Labrador et al., *Cogent Food & Agriculture* (2016), 2: 1210066
http://dx.doi.org/10.1080/23311932.2016.1210066

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ANIMAL HUSBANDRY & VETERINARY SCIENCE | RESEARCH ARTICLE

Effect of garlic powder-supplemented diets on the growth and survival of Pacific white leg shrimp (*Litopenaeus vannamei*)

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Received: 31 December 2015
Accepted: 01 July 2016
First Published: 07 July 2016

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Reviewing editor:
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Abstract: The effect of different concentrations of garlic (*Allium sativum*) powder supplement in fish diet on growth and survival of Pacific white leg shrimp, *Litopenaeus vannamei* fry was investigated. Shrimp were separated into four experimental groups of 0% (Controls), 2, 4 and 6% concentrations of garlic powder. Garlic supplemented diet did not have any significant effect on weight gain (%), final weight (g) and survival rate (%) of *L. vannamei* when compared to shrimp in the Control diet. However, feed conversion ratio was significantly ($p < 0.05$) higher in the Control group (1.94 ± 0.03) than those of the Treated groups. Survival rates of the shrimp fed the diets containing garlic powder were way higher (96–99%) compared to shrimp in the Control diet (91%). It was also observed that the shrimp fed the diet containing 6% garlic powder obtained the highest weight gain (%) of 201.33 ± 0.30% among the other groups. The result of the present study established the potential use of garlic powder as additive in the shrimp diet.

Subjects: Agriculture; Agriculture and Food; Fisheries Science; Food Additives & Ingredients; Nutrition; Zoology

Keywords: *Litopenaeus vannamei*; garlic-supplementation; growth performance;

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Recamar C. Guiñares completed his degree in Fisheries major in Food Processing at the Department of Fish Processing and Marine Biology of the College of Fisheries, MSU-GSC. He authored a scientific paper that involves fish products and by-products analysis and preservation techniques. Gaily Jubie S. Hontiveros is presently working as a faculty member in the Department of Fish Processing and Marine Biology of the College of Fisheries, MSU-GSC. She has completed her BS in Fish Processing Technology and MS in Aquaculture from University of the Philippines in the Visayas.

PUBLIC INTEREST STATEMENT

Fish is a highly nutritive and rich source of animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide an artificial feed, by which fish grow rapidly and attain maximum weight in shortest possible time. One approach is to include new substances into fish diets to improve feed conversion efficiency or elevate general conditions for fish growth and maintenance. The functional use of garlic extracts has been established in both human and animal studies. However, application of which are mostly concentrated on fin fishes. Thus, effort should be made to apply garlic as additive into shrimp diet. To our knowledge, this is the first study in the Philippines that evaluates the effect of garlic on Pacific white leg shrimp.

1. Introduction

The search for new feed additives is still a very important point for aquaculture researchers (Cho & Lee, 2012). To this effect, researches have directed their attentions towards the functional additives that can be possibly extracted from plant sources. The use of herbal plants and their extracts has been previously investigated as part of the ongoing effort to develop safe dietary additives. Plant extracts have been reported to favor various activities like antistress, growth promotion, appetite stimulation, enhancement of tonicity and immunostimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish and shrimp aquaculture due to active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils (Chakraborty & Hancz, 2011; Citarasu, 2010). Several plant extracts are also reported to stimulate appetite and promote weight gain when they are administered to cultured fish (Harikrishnan, Balasundaram, & Heo, 2011; Pavaraj, Balasubramanian, Baskaran, & Ramasamy, 2011; Takaoka et al., 2011). Furthermore, plant extracts have been shown to improve digestibility and availability of nutrients resulting in an increase in feed conversion and leading to a higher protein synthesis (Citarasu, 2010; Nya & Austin, 2009; Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014; Talpur, Ikhwanuddin, & Ambok Bolong, 2013).

