Oxytetracycline Residues in Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

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The distribution of oxytetracycline (OTC) in male and female *Macrobrachium rosenbergii* was examined after the prawns had been given medicated feed containing OTC at levels of 2.5 and 5.0 g/kg of feed for 1 week and nonmedicated feed thereafter. OTC levels in the heads of both male and female prawns were consistently higher than in the muscles. Batches of both male and female prawns treated with higher dosages had significantly higher OTC residues in both head and muscle tissues than the batches treated with lower dosages. During treatment, peak concentrations in the head and muscle from each group were reached within 4−6 days. After drug treatment ceased, 13 days for the head and 10 days for the muscle were required to reduce OTC residues to safe levels in all batches of both sexes and dosages. In practice, to account for variations in water temperature, drug dosage, duration of therapy, and other environmental conditions, a withdrawal period of 21 days is recommended.

Keywords: *Giant freshwater prawn; Macrobrachium rosenbergii; male; female; withdrawal period*

INTRODUCTION

Giant freshwater prawn (*Macrobrachium rosenbergii*) is a very popular food in Thailand. The Thai people prefer it to black tiger shrimp (*Penaeus monodon*) because of its larger size and softer texture. It is a common ingredient in *tomyum gung*, a world renowned Thai dish, and many other seafood dishes. Aside from the local market, it is also in great demand in other countries such as the United States, Japan, and members of the European Union. *M. rosenbergii* is widely distributed in the Indo-Pacific region. Adults usually inhabit freshwater reaches of coastal rivers and lakes (*1*). In Thailand, it is found in the rivers of the central and southern parts of the country. Due to the irregularity of supply from the wild and in order to meet the ever-growing demand in local and export markets, more and more farmers culture it in ponds. About 2000 farmers depended on it for livelihood with the total production of 238478 tonnes in 1999 (The Thai Fisheries Department, unpublished data).

Farmers in Thailand use oxytetracyline (OTC) to protect their shrimp from disease. It is usually administered through medicated feed during the growing period. In other countries, OTC is widely used for treatment of fish (*2*−*8*) as well as shrimp (*9*−*12*). It is a broad-spectrum antibiotic with a high degree of activity against a wide range of Gram-positive and Gram-negative bacteria. It has been proven to be a successful prophylactic against species of *Vibrio*. The Codex Alimentarius Committee set the maximum residue limit (MRL) at 0.10 mg/kg. Problems with residues exceeding the MRL arise when proper withdrawal times are not followed. For *M. rosenbergii*, there is no established withdrawal time, but 14 days is normally used in Thailand following the practice for black tiger shrimp (*13*).

In recent years, preshipment samples rejected due to high OTC levels have decreased significantly in black tiger shrimp but not in *M. rosenbergii*. This may be because appropriate withdrawal times have been established for black tiger shrimp but not for *M. rosenbergii*. This study was thus undertaken to determine the proper withdrawal time for *M. rosenbergii*. Because the males usually grow much larger than the females and their heads are proportionately bigger, differences in drug residue retention between the sexes as well as between the head and abdominal muscle were examined.

MATERIALS AND METHODS

Giant freshwater prawn were purchased from a commercial farm in Nakornpathom province, placed in a Teflon vat, and trucked to the Coastal Aquaculture Development Center in Samutsakhon province, ∼1 h away. The prawns were first segregated by sex: 13 kg of males (230 pieces, average = 56 g/piece) and 18 kg of females (430 piece, average = 41 g/piece); each group was divided into two equal batches, identified as "male A", "male B", "female A", and "female B". Each batch was placed into separate cement tanks measuring 10 × 1.5 × 1 m, which were operated as closed systems with the use of standing freshwater and supplemental aeration. The tanks were located under a shed, which helped to control water temperatures at 28−30 °C. Tank maintenance was done once on the eighth day of the experiment (third day of drug treatment) by removing feces and changing 50% of the water. Prior to the experiment, an acclimatization period of 5 days was arranged to let the prawns recover from the stress of moving to the new environment. During this period the prawns

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were given nonmedicated feed. Prior to the acclimatization period, 5 males and 10 females were tested and confirmed to be free of OTC residues.

