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Green Water Meal as Protein and Carotenoid Sources in Grow-out Diets for Pacific White Shrimp, *Litopenaeus vannamei*

(Tepung Air Hijau sebagai Punca Protein dan Karotenoid di dalam Diet Tumbesaran Udang Putih Pasifik, *Litopenaeus vannamei*)

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ABSTRACT

The present study was conducted to evaluate the potential of green water meal (GWM) as an alternative dietary ingredient for juvenile Pacific white shrimp, Litopenaeus vannamei. *Five isoproteic and isolipidic diets were formulated with 0% (GWM0, control diet), 10% (GWM10), 20% (GWM20), 30% (GWM30) and 40% (GWM40) of GWM replacing fishmeal protein and fed five times daily to triplicate groups of shrimp with an average initial weight of 6.42±0.02 g. In general, growth performance and feed utilization of shrimp fed with GWM10 did not show any significant differences with the control diet. Survival rate was above 88% and not affected by the dietary treatments. The whole-body protein and lipid of the shrimps decreased with the increasing GWM level in the diets. The shrimps fed with the GWM-based diets (GWM10, GWM20, GWM30 and GWM40) presented more intense red/orange colour and contained higher total carotenoid concentration compare with the control diet. The present findings suggested that GWM is an excellent source of carotenoid for shrimp pigmentation and able to replace fishmeal protein at up to 10% replacement level.*

Keywords: Fishmeal replacement; microalgae meal; Pacific white shrimp; pigmentation

ABSTRAK

Kajian ini telah dijalankan untuk menilai potensi tepung air hijau (GWM) sebagai sumber ramuan alternatif dalam pemakanan juvenil udang putih Pasifik, Litopenaeus vannamei. *Lima diet isoproteik dan isolipidik telah dirumuskan dengan 0% (diet kawalan), 10% (GWM10), 20% (GWM20), 30% (GWM30) dan 40% (GWM40) GWM menggantikan protein daripada tepung ikan dan diberi makan lima kali sehari kepada tiga kumpulan udang dengan berat purata awal 6.42±0.02 g. Secara keseluruhannya, prestasi pertumbuhan dan penghadaman makanan untuk udang yang diberi makan dengan diet GWM10 tidak menunjukkan perbezaan yang signifikan dengan diet kawalan. Kadar kelangsungan hidup adalah lebih daripada 88% dan tidak dipengaruhi oleh diet pemakanan. Kandungan protein dan lipid udang menurun dengan tahap GWM yang semakin meningkat di dalam diet. Udang yang diberi makan dengan diet yang berasaskan GWM (GWM10, GWM20, GWM30 dan GWM40) menunjukkan warna merah/jingga yang terang dan mengandungi jumlah kandungan karotenoid yang lebih tinggi berbanding dengan diet kawalan. Hasil kajian ini menunjukkan bahawa GWM adalah sumber karotenoid yang sangat baik untuk pigmentasi udang dan boleh menggantikan protein daripada tepung ikan sehingga 10% tahap penggantian.*

Kata kunci: Penggantian tepung ikan; pigmentasi; tepung mikroalga; udang putih pasifik

INTRODUCTION

Fishmeal has been used as the major source of protein in aquafeeds and contributes considerably to the variable production cost in any aquaculture farms. It is a preferred source of protein due to the balanced profile of essential amino acid and also the presence high palatability compare to other protein sources (Tacon & Akiyama 1997). However, the uncertainty in world production and high price of fishmeal has threatened the growth and sustainability of the aquaculture industry (Tacon et al. 2011). As a result, most of aquaculture nutritionists and the aquafeed industry are focusing on finding cheaper and sustainable alternative protein source that can be incorporated in the aquafeeds (Muin et al. 2015). Several studies were conducted to replace fishmeal in the formulated feeds for Pacific white shrimp, *Litopenaeus vannamei* with various alternative protein sources from either plant or animal origins such as seaweed meal (Silva & Barbosa 2008), soybean meal and peanut meal (Yue et al. 2012), red crab meal (Goytortúa-Bores et al. 2006), yeast (Mc Lean et al. 2006), poultry by-product (Cruz-Suárez et al. 2007), rendered meat and bone meals (Forster et al. 2003) and hemoglobin powder (Chookird et al. 2010). More effort is needed to evaluate the potential of other alternative ingredients to support the rapid growth of the industry.