Garlic has been used as a spice and in traditional medicine. It is rich in calcium, phosphorus, carbohydrates and generally, has a high nutritive value. Garlic also contains many valuable compounds such as iodine salts which have positive effects on the circulatory system, silicates which have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on cholesterolemia, skeletal system and control liver diseases. Garlic also contains many vitamins such as vitamins A, C and B complex as well as linoleic acid (Dra˘gan, Gergen, & Socaciu, 2008). Garlic is a perennial bulb-forming plant that belongs to the genus *Allium* in the family *Liliaceae*. Garlic has been known for centuries as a flavoring agent, traditional medicine, and a functional food to enhance physical and mental health. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder (Shin & Kim, 2004).

In aquaculture, garlic inclusion in fish feeds has been reported to increase growth performance in fish (Metwally, 2009). According to Sheela and Augusti (1992) and Dias, El-Nagar, and El-Hady (2002) garlic has the ability of enhancing catalase activity in serum and lowering the levels of plasma glucose in fish. The non-specific defense system of *Oreochromis niloticus* has been improved by the inclusion of garlic in fish feed (Aly & Mohamed, 2010; Dias et al., 2002). Shalaby, Khattab, and Abdel-Rahman (2006) showed that food intake, specific growth rate and final weight of Nile tilapia (*O. niloticus*) increased when garlic was incorporated in the diet. Several studies were also conducted to evaluate the effects of supplemental garlic on the performance of farmed fish including African catfish (*Clarias gariepinus*) (Agbebe, Ogunmuyiwa, & Herber, 2013; Nwabueze, 2012), rainbow trout (*Oncorhynchus mykiss*) (Breyer, Getchell, Cornwell, & Wooster, 2015; Gabor, Sara, Bentea, Creta, & Baciu, 2012), Nile tilapia (Aly & Mohamed, 2010) and sword fish (*Xiphias gladius*) (Kalyankar, Gupta, Bansal, Sabhlok, & Singh, 2013), *Macrobrachium rosenbergii* (Poongodi, Saravanabhavan, Muralisankar, & Radhakrishnan, 2012), fingerling and juvenile sterlet sturgeon (*Acipenser ruthenus*) (Lee, Ra, Song, Sung, & Kim, 2012; Lee et al., 2014), and *Tilapia zilli* (Jegede, 2012).

As far as the authors know, until now, no trial has been conducted to study the effect of dietary garlic powder (GP) on growth of Pacific white leg shrimp. This study was therefore designed to investigate the effect of varied inclusion levels of dietary GP on its growth performance and survival.

2. Materials and methods

2.1. Garlic powder

Fresh whole garlic, *Allium sativum* were purchased in sufficient quantities from the Local Public Market of General Santos City, Philippines, sun dried for 2 weeks and powdered at required quantities before feed preparation.

2.2. Experimental diets

Experimental diets were prepared by supplementing a basal formulated diet with different levels (0% (Control), 2, 4 and 6%) of GP (Table 1). The ingredients were blended thoroughly in a mixer and pelleted using a pellet-maker equipped with a 3.0 mm die. The pelleted diets were mechanically dried and stored in plastic bags at 4°C until further use.

2.3. Shrimp culture and feeding trial

A total of 320 *L. vannamei* juvenile (2.29 ± 0.35 g) obtained from Charoen Pokphand Hatchery (Barangay Tinoto, Sarangani Province) were acclimatized to the experimental conditions for 30 min. Thereafter, shrimp were randomly allocated to twelve 70 l cylindrical tanks (25 shrimps per tank; four treatments and three replicates). Water temperature, dissolved oxygen content, salinity, ammonia, nitrite, and pH were monitored daily. Each tank was aerated continuously through an air stone connected to a central air compressor. During the rearing experiment (60 days), shrimp were handfed to apparent satiation four times a day (06.00, 09.00, 12.00, and 18.00 h). Uneaten feed was collected 1 h after feeding and dried at 60°C (Hoseinifar, Khalili, Khoshbavar Rostami, & Esteban, 2013). The whole duration of the experiment was conducted in an indoor fish rearing facility at the College of Fisheries, Laboratory and Research Station (CFLRS- Mindanao State University, General Santos City) during the month of January to March 2015.