In black tiger shrimp culture, the use of 3−5 g of OTC mixed with 1 kg of feed was recommended to fight *Vibrio* (*13*). Medicated feed used for shrimp culture in Thailand is normally prepared by either spraying the pellets with a solution of drug dissolved in water or mixing OTC solution with other ingredients before pellets are formed. In this experiment, medicated feed containing OTC concentrations of 2.5 and 5.0 g/kg of feed were produced by mixing OTC solution with other ingredients prior to the extrusion and drying (at 50 °C) process. Prior to mixing, the purity of OTC was tested and found to be 90%. Before use, OTC in the feed was checked to confirm the concentrations. The feed was in pellet form, the same as what is normally used by farmers. Both male and female A batches were fed 2.5 g of OTC/kg of feed (or 75 mgof OTC/kg of shrimp), whereas the B batches were given 5.0 gof OTC/kg of feed (or 150 mg of OTC/kg of shrimp). Feeding was done three times a day (8 a.m., 12 noon, and 8 p.m.) at the rate of 3% of total body weight. Total body weight was adjusted every time prawns were taken for OTC testing or when dead prawns were removed. Medicated feed was administered for 7 days followed by a withdrawal period during which nonmedicated feed was used until the OTC residues reached safe levels of 0.10 mg/kg and below. Samples for OTC testing were taken every day at 10:00 p.m., 2 h after the last feeding. Five males and 7−11 females (due to the variations in size) from each batch were taken daily from the first day of drug treatment until the residues were at safe levels. Samples were frozen at −20 °C and delivered to the laboratory in Bangkok, 1 h away. Prior to OTC analysis, the heads were separated from the muscles and the shells were completely removed from both tissues. The peeled tissues (head or muscle) in the same batch were pooled together, blended, and taken for duplicate analyses.

Analysis of OTC Residue in Prawn Tissues. OTC analysis was done by HPLC technique based on the sample extraction using solid phase extraction (*14*) and fluorescence detection (*15*).

Preparation of Extraction Solutions and Mobile Phase. Na_2EDTA−McIlvaine buffer (pH 4) was prepared by dissolving 15 g of $Na_2HPO_4 \cdot 2H_2O$, 13 g of citric acid, and 3.72 g of Na_2-EDTA in water, with the pH adjusted to 4.0 and diluted to 1 L. Mobile phase was a mixed solvent of 1 M imidazole with LC grade methanol in a ratio of 77:23. The imidazole solution (1 M) was prepared by dissolving 68.08 g of imidazole, 10.72 g of magnesium acetate, and 0.37 g of Na_2EDTA in 950 mL of water, adjusting the pH to 7.2 with glacial acetic acid, and diluting with water to 1 L.

Sample Extraction Procedures and Testing Methods for HPLC Analyses. Five grams of blended prawn tissue (head or muscle) was homogenized with 25 mL of Na_2EDTA−McIlvaine buffer and 3 mL of dichloromethane in a 50 mL screw-capped polypropylene centrifuge tube for 1 min. The homogenate was centrifuged at 3500 rpm for 10 min and filtered through Whatman No. 1 filter paper into a 100 mL beaker. The precipitate was rehomogenized with 25 mL of Na_2-EDTA−McIlvaine buffer, centrifuged, and filtered as before, and the filtrates were combined. The tissue extract was then loaded onto the Sep-Pak C18 and washed with 10 mL of deionized water (a Sep-Pak C18 cartridge was conditioned with 10 mL of methanol and 10 mL of deionized water prior to use). OTC was eluted with 5 mL of methanol in a 100 mL boiling flask and evaporated to dryness under a vacuum by using a rotary evaporator at 40 °C. The residue was dissolved in 5 mL of mobile phase for HPLC analyses. Each test was done in duplicate, and the reported values are the mean of two determinations. Recovery tests were done to test the efficiency of the method. Samples were spiked with OTC standard at 0.1 mg/kg, and the recovery was found at 82−95%. However, the reported values were not corrected by the percentage of recovery.

HPLC Analyses. The filtrate was injected at the volume of 100 μL to HPLC (run with the mobile phase at flow rate of 0.8 mL/min) with the column Symmetry C8 (15 × 3.9 mm id) used. OTC was monitored at 380 nm excitation and 520 nm emission wavelength by fluorescence detector. The external standard method was used for quantification on the basis of peak area measurements.

Statistical Analysis. Test of significance for the analytical study was done using Students' *t* test (*16*).

