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RESEARCH PAPER

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Effects of Carbohydrate and Artificial Substrate Integration on Growth Performance of Mudskipper (*Pseudapocryptes elongatus*)

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Abstract

The carbon and artificial substrate integration was applied in mudskipper (*Pseudapocryptes elongatus*) culture to evaluate its effect on growth and enzyme activity. Wild mudskipper fries (6.9–7.3 cm; 3.88–4.12 g) were obtained in the coastal area of the Mekong delta. Fish were reared in 1 m^3 fiber glass tanks containing 600 L of 15 ‰ seawater for 60 days in outdoor conditions. Mud was added to the tank bottom around 15 cm to simulate the culture condition as would be found in an earthen pond. Molasses (38% Carbon) was added daily to the culture water based on TAN level to balance C/N to 10. The results showed that the survival was in the range of 80.6–85%, and no significance in term of survival was found when comparing treatments. The individual weight of fish was 11.93–14.11 g/ind in range, fish in the culture where carbon and both carbon and substrate addition had significantly better growth and productivity than fish in the culture where molasses and substrates were not applied. The results of this study demonstrate that the growth performance and production of mudskipper significantly increased when the biofloc and substrate integration was applied.

Introduction

Mudskipper (*Pseudapocryptes elongatus,* Cuvier, 1816), a brackish water fish species, is found in the canals or creeks of South East Asia (Bucholtz *et al*., 2009). This fish has a soft texture with a delicious and distinctive flavor (Fani *et al*., 2017), so the demand for this fish is always high, and it is one of the most economically valuable aquatic species to be cultured in salt water to replace shrimp. Mudskipper can be cultured with high density but aeration does not need to be applied to increase oxygen dissolved in water for fish (Minh *et al*., 2010), because they have the capacity to breathe air through their gills (Murdy, 1989). In addition, the culture water for growout pond does not require regular changing as for other fish species. Therefore, nitrogen waste accumulates with higher concentration by day during the culture period, and this may have a negative effect on the growth of fish. A new approach in aquaculture to reduce dissolved waste from the target animals is the biofloc technique which stimulates heterotrophic bacterial growth within the culture system. Heterotrophic bacteria obtain carbon and energy for growth from naturally occurring organic compounds (McGraw, 2012).

In addition, the effectiveness of submersed substrate in the aquaculture system has been reported to give more space for target animals and provides more

room for periphyton to grow, and the resulting periphyton helps to control water quality (Schveitzer *et al*., 2013). The periphyton is also a natural food source for aquatic animals, as has been reported by better growth and the production of a target animal such as hybrid tilapia (Keshavanath, 2004) *Macrobrachium rosenbergii* (Chavez, 2015), *Litopenaeus vannamei* (Schveitzer *et al*., 2013; Dos Santos *et al*., 2019), *Penaeus monodon* (Kumar *et al*., 2019).

The Mudskipper is a herbivorous species, feeding mainly on pennate diatoms (Bucholtz *et al*., 2009). In an intensive culture system, mudskipper is acclimated to feed on floated commercial feed with small size from 0.6 mm to 2 mm, but the effect of biofloc and substrate addition on the growth of mudskipper is still not studied, so that the aim of this study was to evaluate the effect of biofloc and substrate integration of the growth and enzyme activity of mudskipper.

Material and Methods

Experimental Design

Natural mudskipper *P. elongatus* fries (6.90 – 7.30 cm; 3.88–4.12 g) was obtained from the Mekong delta coastal area. Fish were acclimated in four days, and after that the healthy fish were selected for study.

Fish were reared in 12 round fiberglass tanks (1m^3/tank) containing 600 L of 15‰ seawater at a stocking density of 240 ind./m^2 for 60 days in outdoor conditions. Mud was added to the tank bottom at around 15 cm to simulate culture conditions as would be found in earthen ponds. The experiment consisted of 04 treatments, triplicate for each, as follows:

- Treatment 1 (C+S): with carbohydrate and substrate addition
- Treatment 2 (C): with carbohydrate addition
- Treatment 3(S): with substrates additon
- Treatment 4 (Wo): without carbohydrate and substrate addition

Water exchange was performed when NO_2^- in culture medium $\geq$2 mg/L. An aeration was supported during the study.