Green water is a common term used in aquaculture farms to describe the presence of green microalgae in the fish culture tank. Generally, green water consist mainly *Chlorella* sp. (dominant species), *Scenedesmus* sp., *Pediastrum* sp., *Coelastrum* sp., *Planktosphaeria* sp.

and other microorganism such as bacteria, protozoa and zooplankton. Recently, microalgae have been successfully used in aquafeed either as protein substitution for fishmeal or as a valuable addictive (Ju et al. 2012, 2011). Therefore, investigation on the potential use of green water meal as an alternative protein source in formulated diets deserved thorough investigations. In our previous study (Basri et al. 2015), GWM has been successfully used to replace fish meal at a low inclusion level and has successfully enhanced the colouration of the early stage of *L. vannamei* (~1.7 g). It is hypothesized that larger shrimp will have a better tolerance to this plant protein source. The present study aimed to validate this hypothesis.

L. vannamei is considered an economically important species in aquaculture industry for most country in the world (FAO 2012). Generally, colour of *L. vannamei* plays important attributes in determining the market value and shrimp acceptance (Tume et al. 2009). The orange-red colour of the cooked shrimp is dependent upon the presence of carotenoid pigments and associated with the quality and freshness of the shrimp (Latscha 1989). The main carotenoid pigment that present in the external tissue of the shrimp is astaxanthin in which obtained naturally from microalgae in the environment and commonly supplemented in the diets for the cultured shrimp (Boonyaratpalin et al. 2001; Yamada et al. 1990). In the present study, the potential of this green water meal as a substitute of fishmeal in the diets of *L. vannamei* was evaluated to determine the effect on growth performance, survival, feed utilization, body composition and pigmentation of the juvenile *L. vannamei*.

MATERIALS AND METHODS

GREEN WATER

The production of green water meal was carried out based on the method described by Basri et al. (2015). The green water was randomly sampled daily for microalgae identification and cell counting. The green water was observed under a light microscope (Eclipse 80_i, Nikon) to identify the genus of microalgae presence in the green water culture based on the morphological structure illustrated and described by Bellinger and Sigee (2010).

INGREDIENTS AND EXPERIMENTAL DIETS

Five isonitrogenous and isolipidic diets were formulated to contain 40% crude protein and 10% crude lipid fulfilling the requirement of *L. vannamei* (Ju et al. 2008). The diets were formulated to substitute fishmeal protein with the green water meal at 0% (as control diet), 10%, 20%, 30%, and 40% replacement levels which labelled as GWM0, GWM10, GWM20, GWM30 and GWM40, respectively (Table 1).

All experimental diets were prepared in the Aquaculture Feed Laboratory of Borneo Marine Research Institute, UMS. Table 2 shows the proximate composition fishmeal, green water meal and shrimp meal used in the experimental diets. The dry ingredients such as fishmeal, green water meal, shrimp meal, α-Cellulose, cholesterol, vitamin, mineral and CMC were thoroughly mixed manually by hand to obtain homogenous mixture. Then, the dietary lipid source was added to the mixture and well-mixed

TABLE 1. Diets ingredient composition (g/100 g dry weight)

Ingredients	Diets				
	GWM0	GWM10	GWM20	GWM30	GWM40
Fish meal[a]	43.42	37.77	34.74	30.40	26.05
Green water meal	0.00	7.89	15.77	23.66	31.55
Shrimp meal[b]	6.79	6.79	6.79	6.79	6.79
Fish oil[c]	5.50	5.95	6.15	6.47	6.79
Carboxymethylcellulose[d]	5.00	5.00	5.00	5.00	5.00
Vitamin premix[e]	3.00	3.00	3.00	3.00	3.00
Mineral premix[f]	3.00	3.00	3.00	3.00	3.00
Cholesterol[g]	0.50	0.50	0.50	0.50	0.50
Tapioca starch[h]	23.41	21.08	18.74	16.40	14.06
α - Cellulose[i]	9.37	9.02	6.31	4.79	3.26

