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Growth, survival and histological responses of the marine shrimp, *Litopenaeus vannamei*, to three dosage levels of oxytetracycline

W.A. Bray [a,*], R.R. Williams [b], D.V. Lightner [b], A.L. Lawrence [a]

[a] *Shrimp Mariculture Project, Texas Agricultural Experiment Station, Texas A&M University System, 1300 Port Street, Port Aransas, TX 78373, USA*
[b] *University of Arizona, Department of Veterinary Science and Microbiology, 1117 E. Lowell Street, Tucson, AZ 85721, USA*

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Abstract

Toxicity of oxytetracycline (OTC) in prepared feed for penaeid shrimp was evaluated in a 42-day trial with *Litopenaeus vannamei* (initial mean weight, 10.3 g±0.504 S.D.). Four treatments consisted of: (1) 0.0 g/kg OTC (control feed), (2) 4.5 g/kg OTC (1× treatment, maximum recommended dose), (3) 13.5 g/kg OTC (3× treatment) and (4) 22.5 g/kg OTC (5× treatment). Each treatment consisted of 11 replicate tanks, with 8 shrimp per replicate. The test period was three times the recommended OTC label dosing period (14 days). This target animal safety study was conducted under GLP (Good Laboratory Practices) conditions, as part of submissions to the U.S. Food and Drug Administration Center for Veterinary Medicine for approval of therapeutic use of OTC in penaeid shrimp feeds.

Growth rate (weight gain) was found to be OTC-dose-related. There was no significant difference in mean weight gain between control and 1× OTC treatments (9.1 g and 9.2 g, respectively), but growth was strongly depressed in the 3× and 5× treatments (1.50 g and 4.02 g, respectively). There did not appear to be a dose response in mean percent survival of test shrimp (range: 93.2% to 98.9%).

Abnormal soft exoskeletons (=shell, cuticle) were observed in 66.7% to 90.9% of shrimp in the 3× and 5× treatments after 42 days, compared with 0.0% and 9.1% in control and 1× treatments, respectively (from sample $N=3$ per tank=$N=33$ per treatment, 37.5% of individuals stocked). The high numbers of soft exoskeletons observed could be related to a deficiency of calcium/magnesium, principal shell components, due to divalent cation-chelating properties of OTC.

Some dose-related histological changes were apparent in the hepatopancreas (HP) of experimental shrimp sampled after 42 days of OTC feeds. Only slight changes were apparent in the histological presentation of the HPs of the shrimp from the 0× (untreated control) and the 1× treatment levels. In contrast, shrimp sampled from the 3× and 5× OTC treatment levels showed generally reduced levels of lipid droplet storage in the HP, and some necrosis and sloughing of the HP tubule epithelium. The virtually complete absence of HP lipids and the presence of moderate atrophy of the proximal portion of the HP tubules, indicated by markedly reduced tubule epithelial cell height, were the principal characteristics of the HP of shrimp sampled from the 5× OTC group. Mitotic activity in E-cells, a measure of the regenerative capability of the HP, was constant across all experimental groups regardless of the OTC level in the experimental feed.

Mean OTC consumed per shrimp in the 1× treatment was estimated to be 0.09813 g over 42 days, compared with 0.16850 g and 0.29687 g in the 3× and 5× treatments, respectively. Feeds containing OTC at higher levels (3× and 5×) were consumed at much

* Corresponding author. Tel.: +1 361 749 4625; fax: +361 749 5756.
E-mail address: braywa@aol.com (W.A. Bray).

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lower rates than control and 1× treatments, indicating lower palatability and/or toxicity of extreme dosage levels. The 3× and 5× treatments consumed approximately 46% less feed than the control and 1× treatments.

Keywords: *Litopenaeus*; Crustacean; Shrimp; Oxytetracycline; Antibiotic; Toxicity

1. Introduction

Oxytetracycline (OTC) is one of the most common therapeutics used in aquaculture worldwide because of its effectiveness as a broad spectrum antibiotic, its relative safety, low concentration rate in edible tissue and reasonably short tissue elimination time. Its longevity in the marine environment is significantly mediated by complexing with magnesium and calcium, such that only about 5% of OTC is in a form retaining antibacterial activity (Lunestad and Goksoyr, 1990). It is effective against *Vibrio* sp., the most common bacterial problem observed in shrimp culture (Corliss et al., 1977; Corliss, 1979; Lightner, 1983, 1993; Takahashi et al., 1985; Sano and Fukuda, 1987; Mohney et al., 1992; Williams et al., 1992; Weifen et al., 2004), and it is also effective against NHP (necrotizing hepatopancreatitis, caused by a rickettsia-like bacterium which has been a particular problem in southern U.S. shrimp farms (Johnson, 1990; Krol et al., 1991; Frelier et al., 1992, 1993; Lightner et al., 1992; Lightner and Redman, 1994)). However, OTC is only approved in U.S. aquaculture for finfish and lobsters and, presently, it can only be administered in shrimp farms under an INAD (Investigational New Animal Drug) authorization to the University of Arizona by the U.S. FDA. Use of OTC under the INAD venue has been helpful, if not critical, to the fledgling U.S. industry, but the longer-term answer to OTC usage in U.S. shrimp culture is completion of the lengthy FDA approval process.

A number of submissions are required for FDA approval, including proof of efficacy (in vitro studies, clinical trials, field trials), manufacturing stability, animal safety (toxicity and margin of safety), human safety (residue depletion in edible animal crop tissues and microbial food safety) and environmental safety (impact on the environment). Most of the required submissions have been completed in the past two decades, following initiation of the process in 1985 with acceptance of an INAD application from Pfizer, Inc. in collaboration with Marine Culture Enterprises (Laie, Oahu, Hawaii) and the University of Arizona. Steps remaining (for NHP use) are part of the animal safety submission (the present study fulfills part of that requirement) and environmental assessment (evaluation of all available data on sediment and effluent effects), and microbial food safety.

The objectives of the current study were to determine growth, survival and histological response of *Litopenaeus vannamei* to OTC in feed at three levels (4.5 g/kg OTC = maximum recommended dose; 13.5 g/kg and 22.5 g/kg OTC = extreme high dosage levels) for 42 days (three times recommended treatment duration).

2. Materials and methods

2.1. Experimental design

Four levels of oxytetracycline (OTC) in prepared feed were tested in a 42-day trial with subadult *L. vannamei*. Treatments consisted of feeds containing: (1) no OTC (0× treatment, control feed), (2) 4.5 g/kg OTC (1× treatment), (3) 13.5 g/kg OTC (3× treatment) and (4) 22.5 g/kg OTC (5× treatment). Each treatment consisted of 11 replicates of 100 l fiberglass tanks in a randomized block design, stocked with 8 shrimp each. The trial was conducted indoors with a flow-through (once-through) seawater system.

2.2. Test animals

Test shrimp were domesticated stocks of the U.S. Marine Shrimp Farming Program (USMSFP), U.S. Department of Agriculture, mean weight 10.03 g ± 0.504 S.D., from group weights by tank at stocking.

2.3. Test ingredient

Test ingredient oxytetracycline was provided by the manufacturer, Phibro Animal Health, Inc., Fort Lee, NJ, USA, as TM-100 for Fish-Premix Formulation (oxytetracycline mono-alkyl trimethyl ammonium salt). OTC levels in feeds were analyzed in samples at the time of manufacture, and at initiation and termination of the study (Eurofins/Woodson-Tenent Laboratories, Memphis, TN, USA).