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Estimation of withdrawal time for *Oxytetracycline* in *Penaeus monodon* (black tiger prawn) farming in India

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ABSTRACT

Fisheries sector is one of the fastest growing sector in the world in the world as well as India, contributing to national income, export, food and national security and employment. India's fish export has emerged as the largest group in agricultural exports. In India, *Peanaeus monodon* (shrimp) farming has become immensely popular with about 1,57,000 ha under culture and an average annual production of 1lakh tonnes. Farmed shrimp contributes about 60 percent by volume and 82 percent by value of India's total shrimp export. Shrimp farming in India provides employment directly to about 0.3 million people and indirectly to about 0.6 to 0.7 million people mainly in coastal states Despite the progress, the shrimp industry has been facing severe crisis especially from 1994 owing to the outbreak of infectious diseases caused mass mortalities fetching crop losses to about Rs.1, 203 croes. To control the disease outbreaks and secondary infections, the shrimp growers resorted to use indiscriminately several antibiotics, chemicals and drugs. Even as they failed in controlling the diseases, the indiscriminate use of antibiotics caused the problem of tissue residues in the harvested shrimp. To over come the problem of antibiotic residues in the harvested shrimp, Government of India banned several antibiotics and allowed some antibiotics with MRL's. Inspite of these remedial measures the problem of antibiotic residual problem had not been controlled as several reports have confirmed with contamination antibiotics. To address these antibiotic residual problems, a commonly used broad-spectrum antibiotic Oxytetracycline was used for the present study to estimate the withdrawal time. Oxytetracycline (OTC) is an approved antibiotic in India with a Maximum Residue limit of 0.1 ppm in the harvested shrimp, most commonly used in shrimp farming and hatchery operations to control endemic and systemic *Vibrios* through medicated feed. OTC was selected for estimation of withdrawal period for the black tiger prawn *Peanaeus monodon* under given conditions.

Introduction

India is one of the largest producers of shrimp exports of which frozen shrimp contributed around 1,38,085 tonnes, yielded Rs, 4,220 crores, during 2004-05 worth and contributes about 60 percent by volume and 82 percent by value. The major markets for Indian shrimp used to be mainly USA followed by Japan and Europe Union. Among the various exportable fishery products, shrimp occupies the major share and its production through both capture and culture has expanded. India has been one of the fastest growing fishing nations in the world contributing to about 4.3 percent of the world fish production and 7 percent of the world production through aquaculture. India is unique in its physical geography, climate, land and water resources. It is estimated that 1.24 million ha of potential brackish water area is potential for shrimp farming.

In India shrimp *Penaeus monodon* farming has become immensely popular with about 1,57,000 ha under culture and an average annual production of 1 lakh tonnes. About 91 percent of shrimp growers have a holding in between 0-2 ha, 6 percent between 2-5 ha and the remaining 3 percent have an area of 5 ha and above indicating maximum contribution by the small farming system to the total cultured shrimp production in the country. Shrimp farming in India provides employment directly to about 0.3 million people and indirectly to 0.6-0.7 million people mainly in states bordering the east coast states of Andhra Pradesh, West Bengal, Tamil Nadu and Orissa since a decade. At present both area and productions are the highest in Andhra Pradesh (79,760 ha; 51,230 mt) accounting for 50.57 and 50 percent of total cultured shrimp area and the production followed by West Bengal (47,650 ha; 26,800 mt) with 30.27 percent and 26 percent respectively in the west coast where shrimp farming is being practiced (Cyriac, 2002).

Farming of shrimp *Penaeus monodon*, has become an important component of the Indian aquaculture industry commencing the early 1980's and became a significant commercial activity by 1990, mainly due to the use of under-utilized water logged areas and adoption of improved scientific farming practices in various parts of the country. Despite the progress, the shrimp industry has been facing severe crisis especially from 1994 owing to the outbreak of infectious diseases caused in mortalities fetching crop losses to about Rs 1,203 crores and extensive damage to the system. More recently, India is facing a great problem regarding the use of antibiotics and chemicals in shrimp farming. To control the disease outbreaks and secondary infections, the shrimp growers resorted use indiscriminate use of several antibiotic chemicals and drugs. Even as they failed in controlling the diseases, the indiscriminate use of antibiotics caused the production of tissue

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residues in the harvested shrimps. The European Union rejected 12 shipments of shrimps involving 130 tonnes, during the year 2002 and placed four Indian processing plants under black list mainly due to the presence of antibiotic residues. Eighteen out of 32 rejections in 2002,18 were due to antibiotic residues (Ajayan, 2002). A recent case of rejection of a consignment of shrimp exported from India by the UK, due to the alleged presence of antibiotic residues has brought back to focus the debate on the testing standards in India (Arun,2006). Due to the much growing health concern of the developed countries and WTO-SPS measures, shrimp farming in India faced a serious setback due to the rejection of several Indian consignment based on antibiotic residues. The Government of India's agencies like Marine Product Export Development Authority (MPEDA) banned about 20 antibiotics including Chloramphenicol, Nitrofuran etc., in the shrimp culture practices and fixed Maximum Residue Limits (MRL's) for other antibiotics. In spite of these efforts frequent reports of presence of antibiotics above the MRL's.