[a] Danish fish meal, Denmark
[b] Self-produced of whole body shrimp
[c] Dexchem Industries Sdn. Bhd, Malaysia
[d] EMD Chemicals, Inc. San Deigo, CA.
[e] Vitamin premix. Contained (as g/kg): ascorbic acid, 45; inositol, 5; niacin, 4.5; riboflavin, 1;pyridoxine. 1; thiamin mononitrite, 0.92; retinyl acetate, 0.6; cholecalciferol, 0.083; menadione sodium bisulphite, 1.67; biotin, 0.02; folic acid, 0.09; DL-α-tocopheryl acetate, 8; vitamin B12, 0.00135; Dexchem Industries Sdn. Bhd, Malaysia
[f] Mineral premix. Contained (as g/kg): calcium phosphate.H2O (MDCP), 397.65; calcium lactate, 327; ferrous sulphate. H2O, 25; magnesium sulphate.7H2O, 137; potassium chloride, 50; sodium chloride 60; potassium iodide, 0.15; copper sulphate.$5H_2O$, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite.$5H_20$, 0.02. Dexchem Industries Sdn. Bhd, Malaysia
[g] Sigma-Aldrich Corporation, USA
[h] AAA Brand, Bake With Me Sdn. Bhd., Malaysia
[i] Sigma-Aldrich Corporation, USA

TABLE 2. Proximate composition of the main ingredients (g/100 g dry weight) used in the experimental diets

Ingredients	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Ash (%)	Moisture (%)
Fish meal	69.30	9.70	ND	13.63	10.60
Green water meal	39.04	1.56	8.36	5.97	0.24
Shrimp meal	73.63	4.19	ND	10.36	5.70

ND not determined
*Green water meal contained 1.207 mg g-1 of total carotenoid concentration

again. Finally, cooked tapioca starch was mixed with the mixture to obtain moist dough. For pelleting, the dough was screw-pressed through a meat grinder fitted with 2.0 mm diameter die. The resulting spaghetti-like strands were dried in an oven at 45±2°C until the moisture level was less than 10% (3-4 h). A small proportion of the prepared diets then were taken for the proximate composition following method described by AOAC (1997). All prepared diets were stored in labelled plastic bag and kept in a refrigerator at -20°C until use.

EXPERIMENTAL DESIGN AND FEEDING TRIAL

Feeding trial was conducted using a flow-through seawater system located at the Shrimp Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. The system was designed for 3 replicates for each diet treatment using 15 cubical fiberglass tanks (50 L). All the tanks were supplied with constant seawater flow (0.5 L/min) and provided with continuous aeration through airstones (1.5 L/min). The tanks were also covered with netting on top to prevent shrimp escape.

The juvenile *L. vannamei* was obtained from the Shrimp Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah, stocked in a 1000 L tank and fed with commercial shrimp pellet with 42% crude protein and 6% crude lipid (Cargill, Malaysia) for a week during the acclimatization process. A total of 15 juvenile shrimp with a mean initial body weight of 6.42±0.02 g were distributed randomly into each of 15 cubical fiberglass tanks. At the beginning of each feeding trials, 30 juvenile shrimps were randomly sampled and stored in a freezer (-15ºC) until further analysis of initial body proximate composition.

The shrimps were fed 5 times (0700, 1000, 1300, 1600 and 1900 h) with amount of 5-7% of their body weight. Every morning, any dead shrimp was recorded and the remaining uneaten feed was visually estimated prior to bottom cleaning. During the feeding trials, the temperature, dissolved oxygen and salinity ranged from 27-31°C, 5-6 mg/L and 30-32 ppt, respectively. Every 2 weeks, total weight of the shrimps in each tank was measured to adjust the daily amount of feed. The feeding trial was conducted for 56 days, upon termination, the final body weight and total length of the remaining shrimp in all tanks were recorded individually and 10 shrimp from each tank were sampled for whole-body proximate composition, colour comparison and total carotenoid concentration analysis following method described by (Arredondo-Figueroa et al. 2003).

CALCULATIONS

The growth performance, feed utilization efficiency and survival rate of the shrimps fed with the experimental diets were calculated using the following formula:

a. Body weight gain (WG; %) = 100 × (final body weight - initial body weight) / (initial body weight)
b. Specific growth rate (SGR; %/day) = [Ln (final body weight) - Ln (initial body weight)/days] × 100
c. Dry feed intake (DFI; g) = FI × (1 - LDM/100), Where, Lost dry matter (LDM; %) = [(weight of feed before immersion - weight of feed after immersion) / (weight of feed before immersion)] × 100
d. Feed intake (FI; g) = Σi [(feed intake on i^{th} day) / (number of shrimp on i^{th} day)]
e. Feed conversion ratio (FCR) = DFI / weight gain
f. Protein efficiency ratio (PER) = total weight gain (g) / total protein intake
g. Apparent net protein utilization (ANPU; %) = 100 × [(final body protein - initial body protein) / total protein intake]
h. Survival (%) = 100 × (final count of the shrimps) / (initial count of the shrimps)

STATISTICAL ANALYSIS

Data are expressed as means ± standard error. The mean data of growth performance, feeding utilization efficiency, survival rate, whole body composition and total carotenoid concentration were subjected to one-way analysis of variance tests (ANOVA) and Tukey HSD to determine if significant difference ($p<0.05$) existed among the experimental treatments. The statistical analysis was done by using SPSS v 18.0 for Windows platform.

