CAN THO UNIVERSITY
COLLEGE OF AQUACULTURE AND FISHERY

USE OF *ARTEMIA* BIOMASS AND GUT WEED MEAL AS PROTEIN SOURCE IN PRACTICAL DIETS FOR THE BLACK TIGER SHRIMP *(Penaeus monodon)*

By
TA XUAN DUY

A thesis submitted in partial fulfillment of the requirements for
The degree of Bachelor of Aquaculture

Can Tho City, December 2013

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Student sign Supervisor sign

Ta Xuan Duy Dr. Nguyen Thi Ngoc Anh

ABSTRACT

Three separate experiments were carried out to evaluate the potential use of *Artemia* biomass and gut weed (*Enteromorpha* sp.) in practical diet for the tiger shrimp (*Penaeus monodon*). Each experiment had four treatments with three replicates. In experiment 1, *Artemia* biomass was used as protein source to replace 0, 20, 40 and 60% fishmeal protein in practical diets for tiger shrimp. In experiment 2, gut weed was used as protein source to replace 0, 15, 30 and 45% soybean meal protein in the test diet. In experiment 3, combined substitution in all treatments in experiment 1 and 2 that fishmeal protein replaced with *Artemia* biomass protein and soybean meal protein replaced with gut weed protein. The diet without containing gut weed and *Artemia* protein consider as a control. All experimental diets were formulated to be equivalent in crude protein (40%) and lipid (7%), shrimp were fed 4 times a day for 45 days.

The results showed that survival rates of experimental shrimps in three experiments were not affected by the feeding treatments, and attaining more than 80% survival. For experiment 1, a gradual increase in growth performance of the shrimp was achieved on increasing dietary inclusion of *Artemia* protein, and significant difference was found between the control and the 60% fishmeal replacement with *Artemia* biomass protein. For experiment 2, soybean meal protein was substituted with gut weed protein up to 45%, shrimp had similar growth rate compared to the control while at lower substitution levels (15 and 30%) growth of shrimp was significant improved. For experiment 3, shrimp fed the test diets with combined substitution of *Artemia* biomass for fishmeal protein and gut weed for soybean meal showed significantly higher growth rate than in the control. In most cases, feed conversion ratio in the test diets were lower than in the control. These results indicated that both *Artemia* biomass and gut weed can be used as protein sources in practical diets for the tiger shrimp *Penaeus monodon*, indicating the high potential of using locally available of *Artemia* biomass and gut weed in the region.

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FCR:	Feed Conversion Ratio
AT:	Artemia
GW:	Gut weed
TAN:	Total ammonia nitrogen
PL:	Post-larvae

CHAPTER 1

INTRODUCTION

1.1. Introduction

Aquaculture production is highly dependent on commercial feeds that aquafeeds relies on several common input ingredients such as fishmeal, soybean, corn, fish oil, rice bran and wheat powder, for which it competes in the market place with the animal husbandry sector (Rana *et al.*, 2009). Currently, its availability is a major concern for its high cost and scarcity of raw materials. Moreover, in shrimp farming, feed cost is the highest proportion and it accounts for more than 50% of the total production costs (Tacon, *et al.*, 2004; Davis *et al.* 2008). In addition, most feed manufactures are using expensive imported fishmeal and soybean meal as a protein source for aquafeeds resulting in high price. Therefore, assessment of cheaper or more readily available alternative plant protein sources such as seaweed, aquatic plants or by-product from fisheries that may reduce the use of imported ingredients in feeds (FAO, 2003; Rana *et al.*, 2009).

Gut weed (*Enteromorpha* spp.) has a high nutritional value; it contains 9–14% protein; 2–3.6% lipid; 32–36% ash, and n-3 and n-6 fatty acids 10.4 and 10.9 g/100 g of total fatty acid, respectively; the protein of this seaweed has a high digestibility up to 98% (Fleurence, 1999; Aguilera-Morales, *et al.*, 2005). Recent investigations revealed that gut weed belonging to green algae distribute abundantly in the extensive shrimp farms and other brackish water bodies of the Mekong delta, Vietnam (ITB-Vietnam, 2011). This indicates large quantity of gut weed is available for aquaculture feeds. Moreover, several studies reported that gut weed can be used as a direct feed or as ingredient in diets for fish and shrimp (FAO, 2003; Dhargalkar and Pereira, 2005, Nguyen Thi Ngoc Anh *et al.*, 2012).

Artemia biomass has excellent nutritional compositions with 50-60% protein, rich in unsaturated fatty acid and essential amino acids (Lim *et al.*, 2001; Nguyen Thi Ngoc Anh, 2009). Previous studies reported that *Artemia* biomass could be used in different forms (fresh, frozen, dried) as direct feed or as a protein source for replacing fishmeal in practical diets for fish and shrimp (Naegel *et al.*, 2004, Nguyen Thi Ngoc Anh *et al.*, 2010). Additionally, Nguyen Thi Ngoc Anh *et al.* (2011) reported that *Artemia* biomass by-product from *Artemia* cyst production can be used to replace fishmeal protein in the diet for goby (*Pseudapocryptes elongatus*) fingerlings resulted in superior growth performance and better feed utilization compared to a fishmeal control and a commercial feed. According to Nguyen Thi Ngoc Anh *et al.* (2010), *Artemia* biomass- by product from *Artemia* cyst production ponds could be collected between 0.2 and 0.3 ton/ha after termination of the production season in Vinh Chau and Bac Lieu salt fields. This indicates large quantity of *Artemia* biomass is available in this region.

Black tiger shrimp (*Penaeus monodon*) has high economic value, which is important cultured species in the Mekong delta. According to report of Department of Fisheries in 2012, black tiger shrimp farming area is 619,355 ha, production is 298,607 tons. Moreover, the survey results from Vu Nam Son *et al*. (2011) reported that feed cost accounts for large proportion (58%) of the production cost in the intensive shrimp farming; hence using locally available products in the culture region for shrimp feed may contribute to reduce the feed costs and improve economic efficiency. From above issues, evaluating potential use of *Artemia* biomass and gut weed as protein source in practical diets for the black tiger shrimp (*Penaeus monodon*)" was performed.

