ABAH BIOFLUX
Animal Biology & Animal Husbandry
International Journal of the Bioflux Society

Simultaneous replacement of protein, vitamins and minerals with *Chaetoceros calcitrans* paste in the diet of the black tiger shrimp (*Penaeus monodon*) larvae

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Abstract. A 30-day feeding trial was conducted to evaluate whether or not *Chaetoceros calcitrans* paste could simultaneously replace the protein from squid meal, vitamins and minerals in a formulated dry diet for *Penaeus monodon* larvae. Three experimental diets were prepared: Diet 1, the control diet with no algal paste content; Diet 2 containing the algal paste simultaneously replacing 15% of the protein, 33% of the vitamins and 50% of the minerals; Diet 3 containing the paste simultaneously replacing 30% of the protein, 55% of the vitamins and 100% of the minerals. The specific growth rate (SGR) of shrimp larvae fed with Diets 2 and 3 and those with Diet 1 were statistically similar while significantly higher food conversion efficiency (FCE) was observed in shrimp larvae fed Diet 3 than those with Diet 1 or Diet 2. Survival was significantly enhanced in shrimp larvae fed with Diet 2 over those fed with the control diet (Diet 1) and Diet 3. Shrimp larvae fed with either Diet 2 or 3 were significantly longer than those fed the control diet. These findings on the growth and survival of the *P. monodon* larvae indicated that the diatom paste could simultaneously replace the crude protein content of squid meal by 30%, 55% of the vitamins and 100% of the minerals in the dry diet at least for 30 days.
Key Words: Microalgae, diatom paste, algal paste, vitamin supplement, mineral supplement.

Introduction. There have been few studies on the evaluation of microalgae in the diet of shrimp in aquaculture (Cuzon et al 1981; Hasan & Chakrabarti 2009; Ju et al 2009). Cuzon et al (1981) suggested that the defatted Spirulina meal contains an unknown growth factor that resulted in the promotion of growth of *Penaeus japonicus*. Nakagawa & Gomez-Diaz (1995) fed whole *Spirulina* meal to the giant freshwater shrimp (*Macrobrachium rosenbergii*) and noted significant improvement in growth, survival and feed utilization; they suggested this was probably due to the enhancement of protein assimilation. Enhanced shrimp growth was observed following inclusion of 9% whole microalgae meal of *Thalassiosira weissflogii* or of *Nannochloropsis* in the control diet (Ju et al 2009).

Larvae of shrimps are considered herbivoruos zooplankton initially with tendencies of becoming omnivores with a preference for zooplankton after several days. Natural food including phytoplankton that are readily consumed from zoea 1 until postlarvae (PL) 2 (Evans 1992) and *Artemia nauplii* are commonly used in shrimp larval rearing to postlarval stage. Postlarval shrimp consumes natural food organisms such as copepods and most of the time it feeds on detritus, animal remains, diatoms, cyanobacteria and green algae. The current practice in shrimp production is to enhance the growth of natural food in the ponds and provide artificial feed.

In shrimp hatcheries, growers commonly use a combination of live feeds with prepared high-protein dried feeds (Fegan 1992). Current hatchery practice uses *Artemia* in shrimp larvae culture to satisfy nutritional needs (Wilkenfeld 1992; Dhert et al 1993). Microalgae are another natural food of shrimp commonly offered to the early larval stages (Bailey-Brock & Moss 1992). In culture facilities, algae species are selected for cultivation based on ease of culture, cost of culture and dietary value (Treece & Yates 1990).

Diatoms are beneficial in aquaculture due to their low fiber content (Mann & Pruder 1988) and high concentration of polyunsaturated fatty acids (Phillips 1984). Penaeid shrimps consume diatoms in their natural environment (Gleason & Wellington 1988) and in aquaculture ponds (Bombeo-Tuburan et al 1993; Moss & Pruder 1995). The diatoms are being processed in some laboratory including that of the UP Visayas as microalgal pastes. The advantages of algal pastes include stable nutritional composition, reduction in both the labor and expense of maintaining a large live algal production facility, ability to harvest excess algal biomass for use at a later date, maintenance of the nutritional profile without requiring nutrient additions, ease of achieving high concentrations, and ease of suspension in the water column with minimum circulation (Zelaya et al 2007).