RESULTS

GREEN WATER

Several species of microalgae found in the green water were mostly from Chlorophyta division. The identified microalgae species include *Chlorella* sp. *Scenedesmus* sp., *Pediastrum* sp. and *Coelastrum* sp. with *Chlorella* sp. being

the most dominant species which constitutes up to~70% of the total microalgae found in the green water production.

EXPERIMENTAL DIETS

Table 3 shows the proximate composition of the experimental diets for the juvenile *L. vannamei*. The crude protein and crude lipid of the experimental diets were slightly lower than the calculated values (crude protein 38.20 - 40.09%; crude lipid 8.04 - 9.21%). The estimated energy value of the experimental diets ranged from 355.72 (GWM10) to 364.85 (GWM30) kcal/100 g.

GROWTH PERFORMANCE, SURVIVAL RATE AND FEED UTILIZATION EFFICIENCY

The initial body weight (g), final body weight (g), body weight gain (%), and SGR (%/day) of the shrimps fed with the five experimental diets are shown in Table 4. The five groups of shrimps presented an initial mean body weight of 6.42±0.02 g and attained final body weight ranging from 12.35 to 17.24 g. Body weight gain of the shrimps throughout the feeding trial ranged from 90% (GWM40) to 170% (GWM0). The control diet (GWM0) presented the highest value of body weight gain (170.00±5.6%), but was not significantly different from GWM10 (161.14±3.07%). Weight gain of prawn fed GWM20 (129.84±1.50%), GWM 30 (120.04±2.33%) and GWM40 (90.00±4.15%) was significantly lower than the control diet and GWM0 and GWM10. Similar with body weight gain, specific growth rate (SGR) of GWM10 (1.50±0.04%/day) was also not significantly different from the control diet (1.56±0.02%/day).

DFI, FCR, PER, and ANPU of shrimps fed with experimental diets are shown in Table 5. After 56 days feeding trial, the DFI ranged from 16.36 (GWM40) to 19.99 (GWM0) where DFI were decreased with the increasing level of GWM in the diets. In term of palatability, the juvenile *L. vannamei* readily accept the GWM-based diets with inclusion level of up to 10%. Feed intake start to reduce significantly when GWM was included at more 20% replacement level. Feed conversion ratio (FCR) ranged from 1.85 to 2.80 where the best value of FCR was observed in shrimp fed with GWM0 (1.85±0.07) but not significantly differ with GWM10 (1.89±0.04). PER and ANPU revealed similar trends where the values were decreased from GWM20 to GWM40. Highest PER and ANPU values were observed in GWM0 (1.38% and 23.77), but not significantly differ with GWM10 (1.37% and 22.72). Survival rate of the shrimps fed experimental diets are high and ranged from 88.89% to 91.11% (Figure 1).

BODY COMPOSITION OF WHOLE-BODY SHRIMP

Table 6 shows the proximate composition of the whole body shrimp (% wet weight). Initial shrimp whole body composition was 74.46% moisture, 18.08% protein, 1.15% lipid and 2.69% ash. The moisture and crude protein contents of the shrimp fed experimental diets ranged from 75.88 - 78.48% and 15.82 to 17.66 %, respectively. While the crude lipid contents of the whole body shrimp in the present study ranged from 0.30 to 0.54%. The crude ash of shrimp fed with the experimental diets ranged from 2.90 to 3.27% and no significant different was detected. In general, the moisture contents of the shrimp fed experimental diets

