Values shown are concentration means ± standard deviations from 5 tilapia fillet samples.

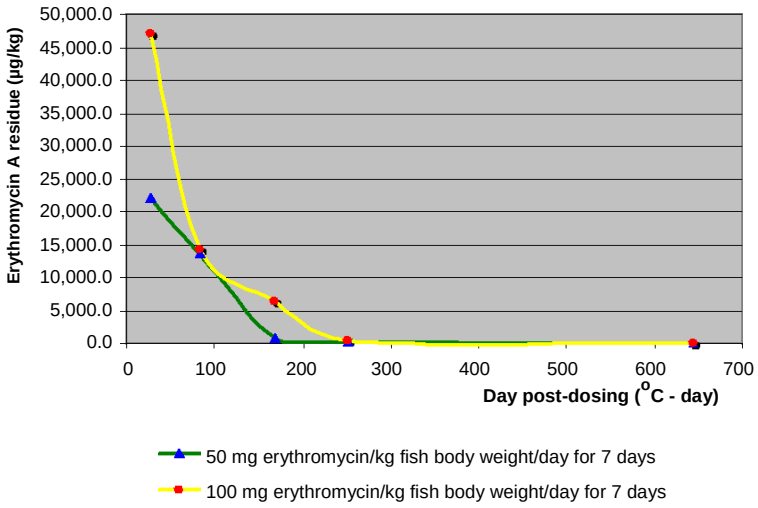

Figure 3. Erythromycin A depletion in tilapia fillet samples treated with 50 and 100 mg.kg^{-1} fish body weight.day^{-1} for 7 days.

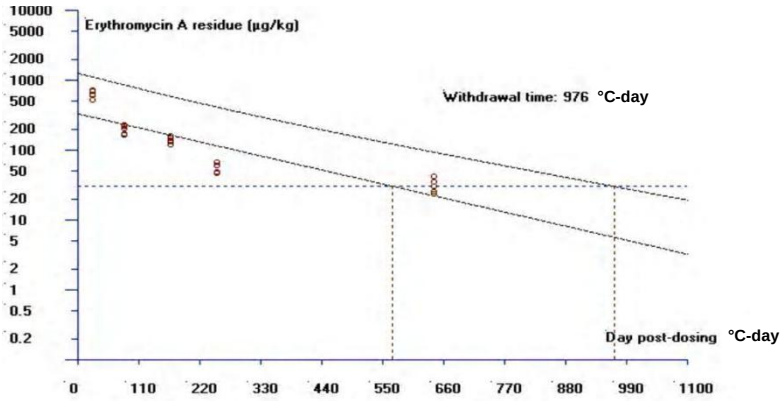

Figure 4. Withdrawal time in muscle prawn treated with erythromycin - Group B.

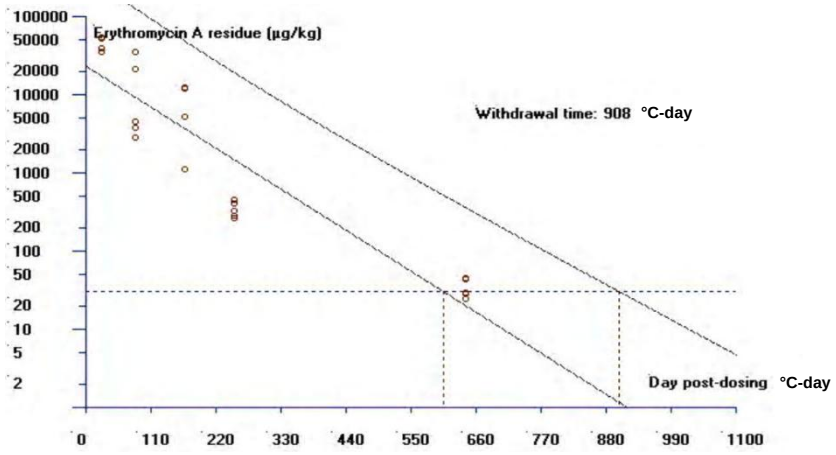

Figure 5. Withdrawal time in muscle tilapia treated with erythromycin - Group A.

