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The Use of Antibiotics in Shrimp Farming

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1. Introduction

Global aquaculture has grow dramatically over the past 50 years to around 52.5 million tones in 2008 worth US$98.5 billon and accounting for around 50 per cent of the world´s food fish supply. Asia dominates this production, accounting for 89 per cent by volume and 79 per cent by value, with China by far the largest producer (32.7 million tonnes in 2008). The rapid growth in the region has been driven by a variety of factors, including pre-existing aquaculture practices, population and economic growth, relaxed regulatory framework and expanding export opportunities.

Aquaculture development in Europe and North America was rapid during the 1980s-1990s but has since stagnated, probably owing to regulatory restrictions on sites and other competitive factors, although as markets for fish and seafood they have continue to grow (Bostock et al., 2010).

In contrast to other animal production sectors, aquaculture is highly dynamic and characterized by an enormous diversity of species raised both in natural and artificial systems (Walter & Winton, 2010). Aquaculture began in Asia with the cultivation of freshwater fish, and use of the cultivation techniques currently extend to all continents, with a great diversity in the species raised (Subasinghe et al., 2009). Approximately 350 different species are raised in farms, including 34 fish, 8 crustaceans, and 12 species of mollusks that have annual production levels exceeding 100,000 tons (Walter & Winton, 2010).

Shrimp cultivation areas have expanded the most. However, this industry faces major problems with bacterial diseases, and large quantities of chemical and antibiotic products are frequently used to counteract this (Le et al., 2005; Tu et al., 2008).

2. Shrimp disease, one of the main threats facing aquaculture

In aquaculture, bacterial diseases have emerged as a serious problem and represent the most important challenge facing this industry (Morales, 2004; Holmstrom et al., 2003). Bacterial microorganisms can also cause destructive infections, such as the diseases caused by bacteria of the *Vibrio* genus and the bacteria that cause necrotizing hepatopancreatitis (NHP). These are the main diseases responsible for infections in shrimp farms (Roque et al.,

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2001). The causative agent of necrotizing hepatopancreatitis (NHP) is a gram-negative, pleomorphic, obligate intracellular pathogen. The predominant is a non-flagellated, rod-shaped, Rickettsia-like form, that occasionally, exhibits a transverse constricted zone indicative of replication by binary fission (Vincent & Lotz, 2007).

Infections due to necrotizing hepatopancreatitis have been virtually eliminated in the Americas, but the International Office of Epizootics (IOE) has stated some concerns due to the potential that this disease possesses that could be extended to the entire world, because the mortalities caused in shrimp due to necrotizing hepatopancreatitis may include up to 95% of the organisms infected in the pond, causing large losses during the crop cycle (Vincent & Lotz, 2007).

The first infection by necrotizing hepatopancreatitis in a shrimp crop was reported on a farm in the state of Texas (United States of America) in 1985, and outbreaks have subsequently occurred in Peru, Ecuador, Venezuela, Brazil, Panama, Costa Rica, and Mexico, causing significant mortality. This disease is also known as granulomatous hepatopancreatitis or Texas necrotizing hepatopancreatitis, and the infection has been identified in species of *Litopenaeus vannamei*, *Litopenaeus setiferus, Litopenaeus stylirostris, Farfantepenaeus aztecas*, and *Farfantepenaeus californiensis* (Vincent & Lotz, 2007).

The symptoms that shrimp infected with necrotizing hepatopancreatitis exhibit include a reduction in food ingestion, lethargy, an empty intestine, a flaccid body, darkening of the gills, an expansion of the chromatophores around the swimmerets leading to a darkened appearance, and marked atrophy of the hepatopancreas (Lightner, 1996).

Bacteria of the *Vibrio* genus constitute the majority of the bacteria isolated from among those that cause disease and death, either when equilibrium is broken or the immune system is suppressed, and these can be due to some factors. Physico-chemical changes like salinity and temperature, and hypoxia have been reported to affect the immune response of shrimp and its susceptibility to pathogen bacteria. Water with low and high pH level, as well as those with low dissolved oxygen have been reported to decrease the total haemocyte count (THC) and phenol oxidase (PO) activity (Li & Chen, 2008).

Systemic vibriosis, bacterial erosion, Zoea II syndrome, and "white ball" are some of the diseases affecting shrimp produced by bacteria of the *Vibrio* genus (Gómez-Gil et al., 2001). Systemic vibriosis, also known as seagull syndrome, affects all farmable species of shrimp, as they may be susceptible to infection under stressful conditions. *Vibrio* species: *harveyi, vulnificus*, *parahaemolyticus*, and *alginolyticus* are all related to this disease (Morales, 2004).

