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Effect of feeding frequency on the growth performance of black tiger shrimp (*Penaeus monodon*) "Nanhai NO. 2" in cement ponds culture

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Abstract

This study examined the growth response of black tiger shrimp, *Penaeus monodon*, reared in indoor cement ponds fed at variable daily feeding frequencies. Five hundred healthy shrimp (1.26±0.37 g initial weight) were stocked in 12 cement ponds (5.5×2.5×1.5m). Three ponds in each group were assigned to four different feeding frequency i.e., two, three, four and six times/day designated as FF1, FF2, FF3 and FF4, respectively. Artificial diet (40% fishmeal protein) was used to feed at eight percent of total shrimp body weight per day for a total period of 56 days. The shrimp length and weight were measured fortnightly and the feeding rate was adjusted according to the new weight. At the end of the trial, all shrimp remained healthy and active with the survival rate of 70-85%. The specific growth rate (SGR) were the highest in groups FF3 (4.21±0.53) and FF4 (4.23±1.04), which were significantly higher than those in FF1 (3.82±0.49) and FF2 (4.02±1.03) groups. Whole body proximate composition (protein, ash, moisture and lipid) remained similar in all the treatment groups. It could be concluded that the black tiger shrimp, *P. monodon*, would be grown in indoor cement ponds effectively by feeding four times day^{-1} to achieve significantly better growth.

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Introduction

A dramatic increase in the human population, expected to exceed 9 billion by 2050 with 22% individuals with age under 40 years (Heilig *et al*., 2012), will pose a threat to food security and insufficiency in the future which may be fulfilled by aquaculture (Godfray *et al*., 2010). For successful aquaculture practice shrimp nutrition feed needs to be managed in order to obtain healthy shrimp while keeping the production cost at the minimum. Good feeding management is sought to improve shrimp growth and production with high feed efficiency and minimum impact on water quality (Tacon *et al*., 2013). Feeding frequency, time, ration and dispersal method constitute the regime of feeding management for intensive culture of shrimp (Tacon *et al*., 2013). Therefore, the supply of feed at an optimal feeding rate would ensure better growth rate, economic returns, and improved total production of shrimp (Silva *et al*., 2007).

Knowledge of optimal feeding regimes with efficient feeding frequency and feeding rate with respect to shrimp body weight can avoid overfeeding as well as insufficient feeding that may cause mortality and increase serious growth and health issues for the shrimp and in the surrounding environment (Jiang *et al*., 2013). For example, this may result in high feed conversion ratio with corresponding low growth and inadequate feed supply would increase competition and cannibalism resulting in low total yield and profitability (Bureau et al., 2006). In the study of aquatic animals such as *Litopenaeus vannamei* (Xut *et al*., 2020), *Penaeus subtilis* (Nunes *et al*., 2000), Channel catfish, Ictalurus punctatus (Noeske *et al*., 1985) and black sea trout, *Salmo trutta labrax* (Başçınar *et al*., 2007), it is shown that the feeding behavior of different species and their selection of a certain feed are different. Therefore, it is necessary to conduct in-depth research on the specific feeding behavior of organisms.

The black tiger shrimp (*Penaeus monodon*, Crustacea, Decapoda, Penaeidae) is an economically and globally important marine species, and very important in the aquaculture industries in China (Jiang *et al*.,2018). At present, there are many reports on feeding frequency of *P. monodon*, but the parental source of these experimental taken from the wild is unknown. In 2017, the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (SCSFRI, CAFS) used the method of cross breeding to create a new *P.monodon* variety named "Nanhai NO. 2" with fast growth and high survival rate. The male and female parents of "Nanhai NO. 2" are both cultured *P. monodon*. The "Nanhai NO. 2" *P. monodon* has not been studied for its growth performance in indoor cement ponds. Therefore, the present study was designed to evaluate the optimum daily feeding frequency for maximum growth in indoor cement ponds with respect to feed consumption and body composition.