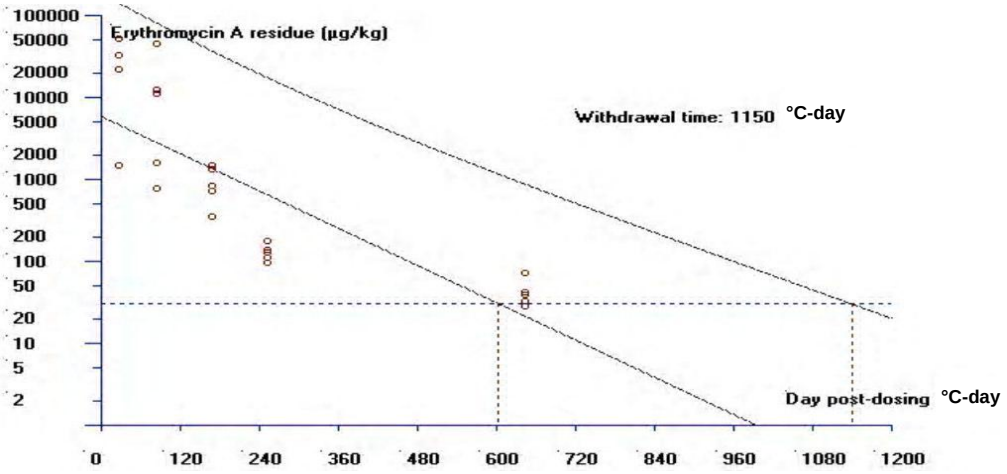

Figure 6. Withdrawal time in muscle tilapia treated with erythromycin – Group B.

Table 4. Degradation of erythromycin at different times in giant prawn muscle samples treated with 50 and 100 mg.kg^{-1}prawn body weight.day^{-1} for 7 days.

Name of Sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)	
				Group A	Group B
Erythromycin base	EBS/0901-RC-002	Erythromycin B	1.0	N.D	N.D
		Erythromycin C	1.0	N.D	N.D
		Erythromycin D	1.0	N.D	N.D
		Erythromycin E	1.0	N.D	N.D
		Erythromycin F	1.0	5.00	5.00
Giant prawn muscle	GP – S1	Erythromycin B	10.0	N.D	N.D
		Erythromycin C	10.0	N.D	N.D
		Erythromycin D	10.0	N.D	N.D
		Erythromycin E	10.0	N.D	2.09
		Erythromycin F	10.0	N.D	N.D
Giant prawn muscle	GP – S5	Erythromycin B	10.0	N.D	N.D
		Erythromycin C	10.0	N.D	N.D
		Erythromycin D	10.0	N.D	N.D
		Erythromycin E	10.0	N.D	5.81
		Erythromycin F	10.0	N.D	3.52

* MDL: Method Detection Limit. ** N. D: Not detected.

concentration of erythromycins A, B, C, and D and some of their derivatives were determined against 21 gram-positive and 15 gram-negative microorganisms. Antibacterial activity was confined to gram-positive and very few gram-negative bacteria. Erythromycin B was somewhat less active than erythromycin A, and erythromycin C and D showed about half that activity or even less. Most other derivatives had negligible activity (Isaac Ongubo Kibwage et al., 1985).

Prawn

The mean concentration of erythromycin in Group A was lower in comparison with that in Group B. However, the eliminating slope of erythromycin residue in Group B

Table 5. Degradation of erythromycin at different times in tilapia muscle samples treated with 50 and 100 mg.kg^{-1} fish body weight.day^{-1} for 7 days.