Bacterial erosion of the shell is present in all penaeid shrimp, juveniles and adults alike. It manifests with the appearance of brown or black stains in areas that have been eroded through the action of chitinolytic bacteria, such as *Vibrio* sp., *Aeromonas* sp., *Spirillum* sp., and *Flavobacterium* sp. The disease is self-limiting and generally disappears when the shrimp molt. If left untreated, it becomes more serious and may become a systemic infection (Morales, 2004).

Zoea II Syndrome causes high mortality rates in the juvenile stage of shrimp. In Ecuador, the causal agent of this disease was found to be *Vibrio harveyi*, although other authors have reported the possible presence of intracellular bacteria. This infection was detected for the

first time in 1993 in farms of *Litopenaeus vannamei* in Ecuador, Mexico, and the United States (Morales, 2004).

"White ball" disease causes the appearance of small balls, arising from desquamated hepatopancreatic cells or to hypertrophied and rounded hepatocytes that appear as spherical formations. It is believed that these balls are caused by toxins produced mainly by *Vibrio* spp. The species of *Vibrio* related to this syndrome are *Vibrio alginolyticus* and *Vibrio harveyi* (Gómez-Gil et al., 2001; Vandenberghe et al., 1999).

A significant limitation to the industry is loss of stock through bacterial disease. Traditional methods to combat disease with antibiotics have been questioned and alternatives have been sought. The modern aquaculture industry demands alternative prophylacts that may help to keep a microbiologically healthy environment, resulting in better production and higher profits. Within this context, the probiotics seem to be a very promising alternative for the management of disease in aquaculture (Sáenz, et al., 2009) Another form is with the reduction in stocking density.

3. Antibiotics used in the cultivation of shrimp

Antibiotics are commonly used in aquaculture during the production cycle, both in the larval and growth phases. The use of antibiotics in aquaculture is associated with environmental and human health problems, including bacterial resistance, persistence of the disease in the aquatic environment, and effects on the biogeochemical composition of the sediment. The accumulation of antibiotic residues in the edible tissues of shrimp may also alter human intestinal flora and cause food poisoning or allergy problems (Ma et al., 2006).

The antibiotics most frequently used in aquaculture to combat bacterial diseases include oxytetracycline, florfenicol, sarafloxacin, and enrofloxacin (Roque et al., 2001; Soto-Rodríguez et al., 2006). Globally, other antibiotics such as chlortetracycline, quinolones, ciprofloxacin, norfloxacin, oxolinic acid, perfloxacin, sulfamethazine, gentamicin, and tiamulin are used (Holmstrom et al., 2003).

3.1 Oxytetracycline

Oxytetracycline is widely employed to treat bacterial infections in aquaculture farms, such as vibriosis and furunculosis (Capone et al., 1996; Prescott et al., 2000; Reed et al., 2006; Wang et al., 2004). It belongs to the tetracycline group, which exerts antimicrobial action against both Gram (-) and (+) bacteria, ricksettsias, mycoplasmas, and others (Gómez-Gil et al., 2001). Tetracyclines are produced by *Streptomyces* spp., which possess determinants for resistance to this class of antibiotics.

Oxytetracycline is a bacteriostatic antibiotic that exerts its antimicrobial effect against protein synthesis, by bonding directly to the S7 protein of the 30S subunit of the bacterial ribosome, thereby impeding the bonding of aminoacyl-tRNA (aminoacyl transfer RNA) to the A-site of the ribosome. This prevents the addition of amino acids to the growing peptide chain (Chambers, 2004; Isidori et al., 2005; Jara, 2007). In order for oxytetracycline to interact with its target site, it needs to pass through the external membrane via passive diffusion through the OmpF and OmpC pores, and through the cytoplasm membrane via an energy-dependent process (Jara, 2007).