To address the issue of antibiotic menace in Indian shrimp farming, a study has been under taken to find out the withdrawal time of the antibiotics from the body of the shrimp.

Material and methods

Estimation of Oxytetracycline withdrawal period in *Penaeus Monodon*

OTC is an approved antibiotic in India with a Maximum Residue Limit of 0.1ppm in the raw material (GOI, 2002). As there is no antibiotic available to control specific microorganisms, Oxytetracycline (OTC), a broad-spectrum antibiotic is most commonly used in shrimp farming and hatchery operations to control endemic and systemic *Vibrios* through medicated feed. Keeping in view of the frequency of its usage and importance in shrimp farming practices, OTC was selected for estimation of withdrawal period for the black tiger prawn *Penaeus monodon* under given conditions. The ambiguity of data on withdrawal period with respect to Indian conditions was the reason for the present study. To fix the critical limits for the identified critical control points, OTC was used for the estimation of withdrawal time, under the given conditions.

Tank Preparation

Six FRP tanks each of 1500 l capacity and measuring 0.75 m in height and 1.8 m in diameter were used for the study. Among the four tanks, three were used for triplicate set of experiments and another three were used for control. 15 days prior to the commencement of experiment, seawater was collected from the nearby rocky beach during high tide time and kept in a separate tank of 2,000 l capacity and allowed for settlement for one week. The upper column of the water was transferred to the six tanks. Small amounts of alum and liquid chlorine were applied for conditioning of the water and proper aeration was provided for all the tanks. The salinity of the water was maintained at 13 ppt, in line with the pond where shrimps were collected.

Shrimp Collection

300±15 numbers of 80 days old shrimps of average body weight of 10g were collected from M/s. Pancham Aqua, Safale, Thane (Dt), Maharashtra. The shrimps were collected from the ponds where antibiotics were not used. The animals were brought under oxygen to the CIFE Aquaculture laboratory, Mumbai. Salinity of pond water at the farm and the transport time were noted. After reaching the experimental station, shrimps were transferred immediately to the experimental tanks at the rate of 50 animals per each tank.

Rearing of Shrimps

Semi-intensive culture practices were followed throughout the experiment. Salinity, pH and Dissolved Oxygen were maintained at optimal levels. Shrimps were acclimatized for 10 days and the medicated feed was started from the 11^{th} day onwards for a period of 7 days and post medication for 18 days. 'The Water Base (TB4)' feed was used for the entire period of study.

Feed Medication

Oxytetracycline Soluble powder (250 mg of OTC active ingredient in each 4 gm veterinary grade of M/S Pfizer Animal health, Navi Mumbai was used for the present study. 5g of OTC dissolved in 45 ml of distilled water was sprayed on 1 kg of feed to obtain OTC concentration of 5 gm / kg of diet. The experimental diet was prepared on a daily basis. To protect leaching of the OTC, pure cod liver oil was added at the rate of 30 ml per kg feed. Feed was given 4 times daily at the rate of 3% body weight during 0630, 1130, 1730 and 2330Hrs. Medicated feed was given for 18 days and non-medicated feed was given for the remaining 18 days of culture. Control tanks received only non-medicated feed throughout the experiment.

Sampling Procedure

Prior to second feeding time, 3 animals from each tank were collected randomly every day from the first day of starting of medication till the end of the experiment. Eighteen animals were sampled every day and the length and weight were measured and the animals were observed for symptoms of disease if any. Animals were sealed in different polythene pouches, labeled and kept at –20°C until further analysis.

Sample Analysis

The samples collected during the medication period (1-7 days) and post-medication period (8-25 days) was analyzed using a High Pressure Liquid Chromatography (HPL.C).

Sample Preparation

The method of Ueno and Aoki (1999) was followed with slight modifications. 1g of muscle tissue pooled from 3 animals from each tank collected each day was homogenized with 5ml cold 30% Methanol containing 0.5% EDTA in 10 ml tube, and centrifuged at 4,000 rpm for 5 minutes. Supernatant was filtered through a syringe filter unit of 0.2m diameter.

