Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading

Effect of garlic peel on growth, hematological parameters and disease resistance against *Aeromonas hydrophila* in African catfish *Clarias gariepinus*(Bloch) fingerlings

Karthik Thanikachalam, Marimuthu Kasi*, Xavier Rathinam

Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Malaysia

ARTICLE INFO

Article history:
Received 19 June 2010
Received in revised form 27 June 2010
Accepted 20 July 2010
Available online 20 August 2010

Keywords:
Garlic peel
Disease resistance
Clarias gariepinus

ABSTRACT

Objective: To evaluate the efficacy of dietary doses of garlic (*Allium sativum* L.) peel on the hematological and disease resistance of African catfish [*Clarias gariepinus(C. gariepinus*)] fingerlings against the infections caused by opportunistic bacterial pathogen *Aeromonas hydrophila*. **Methods:** Powdered garlic peel was incorporated into the diets at (0%, 0.5%, 1.0% and 1.5%) and fed to catfish fingerlings for 20 days. After the feeding trial, biochemical (serum total protein, albumin and globulin), hematological parameters (white blood cells and red blood cells) of the fish were examined. Fish were challenged with *Aeromona hydrophila (A. hydrophila)* after 20 days of post feeding and percentage mortalities were recorded up to 10 days after post challenge. **Results:** Enhanced serum protein, albumin and globulin in fish fed with all the dosages of garlic peel when compared to control group. Significantly highest red blood cell and white blood cell counts were recorded in garlic peel incorporated diet fed groups compared to control group. The results also demonstrate that low survival rate (55.5±11.0)% in control groups and significantly higher survival rates were recorded in all the garlic peel fed groups after challenging with *A. hydrophila*. However no significant impact was noticed with regard to body weight gain, specific growth rate and food conversion ratio of fish fed with different levels of garlic peel inclusion and control group. **Conclusions:** These results indicate that garlic peel enhances the hematological parameters even at a low level (0.5%) incorporation and makes *C. gariepinus* highly immunopotent and more resistant to infection by *A. hydrophila*.

1. Introduction

Aquaculture fish production has increased significantly over the past few decades which has lead to intensive fish culture practices where stressors like over crowding, transport, handling, size grading and poor water quality are common problems. It has been widely demonstrated that farmed fish are more susceptible to various disease agents in intensive farming. In order to improve health conditions in the rearing of aquatic organisms, several alternatives such as improved husbandry, nutrition and water quality; optimal stocking density; and use of vaccines, probiotics[1] and immunostimulants[2] have been proposed. The enhancement of the immune system of fish is considered the most promising method of preventing fish diseases in aquaculture. This enhancement can be achieved with application of vaccines, which enhance the specific immune response of the fish and are considered to be the most effective agents. Further the use of antibiotics and chemotherapeutics to combat fish diseases has several drawbacks including the risk of generating resistant pathogens, bioaccumulation and environmental pollution. The available commercial vaccines are expensive for fish farmers and are highly specific against particular pathogens[3, 4]. In contrast to vaccines, immunostimulants enhance the non-specific immune response of fish[5]. The major components of the innate immune system are macrophages, monocytes, granulocytes and humoral elements, like lysozyme[6]. Several biological and synthetic compounds have been shown to enhance non specific immune system of cultivated fish[7-9]. Immunomodulatory activity of several plants and herbal components have been available and mostly studied in mice, fish, chickens or human cell lines[10, 11].