2.4. Determination of growth performance and survival rate

The growth performance parameters including weight gain (%), average body weight (g) expressed as final weight, survival rate (%), and feed conversion ratio (FCR) were calculated according to the following formulae:

$$\text{Weight gain } (\%) = W_2 - W_1$$

where W_1 is the initial weight (g), W_2 is the final weight (g),

Table 1. Formulation (%) and proximate composition of the experimental diets

	Control	2%	4%	6%
Ingredients:				
Fish meal[a]	44	44	44	44
Soy bean meal	27	27	27	27
Copra meal	12	12	12	12
Cassava meal	6	6	6	6
Corn flour	7	5	3	1
Garlic powder	0	2	4	6
Vegetable oil	3	3	3	3
Vit- min premix[b]	1	1	1	1
Proximate analysis (% DM):				
DM	89.64	89.5	90.01	89.50
Crude protein	21	21.8	23.1	24.2
Crude fat	10.24	10.20	10.28	10.21
Crude fiber	3.3	3.27	3.13	3.3
Ash	3.45	3.50	3.40	3.60

[a]Fish meal (2%); squid meal (15%); corn starch (2%); vitamin and mineral mix (5%).

[b]Vitamins and mineral mixes: Vitamin A (10,000,000 IU), Vitamin D3 (3,000,000 IU), Vitamin E (10,000 IU), Vitamin B1 (400 mg), Vitamin B2 (1,200 mg), Vitamin B6 (1,200 mg), coated Vitamin C (25,000 mg), Folic acid (600 mg), Niacin (6,000 mg), Calcium pantothenate (10,000 mg), Biotin (20,000 mcg), Choline Chloride (10,000 mg), Iron (12,000 mg), Copper (1,200 mg), Iodine (400 mg), Manganese (5,000 mg), Zinc (6,000 mg), Cobalt (20 mg), Selenium (20 mg), Carrier q.s ad to make 1 kg (Source: Progressive Laboratories, 149 Dangay St. Project 7, Quezon City, Manila, Philippines).

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Table 2. Water-quality parameters recorded during the experiment with Pacific white leg shrimp`

Treatment[a]	Salinity		Temperature (°C)		pH		Dissolve oxygen (mg L⁻¹)		Ammonia (mg L⁻¹)		Nitrite (mg L⁻¹)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	24.33 ± 0.58	0.58	31.00 ± 0.00	0	7.30 ± 0.00	0	8.17 ± 0.76	0.76	ND[b]	–	0.30 ± 0.00	0
2% GP	25.00 ± 0.00	0	30.33 ± 0.58	0.58	7.30 ± 0.00	0	7.67 ± 0.29	0.29	ND	–	0.30 ± 0.00	0
4% GP	25.00 ± 0.00	0	30.33 ± 0.58	0.58	7.50 ± 0.35	0.35	6.92 ± 1.01	1.01	0.07 ± 0.12	0.12	0.37 ± 0.12	0.12
6% GP	25.00 ± 0.00	0	31.00 ± 0.00	0	7.40 ± 0.17	0.17	7.17 ± 0.58	0.58	0.07 ± 0.12	0.12	0.30 ± 0.00	0

Notes: Data are expressed as Mean ± SD.

SEM: Standard error of the mean.

[a]Treatments consisting varying levels of garlic powder (GP).

[b]ND–Not Detected.

$$\text{Average body weight (g)} = \frac{\text{Total weight harvested}}{\text{Total number harvested}}$$

$$\text{FCR} = \frac{\text{FO}}{\text{WG}}$$

where FO is the feed provided (g) and WG is the weight gain (g),

$$\text{Survival rate (\%)} = \frac{N_f}{N_0} \times 100$$

where N_0 is the initial number of shrimp and N_f is the final number of shrimp.