HPLC Analysis

All the aliquot samples of experimental tanks and control were taken in a insulated box to Therapeutic Drug Monitoring Laboratory, Sion, Mumbai for HPLC analysis. The mobile phase used was Methanol-0.2 M Oxalic acid (1:9v/v); Seepak 'C18' cartridge was used as column for extraction using UV detector. The standard solution was 100μg/ml kept in water at –20°C. Aliquots of samples (100 μ l) were injected into the HPLC.

Results

The results of the present study on estimation of Oxytetracycline showed (table.1 and Figs.1-4)) that the amount of OTC on day after cessation of medicated feed was 4.842 ppm, which reduced gradually to reach the lowest of 0.001 ppm on day 15.Tissue residues were totally abolished by the 16^{th} .The results of ANOVA (table 2) indicated that there was no significant variation between the control and treatment means of the observed F value with respect to the length – weight relationship

Discussion

The results clearly proved that antibiotic residues in the tissues and a withdrawal period of 15 days. Thus the residue of OTC was totally abolished by the 16^{th} day. Consumption of shrimps within this period would pose human health hazards and

hence the need for the setting up of a critical limit. Based on the present results as also earlier studies (MPEDA, 1996), and taking into consideration the fact that many other antibiotics may also be used, a period of 21 days post medication should be enforced as a corrective or control measure. Selvaraj *et al.,* (2002) suggested the proper withdrawal time must be observed to ensure that the edible tissue in safe when it is offered for sale.

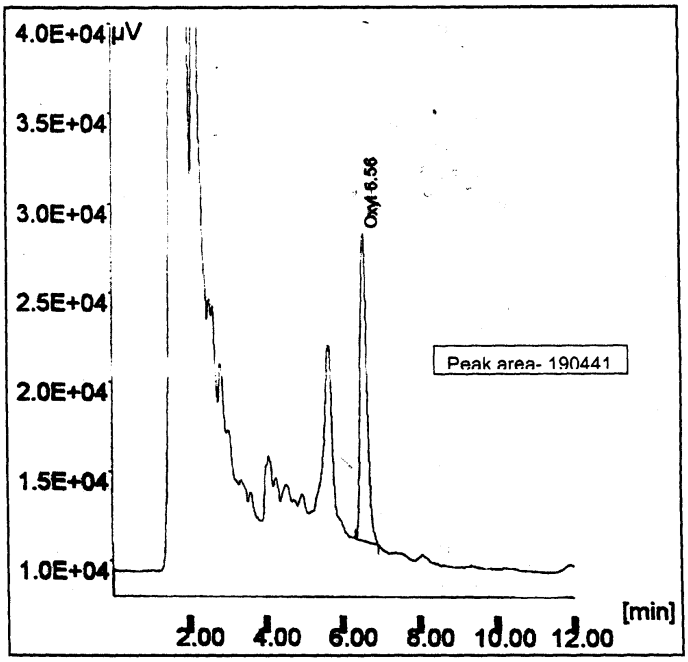

Fig.1.Chromatogram of residual oxytetracycline in the muscle tissue of treated shrimps on 1st day after cessation medicated feed

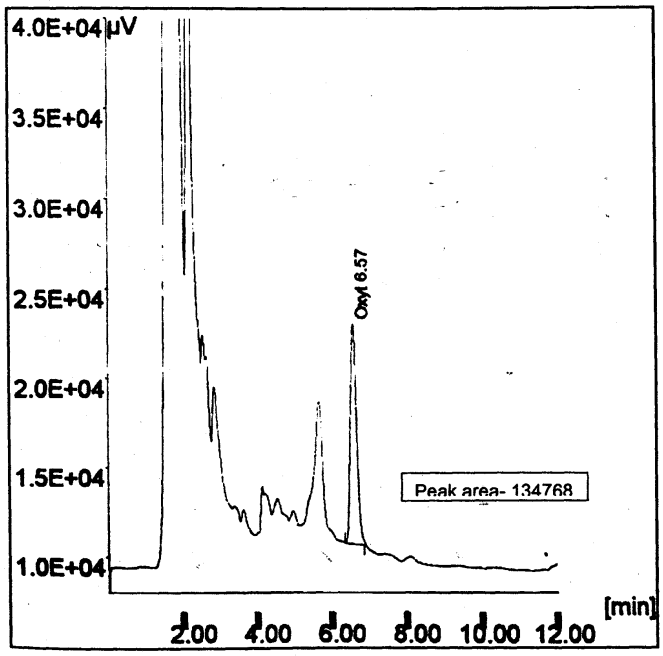

Fig.2 Chromatogram of residual oxytetracycline in the muscle tissue of treated shrimps on 7th day after cessation medicated feed