Name of sample	Identification	Test parameter	MDL (µg/kg)	Result (µg/kg)	
				Group A	Group B
		Erythromycin B	1.0	N.D	N.D
		Erythromycin C	1.0	N.D	N.D
Erythromycin Base	EBS/0901-RC-002	Erythromycin D	1.0	N.D	N.D
		Erythromycin E	1.0	N.D	N.D
		Erythromycin F	1.0	5.00	5.00
	TL - S1	Erythromycin B	10.0	N.D	N.D
		Erythromycin C	10.0	N.D	131.49
Tilapia Fillet		Erythromycin D	10.0	N.D	N.D
		Erythromycin E	10.0	N.D	258.28
		Erythromycin F	10.0	N.D	N.D
	TL - S5	Erythromycin B	10.0	N.D	N.D
		Erythromycin C	10.0	N.D	N.D
Tilapia Fillet		Erythromycin D	10.0	N.D	N.D
		Erythromycin E	10.0	0.30	6.94
		Erythromycin F	10.0	1.37	5.90

* MDL: Method Detection Limit. ** N. D: Not detected.

was faster than in Group A.

Salmon *Oncorhynchus mykiss*, after its erythromycin administration at 100 mg.kg^{-1} trout body weight.day^{-1} for 21 days through medicated feed (water temperature, 11.5°C) gave a withdrawal time of 255°C days (Annarita Esposito et al., 2007). Salmon *Oncorhynchus tshawytscha* through intraperitoneal injection (William et al., 2006) as well as orally administered erythromycin (Fairgrieve et al., 2005), the mechanism of its retention and depletion was also investigated.

The digestive enzymes of tryptase, pepsin, cellulase, amylase, and metabolic enzymes of alkaline phosphatase (AKP), acid phosphatase (ACP), superoxide dismutase (SOD) and glutathione-S-transferase (GST) were dominated in the hepatopancreas of *M. rosenbergii* (11). Only erythromycin F (5 µg/kg) presented in erythromycin base. During medication at dose 100 mg.kg^{-1} prawn body weight.day^{-1} for 7 days via feed, erythromycin has slightly changed to erythromycin E (2.09 µg/kg) after ceasing drug one day. At day 23 of post- treatment, erythromycin E (5.81 µg/kg) and erythromycin F (3.52 µg/kg) was detected and fortunately was not significant to our concern (Table 4). Considering the fact that biotransformation has been kept erythromycin derivatives at safe residue if we tightly obey recommendation of withdrawal time and drug dosage.

Drug residue levels dropped quickly during the first 3 days after treatment termination, then slowly and steadily until a residue level of < 100 µg/kg, considered a safe limit by requirements of FDA and the European Community was attained at day 9 of erythromycin withdrawal. However, a longer withdrawal period (35 days of post- treatment) was recommended to ensure complete drug depletion to satisfy CFIA's concern.

Tilapia

The high metabolic rate of furazolidone, AOZ in Nile tilapia was 22 days at least (Weihai Xu et al., 2006). Mean while, a research of accumulation and clearance of florfenicol in tilapia didn't rule out the withdrawal times (P. R. Bowser et al., 2009s).

When tilapias were medicated with erythromycin base at low dose (Group A), none of derivatives of erythromycin was detected in tilapia muscle at day 1 of post-treatment. At day 23 of post-treatment, erythromycin E (0.30 µg/kg) and erythromycin F (1.37 µg/kg) was not significant to our concern (Table 5).

In case tilapias were fed with erythromycin at higher dose (Group B), two derivatives erythromycin C (131.49 µg/kg) and erythromycin E (258.28 µg/kg) appeared right after ceasing drug treatment. At day 23 of post-treatment, erythromycin E (6.94 µg/kg) and erythromycin F (5.90 µg/kg) was detected and fortunately was also not significant to our concern (Table 5) . . This phenomenon could be explained by intestinal and hepatic enzymes. Maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV,