RESULTS

Figure 1a shows the OTC concentrations in the head and abdominal muscle of female A. In both tissues, OTC was detectable from the first samples, which were taken 14 h after the introduction of medicated feed. From these samples, OTC residues were found at 6.33 and 6.28 mg/kg in head and muscle, respectively. On the sixth day of treatment, OTC levels peaked at 9.28 mg/kg in the head and at 6.84 mg/kg in the muscle and then declined to 6.30 and 4.89 mg/kg, respectively, on the seventh day, the last day of treatment. Similar to female A, OTC residues were detectable in the head and muscle in the first samples of female B at levels of 5.23 and 5.03 mg/kg, respectively (Figure 1b). On the fourth day of treatment, peak OTC values were reached at 18.20 mg/kg in the head and 12.84 mg/kg in the muscle and then dropped the next day and remained at 9.51 and 9.63 mg/kg, respectively, on the seventh day. After drug treatment ceased, OTC levels decreased dramatically in the first 2 days in both tissues of both batches and then gradually decreased thereafter. It took 9 days to reach safe OTC levels in both tissues of female A, whereas in female B, this required 8 days for the muscle and 13 days for the head.

Figure 2 shows OTC concentrations in the head and abdominal muscle of the male batches. Similar to the female batches, OTC was detected in the first-day samples. In male A, OTC levels were found at 2.8 and 2.08 mg/kg in the head and muscle, respectively, and in male B, at 7.51 and 5.33 mg/kg, respectively. On the second day of treatment, OTC levels in male A peaked at 10.20 mg/kg in the head and at 6.04 mg/kg in the muscle, and at the end of treatment, levels were at 3.99 and 2.85 mg/kg, respectively. OTC in male B reached the highest levels at 19.93 mg/kg in the head and 10.91 mg/kg in the muscle on the sixth day and dropped to 17.49 and 10.75 mg/kg on the seventh day. After drug treatment ceased, OTC levels in the head and muscle of both batches declined dramatically during the first 2 days and then decreased gradually thereafter. OTC levels in both head and muscle of male A and also in the muscle of male B reached safe levels after 10 days of withdrawal period, whereas 13 days was required in the head of male B.

DISCUSSION

In this experiment, OTC was first detected in all batches 14 h after the introduction of the drug. This is shorter than the finding in juvenile white shrimp (*Penaeus setiferus*) fed with medicated feed containing 1−10 gof OTC/kg of feed for 3 weeks (*9*), wherein OTC was first detected in shrimp muscle after 48 h of feeding with 1 g/kg of feed and after 24 h feeding with 5 and 10 g/kg of feed.

During the treatment period, it was observed that peak OTC levels in the heads of all female and male batches were significantly higher ($P < 0.05$) than levels in the muscles of the same batch. Similarly, peak levels in both tissues of the batches treated with higher drug dosages were significantly higher ($P < 0.05$) than the