Carbon/Nitrogen (C/N) Manipulation

Molasses (38% carbon) was added to the mudskippers' culture medium to manipulate carbon/nitrogen (C/N) ratio to 10. The amount of molasses added was based on a concentration of total ammonia nitrogen (TAN) in the culture medium.

Feed and Feeding

Commercial feed (De Hues, 40% protein) was provided at 15–20% of fish weight to the mudskipper; the total amount of feed for a day was divided and offered to the fish 04 times/day.

Sample Collection and Analysis

Physio-chemical Parameters

Temperature and pH were measured 2 times per day at 07:00 am and 14:00 pm. Nitrite (NO_2^-) and total ammonia nitrogen (NH_3/NH_4^+) were tested in the morning over 3 days by Sera test-kit (Germany).

Biological Parameters

The weight and length of fish were determined for 30 fish on the first day and the end day of the experiment.

The final number of fish in each replication of each treatment was recorded at the end day of the study.

Survival Rate and Growth Performance

$$\text{Survival (\%)} = 100\ x\ \frac{\text{number of individuals after experiment}}{\text{number of individuals before experiment}}$$

Daily weight gain (g/day): DWG = $\frac{W1-W2}{t}$

Daily length gain (cm/day): DLG = $\frac{L1-L2}{t}$

Specific growth rate of weight (%/day):

$$SGR_w = \frac{100(LnW2-LnW1)}{t}$$

Specific growth rate of length (%/day):

$$SGR_L = \frac{100(LnL2-LnL1)}{t}$$

Protease Assay

The protease enzyme activity determined according to the methodology was described by Beg *et al*. (2002) with several modifications. To prepare the crude enzyme extract, 0.1 g of intestines were homogenized in 0.9 mL of PBS (Phosphate-buffered saline) buffer by a plastic pestle (25 s) in an Eppendorf tube and centrifuged at 10,000 rpm for 10 minutes to obtain the intestinal enzyme solution at 4 ^{0}C. The 100 µL of supernatant was collected and then incubated with 100 µL Casein (1% w/v) for 10 minutes at 37 °C, and then 500 µL TCA (5% v/v) was added to stop the reaction. After 20 minutes, the mixture was centrifuged at 3000 rpm for 10 minutes to determine the protease activity according to the methodology as described by Lowry *et al*. (1951). A protease unit corresponds to the enzyme concentration required to release 1 µg tyrosine/mL/min under standard conditions.

Statistical Analysis

The dataset of each treatment was calculated to get mean and standard deviation by Microsoft Excel software and statistically processed two-way ANOVA factor and the Tukey-HSD test by Statistica 7.0.

Results

Physio-chemical Parameters

The temperature of culture medium varies in the range 27.6–30.5°C (Table 1). The concentration of TAN (NH_4^+/NH_3) in the culture medium is 1.52–1.59 mg/L. Nitrite (NO_2^-) is 0.51–0.59 mg/L.

Growth Performance

The growth results in length are shown in Table 2. The initial length of fish was 6.90–7.30 cm/fish. The final length of fish is shown in Table 2, the addition of molasses and substrate provided produced bigger fish, the growth of fish in length in T1 and T2 was no significantly better as compared to fish in T3 or T4. These results showed that both carbohydrate and substrate addition individually significantly improved on the growth in terms of length of fish, but there is no combined effect on the growth of fish (Table 3). The final weight of fish ranged between 12.26 and 14.51 g, and there was significant difference between the treatments. The addition of molasses and substrate produced higher growth of fish, significantly better than the culture where molasses was not added and substrate was not provided. In addition, the daily weight gain and specific growth rate of fish in the culture where molasses was added and substrate provided was significantly better than fish in the culture where molasses was not added and substrates were not provided, but not significantly better than fish in a solely molasses-added culture. The addition of carbohydrate and substrate improved the higher survival rate of fish, but the increase was not significantly different (P>0.05) when compared to the culture where carbohydrate and substrate were not applied. The fish yield ranged from 2.6–3 kg /m^3. The addition of molasses and substrate increased the production of fish as compared to that without molasses and substrate added. However, the significant increase of production in the molasses and substrate-added as compared to the control, but no significant difference was found when compared to the culture where solely molasses or substrate was added.