*Corresponding author: Marimuthu Kasi, Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Malaysia.
E-mail: aquamuthu2k@yahoo.com

Garlic (*Allium sativum* L.), is a medicinal plant which has been used for thousands of years in Indian ayurvedic medicine. Many beneficial health benefits of garlic are attributed to organosulphur compounds, particularly to thiosulfanates (R-S-S (O)-R)[12]. Garlic has shown hypolipidemic[13], anti-microbial[14], antihypertensive[15], hepatoprotective[16], insecticidal[16] and anti fungal activity [17]. Garlic extract has also been shown to reduce serum cholesterol levels[18]. *Allium* species also been reported to have immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity[19, 20]. Huge quantities of garlic are consumed all over the world for flavouring various types of food and their outer layers not been utilized and discarded as waste. Earlier studies are more focused on the utilization of garlic pulp and its extracts in fish[20]. No information is available on the immunostimulatory effects of garlic peel in aquaculture. Hence the present study is aimed to determine the immunostimulating effect of garlic peel in African catfish [*Clarias gariepinus(C. gariepinus)*] fingerlings. The study was evaluated the effects on biochemical and haematological parameters of the serum/blood of fishes for the first time using garlic peel.

2. Materials and methods

2.1. Fish and their maintenance

African catfish *C. gariepinus* is one of the common fresh water fish species was used in this study. Fingerlings of African catfish [average weight (8±2) g; length (11.30±2.5) cm)] were obtained from a private fish farm, Sungai Petani, Malaysia. The fish were transported in polythene bags with sufficient aeration to the animal house. They were carefully transferred to circular cement tanks (400 L) and left undisturbed overnight. The fish was acclimatized under aerated conditions for a period of 10 days at (28.0±1.5) ℃. Fish were fed with a commercial catfish feed at *ad libitum* twice daily during the acclimatization period.

2.2. Plant material and feed formulation

The plant material, *Allium sativum* was purchased at local supermarket, Sungai Petani, Malaysia. The peels were separated from the garlic bulbs. The peels were washed thoroughly and oven dried at 50 ℃. Then, the peels were ground into fine powder using heavy duty blender. Then the powder was incorporated into fish feed at a rate of 0 g (control), 5 g, 10 g and 15 g per/kg feed to prepare different experimental fish diet. The garlic peel free feed was used as a control diet including fish meal(45.7%), soya bean meal(16.6%), rice bran(11.5%), CMC binder(2.0%), wheat(21.0%), Vitamin mix(2.0%), fish oil(1.2%). The dry ingredients were mixed thoroughly with water for 10 minutes. The resulting dough was pelleted, dried at room temperature for 48 hours and then stored in airtight containers until fed.

2.3. Experimental design and feeding trial

African catfish fingerlings (n=180) were randomly divided into four groups (T1, T2, T3 and T4). Each group of 45 fingerlings was again divided into three equal triplicate subgroups and maintained in 12 plastic troughs (40 L capacity). Each aquarium was supplied with aeration by aquarium aerators. Group T1 was fed with basal diet and treated as the control. The remaining groups were fed with 5 g garlic peel/kg of feed (Group T2), 10 g garlic peel/kg (Group T3) and 15 g garlic peel/kg of feed (Group T4) for 20 days. The fish were fed *ad libitum* twice daily at 9:00 and 17:00. Water exchange (50.0%) was done daily and water quality was monitored throughout the experimental days at weekly intervals. Temperature was (28±1.5) ℃, pH 6.5±1.5, dissolved oxygen concentration (5.2±0.3) mg/L. Specific growth rate (SGR) and feed conversion ratio (FCR) were estimated for both control and experimental groups. The following formula was used to calculate the growth parameters.

SGR (%/day) = $100\times[(\ln W_1 - \ln W_0)/t]$, where W_0 and W_1 are average initial and final body weights, respectively, and t is time (days);

FCR = Food consumed (g) / Weight gain (g)

2.4. Collection of blood and determination of hematological and biochemical parameters

After 20 days of post feeding the feed was withheld from fish for 24 hours prior to collection of blood. Six fish from each subgroup were randomly selected for blood and serum collection. Heparin was used as an anticoagulant. Blood was collected from the caudal vein with a 1 mL plastic syringe ringed with heparin and stored at 4 ℃. The blood was then transferred immediately to a test tube containing heparin solution and shaken gently. The blood was used for determination of RBC and WBC count. Blood samples were also collected without heparin, allowed to clot and centrifuged at 7 000 g for collection of serum and refrigerated. Sera and blood were pooled into four groups for estimation of hematological and biochemical parameters. Serum samples were analyzed for total protein following the method of Lowry *et al*[21], albumin content by Doumas *et al*[22] and globulin content (subtracting albumin from the total protein). Total erythrocyte count (RBC) was performed following the method of Hendricks[23] using a haemocytometer where a total leukocyte count (WBC) was determined following the method of Shaw[24].