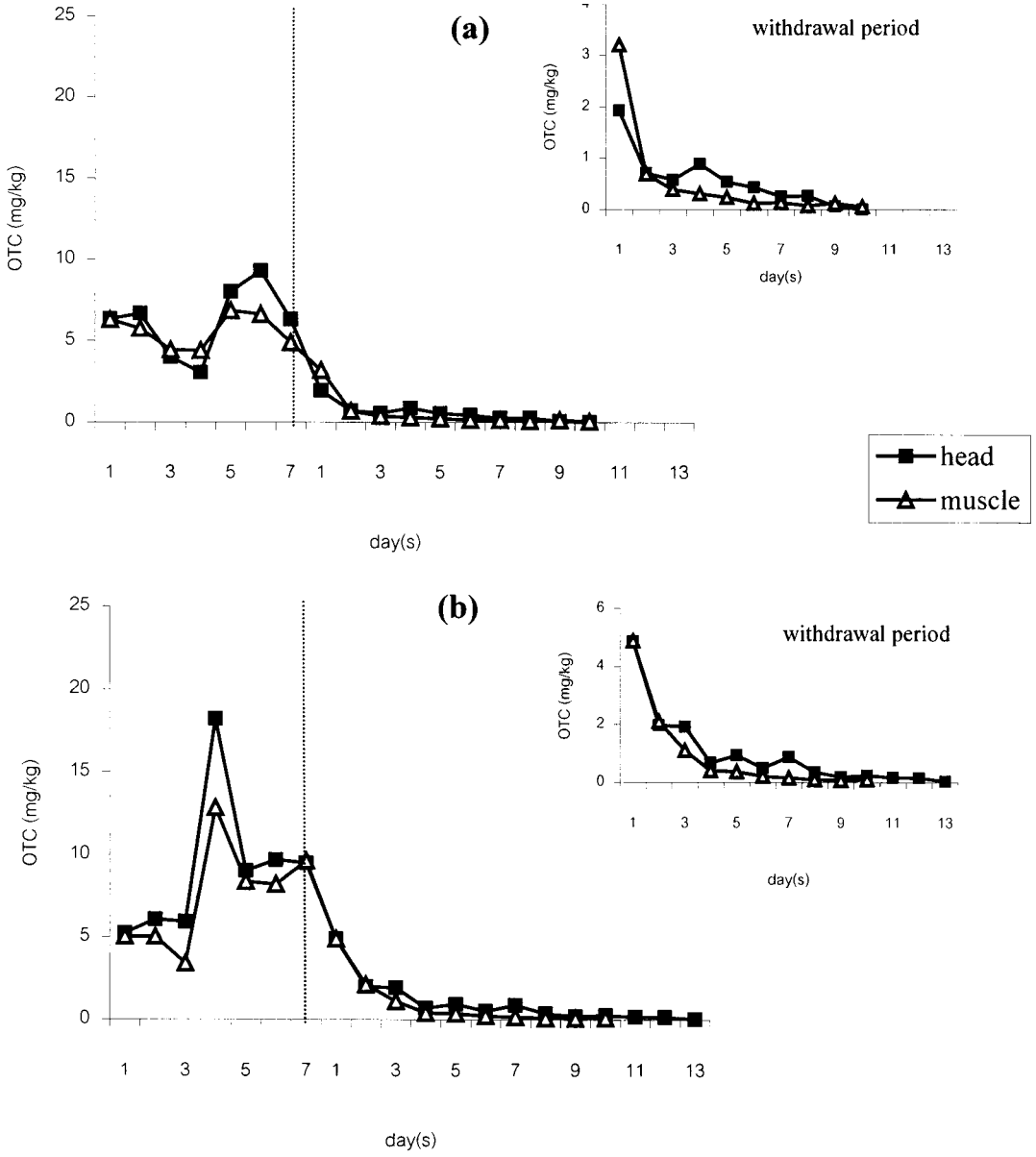

Figure 1. OTC concentrations in heads and muscles of (a) female A and (b) female B treated with medicated feed for 7 days.

levels in the batches treated with lower dosages. This is in agreement with the results from other works (*9*, *17*) wherein the distribution of OTC in different tissues of shrimp depended on the amount of OTC feeding. *P. monodon* were examined after force-feeding at OTC levels of 60 and 80 mg/kg of shrimp and found to retain higher residues at longer times in the hepatopancreas (located in the head) than in the muscle, whereas at 40 mg/kg of shrimp there were no differences between the head and muscle (*17*). This could be explained by the fact that food first enters the digestion system of shrimp via the foregut to the hepatopancreas, where it is absorbed and distributed to the other tissues via the hemolymph. Thus, the hepatopancreas contains higher OTC levels and retains the drug for longer periods (*18*). However, no significant differences ($P < 0.05$) were noted in peak OTC levels in the same tissues between female and male batches treated with the same dosages.

The highest OTC concentrations in the muscles of both female and male batches in this study were found to be much higher than peak levels reported in other works. The highest OTC level at 4.25 mg/kg was detected in juvenile *P. setiferus* after treatment with medicated feed containing 5.0 g/kg of feed for 3 weeks (*9*), whereas 0.89 mg/kg was detected in juvenile *P. monodon* after treatment with medicated feed containing 5.0 g/kg of feed for 1 week (*13*). However, it is difficult to compare the OTC levels from this study to those of other studies because the feed preparation techniques and the type of water used for shrimp culture were different. Medicated feed was prepared by spraying the pellets with a solution of drug dissolved in water, and this coating was easily lost due to leaching from the pellets or binding to the cations in seawater (*13*). This loss could be as high as 50% within 4 h at 28 °C in seawater (*19*). In the present study, loss due to leaching or binding could be minimized by the way OTC in which was mixed directly with the feed as well as the use of freshwater in the culture tanks. Moreover, the use of HPLC, which is acknowledged to be more accurate and to have a lower detection limit than microbiological assay (*20*−*22*), which was used in the other studies, could be another contributor to the higher levels found in this study. Finally, differences in feed formulation,