1.2. Research objectives

- Determine the suitable substitution levels of fishmeal protein with *Artemia* biomass protein in the practical diets for the black tiger shrimp.
- Determine the proper replacement levels of soybean protein with gut weed protein in practical diets for the black tiger shrimp.
- Find out the appropriate replacement levels of combined *Artemia* biomass and gut weed protein for fishmeal and soybean meal protein in practical diets for the black tiger shrimp.

1.3. Research content

- Effect of fishmeal replacement with *Artemia* biomass as a protein source in practical diets on survival and growth of the black tiger shrimp.
- Effect of soybean meal replacement with gut weed as a protein source in practical diets on survival and growth of the black tiger shrimp
- Evaluating combined substitution of *Artemia* biomass and gut weed protein for fishmeal and soybean meal protein in practical diets for the black tiger shrimp.

CHAPTER 2

LITERATURE REVIEW

2.1. *Artemia*

2.1.1. Overview of *Artemia*

Brine shrimp, *Artemia* is crustacean which is a cosmopolitan organism, inhabiting coastal lagoons as well as inland salt lakes where there are no or few predators and competitors. In these hypersaline environments which are not tolerable by other filter feeders, *Artemia* survive thanks to their physiological adaptations. *Artemia* distribution is not continuous; the populations are found throughout the tropical, subtropical and temperate climate zones (Persoone and Sorgeloos, 1980).

Artemia of most strains can reproduce both ovoviviparíously and oviparously. Nauplius production allows a rapid growth, whereas the production of diapause cysts ensures the survival of a population through unfavorable conditions (Persoone and Sorgeloos, 1980). A female should continue to reproduce ovoviviparously as long as there is a good probability that her offspring will reproduce themselves. However, if conditions are such that offspring survival is unlikely, then females should invest in oviparous reproduction, in the expectation that these cysts will hatch under more favorable conditions.

Artemia culture in Vinh Chau solar-saltworks in Vinh Chau has been started since late 1980’s with the main aim to produce cysts. Culture techniques have been improved and culture area has been enlarged year by year and thus the area could produce as high as 50 tons of raw cysts in the early of 1990’s (Nguyen Van Hoa *et al.*, 2011).

2.1.2. **Nutritional value of *Artemia***

The nutritional quality in *Artemia* varies considerably. This variation might be related to the geographical origin of *Artemia* to differences among different batches of cysts from the same origin, and to the methods of analysis and greater changes in biochemical composition might be subjected to different strains of *Artemia* (Leger *et al.*, 1986). The nutritional value of on-grown and adult *Artemia* is superior that of freshly-hatched nauplii, as they have higher protein content and are richer in essential amino acids and fatty acids (Lim *et al.*, 2001; Dhont and Sorgeloos, 2002).

Nguyen Thi Ngoc Anh *et al.* (2009a), evaluated the proximate composition of *Artemia* biomass reared on different feed supplementations in salt ponds for 12 weeks, such as protein: 49.4-57.8%; lipid: 9.8-13.9%; Ash: 14.8-23.7%; fiber: 0.3-0.8% and carbohydrates: 10.6-15.8% dry matter. They reveled that at the same culture period, the contents of protein, lipid, ash, fiber and carbohydrates were not significantly different among treatments. However, mean protein and lipid concentrations tended to decline with the culture period, especially the last week of culture (week 12) showed the lowest values, whereas the ash content increased. Carbohydrates and fiber remained similar or slightly lower than the initial day 5 values.

Castro *et al*. (2009) conducted monthly assessments of protein, fatty acids and amino acids in *Artemia franciscana* cultivated in a Mexican salt pond from March 2004 to February 2005. They reported that the contents of total protein and lipids showed a similar tendency from July to December (maintained values of about 300 mg/g) for protein and 90 mg/g for lipid). With the exception of methionine and arginine, others even indispensable amino acids were detected in the monthly samples, having similar values during the period from July to December. The most common fatty acids determined were the C16, C18, C18:1 and C18:3n6. Both, C20:4n6 and C20:5n3, were observed occasionally, but in high quantities. Moreover, author suggested that when using the four micro algae (*Tetraselmis* sp., *Dunaliella*, sp., *Nannochloris*, sp. and the diatom *Navicula* sp.) as food for the *Artemia* cultured under extensive condition in a pond, improved the biochemical composition and allows using *Artemia* as feed for several aquatic species.

2.1.3. Use of *Artemia* biomass for aquaculture species

Although *Artemia* are mostly used under the form of freshly hatched nauplii, more and more use is made of the juvenile and adult *Artemia* known as biomass, collected from natural salt lakes, man-managed pond productions and intensive culture systems for use in shrimp and fish nursery (Dhont and Sorgeloos, 2002, Nguyen Thi Ngoc Anh, 2009).

In recent years, the development of new aquaculture species with life-stage specific requirements has meant diversification in the use of *Artemia* to include live juvenile and adults as well as frozen or dried *Artemia* biomass (Lim *et al*., 2003; Nguyen Thi Ngoc Anh, 2009). Furthermore, the use of on-grown *Artemia* as a cheaper alternative to the use of nauplii, simple cost-effective production techniques have been developed (Dhont and Sorgeloos, 2002; Lim *et al*., 2003).

Previous study found that dried *Artemia* biomass incorporated in the diets is very suitable for the post-larval white shrimp, *Litopenaeus vannamei* (Naegel *et al*., 2004).

Tran Huu Le *et al*. (2008) compared the uses of live *Artemia* biomass versus trash fish for nursing sea bass (*Lates calcarifer*) in earthen pond in Soc Trang. Results showed that after 30 days of culture, survival and growth of sea bass fed single live *Artemia* biomass were highest compared to other feeding treatments.

Nguyen Thi Hong Van *et al*. (2008), assessed fives types of *Artemia* biomass obtained from different culture conditions consisting of four live biomass and a frozen biomass for feeding *Penaeus monodon* postlarvae in 6 weeks. They obtained highest survival in shrimps fed on frozen *Artemia* (63.3 ±4.2%, following by fresh algal eaten *Artemia* (45.8 ±1.2%) and the lowest survival was found in shrimp fed on *Artemia* harvested at the end of culture season. However, their study also revealed that nutritional qualities of *Artemia* biomass in term of essential fatty acid did not play pronounced effects on growth performances and survivals in tiger shrimp.