TABLE 3. Proximate composition of the experimental diets

	Diets				
	GWM0	GWM10	GWM20	GWM30	GWM40
Moisture (%)	9.45	10.29	10.26	10.34	10.30
Ash (%)	11.48	11.08	11.11	10.86	10.83
Crude protein (%)	39.32	38.75	40.09	38.76	38.20
Crude lipid (%)	8.85	8.04	8.89	9.21	9.11
Crude fiber (%)	9.81	10.04	9.51	9.44	9.47
NFE	30.54	32.09	30.4	31.73	32.39
Gross energy (kcal/100 g)	359.09	355.72	361.97	364.85	364.35

NFE, nitrogen free extract

TABLE 4. Growth performance of shrimp fed with the experimental diets

Diets	Initial weight (g)	Final weight (g)	Weight gain (%)	SGR (%/day)
GWM0	6.39±0.06^{a}	17.24±0.37^{c}	170.00±5.6^{c}	1.56±0.02^{c}
GWM10	6.46±0.01^{a}	16.88±0.18^{c}	161.14±3.07^{c}	1.50±0.04^{c}
GWM20	6.37±0.03^{a}	14.65±0.09^{b}	129.84±1.50^{b}	1.32±0.04^{bc}
GWM30	6.35±0.02^{a}	13.97±0.12^{b}	120.04±2.33^{b}	1.20±0.03^{ab}
GWM40	6.50±0.06^{a}	12.35±0.16^{a}	90.00±4.15^{a}	0.97±0.10^{a}

$^{a-c}$ Mean values with different superscript within column are significantly different ($p<0.05$).
SGR, specific growth rate

TABLE 5. Feed utilization efficiency of shrimp fed with the experimental diets

Diets	DFI (g/shrimp)	FCR	PER	ANPU (%)
GWM0	19.99±0.20^{d}	1.85±0.07^{a}	1.38±0.05^{c}	23.77±0.98^{c}
GWM10	19.68±0.04^{d}	1.89±0.04^{a}	1.37±0.03^{c}	22.72±0.23^{c}
GWM20	18.21±0.06^{c}	2.20±0.03^{b}	1.13±0.02^{b}	18.33±0.52^{b}
GWM30	17.44±0.08^{b}	2.29±0.05^{b}	1.13±0.02^{b}	15.75±0.25^{a}
GWM40	16.36±0.07^{a}	2.80±0.10^{c}	0.94±0.03^{a}	14.09±0.53^{a}

DFI, dry feed intake
FCR, feed conversion ratio
PER, protein efficiency ratio
ANPU, apparent net protein utilization
$^{a-d}$ Mean values with different superscript within column are significantly different (p<0.05)

TABLE 6. Proximate composition of shrimp (% wet body weight) fed with experimental diets

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
GWM0	75.88±0.61^{ab}	17.66±0.08^{d}	0.54±0.01^{c}	2.99±0.05^{a}
GWM10	76.50±0.16^{bc}	17.29±0.12^{cd}	0.52±0.01^{c}	3.09±0.15^{a}
GWM20	76.62±0.19^{bc}	17.03±0.20^{c}	0.54±0.02^{c}	3.27±0.04^{a}
GWM30	78.48±0.25^{d}	15.82±0.13^{a}	0.42±0.01^{b}	2.90±0.06^{a}
GWM40	77.99±0.31^{cd}	16.39±0.03^{b}	0.30±0.03^{a}	3.03±0.05^{a}

Whole body proximate for initial shrimp was 74.46% moisture, 18.08% protein, 1.15% lipid and 2.69% ash.
$^{a-d}$ Mean values with different superscript within column are significantly different (p<0.05)

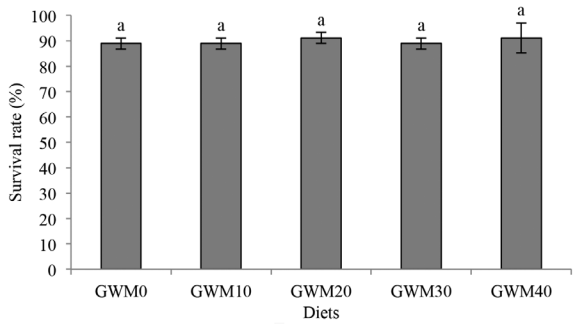

FIGURE 1. Survival rate of shrimp fed with the experimental diets

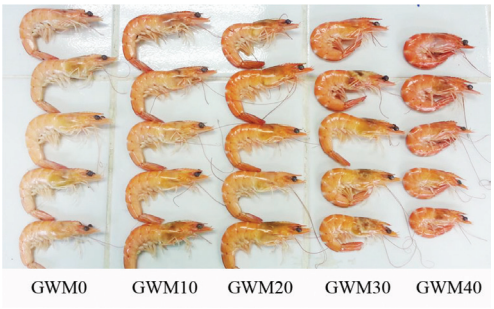

FIGURE 2. Colour of boiled-shrimp fed with the experimental diets

seem to increase with increasing level green water meal in the diets. Meanwhile, crude protein and crude lipid decreased with the increase of GWM in the diets.