3.2 Enrofloxacin

Enrofloxacin was developed as an antimicrobial agent during the 1980s for exclusive use in veterinary medicine and has proven to be effective in the treatment of bacterial diseases that affect aquaculture organisms. Enrofloxacin is a derivative of nalidixic acid. It has a basic dihydroquinoline (4-quinolone ring) chemical core with an ethyl group at the 4th position, favoring its absorption and availability. It is primarily lipophilic and has a low molecular weight, favoring tissue penetration. The mechanism of enrofloxacin acts at the level of the cellular nucleus, inhibiting DNA synthesis. During the multiplication phase of the bacteria, the DNA folds and unfolds alternately. This process is controlled by the enzyme DNA gyrase, which is inhibited by enrofloxacin, causing a collapse of bacterial metabolism and preventing the genetic information from being copied, thus causing the bacteriocidal effect (Williams et al., 2002).

The information related to this antibiotic for the most widely grown shrimp species such as *Litopenaeus vannamei* is scarce, but pharmacokinetic studies on enrofloxacin have been carried out using other species, such as crab (*Scylla serrata*), tilapia (*Oreochromis niloticus*), black shrimp (*Penaeus monodon*), Chinese shrimp (*Penaeus chinensis*), and European seabass (*Dicentrarchus labrax*) (Intorre et al., 2000; Tu et al., 2008; Wen et al., 2007; Xu et al., 2006). It is important to note that the pharmacokinetic results for enrofloxacin obtained for these species should not be extrapolated to other aquatic species, because each organism possesses a different metabolism, and the cultivation conditions may have a significant influence over the kinetic behavior displayed by the antibiotic.

None of the fluoroquinolones included enrofloxacin is approved for use in shrimp in the United States. Their potential use in other countries, such as Mexico, as well as the potential for extra-label use in the United States provides a need for efficient methods to monitor food supplies for the presence of fluoroquinolones residues (Schneider et al., 2005).

3.3 Ciprofloxacin

Ciprofloxacin is the main metabolite of Enrofloxacin and is active against a broad spectrum of aerobic Gram (-) bacteria, including enteric pathogens such as *Pseudomonas* and *Serratia marcescens*. It is also active against Gram (+) pathogens, even when these bacteria have developed resistance to other antibiotics, such as penicillin (Wen et al., 2007). It is not active against anaerobic bacteria and may be used occasionally, in combination with other antibacterial agents, for the treatment of mycobacterial infections.

The antibacterial effects of ciprofloxacin arise from its inhibition of Topoisomerase IV and bacterial DNA gyrase, which act by cleaving the DNA of the bacterial chromosome and rejoining the ends once a superhelix is formed (Banerjee et al., 2007). When these enzymes are inhibited, bacterial cell multiplication is interrupted.

3.4 Florfenicol

This fluorinated antibiotic, derived from thiamphenicol, is a potent and broadly acting bacteriostatic agent. It is effective in the treatment of infections caused by *Pasteurella piscicida, Aeromonas salmonicida, Vibrio anguillarum*, and *Edwardsiella tarda*. Its chemical

structure is very similar to that of chloramphenicol, and florfenicol is effective against bacteria that have developed the ability to deactivate other drugs, such as thiamphenicol and chloramphenicol. Pharmacokinetically, florfenicol use has been reported among some species of fish such as Atlantic salmon (*Salmo salar*), in which a bioavailability of more than 95% is present, exhibiting a good distribution among all of the organs and tissues. Its half-life in fish is less than 15 h (Yanong & Curtis, 2005). However, published information for shrimp is scarce, meaning that the kinetic behavior of this compound among these crustaceans has not yet been completely elucidated.

3.5 Sarafloxacin

This is a white or slightly yellow crystalline solid with the chemical name 6-fluoro-1-(4-fluorophenyl)-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid. Its solubility is 0.034 mol/L at pH 1, and its molecular weight is 385 (King et al., 2000; Oliphant et al., 2002). Two fluoroquinolones have been approved by the Food and Drug Administration (FDA) in the United States for use in the production of animal-derived food products. Sarafloxacin was approved in August 1995 for the treatment of infections caused by *Escherichia coli* in poultry (turkeys and chickens) but, along with other fluoroquinolones, has not been authorized by the Food and Drug Administration for use as a therapeutic agent in aquaculture (Roybal et al., 2002; Nakata et al.,2005).

The normal dosage used in farm animals is 10 mg kg^{-1}, and the drug is administered in drinking water. A Maximum Residue Level (MRL) has not been established for cow's milk or chicken muscle (King et al., 2000; Oliphant et al., 2002).

3.5.1 1st Generation quinolones

These are used exclusively as urinary antiseptics because they do not have sufficient serum levels and are actively eliminated with urine. All are administered orally.