Protease Activity

Protease activity in fish increased in the culture where molasses was added only or a combination of molasses with substrate added, but protease activity was not increased when substrate was provided alone in the culture (Figure 1). In addition, the protease activity increased significantly in the culture where molasses was added.

Discussion

Mudskipper (*P. elongatus)* is a herbivorous species, feeding mainly on pennate diatoms (Bucholtz *et al*., 2009). In an intensive culture, mudskippers are always

Table 1. The culture water parameters.

Parameters	Temperature (°C)		pH		TAN (mg/L)	NO_2^- (mg/L)
	7:00	14:00	7:00	14:00		
NT1 (C+S)	27.8±1.0	30.5±1.4	7.8±0.5	7.7±0.4	1.59±1.05	0.59±0.53
NT2 (C)	27.7±1.1	30.2±1.3	7.8±0.5	7.7±0.4	1.58±1.06	0.57±0.55
NT3 (S)	27.7±1.2	30.4±1.4	7.8±0.5	7.6±0.5	1.52±1.08	0.51±0.46
NT4 (Wo)	27.6±1.1	30.1±1.4	7.8±0.6	7.6±0.5	1.55±1.10	0.52±0.53

Table 2. Growth performance in term of length of fish. Values are mean±standard deviation (n=30). Different superscripts in the same row denote significant differences (P<0.05).

Treatment	**T1 (C+S)**	**T2 (C)**	**T3 (S)**	**T4 (Wo)**
L_{int} (cm)	6.90±0.80	6.90±0.90	7.20±1.60	7.30±1.20
L_{final} (cm)	15.35±0.86^a	15.13±1.79^a	15.18±0.79^a	15.08±0.79^a
DLG (cm/day)	0.14±0.01^b	0.13±0.01^{ab}	0.13±0.01^{ab}	0.13±0.01^a
W_{int} (g)	4.12±1.17^a	3.88±1.00^a	4.00±1.31^a	4.02±1.17^a
W_{final} (g)	14.51±2.06^c	13.58±2.92^{bc}	13.02±2.84^{ab}	12.26±2.25^a
SGR(%/day)	2.09±0.14^c	2.07±0.24^{bc}	1.85±0.17^a	1.94±0.26^{ab}
DWG(g/day)	0.17±0.02^c	0.16±0.03^{bc}	0.13±0.02^a	0.15±0.03^{ab}
Survival (%)	84.9±2.9^a	86.0±1.2^a	80.7±3.5^a	82.8±2.7^a
Production (kg)	3.03±0.19^b	2.96±0.03^{ab}	2.81±0.12^{ab}	2.57±0.25^a

Table 3. p-value of experimental factors on mudskipper performance. *** denotes P < 0.001; **: P < 0.01 and *: P < 0.05).

P-value	Source of variation		
	Substrate (S)	Carbohydrate (C)	S*C
L_{final}	0.0000****	0.002**	0.8449
W_{final}	0.0000****	0.0000****	0.0208*