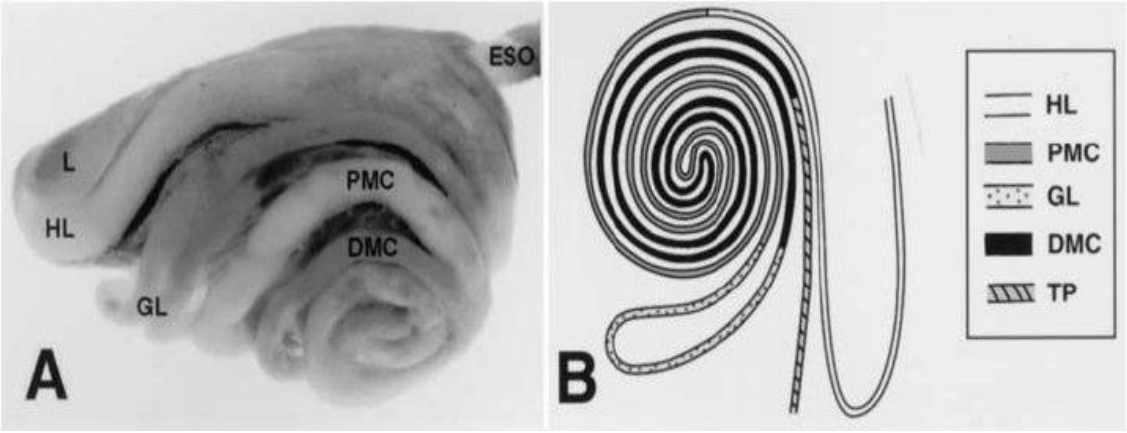

Figure 7. Photomicrograph (A) and schematic drawing (B) of five intestinal segments of tilapia. HL, hepatic loop; PMC, proximal major coil; GL, gastric loop; DMC, distal major coil; TP, terminal portion of the intestine

IV, lipase, non-specific esterases, and alkaline phosphatase were their intestinal enzymes participated in erythromycin metabolism.

Maltase, leucine aminopeptidase, dipeptidyl aminopeptidase IV, lipase, non-specific esterases, and alkaline phosphatase were present at specific sites along the first four intestinal segments (Bundit et al., 2000). Strong reaction for maltase was present in the third intestinal segment, while aminopeptidases and alkaline phosphatase were detected in the first three parts. The most intense activity for lipase was present in the first two parts, while non-specific esterases were observed in the first four portions. Activities of all these enzymes were demonstrated in the brush border. Non-specific esterases were also present in the cytoplasm of the enterocytes. In addition to its brush border localization in the cranial segments, dipeptidylaminopeptidase IV was also observed in the basal lamina of all segments, including the terminal segment. The first four regions played the most important role in both digestion and absorption of erythromycin (Figure 7).

Parallel with intestinal enzymes, hepatic biotransformation enzymes in tilapia such as CYP1A protein, 7-ethoxyresorufin O -deethylase (EROD), glutathione S-transferase (GST), UDP-glucuro-nasyl transferase (UDP-GT) and lipogenic enzyme were also dominated and highly correlated with erythromycin demethylase (Bernard Kwaku-Mensah Gadagbui et al., 1996). They eliminated erythromycin derivatives to minor level at date 23 post-dosing.

Our study provides preliminary data for a prudent use of the antimicrobial drug erythromycin in Nile tilapia, in order to guarantee safety in foods for the consumers and to improve fish farming management. The withdrawal time of erythromycin in Nile tilapia was recommended 33 days or 42 days at least depend on dosage of chemotherapy.

Conclusion

There were lots of researches about toxicity and carcinogenicity of erythromycin A and erythromycin derivatives on mice, rat, dog, and even on human. However, studies about toxicity and carcinogenicity of erythromycin A and erythromycin derivatives in aquatic animals were scarcely investigated. Bio-transformative forms of erythromycin B, C, D, E, F were always in mind of human whether they could be transformed from parental drugs in aquaculture or not. Whether could they create harmful risks to human health? So this research confirmed an obvious evidence that bio-transformative forms of erythromycin appeared through endo-enzymatic mechanism and quickly decomposed to minor level. This would set a basic foundation that there will be no risk of toxicity and carcinogenicity of erythromycin B, C, D, E, and F in aquaculture if farmers strictly follow the recommended veterinary dose.

ACKNOWLEDGEMENT

The technical assistance of Dr. Nguyen Ba Hoai Anh was highly appreciated.

REFERENCES

Ahmed HA (2003). Bacterial flora of freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in concrete tanks in Saudi Arabia. J. Appl. Aqua., 14: 113-124.