SHRIMPS COLOUR AND TOTAL CAROTENOID CONCENTRATION

There was a distinct difference in intense red/orange colouration of the shrimps fed with the experimental diets (Figure 2). Shrimp fed with GWM-based diets appeared to be more red/orange compare to the control diet and the reddest shrimp observed in shrimp fed with GWM40. The total carotenoids also confirmed the visual observation, where the amount of total carotenoid increased with the increase of GWM in the diets. Feeding shrimp with GWM had increased the total carotenoid concentration from 8.09 (control) to 32.78 µg/g (GWM40) at the end of the feeding trial. This was an increase of about 305% in total carotenoid concentration of the experimental shrimp (Figure 3).

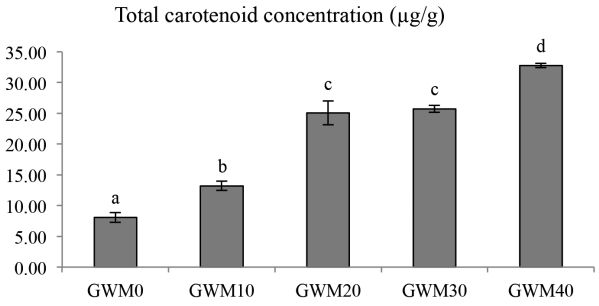

FIGURE 3. Whole-body total carotenoid concentration (µg/g) of shrimp at the end of the experiment

DISCUSSION

A few studies reported the replacement of fishmeal with alternative protein from algae sources in the diet of various aquaculture species such as dried *Chlorella* spp., *Scenedesmus* spp. and *Hydrodictyon reticulatum*

in *Oreochromis niloticus* diets (Appler 1985; Badwy et al. 2008), *Spirulina platensis* in *Cyprinus carpio* (Nandeesha et al. 1998), *Nannochloropsis* sp. and *Isochrysis* sp. in *Gadus morhua* (Walker & Berlinsky 2011) and *Spirulina* in *Pangasianodon gigas* (Tongsiri et al. 2010). Specifically, limited studies were reported on the use of algae meal as protein sources in *L. vannamei* diets, except for those reported using *Spirulina platensis* (Macias-Sancho et al. 2014) and *Haematococcus pluvialis* (Ju et al. 2012).

Similar to our previous findings (Basri et al. 2015), replacement of fish meal protein with GWM at 10% replacement level was tolerable by the shrimp. However, when fed with GWM20, GWM30 and GWM40, the growth of the shrimps were reduced. Hasan and Chakrabarti (2009) stated that only about 10-15% of dietary protein requirement can be met by algae protein. Higher inclusion of algae meal in the diet was suggested to cause the poor palatabilty and thus decreased the growth performance of the cultured species. On the contrary, defatted pure microalgae (*Haematococcus pluvialis*) meal was successfully substitute the fishmeal protein up to 50% replacement level in the diet of juvenile *L. vannamei* (Ju et al. 2012). Besides that, *Spirulina platensis* meal was reported to be able to replace fishmeal at up to 75% replacement level in *L. vannamei* with improvement in the immune system (Macias-Sancho et al. 2014).

In other study, microalgae meal from a combination of *Nannochloropsis* sp. and *Isochrysis* sp. can only replace the fishmeal protein at 15% replacement level in the diet of Atlantic cod, *Gadus morhua* (Walker & Berlinsky 2011). They found, the growth performance and feed intake of the Atlantic cod were significantly reduced as algal incorporation in the diet increased and suggested this was due to the poor palatability of the diet (Walker & Berlinsky 2011). Meanwhile, in the study conducted by Tongsiri et al. (2010), the Mekong giant catfish, *Pangasianodon gigas* can only tolerate up to 10% of fishmeal protein replacement with *Spirulina* meal in their diet. In general, most study on fishmeal replacement using microalgae meal yielded various successful inclusion levels and mostly using the pure microalgae meal such as *Spirulina* spp., *Nannochloropsis* sp. and *Isochrysis* sp. (Tongsiri et al. 2010; Ungsethaphand et al. 2010; Walker & Berlinsky 2011).