3.5.2 2nd Generation quinolones

These are monofluoride quinolones. Compared to 1st generation quinolones, these exhibit more potent activity, a greater bacterial spectrum, a longer half-life, and, with the exception of norfloxacin and enrofloxacin, they achieve good serum levels, making it possible to treat systemic infections. Ciprofloxacin, Ofloxacin, and Perfloxacin can be administered both orally and parenterally.

3.5.3 3rd Generation quinolones

These are bi- and trifluoride fluoroquinolones. Some have a greater half-life and others have a greater antibacterial spectrum, in some cases including Gram (+) bacteria (mainly streptococci), intracellular bacteria (Chlamydia, mycoplasma, mycobacteria, etc.), and anaerobic bacteria.

3.5.4 4th Generation quinolones

This group has improved activity against Gram (+) bacteria and anaerobic bacteria (King et al., 2000; Oliphant et al., 2002).

4. Studies on the accumulation and elimination of enrofloxacin and oxytetracycline antibiotics among *Litopenaeus vannamei* shrimp

The exposure of the consumer to antibiotic residues in seafood is of great importance for health. Information related to residues of oxytetracycline, enrofloxacin and its metabolite ciprofloxacin in *Litopenaeus vannamei* tissues is very scarce, even though this species is among the most highly valued for its commercialization and oxytetracycline and enrofloxacin are frequently employed when farming this species.

However, pharmacokinetic and bioavailability studies of antimicrobial agents in farmed shrimp are important in order to determine optimal dosage regimens and formulations, to establish safe withdrawal periods, and to minimize the environmental effects of the drug used in aquaculture.

The low analyte concentrations normally present (ng g^{-1}, ng mL^{-1}), the complexity of matrices and the diverse physico-chemical properties that antibiotics may present make their determination difficult, and highly sensitive, selective methods are necessary for monitoring antibiotics in the aquatic organisms (Hernández et al., 2007). The liquid chromatography is the most widely used method, for quantitative analysis of antibiotics.

The purpose of introduce the chemical method about high-performance liquid chromatography was precise quantitative determination of antibiotics residues in the muscle tissues and hepatopancreas to shrimp. Following this analytical approach, the requirements for veterinary monitoring concerning to antimicrobials residues would be fulfilled. Previously only microbiological methods were used, which gave only orientation results.

In a study performed in our laboratory (unpublished data), an attempt was made to determine the accumulation and elimination of enrofloxacin and its metabolite ciprofloxacin in a crop of *Litopenaeus vannamei* using high-performance liquid chromatography after administering a diet medicated with enrofloxacin at a level of 200 mg kg^{-1} for 14 days. Subsequently, a diet without the antibiotic was administered for 16 days. The study was carried out under controlled laboratory and farm conditions. It was found that the maximum concentrations (C_{max}) reached for enrofloxacin in the muscle and hepatopancreas under laboratory conditions were 0.54±0.26 µg g^{-1} and 3.52±1.9 µg g^{-1}, respectively. For ciprofloxacin, levels of 0.18±0.13 µg g^{-1} and 1.05±0.20 µg g^{-1} were reached in the muscle and hepatopancreas, respectively.

In the farm study, C_{max} enrofloxacin levels of 0.36±0.17 µg g^{-1} and 1.60±0.82 µg g^{-1} were reached in the muscle and hepatopancreas, respectively. For ciprofloxacin, these were 0.03±0.02 and 0.36±0.08 µg g^{-1}, respectively. Once the medicated diet was suspended, enrofloxacin and ciprofloxacin residues in the tissues decreased, requiring four to ten days for the levels of both antibiotics to be undetectable in the muscle and six to fourteen days for elimination from the hepatopancreas.

Under controlled conditions, the greatest accumulations of the antibiotic and its metabolite were reached, showing a reduction of 33% in the muscle and 55% in the hepatopancreas when compared with the levels reached under farm conditions. For ciprofloxacin, the reduction was 66% in both tissues. It is important to relate the accumulations levels reached with the Minimal Inhibitory Concentration of the drug that can treat bacterial infections

among shrimp, to determine whether this antibiotic is effective for the control of these diseases.

Oxytetracycline, another of the most widely used antibiotics both in fish and shrimp farms, was studied in relation to its accumulation in shrimp tissues (muscle and hepatopancreas), and this was related to its effect in the inhibition of *Vibrio* bacteria through determination of the Minimal Inhibitory Concentration. In addition, the time of elimination of the antibiotic from shrimp tissues was established.