Materials and Methods

Feed formulation and preparation

Feed ingredients were procured from the local market in Guangzhou. Fishmeal was the major protein source used in the diet and the protein level was maintained at 40%. Soybean meal, and peanut meal were also used as protein source. The formula was designed according to the principle of equal energy and unequal protein. All feed materials were crushed through 80 mesh sieve, mixed and then stirred with water to make 1.00 and 1.50 mm diameter pellet feed, baked in 90 ℃ oven (put some water in the oven) for 2 hours, dried in the air-conditioned room. Feed was stored under dry condition in a freezer until utilized. The dried feed samples were analyzed for percent proximate composition of dry matter, crude fat, crude protein, crude fiber and moisture contents (**Table 1**).

Table 1 Composition and nutrient content of experimental diets

Ingredients（%）	Diet
Fish meal	30.00
Soybean meal	18.00
Peanut meal	10.00
Wheat flour	21.99
Beer yeast	3.00
Shrimp head meals	5.00
Soybean protein concentrate	4.80
Soybean lecithin	1.00
Fish oil	1.00
Soybean oil	0.50
CAscorbic Phosphate ester	0.10
Cholesterol	0.50
Vitamin premix[a]	1.00
Mineral premix [b]	1.00
$Ca(H_2PO_4)_2$	1.00
Methionine	0.1
Carboxymethylcellulose	1.00
Y_2O_3	0.01
Sum	100.00
Proximate composition	
Moisture（%）	8.32
Crude protein（%）	39.65
Crude lipid（%）	5.70
Ash（%）	11.89

Note：1.Vitamin premix ($g \cdot kg^{-1}$):V_A 2.5; V_D 6.25; V_E 75; V_K 2.5; V_{B1} 0.25; V_{B2} 1.0; V_{B3} 5.0; V_{B6} 0.75; V_{B12} 2.5; folic acid 0.25; biotin 2.5; inositol 379; cellulose 500; 2.Mineral premix ($g \cdot kg^{-1}$)：KCl, 90; KI 0.04，NaCl, 40g; $CuSO_4 \cdot 5H_2O$, 3;$ZnSO_4 \cdot 7H_2O$, 4; $CoSO_4 \cdot 7H_2O$, 0.02; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 3; $MgSO_4 \cdot 7H_2O$, 124; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 500; $CaCO_3$, 215.

Experimental design

Penaeus monodon juveniles were selected variety by SCSFRI, CAFS and initially stocked in indoor cement ponds for 20 days adaptation. During this time, juveniles were adapted to the diet offered. Active shrimp juveniles in uniform size (mean body weight 1.26±0.37g; n=6000) were distributed (500 shrimp/cement pond) in four groups of triplicate indoor cement ponds (5.5m×2.5m×1.5m). The shrimp in four diet groups were named (FF1, FF2, FF3 and FF4) which were fed two, three, four- and six-times day^{-1} at 8% BW, respectively for 56 days during May 1st to June 25th, 2019 (**Table 2**).

Table 2 Average initial weight, length and other parameters in each feeding group

Treatments	Number of replicates	Feeding Frequency/day	Stocking per pond	Average initial weight (g)	Average initial length (cm)
FF1	3	2	500	1.24±0.31^a	5.16±0.48^a
FF2	3	3	500	1.28±0.35^a	5.03±0.51^a
FF3	3	4	500	1.25±0.21^a	5.22±0.33^a
FF4	3	6	500	1.26±0.29^a	5.20±0.43^a

Note：Values are mean±SD. Values marked with different alphabetical letters in each experimental group indicate significant differences.

Shrimp counts and individual weights were taken fortnightly and the amount of feed was adjusted according to the new weight. Shrimp growth was ascertain on the basis of weight gain (WG=Average final weight–Average initial weight), Average daily weight gain [ADG=(Average final weight–Average initial weight)/cultured days] and specific growth rate [SGR= [(Ln(Total final weight) – Ln(In Total initial weight)]/cultured days×100].

Proximate composition of shrimp diet and shrimp were carried out using standard methods (AOAC, 1995) including moisture (drying at 60 º C until achieving constant weight), ash (drying at 550 º C for 24 h), crude protein (Foss, 2300) and crude fat (Foss, 2050). From the initial stock 100 individuals were separately euthanized and stored at -20˚C for the entire carcass composition analysis. Similarly, at the end of feeding trial, 20 shrimp from each pond were randomly collected and anesthetized for subsequent final shrimp carcass composition.

