Asian Fisheries Science **18** (2005): 25-31
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2005.18.1.003
Asian Fisheries Society, Manila, Philippines

Influence of Dietary Intake of Levamisole on Growth and Survival of *Macrobrachium rosenbergii* (Palaemonidae, de Man)

N. BARUAH[1] and K.P. PRASAD[2]

[1]Regional Agricultural Research Station
Assam Agricultural University
Nagaon, Assam
India

[2]Central Institute of Fisheries Education (ICAR
Mumbai-400061
India

Abstract

An inclusive indoor experimental trial was carried out to determine the influence of levamisole, an antihelminthes, as a growth facilitator in the culture of scampi (*Macrobrachium rosenbergii,* Palaemonidae). A purified diet was prepared with 40% protein, containing levamisole at 0 mg (as control), 125 mg and 250 $mg \cdot kg^{-1}$ dry weight. Nine plastic tanks were stocked with a group of 32 animals (PL20-30) each and fed the trial diet for three months. Animals were regularly weighed at the end of each month. No significant difference was noticed in mean growth and survival ($P > 0.05$) of the animals between treatments. However, significant differences ($P < 0.05$) were observed in the growth parameters like percentage of weight gain, percentage of SGR and FCR among the control and levamisole treated animals. Survival percentages of the animals treated with levamisole were better than the control.

Introduction

The looming incidences of scores of diseases like white spot syndrome disease (WSSD), yellow head disease (YHD), soft shell disorder (SSD) and luminescent bacterial disease (LBD) that confronted shrimp farming have compelled aquaculturists to find suitable alternatives. Lately, scampi culture has emerged as one of the fastest growing sectors of aquaculture. With the change of palate and increased appetite for scampi in the international market, competitions have been flocking in towards the intensive farming or “crowded farming” in India. Jam-packed culture to execute the dollar-dream in haste often results in stunted growth and high mortality of this farmed animal. To sustain this pursuit, apt management of diet with adequate growth facilitators like levamisole is imperative to serve the job of stress busting. They boost production by facilitating built in protection to dispel invading pathogens for faster individual growth and equally cut down the heavy expenditure incurred on health security. They reinforce immune system and thereby minimize the chances of secondary infections in wounded or newly moulted vulnerable animals. Optimal utilization of levamisole in crustaceans has reportedly aided in increased growth rate and better survival in shrimps (Newman 1996; Song et al. 1994). The management of feed with appropriate additives is very significant to attain high growth rate and thereby maximum farm production in shrimp farming. (Nair and Sridhar 1994).

The synthetic phenylimedazolthiazole or levamisole has been extensively utilized in veterinary and human medicine as antihelminthes and has immunomodulating effects in fish (Anderson 1992). It is reported that carp fingerlings treated with levamisole showed increased growth and reduced mortality (Siwicki and Korwin-Kossakowski 1988). This trial was conducted to ascertain the implications of antihelminthes in the culture of scampi to facilitate stimulated growth and better survival.

Materials and Method

A trial diet was prepared at 40% protein content (Tables 1 and 2). First dry ingredients and levamisole were thoroughly mixed. Then both oils were added to the mixture and made into dough with lukewarm distilled water containing 5 g of gelatin to act as a binder. Control diet was made free of levamisole. Pellets were made using a hand pelletizer (1- 2 mm die) and then cut into small pieces (0.5 cm). Pellets were air dried overnight and stored in airtight bottles under refrigeration at 4°C. Pellets were made once every fortnight to meet the need of the animals.

Table 1. List of ingredients

Ingredients	Quantity (g·100 g)
Vitamin – free casein (SRL)	30
Cellulose powder (Hi media)	10
Dextrin (Qualigens)	30
Cod liver oil (Seven seas)	5
Sunflower oil (Dhara)	5
Vitamin –mineral mix (Sup Levite-M, Sarabhai)	5
Gelatin (Hi – media)	15

Scampi post larvae of 20-30 stages (initial mean weight 0.60 g ± 0.10 g) were collected from the wild and stocked in triplicate in nine plastic tanks of 50 l capacity. Each tank contained 32 animals irrespective of their individual size. Animals were fed two times a day at 6 % of their average body weight for three months. The pelleted diets containing levamisole hydrochloride at 0 mg (as control), 125 mg and 250 mg·kg^{-1} dry feed were supplied. Animals were regularly weighed at the end of each month. Tanks were thoroughly cleaned to maintain hygiene. Leftover feed (approximately one third of daily feed supplied) and excreta were siphoned out regularly from the tanks. Excess amount of feed was supplied to check on the possible cannibalistic behaviour of the animals. Around 80% of water was exchanged daily from the tanks and was carried out in the morning hours to minimize the shock of fluctuation of water temperature. Uninterrupted aeration was provided to the animals. Broken asbestos pieces, PVC pipes and other means of shelters like small bottles and conch shells were provided in each tank to avoid cannibalism. Physico-chemical parameters of water were not controlled.