Nguyen Thi Ngoc Anh (2009b), evaluated the potential use of *Artemia* biomass as protein source in practical diets for postlarval (*Macrobrachium rosenbergii*) in 30 days. The experimental diets (approximately 40% crude protein) were formulated by replacing levels of the fishmeal protein difference either with dried or frozen *Artemia* (0, 25, 50, 75 and 100%). They reported that a gradual increase in survival and growth of the prawns was achieved with increasing dietary inclusion of *Artemia* protein. These results indicated *Artemia* biomass may totally replace fishmeal in prawn diets. Similar findings were also reported by Nguyen Thi Ngoc Anh (2011), *Artemia* biomass can be used either as direct feed or as ingredient in formulated feeds for hatcheries and nurseries of brackish cultured species (mud crab, goby, black tiger shrimp) which enhance survival rate, growth and shorten the rearing time.

2.2. Gut weed

2.2.1. Overview of gut weed

Figure 1. Morphology of *Enteromorpha* sp.

The genus gut weed *Enteromorpha* belong to green macroalgae (Chlorophyta), the phallus of *Enteromorpha* with tubular and elongate fronds that may be branched flattened or inflated. They are bright green in color. The fronds of a species may vary in appearance due to changes in environmental conditions, which further confuses identification, and microscopic examination of cell details is often required to identify a species with certainty (Nguyen Van Tien, 2007).

Gut weed *Enteromorpha* are distributed worldwide, in different environments. They can tolerate different salinities ranging from freshwater to seawater and can be found in salt streams. They can grow on the ocean coast, in the brackish and fresh water inland. *Enteromorpha* can also grow on many types of substrate: sand, mud or rock, even wood, concrete or metal type or free development without substrates. *Enteromorpha* can also develop in coastally tidal areas. It can also grow with some types of seaweed and other algae in many different habitats (Kirby, 2001).

In Vietnam, gut weed *Enteromorpha* sp. were found abundantly in the brackish water bodies in the Mekong delta, Vietnam such as the extensive farms, abandoned ponds, discharged canals, rice fields (ITB-Vietnam, 2011).

2.2.3. Nutritional value of gut weed

Several studies reported that the nutritional value of seaweed depends on species, development stages, seasonal and geographic regions and are affected by the environmental factors such as salinity, temperature, nutrients (Banerjee *et al*., 2009; Nguyen Thi Ngoc Anh *et al*. 2012).

Aguilera-Morales *et al*. (2005) studied on the nutritional composition of gut weed *Enteromorpha* spp, they found that gut weed have 9-14% protein, fatty acid content n3 and n6, respectively, 10.4 and 10.9 g/100 g in total fatty acids and are rich in amino acid and protein digestibility of gut weed are high (98%).

The findings of Banerjee *et al*. (2009) on biochemical composition of three kinds of seaweeds *Ulva lactuca*, *Enteromorpha intestinalis*, and *Catenella repens* Indian river showed the species of Chlorophyceae class such as *Ulva lactuca*, *Enteromorpha intestinalis* is rich in protein, lipid, carbonhydrate, and astaxanthin. *Enteromorpha intestinalis* has the highest average protein and astaxanthin, respectively, 10.4%, 149.57 ppm compared to *Catenella repens* (9.47% protein, astaxanthin 138.27 ppm) and *Ulva lactuca* (protein 9.25% and 127.84 ppm astaxanthin).

Nguyen Thi Ngoc Anh *et al.* (2012), found that the nutritional composition of gut weeds (*Enteromorpha* spp.) had variations in different developmental stages, in which the nutritional values of young stage were comparable to or better than the adult one and both were superior to those of the senescent stage. The proximate composition and amino acid profiles of gut weeds were also determined at different salinity ranges (the lowest, intermediate and highest ranges) for each habitat. In Soc Trang, gut weed samples at three salinity ranges (1-2 ppt, 5-6 ppt and 10-12 ppt) were analyzed, these results exhibited that the wet/dry ratio decreased with increasing of salinity while the total lipid and ash contents increased with salinity, and other components (protein, fiber and carbohydrates) showed slightly changes. The total amino acid collected at salinities of 1-2 ppt and 5-6 ppt were similar and both were better than the one harvested at salinities of 10-12 ppt. Samples of gut weeds recorded from Bac Lieu at four salinities ranging between 10-12 ppt; 15-17 ppt, 20-22 ppt and 25-27 ppt, analysis results revealed that the wet/dry ratio, total lipid and ash contents followed the same pattern as observed for Soc Trang habitat. The protein contents of these samples varied in different ways, the lowest and highest protein contents were found in the 15-17 ppt and 25-27 ppt samples, respectively, while protein values in the 10-12 ppt and 20-22 ppt samples were almost equal; the carbohydrate levels reduced with increasing salinity. Moreover, protein of gut weed posively correlated with nutrient contents in the water bodies. They concluded that gut weeds found in the study areas had high nutritional values which can be used as feeds for aquaculture species.

2.3. Use of gut weed *Enteromorpha* in aquaculture feed

Yousif *et al.* (2004) studies on growth response and carcass composition of rabbitfish *(Siganus canaliculatus)* fed diets supplemented with dehydrated seaweed, *Enteromorpha* sp. They found that the best results of all parameters were achieved in the fish fed control diet combined with the fresh *Enteromorpha*, especially, lipid content increase in the group of fish supplemented with fresh *Enteromorpha.*

Cruz-Suarez *et al.* (2006) reported that growth of the *Litopenaeus vannamei* was greater in the group fed pellets containing *Enteromorpha* than those with *Macrocystis* or *Ascophyllum*. Similarly, feed with *Enteromorpha* produced the best feed conversion ratio (1.78) at 28 days. Besides, shrimp has dark red color after cooking because of the high carotenoid levels typical of *Enteromorpha.*

According to report of Corpetino *et al.* (2009), *Ulva clathrata* was highly efficient in removing the main inorganic nutrients from effluent water. Besides, *U. clathrata* inhibited phytoplankton growth and nutrient removal by *U. clathrata* better than other processes such as phytoplankton and bacterial assimilation, ammonia volatilization and nutrient precipitation.

Asino *et al.* (2010) studied on evaluation of *Enteromorpha prolifera* as a feed component in large yellow croaker (*Pseudosciaena crocea*) diets. Author reported that the feed efficiency ratio (FER) in fish fed the diet with 5% *E. prolifera* was higher than other groups. Supplementation levels of *E. prolifera* can reach at least 15% without affecting the growth and still maintain a high survival rate for juvenile large yellow croaker.