Physio-chemical parameters (temperature, salinity, dissolved oxygen and pH) of the experimental site were recorded once in a week at low, mid and high tides which remained within the normal range. Daily feeding schedule for all the treatments groups are presented in (**Table 3**).

Table 3 Feeding frequency and the time of the day for each feeding group

Feeding time	Feeding frequency			
	FF1	FF2	FF3	FF4
6:00				×
7:00	×	×	×	
9:00				×
12:00		×	×	×
15:00				×
17:00	×	×	×	
18:00				×
21:00				×
22:00			×	

Statistical analysis

The triplicate data of each experimental group were presented as total mean standard deviation (SD). One Way Analysis of Variance (ANOVA) was used to assess the differences in growth performance between treatment groups receiving daily feed at different frequencies. For significant differences among treatment groups the Tukey HSD test was applied. The statistical analysis was performed through SPSS ver.18.

Results

Water physio-chemical parameters were within acceptable range and suitable for cage culture of *P. monodon*. The average values of temperature (28.30±0.52°C), pH (8.30±0.25), salinity (29.50±1.30‰) and dissolved oxygen (6.20±0.20 mg/L) were recorded. The shrimps remained healthy and active during the whole experimental period and the survival remained between 70% to 85%. Lower mortality rate in ponds was the indication of healthy environments for culture. Growth such as percent weight gain (WG), average daily weight gain (ADG) and specific growth rate (SGR) were shown in **Table 4**.

Table 4 Weight gain, Average daily weight gain and Specific growth rate

Parameters	Feeding groups	Experimental duration (days)				
		1-14	15-28	29-42	43-56	1-56
Weight gain (g)	FF1	1.18±0.25	3.65±0.51	1.97±0.14	2.49±0.43	9.29±1.28[c]
	FF2	1.36±0.18	3.77±0.62	1.74±0.28	4.01±0.58	10.88±1.68[b]
	FF3	2.55±0.30	3.95±0.14	1.66±0.43	3.83±0.46	11.99±2.46[a]
	FF4	2.61±0.41	3.97±0.28	1.63±0.26	4.01±0.57	12.22±2.84[a]
ADG ($g·day^{-1}$)	FF1	0.08±0.00	0.26±0.06	0.14±0.02	0.18±0.00	0.17±0.05[c]
	FF2	0.10±0.01	0.27±0.04	0.12±0.03	0.29±0.12	0.19±0.01[b]
	FF3	0.18±0.02	0.28±0.02	0.12±0.01	0.27±0.08	0.21±0.06[a]
	FF4	0.19±0.02	0.28±0.05	0.12±0.00	0.29±0.14	0.22±0.04[a]
SGR (%/day)	FF1	4.78±0.81	6.57±1.06	2.01±0.28	1.93±0.14	3.82±0.49[c]
	FF2	5.17±1.13	6.34±1.58	1.72±0.32	2.86±0.51	4.02±1.03[b]
	FF3	7.94±1.54	5.09±0.46	1.39±0.16	2.44±0.63	4.21±0.53[a]
	FF4	8.02±2.01	5.04±1.27	1.35±0.25	2.52±0.48	4.23±1.04[a]

Note : Values are mean±SD. Values marked with different alphabetical letters in each experimental group indicate significant differences.

At the end of the experiment, WG values in group FF1 (9.29±1.28g) and FF2 (10.88±1.68g) were lower compared to FF3 (11.99±2.46g) and FF4 (12.22±2.84g). There were no significant differences (P>0.05) in weight gain in treatment groups FF3 and FF4. However, they were significantly different from FF1 and FF2 groups. The ADG values increased gradually in all groups and the lowest final ADG was recorded in treatment FF1 (0.17±0.05), followed by FF2 (0.19±0.01). The highest ADG was recorded in FF3 (0.21±0.06) and FF4 (0.22±0.04). The high ADG values recorded in FF3 and FF4 were significantly different (P<0.05) from that of FF1 and FF2. Similarly, the specific growth rate (SGR; %/day) values were lower in FF1 (3.82±0.49) and FF2 (4.02±1.03) groups. The significantly higher (P<0.05) SGR values were detected in FF3 (4.21±0.53) and FF4 groups (4.23±1.04). The initial and final proximate compositions of shrimp body shown in **Table 5**.