All the trails were carried out in triplicate. The average body weight was expressed as mean (± S.Em.) body weight. One-way ANOVA was applied to analyze the data statistically at 0.05 level of probability.

Results and Discussion

The dietary application of levamisole for three months did not show any significant difference ($P > 0.05$) on the mean weight of the experimental subjects (Table 3). The results indicate that levamisole stuffing

Table 2. Composition of Vitamin-Mineral Mixture

Composition	Quantity (per 2.5 Kg V-M mix)
Vitamin A	50.00.000 I. U.
Vitamin D_3	10,00.000 I. U.
Vitamin B_2	2.0 g
Vitamin E	750.0 Units
Vitamin K	1.0 g
Calcium Pantothenate	2.5 g
Nicotinamide	10.0 g
Vitamin B12	6.0 g
Choline Chloride	150.0 g
Calcium	750.0 g
Manganese	27.5 g
Iodine	1.0 g
Iron	7.5 g
Zinc	2.0 g
Copper	2.0 g
Cobalt	0.45 g

diet failed to make any remarkable influence on growth. Although significant statistical difference was not observed in the mean body weight, the preliminary findings of growth indices were very encouraging. Appreciable difference of percentage in (Table 4) weight gain among the animals with different treatment levels was observed. This elementary study necessitates more trials with more replications for a considerable period to find out the exact influence of levamisole on the growth of scampi.

Growth parameters like percentage of weight gain (WG), food conversion ratio (FCR) and percentage of specific growth rate (SGR) extensively used in nutritional studies of aquatic animals (De Silva and Anderson 1995), showed a significant growth difference ($P < 0.05$). All the parameters estimated from each group treated with levamisole showed an elevated growth rate than control with prominent difference (Table 4). The results ensure that levamisole could be applied as a growth facilitator in the diet of scampi. The significant differences of FCR, SGR and WG confirm the positive aspects of use of anti-helminthes in scampi culture. But more elaborated tests are essential to corroborate the promising potentialities of levamisole commercially.

The survival rate of the scampi receiving dietary levamisole at both the levels of treatments did not show any significant difference ($P > 0.05$) (Table 5). But the performance of survival of scampi (b, c) indicates a considerable difference in the survival percentages than the control (a).

Table 3. Mean body weight of scampi

Day	Levamisole (mg·kg)	Average weight (g)
0	0	0.739±0.136
	125	0.640±0.051
	250	0.511±0.053
30	0	0.983±0.567
	125	0.763±0.064
	250	0.600±0.086
60	0	1.084±0.275
	125	0.873±0.078
	250	0.721±0.113
90	0	1.164±0.192
	125	1.322±0.204
	250	1.051±0.047

Table 4. Different growth parameters of scampi

Day	Levamisole ($mg \cdot kg^{-1}$)	Percentage of weight gain	SGR	FCR
90	0	57.51	0.50	6.26
	125	106.56*	0.81*	3.38*
	250	105.68*	0.80*	3.41*

*denotes statistically significant differences (P<0.05) between control and levamisole treated groups

The main impediment of application of levamisole as a growth facilitator in fish and crustacean is the determination of affective dose. The right dose of levamisole is very critical to get appreciable results in scampi culture. The efficacy of this drug strictly depends on precise dose (Anderson 1992). Fishes fed with diets containing levamisole reportedly elevated body weight, growth rate and better survival than without levamisole (Mulero et al.1998).

There is tremendous emphasis to develop feed additives to enhance production in short culture periods by facilitating faster weight gain, higher survival rate and improvement of overall health status, specially in high stocking densities. Furthermore, limited resources available in this field restricts the in depth analysis, particularly use of levamisole in crustaceans. Most of the experiments on levamisole were carried out as immuno-modulator in fish. It is reportedly known to effectively work in most of the fishes, but still defined dose remains an issue (Anderson 1992). The amount of levamisole retained in the subjects body or the amount excreted with faeces and leached out in water from the feed pellets are debatable questions to draw a distinctive conclusion. Sparse previous research in crustaceans restricts broad confirmation of results in this experiment.