2.5. Culture of pathogen

Aeromonas hydrophila(A. hydrophila) (ATCC 49140) was obtained from ALLEIGHTS Sdn. Bhd, Malaysia and cultured in nutrient broth (Himedia) for 24 hours at 37 ℃. The broth culture was centrifuged at 3 000 g for 10 min. The supernatants were discarded and the pellets were re suspended in phosphate-

buffered saline (PBS 7.4), and the OD of the solution was adjusted to 0.5 at 456 nm, which corresponded to 1×10^7 cells mL^{-1}. The bacterial suspensions were then serially diluted using standard dilution technique with PBS and used for the challenge experiment. After 20 days of feeding, 9 fish from each subgroup were intraperitoneally injected with 0.1 mL of suspended bacteria. Mortality was recorded until 10 days following infection.

2.6. Statistical analysis

Data for growth (SGR and FCR), hematological and biochemical parameters were analysed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan's multiple range test (DMRT) using SPSS version 11 (Duncan, 1955). Significance was tested at 5% level.

3. Results

3.1. Fish growth and feed efficiency

The growth parameters of African catfish *C. gariepinus* fingerlings fed with various dietary incorporation of garlic peel are presented in Table 1. The dietary inclusion of garlic peel had no significant ($P>0.05$) impact on weight gain, specific growth rate and feed conversion ratio of *C. gariepinus* fingerlings when compared to garlic peel fed groups and control group. No mortalities were observed in all the treatment groups and control group during the feeding trial.

3.2. Blood/serum biochemical and hematological parameters

The serum/blood biochemical and hematological parameters are shown in Table 2. Significantly higher protein content was recorded in the 1.0% and 1.5% garlic peel fed treatments than the control. The higher amount of albumin and globulin was observed in all the three garlic peel incorporated diet fed groups when compared to control group. However no significant difference was noticed within dietary inclusion of garlic peel fed groups. The red blood cells are significantly higher in garlic peel fed groups than control group. Significantly highest white blood cell count was recorded in 1.5% garlic peel incorporated diet fed group whereas no significant difference was noticed among the other garlic peel inclusion group T2, T3 and control groups.

3.3. Disease resistance and survival

After challenging fish with *A. hydrophila*, the mortality was recorded for 10 days. There was no mortality of fish up to 24 hrs. The fish fed with different percentages of garlic peel showed significantly ($P<0.05$) higher survival rate when compared with control. The highest survival rate (96%) was recorded in fish fed with 1.5% of garlic peel (Figure 1). However no significant difference was noticed among the three levels of garlic peel incorporated fed groups ($P>0.05$).

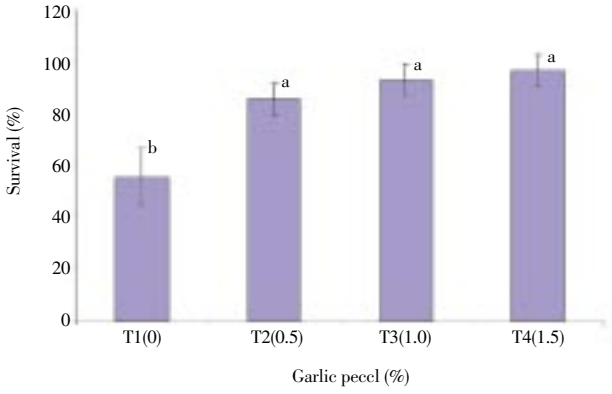

Figure 1. Effect of different levels of garlic peel inclusion on survival rate of African catfish fingerlings after challenging with bacteria *A. hyrophila* (values are mean ± SD).
a: $P>0.05$, b: $P<0.05$.