2.5. Analytical analysis

The chemical composition of the formulated diets (dry matter) was analyzed following the standard methods of AOAC (1990): moisture content (procedure 930.15); ash content (procedure 942.05); protein content (procedure 954.01); and fat content (procedure 920.39). Water quality was monitored by analyzing for pH, temperature, dissolved oxygen (DO), salinity, ammonia, and nitrite following standard methods (APHA, 1998).

2.6. Statistical analyses

Average values of three determinations were evaluated and the statistical significant differences among treatment mean values were analyzed by Analysis of Variance (ANOVA) of the IBM SPSS version 20 for windows, following Duncan Multiple Range Test (DMRT) set at 5% grade of significance to identify what mean values were different. Regression analysis was determined using Microsoft Excel 2010.

3. Results

The water quality parameters recorded in the different treatment tanks during the experiment are summarized in Table 2. Statistical analyses revealed that water quality parameters did not vary significantly ($p > 0.05$) with the dietary inclusion of *A. sativum* powder at increasing levels. However, it was found to be at optimum level for growth and survival of *L. vannamei.*

The growth performance parameters of Pacific white leg shrimp fry fed experimental diets containing different levels of GP are presented in Table 3. At the end of the experiment (60 days), no significant differences were observed in the final weights, WG, and SR of Pacific white leg shrimp fry fed the GP-supplemented diets and the control diet ($p > 0.05$; Table 3). The result of the final weights further indicates that 6% GP supplementation gained the highest weight (4.47 ± 0.30). Survival rate of the GP supplemented groups, on the other hand, showed highest result when compared to the Control, having 4% GP supplementation the highest SR (99 ± 2.31%).

Table 3. Growth performance parameters and survival rate of Pacific white leg shrimp fry fed diets supplemented with varying levels of garlic powder (GP) for 60 days (Mean values with their standard errors)

	Control		2%		4%		6%	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Initial weight (g)	2.51	0.02	2.47	0.03	2.47	0.02	2.46	0.02
Final weight (g)*	3.92	0.24	4.54	0.49	4.33	0.18	4.47	0.3
Weight gain (%)§	140.67	0.24	207.33	0.49	186	0.18	201.33	0.3
Survival rate(%)†	91	6.11	96	0	99	2.31	96	0
FCRφ	1.94[a]	0.03	1.29[b]	0.24	1.42[b]	0.29	1.27b	0.16

Note: FCR, feed conversion ratio.

*$\alpha = 0.05$; *p*-value = 0.16.

§$\alpha = 0.05$; *p*-value = 0.15.

†$\alpha = 0.05$; *p*-value = 0.85.

φ$\alpha = 0.05$; *p*-value = 0.012.

[a, b]Mean values within a row with unlike superscript letters were significantly different ($p < 0.05$).

Calculation of feed conversion ratio at the end of the experiment revealed that non- supplementation of dietary GP (Control) significantly affect the feed conversion ratio of *L. vannamei* fry ($p < 0.05$; Table 3). *L. vannamei* fry fed the Control diet exhibited the highest FCR (1.94 ± 0.03).

4. Discussion

As of the moment, to the best of our knowledge, this is the first study to investigate the effects of dietary GP supplementation on the growth performance and survival of Pacific white leg shrimp fry. The results of the present study showed that 2, 4 or 6% GP supplementation had no significant effects on the growth performance of *L. vannamei* fry. These results are not in accordance with those of previous studies in sea bass (*Dicentrarcus labrax*) (Saleh, Michael, & Toutou, 2015), Nile tilapia (*O. niloticus*) (Dias et al., 2002; Metwally, 2009; Shalaby et al., 2006), *Channa orientalis* (Charjan & Kulkarni, 2013), *C. gariepinus* (Nwabueze, 2012), *T. zillii* (Jegede, 2012), juvenile sterlet sturgeon (*A. ruthenus*) (Lee et al., 2012), fingerling sterlet sturgeon (*A. ruthenus*) (Lee et al., 2014), ecotype cichlid (Megbowon et al., 2013) and orange—potted grouper (*Epinephelus coioides*) (Guo et al., 2012). Aly and Mohamed (2010) also reported that dietary GP supplementation had no significant effects on the growth performance of Nile tilapia after feeding with 10 and 20 g kg^{-1} GP-supplemented diet for 1–2 months. The differences observed in the results of these studies might be due to the different methods of prebiotic administration, dosage levels, different intestinal morphology, gut microbiota and life stages (Hoseinifar, Zare, & Merrifield, 2010 as cited by Hoseinifar, Soleimani, and Ringø (2014)). After the 60 days feeding trial, shrimp fed GP supplemented diet (6% GP) exhibited lowest FCR value (1.27 ± 0.16). Accordingly, the closer the value of FCR to 1, the better is the feed utilization. The FCR values are incomparable with the FCR values of white leg shrimp fed marine micro-algae as feed protein source (Kiron, Phromkunthong, Huntley, Archibald, & De Scheemaker, 2011).