The results are agreeing with the studies of Onkong et al (2000) who reported that under practical conditions where farmers use OTC at the rate of 5 g/kg of black tiger shrimp feed, the longest elimination of the drug from any tissue was 18 days, and suggested that the withdrawal period between the use of OTC and harvesting should be no less than 21 days. Selvin and Lipton (2004) reported that *Penaeus monodon* fed for 7 days with 50-100 mg/kg shrimp of OTC were examined for residual accumulation and depletion. A residue of 3.47 µg/g shrimp tissue was found after 1 day of post dosing and a substantial quantity of residue 1.56 µg/g was observed up to 15 days of post-treatment. By the 20th day of experiment, the OTC concentration was found in trace quantity 0.42 µg/g or below detectable limit. The present study results endorse the recommendations of Shankar and Mohan, 2002; and MPEDA, 1996.

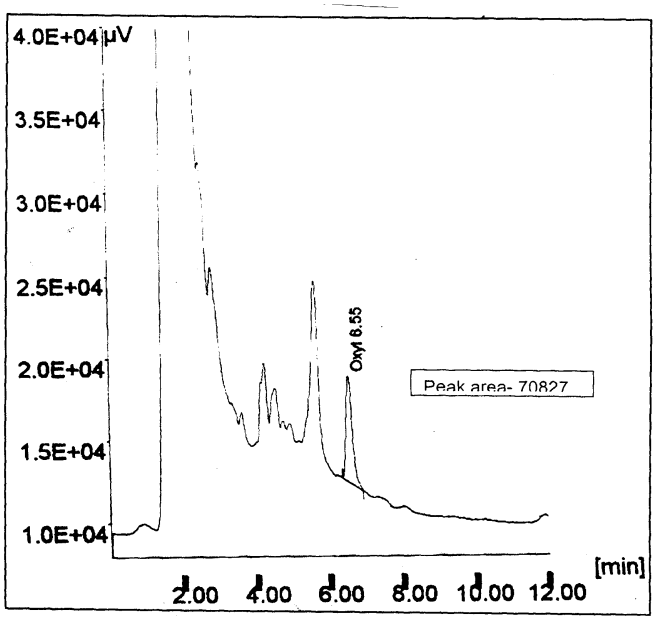

Fig 3. Chromatogram of residual oxytetracycline in the muscle tissue of treated shrimps on 15th day after cessation medicated feed

Keeping in view of the international trade perspectives and WTO-SPS agreement, it is essential to adopt the organic farming, as it protects the health of consumers by reducing the overall exposure to toxic chemicals from pesticides that can accumulate in the ground, air, water and food supply. As organic aquaculture does not use toxic and persistent antibiotics and pesticides and also helps to prevent top soil erosion, improves soil fertility, protects ground water and conserves energy. It is essential to control the prohibited antibiotics and chemicals by the Government. The good management practices (GMP's) include stocking disease free and high healthy stock, combine continued assessment of water exchange, use of good quality feed without over feeding, and use sedimentation and recycling ponds (MPEDA, 1996). Preventive healthy programs at aquaculture facilities include the establishment of a population of healthy, disease-free animals. Good monitoring and record-keeping systems to be implemented. Close surveillance for diseases can provide advance warning of potential problems. Programs such as HACCP at farm level (the authors have developed a HACCP framework applicable at shrimp farms specifically to address the antibiotic residue menace) and farm bio-security can then be established to promote animal health and fight diseases (More and Frelier, 2002; FAO, 2001). However it is imperative that the ban on indiscriminate usage of antibiotics shrimp farming operations is strictly enforced

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Table 1. Concentration of Oxytetracycline (OTC) detected from shrimp muscle tissues using HPLC

S.No	Experiment	OTC Concentration During Post-Medication Period (Day)				
		1st	7th	14th	15th	16th*
1	Control	0.000	0.000	0.000	0.000	0.000
2	Treatment	4.842	2.563	0.012	0.001	0.000

16th Day – 24th October 2004

Table 2. Analysis of Variance of length and weight of shrimps treated for estimation of Oxytetracycline withdrawal period

Anova: Weight						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
W=T1	25	303.6	12.144	2.403667		
W=T2	25	323.14	12.9256	2.690551		
W=C	25	305.59	12.2236	2.751649		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.250323	2	4.625161	1.768509	0.177916	3.123901138
Within Groups	188.3008	72	2.615289			
Total	197.5511	74				
Anova: Length						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
L=T1	25	296.74	11.8696	0.24357		
L= T2	25	305.78	12.2312	0.83294		
L=C	25	301.38	12.0552	0.68634		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.63482	2	0.81741	1.39106	0.25542	3.12390114
Within Groups	42.3084	72	0.58762			
Total	43.9432	74				

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