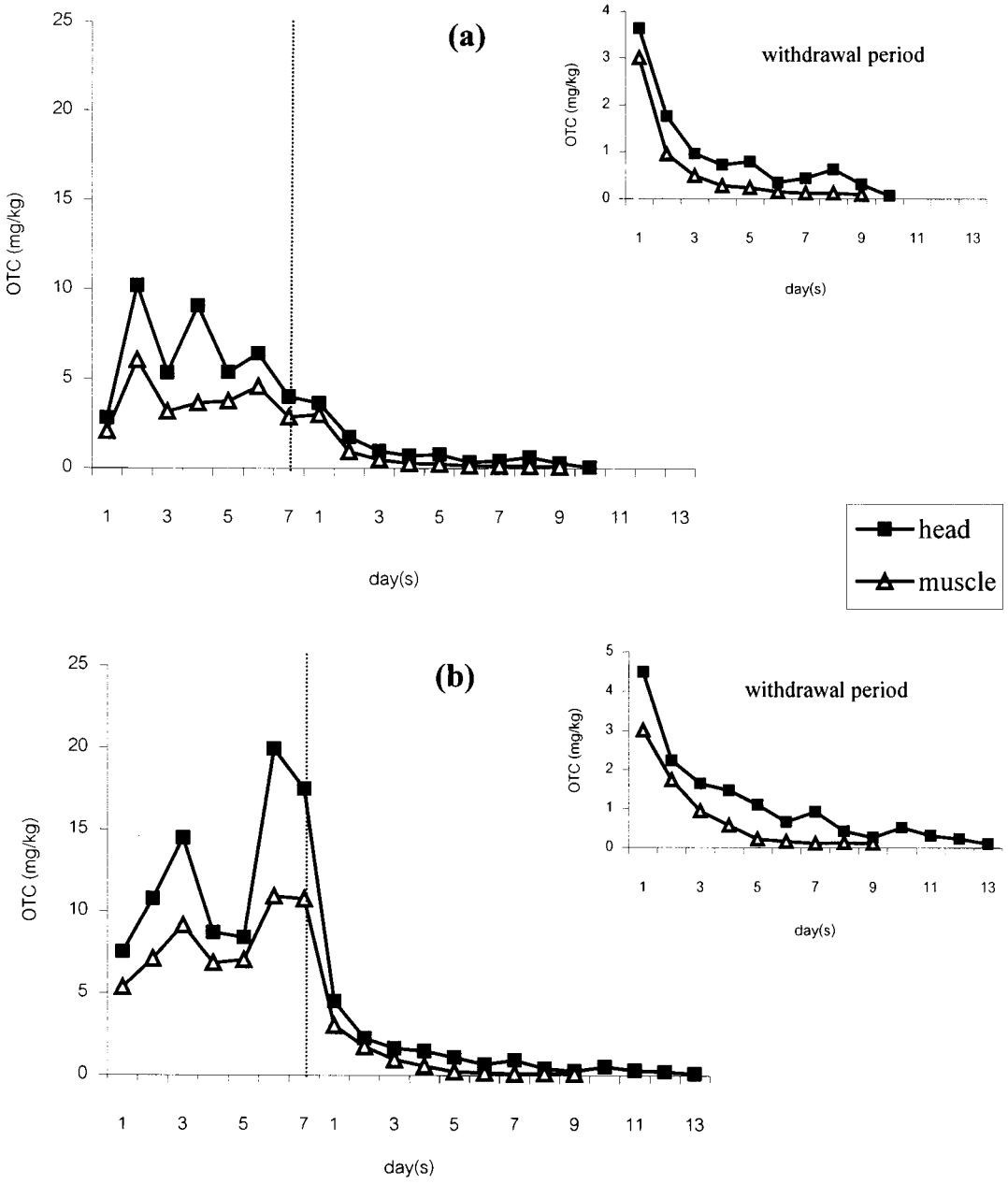

Figure 2. OTC concentrations in heads and muscles of (a) male A and (b) male B treated with medicated feed for 7 days.

water temperature, and other environmental conditions may affect feeding rate and drug absorption. With regard to the minimal inhibition concentration (MIC) of OTC against *Vibrio* sp., which was recommended at 0.1 to 12.5 mg/kg of shrimp (*10*), it appeared that the OTC peak values in muscle tissues of all batches in this study fell within the range.