Live food is expensive and variable in production and in nutritional quality (Kuban et al 1985; Leger et al 1986); attempts have been made to develop dry diets to replace or supplement live feeds (Jones et al 1987; Fegan 1992; Jones et al 1993). Dry feeds are typically fortified with vitamins and minerals, and have 10–30% higher protein content than feeds used in grow out (Samocha & Lawrence 1992). Formulated artificial feeds can replace live feed (Jones et al 1987) and algae (Kanazawa 1990), in most cases they are only used as partial (50–70%) replacement (Jones et al 1993). The present study was designed to evaluate the partial replacement of the crude protein of squid meal by *Chaetoceros calcitrans* paste in the dry formulated diet, as well as replacing part of the vitamins and minerals, on the postlarval shrimp survival, growth and feed conversion efficiency (FCE).

Material and Method

Macroalgae culture. The algal species used in the study was a diatom, *C. calcitrans*. The stock cultures were maintained using the Tongkang Marine Research Laboratory (TMRL) media under axenic conditions (Coutteau & Sorgeloos 1992). The algae was cultured in batches and maintained at 25°C and constant illumination. Mild aeration using diffuser stones were provided. Algae were harvested during the log phase of growth and cells were counted using a haemocytometer under the microscope. TMRL media was also used for mass culture indoor while urea, ammonium phosphate and ammonium sulphate were used for mass production outdoor. The temperature for *C. calcitrans* culture was maintained at 25-30°C, salinity at 20-35 ppt and light intensity at 15-25 Klx.

Preparation of C. calcitrans paste. Live *C. calcitrans* were harvested using the electrolytic method of harvest currently being evaluated by the U. P. Visayas-Department of Science and Technology National Aquafeeds Program. The microalgae were concentrated and filtered through a cloth (less than 2 µm mesh size) producing a paste (Figure 1). The algal paste was subjected to proximate analysis (AOAC 1996).

Composition of experimental diets. The ingredients used and the feed preparation were as described by Millamena et al (2002). *C. calcitrans* paste was incorporated replacing the protein equivalent of 15% or 30% of that contributed by squid meal (Table 1). This meant that 15% or 30% of the crude protein content of the squid meal was replaced by an equivalent crude protein content of the microalgae. Three diets were prepared, namely, a control diet (Diet 1) with no microalgal paste content; Diet 2 containing 90 g kg^{-1} algal paste replacing 15% of protein of squid meal, 33% of the vitamins and 50% of the mineral

premix; and Diet 3 containing 180 g kg^{-1} algal paste replacing 30% of protein, 55% of vitamins and 100% of mineral premix.

Figure 1. The experimental set up of the feeding trial (photo above) and the C*haetoceros calcitrans* paste (photo below) to evaluate it in a simultaneous protein, vitamin and mineral replacement in the diet of *Penaeus monodon* larvae.

During diet preparation, all dry ingredients were mixed together followed by all the liquid ingredients. *C. calcitrans* paste was added before adding the cooked flour; all the ingredients were mixed manually until it formed into dough. The mixture was then passed through a pelletizer 3-4 times to ensure even mixing of the ingredients and oven dried at 60°C for 72 h to a moisture content of about 10% or less. The pellets were then cut and ground into powder of the desired particle size and kept at 4ºC until use.

Table 1

Composition (g kg^{-1} diet) of experimental diets containing different levels of *Chaetoceros calcitrans* paste*1 replacing the protein content of the squid meal

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*
Squid meal	300.0	255.0	210
C. calcitrans paste	-	90.0	180
Acetes sp.	350.0	350.0	350
Bread flour	110.0	110.0	110.0
K-carrageenan	5.0	5.0	5.0
Celufil	21.5	12.0	-
Cod liver oil	80.0	80.0	80.0
Soybean lecithin	25.0	25.0	25.0
Cholesterol	10.0	10.0	10.0
Vitamin mix	60.0	40.0	27.0
B-carotene	2.5	2.5	2.5
Mineral mix	40.0	20.0	-
BHT*2	0.5	0.5	0.5
Total	1000.0	1000.0	1000.0

*1 - The computation was based on squid meal and *Chaetoceros calcitrans* paste containing 40% and 20% crude protein; *2 - Butylated hydroxytoluene.