Annarita E, Laura F, Dario L, Luigi M, Ettore C, Emilio G (2007). Orally administered erythromycin in rainbow trout (*Oncorhynchus mykiss*): Residues in edible tissues and withdrawal time. Anti. l Ag. Chem., 51: 1043-1047.

Be LM (2002). Investigation on diseases of giant freshwater prawn (Macrobrachium rosenbergii) in ponds and rice-prawn farming systems in A Giang province. *Msc. thesis* (in Vietnameses).

Bernard KMG, Marian A, Anders G (1996). Species characteristics of hepatic biotransformation enzymes in two tropical freshwater tilapia (Oreochromis niloticus) and mudfish (Clarias anguillaris). Comp. Biochem. Physiol. Part C: Pharmacol., Toxicol. Endocrinol., 114: 201-211.

Bundit T, Bonnie JS, Thomas C, Stephen AS (2000). Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. *Aqua.*, 182: 317-327.

Dat NT (2002). Investigation on parasite and bacteria diseases in giant freshwater prawns (Macrobrachium rosenbergii) cultured in pond and rice-prawn with low density. *Msc. Thesis* (in Vietnamese).

European Agency for the Evaluation of Medicinal Products (EAEMP) (2009). Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: marker residue depletion studies to establish product withdrawal periods. EMEA, London, United Kingdom. http://www.ema.europa.eu/pdfs/vet/vich/46319909en.pdf

Fairgrieve WT, Masada CL, McAuley WC, Peterson ME, Myers MS, Strom MS (2005). Accumulation and clearance of orally administered erythromycin and its derivative, azithromycin, in juvenile fall Chinook salmon *Oncorhynchus tshawytscha*. Dis. of Aqua. Org., 64: 99-106.

Isaac OK, Jos H, Eugene R, Hubert V, Ludo V, Maurice D, Claude VP, Pierre P, Georgette L (1985). Antibacterial activities of erythromycins A, B, C, and D and some of their derivatives. Anti. Ag. Chem., 28: 630-633.

Lalitha KV, Surendran PK (2006). Microbiological quality of farmed tropical freshwater prawn *(Macrobrachium rosenbergii).* J. Aqua. F. Pro. Tech., 15: 71-82.

Na L, Yunlong Z, Jian Y (2008). Effects of water-borne copper on digestive and metabolic enzymes of the giant freshwater prawn *Macrobrachium rosenbergii.* Arch. Environ. Contam. Tox., 55: 86-93.

Nagla FG, Safinaz GMI, Khalil RH, Soliman MK (2005). Studies on *Edwardsiella* infection in *Oreochromis niloticus*. Egypt. J. Aqua. Res., 31: 460-471.

Bowser PR, Kosoff RE, Chen CY, Wooster GA, Getchell RG (2009). Florfenicol residues in Nile tilapia after 10-d oral dosing in feed: effect of fish size. J. Aqua. AniM. l H., 21: 14-17.

Shih-Chu C, Yu-De L, Li-Ling L, Pei-Chi W (2001). *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobranchium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. Dis. Aqua. Org., 45: 45-52.

Tran TTH, Dang THO, Nguyen TP (2002). Study on diseases in giant freshwater prawns (*Macrobrachium rosenbergii*): A review.

Weihai X, Xiaobin Z, Xinting W, Liping D, Gan Z (2006). Residues of enrofloxacin, furazolidone and their metabolites in Nile tilapia *Oreochromis niloticus*. Aquaculture, 254: 1-8.

William TF, Cyndy LM, Mark EP, McAuley WC, McDowell GC, Strom MS (2006). Concentrations of erythromycin and azithromycin in mature Chinook salmon *Oncorhynchus tshawytscha* after intraperitoneal injection, and in their progeny. Dis. Aquac. Org., 68: 227-234.

Winton C, Jiam-Chu C (1998). Isolation and characterization of an *Enterococcus* like bacterium causing muscle necrosis and mortality in *Macrobrachium rosenbergii* in Taiwan. Dis Aquac. Org., 34, 93-101.