To perform this study, ponds on a farm of *Litopenaeus vannamei* were employed. The shrimp were treated for 14 days with food that contained a theoretical oxytetracycline concentration of 5000 mg kg^{-1}. Next, an antibiotic-free diet was administered for 16 days. The shrimp were sampled every third day. *Vibrio* bacteria were isolated in the muscle and hepatopancreas of the shrimp, counted and expressed as CFU, and strains belonging to the *Vibrio* genus were identified at the molecular level.

The results obtained show an average C_{max} for oxytetracycline in the shrimp tissues of 31.32±3.44 µg g^{-1} in the muscle and 274.81±62.35 µg g^{-1} in the hepatopancreas. The accumulation levels reached in some of the tissue samples in the treatment stage were greater than the Minimal Inhibitory Concentration determined for oxytetracycline, in the range of 0.75-100 µg mL^{-1}. *Vibrio parahaemolyticus* was identified in 48% of the strains analyzed in the crop system, and no strain was positive for toxigenic *Vibrio cholerae* O1.

The withdrawal times necessary for the oxytetracycline residues to be eliminated were ten days for the hepatopancreas and sixteen days for muscle. Under controlled conditions in the laboratory, a diet was administered to *Litopenaeus vannamei* shrimp that contained 5000 mg Kg^{-1} of oxytetracycline for 14 days. The accumulation levels (C_{max}) for oxytetracycline were 33.54±11.19 µg g^{-1} in the muscle (Fig. 1), 194.37±16.11 µg g^{-1} in the hepatopancreas (Fig. 2), and 18.79±5.87 µg mL^{-1} in the hemolymph. The elimination time for oxytetracycline were six to ten days for all tissues.

The results obtained in the application of these antibiotics demonstrates the importance of applying appropriate therapies with antibiotics, seeking greater effectiveness for the control of bacterial infections, and highlighting the importance of respecting the withdrawal times for each antibiotic, with the purpose of eliminating the residual presence of these compounds from the edible tissues and from the cultivation system in general (to decrease the development of antibiotic resistance in the bacteria).

5. Factors to consider when using antibiotics in aquaculture

Antibiotics must not be used as a preventative measure, since bacteria very rapidly develop resistance to them, leading to their ineffectiveness. Chemical agents should only be applied if there is an appropriate diagnosis of the situation and always under previously established control protocols. To evaluate the impact of antibiotic administration, the usage standards, the hydrology of the area, and the physical-chemical properties of the water should be known (Páez-Osuna et al., 2003; Nakata et al., 2005). Best practices in aquaculture management should be prioritized to avoid the entrance of pathogens into the shrimp cultivation systems, and antibiotics should only be administered as a last resort (Chávez-Sánchez & Higuera-Ciapara, 2003).