The studies conducted by Mulero and his team (1998) on the marine teleost gilthead sea bream (*Sparus aurata* L.) substantially supports the elevation of growth and survival in the subjects, so are in the case of growth parameters, in this experiment. The growth parameters of this trial suggest that subjects are likely to form better corporal immunity to facilitate faster growth and minimum mortality. It is a universally accepted fact that unless an animal is healthy enough to make better use the available food resources; it cannot guarantee high growth and better survival. Levamisole creates an environment to facilitate heightened production. There is no clear explanation why levamisole is so dose sensitive since it is widely assumed as a growth enhancer (Mulero et al. 1998) therefore, further research would be forthcoming.

In this experiment, the rearing environments and feed supplied were almost identical except the addition of levamisole at two treatment levels (b, c). It may be presumed that other factors like moulting, metabolism,

Table 5. Treatments showing survival percentage of prawns

Day ($mg{\cdot}kg^{-1}$)	Levamisole	No. of survivors			Percentage of survival (%)
		Rep.1	Rep.2	Rep.3	
90	0	27	24	26	80.22^a
	125	26	28	29	86.47^b
	250	30	28	28	89.59^c

experimental routine handling, and feeding efficiency might have substantial effects on the growth pattern of scampi (Balazs and Ross 1976). Besides, individual health status and specific growth rate of each animal play a vital role on growth performances (Roubach and Saint-Paul 1994). Individuals within a group may grow at different pace due to genetic variability (De Silva and Anderson 1995). The ration size and feeding frequency are also critical factors in determining the growth of animals (Nair and Sridhar 1994). Apart from all identical conditions, the critical factor that directly influenced the growth and survival of the experimental animals was feed. Considering the influential growth parameters at both levels, it elucidates that levamisole had a limited impact on scampi if not substantial.

A precise knowledge of the relationship among levamisole, feed requirements and body weight of cultured animals is essential to attain the desired growth. Deterioration of water quality with the dumping of excess feed and ensuing excreta might have worsened the living conditions of the animals. Moreover, the duration of moulting period, growth inhibiting hormones and cannibalism affect normal growth and survival invariably. Nevertheless, application of levamisole would be helpful to effectively minimize the natural mortality of the farmed scampi. This experiment evinces a positive tilt towards the dietary application of levamisole in scampi culture but further comprehensive and field trials are highly warranted to establish its efficacy as a growth facilitator. Future studies that employ similar methodologies, however, may shed more light on whether dietary ingestion of levamisole really increases, decreases or does not affect the growth and survival of scampi at lower, higher or differing doses.

Acknowledgments

The authors acknowledge Dr. K.V. Rajendran, Senior Scientist, CIFE, Mumbai and Ms Ritu Geu Goswami, Assistant Professor, Department of Food and Nutrition, Central Agricultural University, Tura for their valuable suggestions and critical review of the manuscript and the Indian Council of Agricultural Research for the adequate funding to carry out this experiment.

References

Anderson, D. P. 1992. Immunostimulants, adjuvants and vaccine carriers in fish. Applications to aquaculture. Annual Review of Fish Diseases, pp. 281-307.

Balazs, G. H and E. Ross. 1976. Effect of protein source and level on growth and performance of the captive freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 7:299-313.

De Silva, S. S. and T. A. Anderson. 1995. Methods used in studies on nutrition. *In*: Fish Nutrition in Aquaculture. Chapman and Hall, London, 279-287 p.

Mulero, V., M. A. Esteban, J. Munoz, and J. Meseguer. 1998. Dietary intake of levamisole enhances the immune response and disease resistance of the marine teleost gilthead sea bream (*Sparus aurata* L.). Fish and Shellfish Immunology 8: 49-62.

Nair, R. and M. Sridhar. 1994. Role of ration size and feeding frequency in shrimp culture. Fishing Chimes 14 (9): 46-47

Newman, S. G. 1996. Non-specific immunostimulants to prevent shrimp disease. Fisheries world April: 4-8.

Roubach, R and U. Saint –Paul. 1994. Use of fruits and seeds from Amazonian inundated forests in feeding trials with *Colossoma macropomum* (Cuvier, 1818) (Pisces Characidae). Journal of Applied Ichthyology 10(2-3): 134-140.

Siwicki, A. K. and M. Korwin-Kossakowski. 1988. The influence of levamisole on the growth of carp (*Cyprinus carpio* L.) larvae. Journal of Applied Ichthyology 4(4): 178-181.

Song, Y. L., C. Z. Lin and H. J. Tsai. 1994. Immunostimulation of giant freshwater prawn (*Macrobrachium rosenbergii*) to induce microbial substances. In: International symposium on aquatic animal health, University of California, School of Veterinary Medicine, Davis, USA, 4-8 September 1994, pp.59 (Summary only).

Manuscript received 23 August 2003; Accepted 10 January 2004