OTC levels were noted to decline on the third day for female A, female B, and male A and on the fourth day for male B. This may be due to the maintenance work done on the culture tanks on the third day of treatment, which could have caused stress to the prawns, thereby reducing food intake. The tank of male B was cleaned later in the afternoon, which may explain why the drop in OTC levels in male B came a day later. It was found that stress from the change in environmental conditions could have lowered feeding rates or affected drug absorption, giving lower retention levels (*9*). Another possible reason is that the water change caused molting, which consequently reduced feed intake of the prawns. It appeared that there was fluctuation in OTC levels in all batches during drug treatment which could be due to the individual variation in residue levels within each set of prawns sampled at the same time (*23*−*25*) that was affected by individual feed intake (*24*).

From this experiment, after treatment with a dosage of 2.5 g of OTC/kg of feed, the appropriate withdrawal period for both sexes and in both tissues is ∼9−10 days. For the higher dosage of 5 g of OTC/kg of feed, the withdrawal period for the muscle in both sexes is also10 days, but a longer period of 13 days is required for the head. The withdrawal periods found in this experiment indicate longer times than the findings in juvenile *P. monodon* (*13*), in which shrimp administered with 1, 3, or 5 g ofOTC/kg of feed for 7 days needed 5−7 days to clear the OTC residue. To account for variations in water temperature, drug dosage, and duration of therapy, the withdrawal period was extended by a few more days to a total of 14 days. Similarly, the withdrawal period needed for *P. setiferus* was also 14 days when drug

concentrations in feed were 1.0 and 5.0 g/kg of feed but longer for 10.0 g/kg of feed (*9*). In view of the controlled conditions in this study, which may not reflect actual conditions in commercial practice, and following the same principle with *P. monodon*, the appropriate withdrawal period for *M. rosenbergii* should be ~15−17 days for the muscle and ~21 days for the head. Considering the tradition in Thailand, other Asian countries, and some Western countries of cooking and eating the whole shrimp, head included, the longer period of 21 days should be adopted. A withdrawal time of 21 days is closer to the withdrawal time of 25 days required for Japanese Kuruma shrimp (*Penaeus japonicus)* treated with oxytetracycline hydrochloride at 50 mg/kg of shrimp per day (*26*). This would indicate that the current practice by farmers of a 14 day withdrawal period for *M. rosenbergii* is not sufficient. This could also explain why there are several instances of rejections of *M. rosenbergii* in samples submitted to the Department of Fisheries for export certification.

In conclusion, the proper withdrawal time for *M. rosenbergii* should be 21 days. There was no difference in drug absorption and residue retention between the males and females. Finally, between the two tissues (head and muscle), drug residue retention was longer in the head when the higher dosage was used, but there was no difference with the lower dosage.

LITERATURE CITED

(1) Sandifer, A.; Smith, I. Freshwater Prawns. In *Crustacean and Mollusk Aquaculture in the United States*; Huner, V., Brown, V., Eds.; AVI Publishing: Westport, CT, 1985; pp 65−89.

(2) Herman, L.; Corliss, D.; Bullock, L. Oxytetracycline residues in different tissues in trout. *Technical Paper 37 of the Bureau of Sport Fisheries and Wildlife*; U.S. Department of the Interior: Washington, DC, 1969; 6 pp.

(3) Snieszko, S.; Bullock, L. Columnaris Disease of Fishes. *Fish Disease Leaflet 45*; U.S. Department of the Interior, Fish and Wildlife Service: Washington, DC, 1976; 5 pp.

(4) Scott, W. Antibiotics—points to watch. *Fish Farmer* **1985**, *8* (5), 25−27.

(5) Jacobsen, D. Withdrawal times of freshwater rainbow trout, *Salmo gairdneri* Richardson after treatment with oxolinic acid, oxytetracycline and trimethoprim. *J. Fish Dis*. **1989**, *19*, 89−96.

(6) Bjorklund, H.; Bylund, G.; Temperature-related absorption and excretion of oxytetracycline in rainbow trout (*Salmo gairdneri* R.). *Aquaculture* **1990**, *84*, 363−372.

(7) Aoyama, G.; McErlane, M.; Erber, H.; Kitts, D.; Burt, M. High-performance liquid chromatographic analysis of oxytetracycline in chinook salmon following administration of medicated feed. *J. Chromatogr*. **1991**, *588*, 181−186.