Experimental larval shrimp and set up. The experiment conducted covered the early life cycle of *Penaeus monodon* from post-larvae (PL) 15 to PL45 stage wherein artificial diets were already acceptable to the larvae. Shrimp larvae were obtained from a local commercial hatchery in Tigbauan, Iloilo Philippines. The larvae were acclimatized, and fed with basal shrimp diet in a 10-ton capacity tank at the Multi-Species Hatchery of the U.P. Visayas for 7 days. A randomly selected sample of shrimps were screened by One-Step Polymerase Chain Reaction (PCR) for the presence of White Spot Syndrome Virus (WSSV) prior to the experiment and found to be free from the virus.

The experiment was conducted using nine 100 L-capacity round fiberglass tanks filled with chlorine-treated seawater placed under a roofed outdoor facility (Figure 1). Water change at 20-30% was done every 2 days to minimize stress to the larval shrimps. Water parameters including salinity (30-36 ppt) and pH (7.5-8) were monitored between 8:00 AM to 9:00 AM daily while temperature (28.5-32.0ºC) and dissolved oxygen (>5 mg/L) were monitored three times a day (8:00-9:00 AM, 12:00-1:00 PM and 2:00-3:00 PM). Total ammonia nitrogen (TAN) (0-0.02) and nitrite (0-0.15 ppm) were measured once a week. Each tank was covered with black plastic sheet all throughout the culture period to maintain the temperature and to minimize strong light going into the tanks. Tanks were provided with moderate aeration and uneaten feeds and feces were siphoned off every day. Feeding was done 3 times daily (8:00 AM, 12:00 PM and 4:00 PM) and were adjusted based on the estimated weight of the shrimps. *Artemia nauplii* were added to the culture at a density range of 0.25-1.0 individuals mL^{-1} day^{-1} every 2 days since it has been established that larval shrimps would not grow in the total absence of live food (Wilkenfeld 1992).

The initial and final biological measurements such as specific growth rate (SGR), efficiency of feed utilization (FCE), survival rate and length were conducted on the day of stocking and at day 30 of the culture period. SGR and FCE were computed using the following formulas:

Specific growth rate (SGR, % day-1) = (lnWf – lnWi) x 100/days of culture

Feed conversion efficiency (FCE, %) = (Wf – Wi) x 100/feed given

Where:

Wi - initial average body weight

Wf - final average body weight.

Statistical analysis. All the data obtained from the trial including SGR, FCE and survival rate were analyzed using the software Statistical Package for the Social Sciences (SPSS) version 16.0. Survival data were cosine transformed before further analysis. These were tested for normality of distribution using Shapiro-wilk test and homogeneity of variance using Levene's test before performing one-way analysis of variance (ANOVA). If there were significant differences at alpha level of 0.05 in the parameters observed, a post-hoc analysis (Tukey's test) was performed to rank the treatment means.

Results and Discussion. The specific growth rate (SGR) of shrimps fed diets with and without *C. calcitrans* paste were statistically similar (Table 2) while there was significantly higher food conversion efficiency in shrimps fed Diet 3 (i.e. algal paste replaced 30% of the protein, 55% of the vitamins and 100% of the minerals). Survival was enhanced in shrimps fed Diet 2 (i.e. algal paste replaced 15% of the protein, 33% of the vitamins and 50% of the minerals) over those fed with the control diet (Diet 1) and Diet 3. These findings on the growth and survival of the *P. monodon* larvae indicated that the *C. calcitran* paste could partially replace the crude protein content of squid meal and at the same time could replace considerable amounts of the vitamins and minerals in the dry formulated diet. Also, shrimps fed with Diet 2 were significantly longer than those fed the control diet, Diet 1 (Figure 2). This is in agreement with the results of Da Silva & Barbosa (2008) wherein the mean total length of *P. monodon* increased as the level of microalgae inclusion in the diet increased.