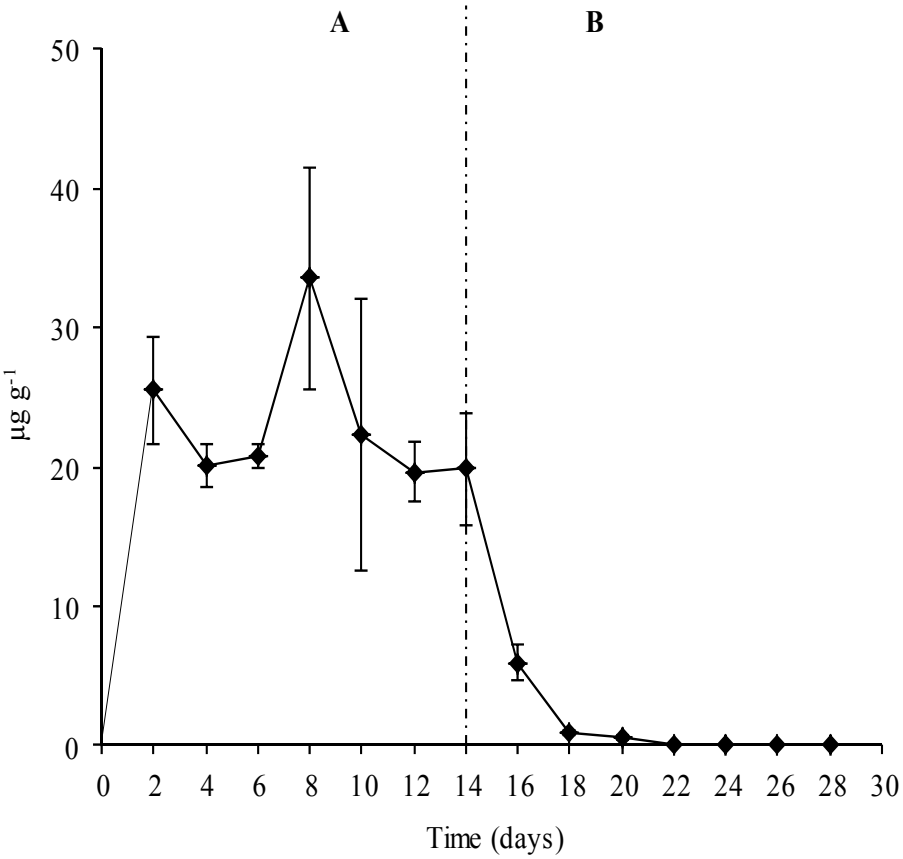

Fig. 1. Muscle oxytetracycline levels -time profiles in the shrimp *Litopenaeus vannamei* after an oral OTC dosage through a medicated feed. The vertical bars represent the Standard Error (n=3). A: treatment period, B: withdrawal period.

The most frequent administration route for antibiotics in shrimp is oral, in which the antibiotic is incorporated in the feed with subsequent exposure to the extremely aggressive aquatic environment. For this reason, it is important that the antibiotic be contained within a pellet to maintain its stability and protect it from factors such as leaching and binding to trivalent and divalent cations (Cabello, 2004).

It must be certain that the shrimp will eat the food when the antibiotic therapy is applied, because the disease will otherwise not be treated, the environment will be contaminated, and the emergence of bacterial resistant strains will be favored. The consumption of food by the farmed organisms may decrease during the molting period, due to environmental factors, or factors related to the infections, reducing the quantity of the antibiotic ingested (Cuzon et al., 2004).

The water temperature of the farm ponds is a critical point to consider, because parameters such as the maximum concentration, distribution volume, and rate of elimination of the

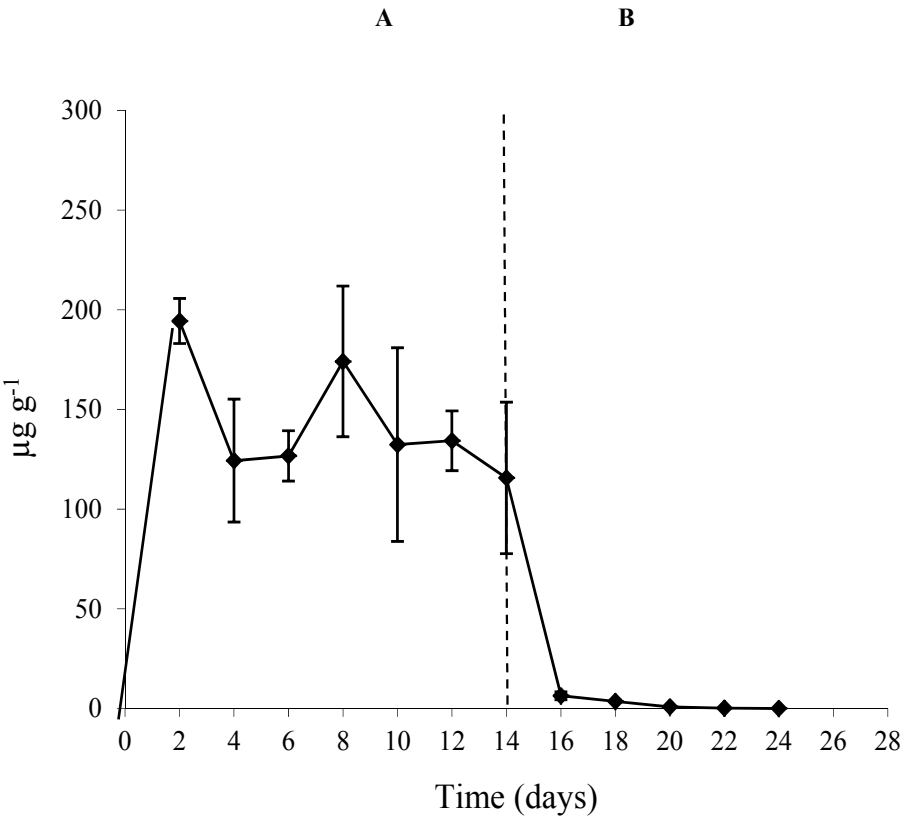

Fig. 2. Hepatopancreas oxytetracycline levels –time profiles in the shrimp *Litopenaeus vannamei* after an oral OTC dosage through a medicated feed. The vertical bars represent the Standard Error (n=3). A: treatment period, B: withdrawal period.

antibiotic may be affected. The pH, oxygenation, salinity, stage of disease, climatic changes, and presence of natural food in the ponds are other factors that affect antibiotic therapies among aquatic organisms (Chávez & Montoya, 2004; Montoya, 2002).