Table 1
Growth parameters of African catfish fingerlings fed with various dietary incorporation of garlic peel.

Treatments	Initial weight (g)	Final weight (g)	Weight gain(g)	SGR (%/day)	FCR(%)
T1(control)	8.77±0.18	18.05±0.85	9.28±0.67	3.60±0.13	2.14±0.47
T2 (0.5%)	8.71±0.30	17.67±0.49	8.95±0.52	3.53±0.16	2.33±0.38
T3 (1.0%)	8.88±0.27	18.09±0.52	9.22±0.25	3.56±0.10	2.10±0.17
T4 (1.5%)	8.79±0.01	17.99±0.76	9.20±0.75	3.57±0.20[a]	1.75±0.55

Table 2
The biochemical and hematological parameters of fingerlings of African catfish fed with different levels of garlic peel incorporated diets.

Dietary treatments	Protein ($g\cdot dL^{-1}$)	Albumin($g\cdot dL^{-1}$)	Globulin($g\cdot dL^{-1}$)	RBC (1 000 cells mm^{-3})	WBC (1 000 cells mm^{-3})
T1(control)	5.29±0.10*	0.19±0.05*	5.09±0.05*	418±29*	20±9*
T2 (0.5%)	5.48±0.10*	0.28±0.05	5.19±0.15	490±9	30±7*
T3 (1.0%)	5.64±0.05	0.29±0.10	5.34±0.05	470±30	28±3*
T4 (1.5%)	5.63±0.03	0.30±0.01	5.33±0.30	515±50	41±4

*: $P<0.05$.

4. Discussion

The use of natural immunostimulants in aquaculture has been increasing rapidly for the prevention of diseases and also to avoid the indiscriminate use of hazardous antibiotics [3, 4]. Herbal immunostimulants are biocompatible, biodegradable and safe for the environment and human health[25]. Hence the present study investigated the effect of garlic peel as an immunostimulant in African catfish. Survival of the fish was not significantly (P>0.05) affected by the experimental diets. Weight gain, SGR and FCR of African catfish fed with experimental diet containing different levels of garlic peel was also not significantly different (P>0.05) than those of fish fed with control diet. The present study corroborates with the study results of Sahu *et al*[20] in *Labeo rohita* fingerlings fed with 1 g, 5 g and 10 g of garlic bulb/kg feed and revealed that no significant difference in SGR and FCR of fish fed with garlic bulb and control diet.

Leucocytes play an important role in non specific or innate immunity and their count can be considered as an indicator of the health status of fish[26]. Significantly increased (P< 0.05) total white blood cell counts following 20 day garlic peel post feeding trial supports the anti-infection properties of garlic peel[27]. This result is also supported by another study by Choudhury *et al*[28] found that there was an increase in the WBC count when *Labeo rohita* juveniles were treated with immunostimulants like levamisole and ascorbic acid[28]. The erythrocyte count was higher in garlic peel fed groups when compared to control groups. The erythrocyte count increased with the administration of garlic peel, which might indicate an immunostimulant effect. The findings conform to those by Duncan and Klesius[29] who reported that the number of erythrocytes was significantly greater in channel catfish fed with a diet containing b-glucan.

The serum total protein after 20 days of feeding with garlic peel increased (P<0.05) compared to the control diet. Siwicki[30] observed an increase in total protein content after feeding of b-glucan (0.2%) and chitosan (0.5%) in the diet. The serum proteins like albumin and globulin are the major proteins, which play a significant role in the immune response of fish. Globulins like gamma globulin are absolutely essential for maintaining a potential immune system. Serum albumin and globulin values in fish fed with garlic peel were higher than the control. Increase in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response of fish[31].