(8) Uno, K.; Aoki, T.; Ueno, R. Pharmacokinetic study of oxytetracycline in cultured rainbow trout, amago salmon, and yellowfin. *Nippon Suisan Gakkaishi*. **1992**, *58* (6), 1151−1156.

(9) Corliss, P. Accumulation and depletion of oxytetracycline in juvenile white shrimp (*Penaeus setiferus*). *Aquaculture* **1979**, *16*, 1−6.

(10) Takahashi, Y.; Itami, T.; Nakagawa, A.; Nishimura H.; Abe, T. Therapeutic effects of oxytetracycline trial tablets against vibriosis in cultured Kuruma prawn *Penaeus japonicus* Bate. *Bull. Jpn. Soc. Sci. Fish*. **1985**, *51*, 1639−1643.

(11) Higuera-Ciapara, I.; Brown, H.; Jauncey, K. Effect of oxytetracycline and sulphamethazine on weight gain and survival of *Penaeus monodon* under stress. In *Chemotherapy in Aquaculture from Theory to Reality*; Michel, C., Aldermann, J., Eds.; Paris, France, 1992.

(12) Choo, P. Antibiotic use in aquaculture: the Malaysian perspective. *INFOFISH Int*. **2000**, *2*, 24−28.

(13) Chanratchakool, P.; Limsuwan C. Accumulation of oxytetracycline in tiger shrimp *Penaeus monodon* (Fabricus). *Thai Fish. Gaz*. **1991**, *44* (1), 31−33.

(14) Sokol, J.; Matisova, E.; Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction. *J. Chromatogr*. **1994**, *669*, 75−80.

(15) Yutaka, Y.; Noriko, F.; Ryoukichi, A. Determination of residual tetracycline in meat by HPLC. *J. Food Hyg. Jpn*. **1989**, *30* (1), 42−47.

(16) Laitinen, H.; Harris, W. Statistics. In *Chemical Analysis*; Laitinen, H., Harris, W., Eds.; McGraw-Hill: New York, 1975; pp 542−545.

(17) Chaiyen, A.; Limsuwan, C.; Chanratchakul, P.; Chinabut, S. Study on distribution of oxytetracycline in Black Tiger Shrimp (*Penaeus monodon Fabricius*). Paper presented at the 31st Kasetsart University Annual Conference, Feb 3−6, 1993.

(18) Lockwood, M. *Aspect of Physiology of Crustacean*; Freeman: San Francisco, CA, 1967; 570 pp.

(19) Higuera-Caipara, I.; Brown, H.; Jauncey, K. Leaching of oxytetracycline from pellets shrimp feeds. Bacterial disease of fish. Presented at the Scientific Symposium, University of Stirling, Scotland, U.K., June 26−29, 1990.

(20) Murray, J.; McGill, A.; Hardy, R. Development of a method for the determination of oxytetracycline in trout. *Food Addit. Contam*. **1987**, *5* (1), 77−83.

(21) Onji, Y.; Uno, M.; Tanagawa, K. Liquid chromatographic determination of tetracycline residues in meat and fish. *J. Assoc. Off. Anal. Chem*. **1984**, *6*, 1135−1137.

(22) Ashworth, B. Liquid chromatographis assay of tetracyclines in tissue of food-producing animals. *J. Assoc. Off. Anal. Chem*. **1985**, *5*, 1013−1018.

(23) Salte, R.; Lieslol, K. Drug withdrawal from farmed fish. *Acta Vet. Scand*. **1983**, *24*, 418−430.

(24) Nordlander, I.; Johnsson, H.; Osterdahl, B. Oxytetracycline residues in rainbow trout analysed by a rapid HPLC method. *Food Addit. Contam*. **1987**, *4* (3), 291−296.

(25) Suwannaruk, W.; Brillantes, S.; Promkhum, R. Determination of oxytetracycline in different parts of freshwater shrimp (*Macrobrachium rosenbergii*). *Thai Fish. Gaz*. **2000**, *53* (5), 471−477.

(26) Nakazawa, H. Overview of antibiotics used for agriculture and residual analysis in Japan. In *Chemical Analysis for Antibiotics Used in Agriculture*; Oka, H., Nakazawa, H., Harada, K., MacNeil, D., Eds.; AOAC Publications: Washington, DC, 1995; pp 2−29.

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