Recent investigation on using gut weeds (*Enteromorpha* sp.) protein to replace fishmeal protein in the diets for Tilapia. Author found that replacement level of gut weeds protein up to 40% had no adverse effects on survival, growth performance and feed utilization efficiency (Dam Phuoc Hien, 2012).

Dinh Thi Kim Nhung found that gut weed (*Enteromorpha* sp.) could be considered as good candidate to replace soybean meal protein up to 40% in the diets or in co-culture with white leg shrimp (*Litopenaeus vannamei*).

2.4. Black tiger shrimp

2.4.1. Classification

Phylum: *Arthropoda*
Class: *Malacostraca*
Order: *Decapoda*
Family: *Penaeidae*
Genus: *Penaeus*
Species: *Penaeus monodon*

2.4.2. Morphology

Females can reach approximately 33 centimeters (13 in) long, but are typically 25–30 cm long and weight 200–320 grams males are slightly smaller at 20–25 cm long and weighing 100–170 g.

2.4.3. Nutritional requirement

Protein and amino acid

Protein is the most important ingredient in foods, plays a vital role in the construction of the body, providing energy and essential amino acids. Post larvae need about 40% protein. Commercial shrimp need protein content between 35-40%. Meanwhile brood stock need feed with high protein content of about 45-50%. There are 10 essential amino acids for shrimp include methionine, arginine, threonine, tryptophan, histidine, osoleusine, leusine, valine, phenylanine. The ratio of amino acids in foods as close to the ratio of amino acids in the shrimp body which resulted in better growth (Wouter *et al.*, 2001).

Lipid

Fat plays an important role for shrimp by providing more energy, highly unsaturated fatty acid molecule, phospholipids and vitamins. The fat content of the food needed for the shrimp about 6 to 7.5%. Sources of fat is best from marine animals such as squid, fish oil, food... Besides, feeding have 1% cholesterol shrimp will grow faster, better feed conversion, high absorption of feed efficiency and high survival rate. In addition, lecithin is also essential for shrimp, feed containing 4% of lecithin from soybean meal helps shrimp grow faster. In particular, lecithin is also essential for brood stock culture.

Cacbohydrates

Carbohyrate have an important role in the diet of shrimp in the supply of energy, which helps absorb protein has adhesive function. Carbohydrate content in the diet is about 10-20%.

Vitamin and minerals

Vitamins and minerals are essential in regulating body processes. Vitamin B helps the absorption of protein, carbohydrate and fat are better, vitamins A and C help the body has good resistance to disease. Vitamin D along with the minerals, calcium, and phosphorus help build the shell of the shrimp. All the vitamins and minerals needed in small amounts, but necessary for a complete feed. Ratio of phosphorus and calcium should be in the range 1:1-1.5:1. The calcium level in the diet does not exceed 2%.

CHAPTER 3

MATERIAL AND METHOD

3.1. Time and study site

The study was performed from April to September, 2013 at the College of Aquaculture and Fisheries, Can Tho University.

3.2. Study object

- Gut weed (*Enteromorpha* sp.)
- *Artemia* biomass
- Black tiger shrimp (*Penaeus monodon*)

3.3. Material research

3.3.1. Sources of experimental shrimp, *Artemia* and gut weed

- Gut weed (*Enteromorpha* sp.) was collected from the discharge canal from the intensive shrimp ponds in Bac Lieu province.
- *Artemia* biomass (by-product from cyst production) was collected from commercial *Artemia* cyst-oriented ponds in Vinh Chau at the end of the culture cycle.
- Shrimp postlarvae were purchased from the commercial shrimp hatchery in Can Tho city.

3.3.2. Materials and chemicals

- Refractometer, temperature and pH meter,
- Electronic balance, pumps, aerator …
- Formalin, chlorine, iodine, natri thiosufat, sodium bicarbonate.

3.4. Research methodologies

3.4.1. Experimental diets

Ingredients used for experimental feeds such as gut weed meal, *Artemia* biomass meal, fishmeal, soybean meal, rice bran, cassava powder, premixed vitamin, squid oil and gelatin. All test diets were formulated to be approximately isonitrogenous (40%) and isolipidic (7% dietary protein). The 'SOLVER' program in Microsoft Excel was used to establish the formulated feeds.

Proximate analysis (moisture, crude protein, total lipid, fiber and ash) of the ingredients and experimental diets will be determined according to the standard methods of AOAC (1995). Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber and ash from 100% (Table 1).

Table 1. Proximate composition (% of dry matter) of the ingredients used in three experimental diets

Ingredients	Moisture	Protein	Lipid	Ash	Fiber	NFE
Fishmeal	11.08	58.14	9.17	21.36	0.56	10.77
Soybean meal	10.43	44.32	2.23	8.25	0.27	44.93
Artemia biomass meal	8.72	58.45	10.35	19.71	0.10	11.40
Gut weed meal	6.19	25.44	2.16	24.17	2.14	46.08
Rice bran	9.86	8.52	8.15	21.32	2.33	59.68
Cassava powder	10.87	5.14	1.77	0.69	0.87	91.53

- **Experiment 1:** Four experimental diets were formulated by replacing 0%, 20%, 40% and 60% of the fish meal protein in a standard diet with *Artemia* biomass protein (Table 2).
- **Experiment 2:** Four experimental diets were formulated by replacing 0%, 15%, 30% and 45% of the soybean meal protein in a standard diet with gut weed protein (Table 3).
- **Experiment 3:** Four experimental diets of which diet without containing *Artemia* biomass and gut weed powder as a control treatment. 3 other diets were formulated by combined substituting fishmeal protein with *Artemia* biomass protein and soybean protein replaced with gut weed protein followed in the order: 20% *Artemia* protein+ 15% gut weed protein, 40% *Artemia* protein+ 30% gut weed protein and 60% *Artemia* protein+ 45% gut weed protein (Table 3).

3.4.2. Experimental design

Experiment 1: Effect of fishmeal replacement with *Artemia* biomass as a protein source in practical diets on survival and growth of the black tiger shrimp (*Penaeus monodon*).