Table 2

Growth, feed efficiency and % survival of larvae *Penaeus monodon* fed diets with C*haetoceros calcitrans* paste

Parameter	*Diet 1*	*Diet 2*	*Diet 3*
SGR (% day^{-1})	2.76 ± 0.00^{a}	2.76 ± 00^{a}	2.77 ± 00^{a}
FCE	0.85 ± 0.80^{b}	0.74 ± 0.19^{b}	1.36 ± 0.32^{a}
Survival (%)	68.33 ± 8.33^{b}	95.00 ± 2.89^{a}	78.33 ± 10.93^{b}

The diatom *C. calcitrans* is normally used in aquaculture especially in the shrimp culture and it was expected to be well accepted by the experimental shrimps. This expectation was confirmed in the present study. *C. calcitrans* as mono species feed or within mixed diets for larval animals have been shown to be an excellent nutritional package that could be introduced directly or indirectly to the larval animals (Jeffrey et al 1992; Brown et al 1997). It contained 20% crude protein as was measured in the present study (Table 2) which is the minimum percentage required for an ingredient to be considered as a protein supplement. However, it contained so much ash (about 68%) which indicated that it could be a replacement for dietary minerals. It supplements vitamins in shrimp hatcheries (Hemaiswarya et al 2011), particularly essential vitamins that are similar to baker's yeast and liver (Becker 2007). *C. calcitrans* contains high amount of fatty acids primarily 14:0, 16:0, 16:1 and 20:5n3 (Reitan et al 1994) (0-4%) and has high EPA content (Jeffrey et al 1992).

Table 3

Analyzed proximate composition of *Chaetoceros calcitrans* paste

Composition (% Dry matter)	*Chaetoceros calcitrans paste*
Dry matter	7.95 ± 0.26
Ash	67.72 ± 1.84
Crude fat	7.74 ± 0.17
Crude protein	20.03 ± 1.66