Table 5 Initial and the final whole body proximate composition

Parameters	Initial values	Final proximate composition of the shrimps in the experimental feeding groups			
		FF1	FF2	FF3	FF4
Moisture	74.24±7.24	76.14±6.32	77.16±7.02	76.82±5.11	76.05±6.88
Protein	70.35±6.84	69.32±5.65	68.64±5.19	69.57±6.39	70.15±4.35
Lipids	4.95±1.57	5.18±1.12	5.61±0.67	5.34±1.23	5.07±1.36
Ash	17.14±3.51	16.03±2.36	16.71±2.67	17.04±1.97	16.19±2.71

The body composition of *P. monodon*, was not influenced by the feeding frequency in indoor cement ponds. Shrimp body composition, moisture, protein, lipid and ash contents were not significantly different (P>0.05) from their respective initial values.

Discussion

The present study has optimized daily feeding frequency for black tiger shrimp "Nanhai NO. 2" *P. monodon* in indoor cement ponds for which no previous information is available as to evaluate this artificial breeding species as a candidate for aquaculture. We observed that hand feeding at reasonable rates (four times a day) would curtail feed losses and negative environmental impact. The water quality parameters (temperature, dissolved oxygen, pH and ammonia concentration) in and around ponds remain within the acceptable range for shrimp culture. Similarly, the shrimp remained healthy throughout the trial period with very low mortality and no negative competition for feed and/or cannibalism was detected.

For the growing aquaculture industry feed and feeding frequency for different shrimp and fish species is a challenge for feed being the main regulator of the total production cost (Silva *et al*., 2007). For outdoor ponds, shrimp feed share 40 to 55% of the total production cost (Ullman *et al*., 2019). Therefore, feeding frequencies and feeding ratios are of key importance in aquaculture, which not only determines the feed requirement for maximum growth and shrimp survival, but also establishes the production cost for a successful aquaculture practice (Aydin *et al*., 2011; Kaiser *et al*., 2011).

It is generally accepted that cultured organisms' size, age and culture conditions, including the amount of feed provided, quality of feed and water temperature, defines the feeding frequency for cultured organisms in culture to have a maximum growth response (Puvanendran *et al*., 2003). Small size shrimp have higher energy demands and they must be fed frequently (Velasco *et al*., 1999). Similarly, feed consumption and growth of larger shrimp is dependent on feed supply and to a certain limit of daily feeding frequency (Bardera *et al*., 2018). In the present study by increasing feeding frequency in black tiger shrimp "Nanhai NO. 2" *P. monodon* increases the growth up to four times a day (FF3) and further increment in feed supply (FF4; six times/day) had no positive effect on growth and only the feed consumption was increased. Shrimp in group FF1 and FF2, with lower feeding frequency, had a significantly lower growth performance. This is also evident for other shrimp and fish species , for instance, *L. vannamei* juvenile of 1.52±0.37 g daily revealed better results at six times day^{-1} reared in biofloc-based zero-exchange intensive systems (Xu *et al*., 2020) ; *P. monodon* juvenile responded well at high frequency (five times day^{-1}.)(Arnold *et al*., 2016); Flounder (*Paralichthys olivaceus*) fed two or three times/day showed better growth compared to fish fed once in two days (Lee *et al*., 2000); According to Shelbourn *et al*. (1973) Sockeye salmon (*Oncorhyncus nerka*) had better growth when fed continuously for 15 h/day. Researchers used hand feeding method at a slow rate that kept the fish satisfied with less stress, which yields uniform size healthy fish (Başçınar *et al*., 2001). Frequent feeding also provides a chance to subordinate fish in a group to fetch food as it is known that healthier and more active, dominant fish consume most of the feed and subsidiary fish eat nothing or a little (Jobling and Baardvik., 2010; Damsgård *et al*., 1997). It may be concluded from the present finding that black tiger shrimp "Nanhai NO. 2" *P. monodon*, was assessed for the first time and that it may be grown in indoor cement ponds effectively by feeding four times day^{-1} at 8% BW (40% protein diet) which would yield significantly better results under given rearing conditions.

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