Experiment consisting of 4 feeding treatments was set up randomly with three replicates per treatment as follows:

- Treatment 1: 0% *Artemia* protein (control, 0% AT)
- Treatment 2: 20% *Artemia* protein replacement for FM protein (20% AT)
- Treatment 3: 40% *Artemia* protein replacement for FM protein (40% AT)
- Treatment 4: 60% *Artemia* protein replacement for FM protein (60% AT)

Table 2. Composition of ingredients (g/100 g dry matter) and proximate composition of experiment 1

Treatment	0% AT	20% AT	40% AT	60% AT
Fishmeal	44.50	35.61	26.69	17.80
Soybean meal	29.19	29.19	29.19	29.19
Artemia biomass meal	-	8.84	17.71	26.56
Rice bran	3.80	3.65	3.50	3.80
Cassava powder	16.85	17.14	17.44	17.30
Squid oil	1.16	1.07	0.98	0.85
Lecithin	0.50	0.50	0.50	0.50
Premix -Vitamin	2.00	2.00	2.00	2.00
Gelatin	2.00	2.00	2.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Proximate analysis of experiment 1				
Moisture	10.16	10.54	11.09	11.13
Protein	40.68	40.38	40.15	40.02
Lipid	6.98	7.02	7.03	6.95
Ash	14.28	14.15	14.06	14.20
Fiber	2.92	2.52	2.36	2.18
NFE	35.13	35.94	36.39	36.64
Calcium	2.17	2.46	2.77	3.13
Phosphorus	1.32	1.36	1.28	1.31
Energy (kgcal/g)	**4.43**	**4.45**	**4.46**	**4.46**

Experiment 2: Effect of soybean meal (SB) replacement with gut weed as a protein source in practical diets on survival and growth of *P. monodon*.

Experiment 2 composed four feeding treatments were randomly designed with three replicates for each treatment as follows:

- Treatment 1: 0% gut weed protein (control, 0% GW)
- Treatment 2: 15% gut weed protein replacement for SB protein (15% GW)
- Treatment 3: 30% gut weed protein replacement for SB protein (30% GW)
- Treatment 4: 45% gut weed protein replacement for SB protein (45% GW)

Table 3. Composition of ingredients (g/100 g dry matter) and proximate composition in experiment 2

Treatment	0% GW	15% GW	30% GW	45% GW
Fishmeal	44.50	44.50	44.50	44.50
Soybean meal	29.19	24.82	20.44	16.07
Gut weed meal	0.00	7.63	15.23	22.88
Rice bran	3.80	8.18	8.76	7.49
Cassava powder	16.85	9.51	5.76	3.67
Squid oil	1.16	1.32	1.39	1.00
Lecithin	0.50	0.05	0.05	0.05
Premix -Vitamin	2.00	2.00	2.00	2.00
Gelatin	2.00	2.00	2.00	2.00
Total	**100.00**	100.00	100.00	100.00
Proximate analysis of experiment 2				
Moisture	10.16	10.68	10.82	10.45
Protein	40.68	40.06	39.98	39.82
Lipid	6.98	6.79	6.65	6.72
Ash	14.28	16.28	16.34	17.26
Fiber	2.92	3.21	3.35	3.44
NFE	35.13	33.66	33.68	32.75
Calcium	2.17	2.26	2.59	2.47
Phosphorus	1.32	1.26	1.15	1.08
Energy (kgcal/g)	**4.43**	**4.32**	**4.30**	**4.26**

Experiment 3: Evaluating combined substitution of *Artemia* biomass and gut weed protein for fishmeal and soybean meal protein in practical diets for the black tiger shrimp.

This experiment consisted of 4 treatments in which the control without containing gut weed and *Artemia* protein in the test diet. 3 other test diets were combined substitution that fishmeal protein replaced with *Artemia* biomass protein and soybean meal protein replaced with gut weed protein as followed:

- Treatment 1: without gut weed and *Artemia* protein (control)
- Treatment 2: 20% *Artemia* protein+15% GW protein (20%AT+15%GW)
- Treatment 3: 40% *Artemia* protein+ 30% GW protein (40%AT+30%GW)
- Treatment 4: 60% *Artemia* protein+ 45% GW protein (60%AT+45%GW)

Table 4. Composition of ingredients (g/100 g dry matter) and proximate composition in experiment 3.

Treatment	Control	20%AT+15%GW	40%AT+30%GW	60%AT+45%GW
Fishmeal	44.50	33.37	22.26	11.13
Soybean meal	29.19	24.82	20.44	16.06
Artemia biomass meal	-	11.07	22.14	33.20
Gut weed meal	-	7.63	15.26	22.86
Rice bran	3.80	7.95	11.92	8.54
Cassava powder	16.85	9.91	3.13	3.27
Squid oil	1.16	1.25	0.86	0.94
Lecithin	0.05	0.05	0.05	0.05
Premix - Vitamin	2.00	2.00	2.00	2.00
Gelatin	2.00	2.00	2.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Proximate analysis of experiment 3				
Moisture	10.16	10.27	10.11	10.47
Protein	40.68	40.04	39.97	40.03
Lipid	6.98	7.07	7.11	6.97
Ash	14.28	15.64	16.46	17.98
Fiber	2.92	2.78	3.12	3.24
NFE	35.13	34.47	33.34	31.78
Calcium	2.17	2.51	2.49	2.61
Phosphorus	1.32	1.12	1.19	1.34
Energy (kgcal/g)	**4.43**	**4.38**	**4.33**	**4.25**

Gross energy was calculated based on protein = 5.65; lipid = 9.45 and NFE = 4.20 (kgcal/g)

3.4.3. Experimental system and management

Experiment 1 was conducted in the nursery house (indoor), the volume of culture tanks was 250-L and water volume 150L.

Figure 2. Experimental system

Experiment 2 and 3 were performed outside the seaweed station, the shading net was hanging on the top. The volumes of experimental tanks were 120 L plastic tank with water volume of 80 L.

Figure 3. Experimental system

Experimental shrimps in three experiments were set up the same salinity (10 ppt) and stocking density (30 postlarvae per tank) with the open clear water system and each tank provided continuous aeration.

Shrimp were fed *ad libitum*, 4 times a day at 6:00, 11:00, 16:00 and 21:00. Water exchange was done every 5-7 days, about 50% of the tank volume.

All three experiments were conducted 45 days.

3.3.4. Data collection

Water quality

- Daily water temperature and pH was recorded at 8:00 and 14:00 h using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius).

- The concentration of $NO_{2,}$ NH_4/NH_3 and alkalinity were monitored weekly using test kits (Sera, Germany).