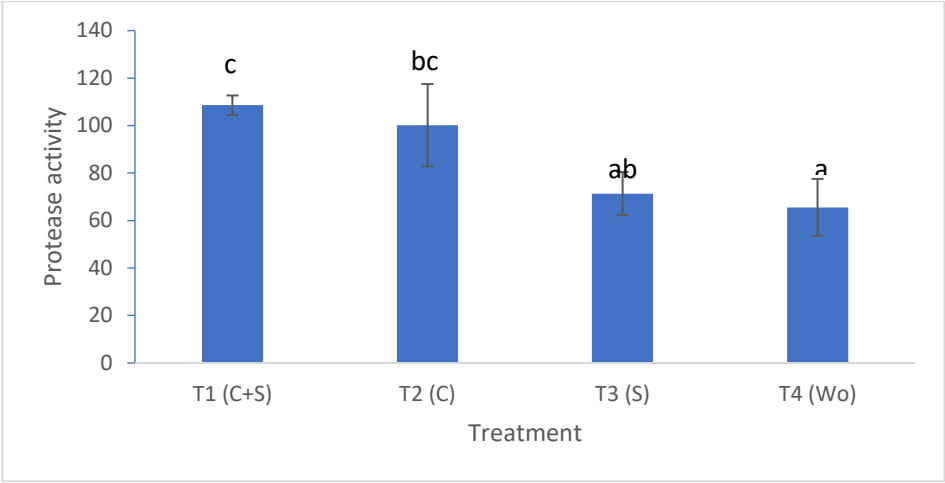

Figure 1. Protease activity in gastrodigestive tract of fish

acclimated to feed on floating commercial feed. However, the mudskipper has the capacity to breathe air through its gills (Murdy, 1989) and is able to tolerate high ammonium concentration in the culture medium, so that culture water is rarely exchanged in the intensive culture system. The biofloc technique has been demonstrated to improve water quality through stimulating bacterial growth (Hari *et al*., 2006). The biofloc may be used as food by the target animals (Avnimelech, 1999; Hari *et al*., 2004) thus resulting in savings on commercial food that has been demonstrated by previous studies on white-leg shrimp *Litopenaeus vannamei* (Burford *et al*., 2004), Nile tilapia *Oreochromis niloticus* (Avnimelech, 2011), and the black tiger shrimp *Penaeus monodon* (Anand *et al*., 2014; Kumar *et al*., 2019). Furthermore, the addition of carbon produced better growth of target animals, which has been demonstrated on white-leg shrimp post larvae, the biofloc producing better growth of shrimp because it stimulated shrimp to utilize more food (Kim *et al*., 2013). The improvement growth by biofloc has been reported on the common carp *Cyprinus carpio* L., where the better growth was obtained in the culture where biofloc was combined with 75% of commercial food as compared to the control provided with 100% commercial food (Najdegerami *et al*., 2015). The addition of submersed substrate in the culture medium helps increase natural periphyton productivity, which is the potential food source for target animals. This has been demonstrated *on Litopenaeus vannamei* (Schveitzer *et al*., 2013) *Penaeus monodon* (Kumar *et al*., 2019). The increased growth rate of fish in case of biofloc (bacterial forming) is generally linked to digestive enzyme activities (Long *et al*., 2015). The increase in digestive enzymes may lead to enhanced digestion and absorption of food, which in turn contributes to the improved survival and growth of fish. Biofloc are considered to be a source of exogenous extracellular enzymes (Verschuere *et al*., 1999; Maki *et al*., 2009). The increase of enzyme activities with the addition of specific bacterial strains in the culture medium has been demonstrated in marine larval shrimp *Litopenaeus vannamei*, *Penaeus* sp. (Rengpipat *et al*., 1998; Zhou *et al*., 2009; Nimrat *et al*., 2012) and *Cyprinus carpio* L. (Najdegerami *et al*., 2015). In the present study the protease activity increased in the culture where biofloc was applied, it resulted in better growth of fish as compared to the culture where substrate was individually provided or without carbon added.

In conclusion, the results of this study demonstrate that the growth performance and production of mudskipper significantly increased when the biofloc and substrate integration were applied to the culture. However, the study still remains to be validated in the earthen pond environment.

Ethical Statement

The data of this study is original work, and it has not been previously published in any journal.

Fish were used and cultured under condition with high applicable for Can Tho University guidlines which adapted from national guidelines on the protection and welfare of experimental animals in Viet Nam (Law on animal health; No. 79/2015/QH13). Before operating fish to get the gut samples for enzyme activity analysis, fish were starved for 24 h, then sampled fish were immersed in ice-water slurry until unconscious.

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Author Contribution

HTT and NTHV conceived of the presented idea, writing the research proposal and conducted a study. VHH was the one who was responsible for collecting fish gut samples and doing the emzyme analysis. At the end of study, all the author members dicussed on the results

and interpret collected data for writing the manuscript. HTT was responsible for writing.

Conflict of Interest

On behalf of authorship, I declare that there was no conflicts of intest on this study.

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