The mean water quality parameters recorded in the different treatment tanks during the two experiments did not vary significantly ($p > 0.05$) with the inclusion of *A. sativum* in diets at increasing levels. However, these values were within the optimum range for the growth of *L. vannamei*. According to Chiu (1988) the optimal range of salinity of Pacific white leg shrimp ranges from 20 to 25 ppt. Liu and Manacebo (1983) also mentioned that the best growth can be achieved from salinity of 22 ppt. In terms of temperature, the optimal level reported ranges from 25 to 30°C (Boyd, 1998). Ponce-Palafox, Martinez-Palacios, and Ross (1997) added that the best growth of pacific white leg shrimp ranges between 20–30°C. Dissolved oxygen and pH optimal level for shrimp culture must be 4.5–6 mg/L and 7.5 to 8.5 ppm respectively. In terms of total ammonia and nitrite, concentration should not be more than 1 mg/L and 0.1 g/L, respectively. Generally, water parameter during the culture period was favorable for the growth of shrimp. Salinity, pH, and ammonia were within the optimum range, while dissolved oxygen was more than the levels recommended for shrimp.

Dietary GP supplementation had increased the survival rate of *L. vannamei* fry compared with the Control. Similarly, improved survival of sea bass (*Dicentrarcus labrax*) (Saleh et al., 2015) has been observed upon GP supplementation. Lower survival rate in the Control group may be due to cannibalism. This usually occurs in some stages of shrimp culture where stronger individuals attacks and devour the weak ones and usually targeting the appendages (Johnson, 1995) and thus contributes to the survival reduction. In this study, survival rate obtained from shrimp fed with GP supplemented diets yield the highest SR (96–99%). This is way higher than the Control (91% survival rate). This indicates that incorporation of GP in shrimp diet had increased the survival of the cultured species. The improved survival rate of the Treatment groups is attributed from the bioactive elements of garlic including sulfur containing compounds such as allin, diallysulphides and allicin (Amagase & Milner, 1993), but further investigations are needed to clarify the underlying mechanisms.

5. Conclusion

In conclusion, dietary GP supplementation had no significant effects on the growth performance, survival rate and water quality parameters of Pacific white leg shrimp fry, but significantly affects the FCR. The positive results obtained encourage conducting further research on the administration of GP on Pacific white leg shrimp or other economically important species but with longer duration of culture period. Determination of the mechanisms of action and optimal inclusion levels is a topic recommended for further research.

Acknowledgement

The authors are grateful for the kind help extended by the Faculty and Staff of the College of Fisheries, Mindanao State University, General Santos City, Philippines, in the absence of their valuable comments and suggestions, the present study would not be possible and successful.

Funding

The authors received no direct funding for this research.

Competing Interests

The authors declare no competing interest.

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Citation information

Cite this article as: Effect of garlic powder-supplemented diets on the growth and survival of Pacific white leg shrimp (*Litopenaeus vannamei*), Jack Robert P. Labrador, Recamar C. Guiñares & Gaily Jubie S. Hontiveros, *Cogent Food & Agriculture* (2016), 2: 1210066.

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