Shrimp sampling

- For initial weight and length of shrimp postlarvae, 30 individuals were randomly taken from the conditioning tank to measure individual weight and total length.
- Shrimp sampling was conducted every fifteen days. 10 shrimp were randomly taken from each tank and group weighed using electronic balance and then the shrimp are returned to the original tanks.
- For final weight and length of shrimp was measured individually and counted to calculate survival at the termination of experiment.

Growth performances of shrimp

Growth performance data of experimental shrimp consisting of weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) and feed conversion ratio (FCR) and survival were calculate using the following equations:

Weight gain (g) = Final weight - Initial weight

DWG (g/day) = (final weight - initial weight)/Days of culture

SGR (%/day) = (final weight - initial weight)/Days of culture x 100

FCR = Feed provided (dry weight)/Weight gain (wet weight)

Survival (%) = Final number of shrimp/Initial number of shrimp x 100

3.4.5. Statistical analysis

The data of survival and specific growth rate of shrimp are normalized through arcsine transformation before statistical analysis. For all treatments, results were analyzed statistically with one-way ANOVA analysis of variance to find the overall effect of the treatment (SPSS, version 14.0). DUNCAN test was used to identify significant differences between the mean values at a significant level of $P<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Water quality parameters

Average water temperature, pH and alkalinity of three experiments were presented in Table 4. Experiment 1, daily mean temperature and pH and alkalinity were in the ranges of 25.8-26.9^oC, 7.7-8.0 and 91-93 mg $CaCO_3$/L, respectively. Experiment 2, daily mean temperature and pH and alkalinity fluctuated between 26.2-27.8^oC, 7.8-8.0 and 90-93 mg $CaCO_3$/L, respectively. Experiment 3, daily mean temperature and pH and alkalinity varied in the ranges 26.0-27.2^oC; 7.6-7.9 and 102-105 mg $CaCO_3$/L, respectively. Generally, temperature, pH, and alkalinity in each experiment were almost the same among feeding treatments.

Table 5. Average water temperature, pH and alkalinity in three experiments

Treatment	Temperature (^oC)		pH		Alkalinity (mg $CaCO_3$/L)
	7: 00	14:00	7: 00	14:00	
Experiment 1					
0% AT	25.8 ± 0.8	26.8 ± 0.8	7.7 ± 0.2	7.8 ± 0.2	91 ± 13.1
20% AT	25.7 ± 0.8	26.7 ± 0.7	7.8 ± 0.2	7.9 ± 0.2	92 ± 12.2
40% AT	25.8 ± 0.8	26.9 ± 0.7	7.8 ± 0.2	8.0 ± 0.1	91 ± 11.5
60% AT	25.8 ± 0.7	26.8 ± 0.9	7.8 ± 0.1	8.0 ± 0.1	93 ± 12.7
Experiment 2					
0% GW	26.2 ± 0.7	27.4 ± 0.9	7.8 ± 0.2	7.9 ± 0.1	91 ± 11.5
15% GW	26.4 ± 0.7	27.5 ± 0.8	7.8 ± 0.2	7.9 ± 0.2	93 ± 11.1
30% GW	26.4 ± 0.6	27.8 ± 1.0	7.8 ± 0.2	7.9 ± 0.2	90 ± 10.7
45% GW	26.4 ± 0.7	27.6 ± 0.9	7.9 ± 0.1	8.0 ± 0.1	92 ± 10.5
Experiment 3					
Control	26.1 ± 0.8	27.1 ± 0.9	7.6 ± 0.1	7.9 ± 0.2	102 ± 13.8
20%AT+ 15%GW	26.0 ± 0.8	27.1 ± 0.8	7.7 ± 0.1	7.9 ± 0.1	103 ± 14.9
40%AT+ 30%GW	26.1 ± 0.8	27.2 ± 0.9	7.6 ± 0.2	8.0 ± 0.2	105 ± 14.1
60%AT+ 45%GW	26.0 ± 0.7	27.0 ± 0.9	7.7 ± 0.1	7.9 ± 0.1	105 ± 12.7

Whetstone *et al*. (2002) studied the effect of temperature on growth of the black tiger shrimp (*P. monodon*). Authors reported that temperature had significant effects on performances of shrimp *P. monodon*, this species can live at temperatures from 23-34°C, optimal range is from 26-29°C, and the daily temperature change was not more than 5°C. Moreover, other study confirmed the optimum range of temperature for the black tiger shrimp was between 26 and 33ºC (Ramanathan *et al*., 2005).

pH is one of the vital environmental characteristics, which decides the survival and growth of shrimp under culture; it also affects the metabolism and other physiological process of shrimps. For *P. monodon* culture, pH should be maintained between 7.5 and 8.5 (Ramakrishnareddy, 2000). However, Ramanadhan *et al.* (2005) suggested that the optimum range of pH 6.8 to 8.7 should be maintained for maximum shrimp growth and production.

Alkalinity is the buffering capacity of the pond water. The higher the alkalinity, the better is the stabilization of the pond system. For successful culture of *P. monodon*, alkalinity is recommended to be >80 mg mg $CaCO_3$/L/L and in the range 80-100 mg$CaCO_3$/L is safe for growth of black tiger shrimp (Hansell, 1993). Alkalinity should be kept above 100mg $CaCO_3$/L; ideally above 120 mg$CaCO_3$/L (Boyd, 2005). In the present study, alkalinity in three experiments fluctuated between 90-105 mg$CaCO_3$/L that indicates a suitable range for growth of tiger shrimp.