The use of pharmacological agents, antibiotics, and other chemical agents should be considered as methods of last resort in shrimp farming and aquaculture in general. None of the antibiotics is approved for use in shrimp in the United States. The medicated feed is used in an extralabel manner only for treatment of minor species as defined in the Code of Federal Regulations (21 CFR 514.1(d)(1)(ii). In an aquatic species, the extralabel use of medications added to feed in limited to products approved for use in other aquatic species (FDA, 2001).

6. The development of bacterial resistance

Drug resistance in when a formerly effective drug dose is no longer effective. This can be a natural resistance or an acquired resistance. Resistance arises mainly by natural selection,

the replication of a naturally resistant strain after the drugs has killed all of the susceptible strains. Since mutagenic drugs generally are not used, resistance by drug-induced mutation seldom occurs. Drug resistance also can develop from gene transfer or gene amplification (Albert, 1985).

The most worrying effect of the use of antibiotics in aquaculture production and its relationship with human health is the generation of resistant bacteria strains and the transfer of this resistance from the aquatic environment to land, where strains that are highly immune to antibiotics may originate that are capable of causing disease among humans. The transfer of resistance may occur through mechanisms as simple as the consumption of seafood products that contain bacteria that are resistant to various antibiotics (Gräslund & Bengtsson, 2001).

It has been demonstrated that the use and abuse of antibiotics has given rise to multiple resistance among microbial populations associated with shrimp production. Various studies have shown that antibiotics persist in the sediment and aquatic environment for several months after their administration (Matyar et al., 2008), and that these may affect native bacterial community in detrimental to the ecosystem since this community plays key roles in biogeochemical processes. Some antimicrobials can inhibit important microbial processes as denitrification or primary production by cyanobacteria (Garcia-Armisen et al., 2011).

The capacity of the microorganisms to reduce sulfates may also be reduced (Páez-Osuna et al., 2003), affecting the quality of the sediment and the environment (Ma et al., 2006), thereby promoting the proliferation of resistant bacterial strains or pathogens (Capone et al., 1996; Hektoen et al., 1995; Tendencia & De la Peña, 2002;), which may place the viability of shrimp crops at risk.

It is estimated that between 15 and 40% of the administered medicated diet is not ingested by the organisms and remains in the substrates. Another part of the medication is not absorbed during its passage through the intestinal tract of the organism and returns to the environment in fecal matter. The amount of antibiotic transferred to the environment varies from 1% (chloramphenicol) to 90% (oxytetracycline) (Capone et al., 1996). Hektoen et al. (1995) reported that approximately 70-90% of the antibiotic used in the therapy of farmed organisms ends up in the environment and sediment, and a high percentage exhibits antibacterial activity. It has been reported that residues of oxolinic acid and oxytetracycline are very persistent under certain conditions, with half-lives exceeding 100 days (Samuelsen et al., 1992).

Three mechanisms of resistance to tetracycline have been described: (1) decreased intracellular accumulation due to either impaired influx or increased efflux by an active transport protein pump; (2) ribosome protection due to production of protein that interfere with tetracycline binding to the ribosome; and (3) enzymatic inactivation of tetracyclines. The most important of these is production of an efflux pump. The pump protein is encoded on a plasmid and may be transmitted by transduction or by conjugation. Because these plasmids commonly encode resistance genes for other drugs, eg, aminoglycosides, sulfonamides and chloramphenicol, tetracycline resistance is marker for resistance to multiple drugs (Tenover, 2006).

Some studies have demonstrated that the concentrations of oxytetracycline in the sediment after therapy may range from 0.4 to 495 $\mu g\ g^{-1}$. Therapeutic dosages of oxytetracycline in fish

may cause sub-lethal effects, including alteration of the levels of immunoglobulin in the serum and suppression of the phagocytic response and macrophages (Uyaguari et al., 2009).