After challenge with *A. hydrophila*, all treated groups showed a significantly (P<0.05) reduced mortality compared to the control group. The best survival rate was observed in the group fed with 1.5% garlic peel. Several studies have been reported that, the survival of infected fish is increased after treatment with various immunostimulants[7], vaccines[32] and probiotics[33]. Feeding carp with chitosan and levamisole reduced the mortality of common carp after challenge with *A. hydrophila*[34]. A similar result was reported after feeding large yellow croaker with glucan and challenging with *Vibrio harveyi*[35]. Citarasu *et al*[36] developed an artemia-enriched herbal diet for *penaeus monodon* with a combination of five herbs, which significantly increased the growth and survival during stress conditions. Several herbs were tested for their growth promoting activities in aquatic animals[5, 26]. It is evident from the present study that garlic peel (*A. sativum*) could enhance fish immunity after incorporation in feed, even at a lower dose of 5 g/kg of feed. The present results suggest that inclusion of garlic peel in the diet would improve the non-specific immunity of fish and prevent bacterial infections in culture systems. This is a basic study provides a new perspective for the use of garlic peel waste as a dietary supplement added to fish food to enhance the disease resistance of fish and prevent from diseases. Further purification of the active compounds and their evaluation may substantially improve the quality as well as their usage in aquaculture as immunomodulators.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Gastesoupe FJ. The use of probiotics in aquaculture. *Aquaculture* 1999; **180**: 147-65.

[2] Jian JC, Wu ZH. Influences of traditional Chinese medicine on non-specific immunity of Jian Carp (*Cyprinus carpio* var. Jian). *Fish Shellfish Immunol* 2004; **16**: 185-91.

[3] Ardo L, Yin G, Xu P, Varadi L, Szigeti G, Jeney Z, et al. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Niletilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture* 2008; **275**: 26-33.

[4] Raa JG, Rorstad G, Engstad R, Roberston B. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. In: Shariff M, Subasinghe RP, Arthur JR. *Diseases in Asian Aquaculture 1992*. Phillippines, Manila: Asian Fisheries Socicty, Fish Health Section; 1990, p. 39-50.

[5] Logambal SM, Venkatalakshmi S, Michael RD. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Hydrobiologia* 2000; **430**: 113-20.

[6] Magnadottir B. Innate immunity of fish (overview). *Fish Shellfish Immunol* 2006; **20**: 137-51.

[7] Sakai M. Current research status of fish immunostimulants. *Aquaculuret* 1999; **172**: 63-92.

[8] Anderson DP, Siwicki AK. Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection or immersion. *Progressive Fish Culturist* 1994; **56**: 258-61

[9] Goetz FW, Iliev DB, McCauley LAR, Liarte CQ, Tort LB, Planas JV, MacKenzie S. Analysis of genes isolated from lipopolysaccharide stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages. *Mol Immunol* 2004; **41**: 1199-210.

[10] Cao LZ, Lin ZB. Regulatory effect of *Ganoderma lucidum* polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells *in vitro*. *Acta Pharmacol Sin* 2003; **24**(4): 312-26.

[11] Lin ZB, Zhang HN. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. *Acta*

Pharmacol Sin 2004; **25**(11): 1387–95.

[12] Block E. The organ sulfur chemistry of the genus Allium implications for the organic chemistry of sulfur. *Angewandte Chemie International Edition* 1992; **31**: 1135–78.

[13] Sumiyoshi H. New pharmacological active of garlic and its constituent (review).*Folia Pharmacologica Japonica* 1997; **110**: 93–7.

[14] Kumar M, Berwal JS. Sensitivity of food pathogens to garlic (*Allium sativum* L.). *J Appl Microbiol* 1998; **84**: 213–5.

[15] Suetsuna K. Isolation and characterization of angiotensin I–converting enzyme inhibitor dipeptides derived from *Allium sativum* (garlic). *J Nutr Biochem* 1998; **9**: 415–9.

[16] Wang BH, Zuzel KA, Rahman K, Billington D. Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. *Toxicology* 1998; **126**: 213–22.