Table 6. Average concentration of TAN and NO_2 in three experiments

Treatment	**TAN (mg/L)**	**NO_2^- (mg/L)**
Experiment 1		
0% AT	0.44 ± 0.30	0.96 ± 0.40
20% AT	0.48 ± 0.26	0.78 ± 0.33
40% AT	0.45 ± 0.31	0.76 ± 0.25
60% AT	0.44 ± 0.29	0.63 ± 0.51
Experiment 2		
0% GW	0.56 ± 0.28	0.53 ± 0.45
15% GW	0.58 ± 0.24	0.52 ± 0.42
30% GW	0.60 ± 0.25	0.53 ± 0.42
45% GW	0.58 ± 0.26	0.55 ± 0.44
Experiment 3		
Control	0.07 ± 0.02	0.73 ± 0.39
20%AT+ 15%GW	0.08 ± 0.05	0.77 ± 0.40
40%AT+ 30%GW	0.08 ± 0.04	0.78 ± 0.46
60%AT+ 45%GW	0.09 ± 0.05	0.79 ± 0.56

The average concentrations of TAN and NO2 in three experiments are shown in Table 6. In experiment 1, the mean concentration of TAN was 0.44-0.48 mg/L and NO_2^- varied from 0.63 to 0.96 mg/L in which the control treatment (0%AT) had higher content of NO_2^- than other feeding treatments. Experiment 2, the mean levels of TAN and NO_2^- were similar and in the ranges of 0.56-0.58 and 0.52-0.55 mg/L, respectively. Experiment 3, the average contents of TAN in all feeding treatments was low and in the range of 0.07-0.09 mg/L and the contents of NO_2^- were between 073-0.79 mg/L.

Whetstone (2002) stated that the toxicity of ammonia and nitrite for shrimp is greatly dependent on environmental factors such as pH, dissolved oxygen, salinity, and temperature. For aquaculture purposes, these factors play an important role in the development, growth, and survival of species exposed to ammonia and nitrite. A suitable concentration of TAN and nitrite for culturing juvenile *P. monodon* (0.27 g) were 3.7 mg/L and 3.8 mg/L, respectively (Chen and Lei, 1990). Other study suggested that the suitable level of TAN for black tiger shrimp is between 0.2-2 mg/L and Boyd *et al.* (2002) confirmed that level of TAN in shrimp pond should not be higher than 3 mg/L.

From the cited literatures above, water quality parameters in the present study were within acceptable range for *P. monodon* growth. Therefore, feeding treatments could be the main factor affecting on the performances of experimental shrimp.

4.2 Shrimp performances

4.2.1. Experiment 1: Effect of fishmeal replacement with *Artemia* biomass as a protein source in practical diets on survival and growth of *P. monodon*

Survival of experimental shrimp after 45 days of feeding trial was not significantly different among treatments (P>0.05), ranging from 81.1 to 82.2% (Table 7).

The final weight of experimental shrimps was in the range of 0.89-0.97 g, of which larger weight of shrimp was found at higher *Artemia* protein inclusion in the test diets. Moreover, growth rate of *P. monodon* in terms of weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR) followed the same pattern as observed for final weight (Table 7). Statistical analysis indicated that significant differences was only seen between the control treatment (0% AT) and the 60% *Artemia* protein replacement level (P<0.05).

Feed conversion ratio (FCR) varied from 1.25 to 1.38, and showed slightly declined with increasing level of fishmeal protein with *Artemia* biomass protein in the diets. There was not statistical differences among feeding treatments (P>0.05).

Table 7. Survival, growth performance and feed conversion ratio in experiment 1

	0% AT	20% AT	40% AT	60% AT
Survival	81.1±0.9^{a}	82.2±3.8^{a}	82.2±5.1^{a}	81.1±5.1^{a}
Final weight(g)	0.89±0.08^{a}	0.91±0.07^{ab}	0.93±0.09^{ab}	0.97±0.09^{b}
Weight gain(g)	0.86±0.08^{a}	0.87±0.07^{ab}	0.90±0.09^{ab}	0.94±0.09^{b}
DWG (g/day)	0.019±0.002^{a}	0.019±0.001^{a}	0.020±0.002^{ab}	0.021±0.002^{b}
SGR (%/day)	7.31±0.21^{a}	7.35±0.17^{ab}	7.41±0.22^{ab}	7.50±0.22^{b}
FCR	1.38±0.05^{a}	1.34±0.02^{a}	1.30±0.10^{a}	1.25±0.09^{a}

Values are mean ± standard deviation. Mean values with different superscripts in the same row are significantly different ($P<0.05$)

Previous study found that dried *Artemia* biomass incorporated in the diets is very suitable for the post-larval white shrimp, *Litopenaeus vannamei* (Naegel *et al.*, 2004). The result in this study is in agreement with the study of Nguyen Thi Ngoc Anh *et al.* (2009), who evaluated the effect of fishmeal replacement with *Artemia* biomass as a protein source in practical diets for the giant freshwater prawn *Macrobrachium rosenbergii* for 30 days. They reported that higher survival and better growth performances of prawn were observed with increasing level of *Artemia* biomass protein included in the experimental diets. Similar findings were also reported by Nguyen Thi Ngoc Anh (2011), *Artemia* biomass can be used either as direct feed or as ingredient in formulated feeds for hatcheries and nurseries of brackish cultured species (mud crab, goby, black tiger shrimp) which enhance survival rate, growth and shorten the rearing time.

Artemia biomass contain high levels of the fatty acid 20:5n3 (eicosapentaenoic acid, EPA) and the ARA (20:4n6) which is important nutritional source for fish and crustacean larvae as these compounds improve the larval growth and pigmentation in fishes and crustaceans (Dhont and Sorgeloos, 2002).

4.2.2. Experiment 2: Effect of soybean meal replacement with gut weed as a protein source in practical diets on survival and growth of *P. monodon*

Survival of shrimp in experiment 2 was in the range of 84.4 to 88.9%. There was not significant differences ($P>0.05$) among feeding treatments (Table 8). This result indicated that using gut weed to replace soybean meal protein in the tiger shrimp diet did not affect their survival.

Table 8. Survival, growth performance and feed conversion ratio of shrimp in experiment 2

Treatment	0% GW	15% GW	30% GW	45% GW
Survival	86.7±5.8^{a}	88.9±1.9^{a}	86.7±1.3^{a}	84.4±5.1^{a}
Final weight(g)	0.94±0.11^{ab}	0.99±0.13^{c}	0.97±0.09^{bc}	0.94±0.10^{a}
Weight gain(g)	0.91±0.11^{ab}	0.96±0.13^{c}	0.94±0.09^{bc}	0.91±0.10^{a}
DWG (g/day)	0.020±0.002^{a}	0.021±0.003^{a}	0.021±0.002^{a}	0.020±0.002^{a}
SGR (%/day)	7.43±0.27^{a}	7.53±0.25^{b}	7.51±0.21^{b}	7.42±0.23^{a}
FCR	1.31±0.03^{b}	1.22±0.01^{a}	1.25±0.01^{a}	1.33±0.01^{b}

Values are mean ± standard deviation. Mean values with different superscripts in the same row are significantly different (P<0.05)

The mean initial weight of postlarvae was 0.03±0.005 g. After 45 days of feeding trial, final weight of experimental shrimp ranged from 0.94 to 0.99 g. Growth parameters in terms of weight gain, specific growth rate (SGR) in the control diet (0% GW) and 45% GW diet had was growth, both were significantly lower than those in treatments of 15%GW and 30% GW (P<0.05). However, daily weight gain (DWG) were not statistical differences (P>0.05) among the test diets (Table 8). Furthermore, it was noted that at the 15% soybean meal replacement level with gut weed protein, growth of shrimp was significantly higher than in the control group.