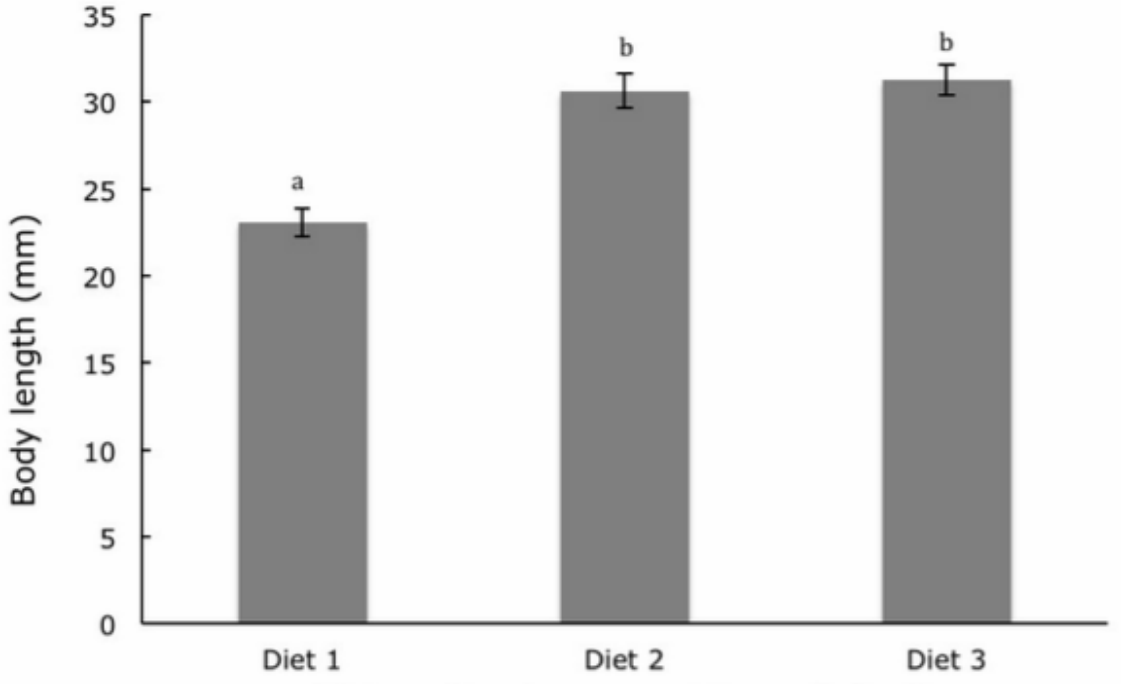

Figure 2. Body length (mm) of *Penaeus monodon* larvae fed with the experimental diets containing *Chaetoceros calcitrans* paste at 0, 90 or 180 g kg^{-1} diet (Diets 1, 2 and 3 respectively) simultaneously replacing protein, vitamins and minerals in the dry diets. Values expressed as Mean ± SEM of 3 replicates per treatment.

Microalgae, in general, contain high quality proteins that are comparable to different vegetable protein in terms of amino acids (Becker 2007). Therefore, microalgae including *C. calcitrans* could replace fishmeal or squid meal as was observed in the present study. Numerous microalgae contain all the essential amino acids (Ortiz et al 2006; Dawczynski et al 2007). Fishmeal contains fish oil in the form of polyunsaturated fatty acids (PUFA) such as omega-3 and omega-6 while microalgae contains eicosapentaenoic acid (EPA), docosahexanoic acid (DHA) and arachidonic acid (ARA) that are found to be an alternative source of fish oil (Miller et al 2005).

The results of the present study was similar with those of Da Silva & Barbosa (2008) in which the microalgae *Hypnea cervicornis* sp. and *Cryptonemia crenulata* at 0%, 13%, 26% and 39% inclusion in the shrimp diet resulted in a significant increase in growth rate as the level of inclusion is increased. Sivakumar et al (2011) have demonstrated that feeding the juvenile shrimp *P. monodo*n with live *Chlorella* sp. resulted in a higher mean total weight and with *Phormidium* sp. in improved survival rate. The improved survival rate of the shrimps when fed *C. calcitrans* in the present study might have stemmed from the effect of the immune-enhancing effect of the microalgae or its antibacterial action or their combination (Seraspe et al 2014).

The improved FCE in shrimps fed diets containing the *C. Calcitrans* paste in the present study might have stemmed from the extracellular polysaccharides of *C. calcitrans* that could serve as a useful binding agent in feed pellet formation (Henry 2013). Although the digestibility of *C. calcitrans* is reported to be lower due to the cellulose in its cell wall, but its overall digestibility of algal carbohydrates has no limitation in using microalgae in feeds (Becker 2004). The findings in the present study were similar to the results of Da Silva & Barbosa (2008) wherein there is an increase in feed conversion ratio (FCR) as the level of microalgal inclusion in the diet of *P. monodon* is increased.

Shrimps fed with Diets 2 and 3 exhibited significantly longer bodies than did those fed with the control diet (Diet 1). This is in agreement with the results of Da Silva & Barbosa (2008) wherein the mean total length of *P. monodon* increased as the level of microalgae inclusion in the diet was increased.

Conclusions. *C. calcitrans* paste could simultaneously replace 30% of the protein from squid meal, 55% of the vitamins and 100% of the minerals in the diet of postlarval *P. monodon*. The replacement resulted in a statistically similar specific growth rate, significantly enhanced feed conversion efficiency, better survival rate and increased body length of shrimps.

Acknowledgements. The authors wish to express their gratitude to the Philippine Department of Science and Technology (DOST) Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) for the funding and the UPV Office of the Research and Extension for (OVCRE) for the publication support.

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Received: 06 January 2015. Accepted: 09 February 2015. Published online: 19 February 2015.
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How to cite this article:
Naorbe M. C., Garibay S. S., Serrano Jr. A. E., 2015 Simultaneous replacement of protein, vitamins and minerals by *Chaetoceros calcitrans* paste in the diet of the black tiger shrimp (*Penaeus monodon*) larvae. ABAH Bioflux 7(1):28-36.