[17] Fromthing RA, Bulmer GS. *In vitro* effect of aqueous extract of garlic *(Allium–sativum)* on the growth and viability of *Cryptococcus neoformans*. *Mycologia* 1978; **70**: 397–405.

[18] Bordia A, Bansal HC, Arora SK, Singh SV. Effect of essentials oils of garlic and onion on ailmentary hyperlipemia. *Atherosclerosis* 1975; **21**: 15–9.

[19] Kyo E, Uda N, Suzuki A, Kakimoto M, Ushijima M, Kasuga S, Itakura Y. Immunomodulation and antitumor activities of aged garlic extract. *Phytomedicine* 1998; **5**: 259–67

[20] Sahu S, Das BK, Mishra BK, Pradhan J, Sarangi N. Effect of *Allium sativum* on the immunity and survival of Labeo rohita infected with *Aeromonas hydrophila*. *J Appl Ichthyol* 2007; **23**: 80–6.

[21] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; **193**: 256.

[22] Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971; **31**: 87–96.

[23] Hendricks LJ. Erythrocytes counts and haemoglobin determinations for the two species of sucker, genus Catostomus from Colorado. *Copeia* 1952; **4**: 265–6.

[24] Shaw AF. A direct method for counting the leucocytes, thrombocytes and erythrocytes of birds blood. *J Pathol Bacteriol* 1930; **33**: 833–5.

[25] Ortuno J, Cuesta A, Rodriguez A, Esteban MA, Meseguer J. Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.). *Vet Immunol Immunopathol* 2002; **85**: 41–50.

[26] Harikrishnan R, Nisha Rani M, Balasundaram C. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. *Aquaculture* 2003; **221**: 41–50.

[27] Iranloye BO. Effect of chronic garlic feeding on some haematological parameters. *Afr J Biomed Res* 2002; **5**: 81–2.

[28] Choudhury D, Pal AK, Sahu NP, Kumar S, Das SS, Mukherjee SC. Dietary yeast RNA supplementation reduces mortality by *Aeromonas hydrophila* in rohu (*Labeo rohita* L.) juveniles. *Fish Shellfish Immunol* 2005; **19**: 281–91.

[29] Duncan PL, Klesius PH. Dietary immunostimulants enhance nonspecific immune responses in channel catfish but not resistance to *Edwardsiella ictaluri*. *J Aquat Anim Health* 1996; **8**: 241–8.

[30] Siwicki AK. Immunostimulating influence of levamisole on non–specific immunity in carp *(Cyprinus carpio)*. *Dev Comp Immunol* 1989; **13**: 87–91.

[31] Wiegertjes GF, Stet RJM, Parmentier HK, Vas Muiswinkel WB. Immunogenetics of disaease resistance in fish: a comparable approach. *Dev Comp Immuno*l 1996; **20**: 357–64.

[32] Bakopoulos V, Volpatti D, Gusmani L, Galeotti M, Adams A, Dimitriadis GJ. Vaccination trials of sea bass, *Dicentrarchus labrax* (L.) against *Photobacterium damsela* subsp. *piscicida* using novel vaccine mixtures. *J Fish Dis* 2003; **26**(2): 77.

[33] Brunt J, Newaj–Fizul A, Austin B. The development of probiotics for the control of multiple bacterial diseases of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis* 2007; **30**(10): 573–9.

[34] Gopalakkanan A, Arul V. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture* 2006; **225**: 179–87.

[35] Ai Q, Mai K, Zhang L, Tan B, ZhangW, Xu W, et al. Effects of dietary β–1, 3–glucan on innate immune response of large yellow croaker, *Pseudosciena crocea*. *Fish Shellfish Immunol* 2007; **22**: 394–402.

[36] Citarasu T, Babu MM, Raja Jeya Sekar R, Marian MP. Developing *Artemia* enriched herbal diet for producing quality larvae in *Penaeus mondon* Fabricius. *Asian Fish Sci* 2002; **15**: 21–32.