Regarding feed conversion ratio (FCR), the data showed similar pattern as observed for growth performance. FCR in all feeding treatments was between 1.22 and 1.33 of which in the treatments of 15% GW and 30%GW had relatively lower values compared to the control. Statistical differences were not found among feeding treatments.

Cruz-Suarez *et al.* (2006) reported that growth of the *Litopenaeus vannamei* was greater in the group fed pellets containing *Enteromorpha* than those with *Macrocystis* or *Ascophyllum*. Similarly, feed with *Enteromorpha* produced the best feed conversion ratio (1.78) at 28 days.

The results in the present study are in agreement with the study of Dinh Thi Kim Nhung (2013) who assessed soybean meal protein substitution with gut weed protein at the rates of 20%, 40% and 60% in practical diets for white leg shrimp postlarvae. Author reported that survival rate of white leg shrimp was not affected by the feeding treatments ranging from 81.1 to 87.8%. For growth rate and feed conversion ratio, at 40% soybean meal protein replaced by gut weed shrimp had similar values to those in the control.

4.2.3. Experiment 3: Effect combined substitution of *Artemia* biomass and gut weed protein for fishmeal and soybean meal protein in practical on survival and growth of *P. monodon*

Table 9 showed that after 45 days of feeding trial, survival of experimental shrimp varied from 81.1 to 86.7% and they were not significantly different among treatments (P>0.05).

The results showed that when using *Artemia* biomass to replace fishmeal and gut weed replace soybean meal in the diet, growth rate of shrimps were better than those in the control. Final weight of experimental shrimp ranged from 0.97 to 1.08g. Growth parameters such as weight gain and specific growth rate (SGR) were significantly higher (P<0.05) than in the control (Table 9).

Table 9. Survival, growth performance and feed conversion ratio of shrimp in experiment 3

Parameters	Control	20%AT+ 15%GW	40%AT+ 30%GW	60%AT+ 45%GW
Survival	84.4±5.1^{a}	83.3±5.8^{a}	86.7±3.3^{a}	81.1±5.1^{a}
Initial weight (g)	0.02±0.001	0.02±0.001	0.02±0.001	0.02±0.001
Final weight (g)	0.97±0.10^{a}	1.07±0.14^{b}	1.08±0.15^{b}	1.03±0.11^{ab}
Weight gain(g)	0.95±0.10^{a}	1.05±0.14^{b}	1.06±0.15^{b}	1.01±0.11^{ab}
DWG (g/day)	0.021±0.002^{a}	0.023±0.003^{a}	0.023±0.003^{a}	0.023±0.002^{a}
SGR (%/day)	8.62±0.22^{a}	8.83±0.26^{b}	8.85±0.29^{b}	8.76±0.24^{ab}
FCR	1.37±0.03^{c}	1.26±0.03^{ab}	1.22±0.05^{a}	1.30±0.02^{b}

It was found that experimental diets containing up to the level of 60% AT+45% GW, shrimp growth was relatively higher than the control treatment but significant difference was not observed (P>0.05).

Considering feed conversion ration (FCR), the result in Table 8 exhibited that FCR was highest (1.37) in the control treatment and significant differences (P<0.05) from other feeding treatments (1.22-1.30).

This experiment indicated that when combined substitution of *Artemia* biomass for fishmeal up to 60% and gut weed for soybean meal up to 45% in the diet, growth performance of P. *monodon* was significantly improved and feed conversion ratio was reduced.

It was found that fatty acids play important roles in the nutrition of crustaceans. Their main functions are as a source of energy and for the maintenance of the functional integrity of bio-membranes. The highly unsaturated fatty acid (HUFA) content of artificial diets has been found to have a strong impact on the survival, growth, feed conversion ratio (Leger, *et al*., 1986). Previous studies reported that *Artemia* biomass contain high level of essential unsaturated fatty acids such as eicosapentaenoic acid (EPA), arachidonic acid (ARA), Linolenic acid (LNA)… are rich in protein, pigmentation which is vital nutritional source as these compounds improve the shrimp growth (Leger, *et al*., 1986; Dhont and Sorgeloos, 2002). According to Nguyen Thi Ngoc Anh *et al.* (2009) diet containing *Artemia* biomass meal enhance palatability and more attractive to the prawns than the fishmeal control diet. Furthermore, Aguilera-Morales *et al*. (2005) found that gut weed *Enteromorpha* spp also have high nutritional values: 9-14% protein, fatty acid content n3 and n6, respectively, 10.4 and 10.9 g/100 g in total fatty acids and are rich in amino acid and protein digestibility of gut weed are high (98%). Similar finding were reported by Nguyen Thi Ngoc Anh *et al*. (2012), gut weed contain 9 essential amino acids and 9 non-essential amino acids and are rich in protein, fatty acid which is suitable food for fish and shrimp.

From literature cited above, it can explain diet containing both *Artemia* biomass and gut weed to improve growth rate of *P. monodon* and reduce feed conversion ratio.

Chapter 5

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

- Using *Artemia* biomass and gut weed *Enteromorpha* sp. as protein source in the diet did not affect survival of shrimp *P. monodon*.
- *Artemia* biomass can substitute fishmeal protein up to 60% in the diet for shrimp *P. monodon*.
- Gut weed *Enteromorpha* sp. may replace soybean meal protein up to 45% in the diet for shrimp *P. monodon*.
- Combined substitution of *Artemia* biomass and gut weed in the *P. monodon* diet obtained similar results as observed for single replacement.

5.2. Recommendation

Evaluating *Artemia* biomass and gut weed *Enteromorpha* sp. as protein source at higher level in the practical diet to find out the maximum appropriate percentage substitution for the tiger shrimp diet.

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