International regulations regarding the use of antibiotics in aquaculture have established a list of prohibited products (Stolker & Brinkman, 2005). Shrimp with traces of these products are subject to measures against their importation. The strongest restrictions are on the use of chloramphenicol, dimetridazole, furazolidone, nitrofurazone, other nitrofurans, and fluoroquinolones, and these antibiotics should not be used at any stage of the production process (Defoirdt, et al., 2007; Tittlemier et al., 2007).

Epidemiological and molecular assays have indicated that genes mediating resistance might be transmitted from aquatic bacteria to bacteria capable of producing infections among humans and terrestrial animals. This demonstrates that the aquatic and terrestrial compartments lack borders with respect to the flow of resistance genes and that the resistance phenomenon is global, because the use of antibiotics in an environment will have, over time, repercussions in other, apparently distant, ecosystems (Cabello, 2002; Rhodes et al., 2000). To decrease the contamination of the environment and bacterial resistance, appropriate aquaculture production practices must be carried out, and biosecurity measures must be applied to reduce outbreaks of disease and the propagation of pathogenic agents (Kemper, 2008).

Global efforts are needed to promote more judicious use of antibiotics in aquaculture and the new strategies to control phatogenic bacteria are needed to make the industry more sustainable. However, it is not always economically feasible to culture the organism in the most optimal conditions, so there will always be a risk to infection and a need for effective biocontrol techniques (Defoirdt, et al., 2007).

It is important to highlight that the application of highly sensitive analytical methodologies is indispensably in measuring the concentrations of antibiotics and their metabolites with certainty in the distinct tissues of aquaculture products. This would help in establishing regulations that protect the environment, generating products that are safe for human consumption, and allowing the growth of aquaculture.

A practical use of the pharmacokinetic data is the possibility to design dosage regimens in which levels of a specific drug can be maintained above the Minimum Inhibitory Concentration and below toxic effects by means of repeated dosages. However, this method requires information on Minimal Inhibitory Concentration established for bacterial pathogens of interest. Although there are reports on available Minimal Inhibitory Concentration for bacterial strains potentially pathogens to shrimp species, they reveal a wide range of values. Takahashi et al., (1985) reported that Minimal Inhibitory Concentration of oxytetracycline against 49 strains of *Vibrio sp*., range from 0.1 to 12.5 μg mL^{-1}. Monhey et al., (1992) found the Minimal Inhibitory Concentration to be in the range of 2.0 μg mL^{-1} or less for *Vibrio* isolated mainly from American shrimp. Furthermore, Roque et al., (2001) in their study of 144 isolated of *Vibrio* reported a Minimal Inhibitory Concentration of 304.0 μg mL^{-1} for oxytetracycline with a range from 0.26 to 1064 μg mL^{-1}. Given the wide range of Minimal Inhibitory Concentration it is recommended to isolate local bacterial strains and evaluate their Minimal Inhibitory Concentration. Additionally, it still requires performing such studies in natural farming conditions (Gómez-Jimenez et al., 2008).

7. Conclusion

Scientific studies have been conclusive with respect to the health risk that the massive and unlimited use of antibiotics in aquaculture represents. When health certifications are implemented for aquaculture products demanded by domestic and foreign markets, the control over the use of these compounds needs to be increased, together with other aspects of primary importance such as food safety, protection of the environment, and the health of farmed organisms. These aspects should be considered and resolved through the implementation of Best Management Practices.

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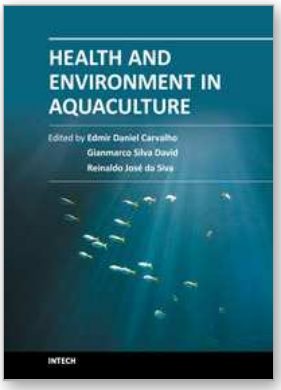

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Aquaculture has been expanding in a fast rate, and further development should rely on the assimilation of scientific knowledge of diverse areas such as molecular and cellular biology, and ecology. Understanding the relation between farmed species and their pathogens and parasites, and this relation to environment is a great challenge. Scientific community is involved in building a model for aquaculture that does not harm ecosystems and provides a reliable source of healthy seafood. This book features contributions from renowned international authors, presenting high quality scientific chapters addressing key issues for effective health management of cultured aquatic animals. Available for open internet access, this book is an effort to reach the broadest diffusion of knowledge useful for both academic and productive sector.

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