The apparently lower growth performance of shrimp fed with diets containing higher inclusion levels of green water meal may be attributed by several factors. One of the factors is most likely due to insufficient essential amino acid such as lysine and tryptophan in green water meal. *Chlorella* sp. is the dominant microalgae in the GWM used in the present study. In general, algae are considered as high nutritional value food, but most of them contained low proportion of essential amino acid especially lysine, a limiting factor in the animal diets (Brown et al. 1997). Specifically, *Chlorella* sp. was reported to contain a lesser proportion of tryptophan, also one of the essential amino acids in the diets of aquatic animals (Brown & Jeffrey 1992). Another factor caused the lower performance of the shrimp maybe due to the low digestibility of the microalgae which is similar to those of other plant sources. Most of the aquatic plants including algae contained higher percentage of carbohydrate which account up to 40% and characterized by complex chemical structure. Therefore, increased algal meal in the diets may cause the low digestibility due to the presence of indigestible complex carbohydrate from the algal materials (Appler 1985).

In this present study the utilization of GWM in larger juvenile shrimp was improved compare to the smaller juvenile shrimp in our previous study using early juvenile as the target species (Basri et al. 2015). The poorer GWM utilization compare to the previous study might due to lower digestibility by the less developed digestive system of the smaller juvenile shrimp. Furthermore, larger sizes of organisms were reported to better utilize the plant protein source (Shapawi et al. 2013).

In term of palatability, the juvenile *L. vannamei* seems to readily accept the GWM-based diets with inclusion level of up to 10%. Feed intake start to reduce significantly when GWM was included at 20% replacement. Similarly, palatability was also affected with the increasing level of microalgae meal in the diets of *L. vannamei* (Ju et al. 2012). PER and ANPU were decreased as increasing GWM in the diets, even though no significant difference detected between the control diet and GWM10. Lower PER and ANPU values in shrimp fed with more than 20% GWM replacement were probably due to the reduced growth rate and the lower whole-body protein content (Shapawi et al. 2007). In the present study, survival rate of the shrimps fed experimental diets were more than 88%, indicating that the inclusion of green water meal in the diets did not affect the survival rate of the *L. vannamei*.

The proximate composition analysis of the whole body shrimp shows that the crude protein and crude lipid of the juvenile shrimp seem to decrease as the level of GWM increased in the diets. The low protein and lipid content of shrimps fed with higher inclusion of GWM in the diet (GWM30 and GWM40) might be due to the utilization of own stored body protein and lipid as consequences of the low feed intake. Similar finding were also reported previously, where the muscle lipid level of the Red sea bream, *Pagrus major* was significantly reduced in the diet containing *Spirulina* sp. compare to diet without *Spirulina* sp. (Mustafa et al. 1994). Diets containing algae protein from *Hydrodictyon reticulatum* (Appler 1985) and *Cladophora glomerata* (Appler & Jauncey 1983) also displayed the same effect, where low lipid content was observed in the Nile tilapia muscle. In contrast, replacement of fish meal with *Haematococcus pluvialis* meal in the diets of Pacific white shrimp did not affect the whole-body proximate composition (Ju et al. 2012).

When boiled or cooked, the individual carotenoid prosthetic group from the carotenoproteins in shrimp body was denatured and thus produced red/orange body

colour (Okada et al. 1994). The intensity of the boiled-shrimp colour is dependents on the amount of deposited carotenoid (Ponce-Palafox et al. 2006). In the present study, the colour intensity of the cooked shrimp was visually differentiated. Total carotenoid analysis also confirmed the visual observation where increased inclusion level of GWM in the diet resulted in higher TCC in shrimp compare to the control treatment. Therefore, the present study suggests that increasing level of GWM in the diets cause better pigmentation to the shrimp. Pigment content of microalgae in the GWM may be deposited directly or indirectly in the tissue of the shrimp (Yamada et al. 1990). Several authors have reported that supplementation or inclusion of microalgae meal in the fish or shrimp diet such as *Haematococcus pluvialis* (Ju et al. 2011), *Chlorella vulgaris* (Gouveia et al. 2003), *Dunaliella salina* (Supamattaya et al. 2005) and *Spirulina platensis* (Sun et al. 2012) have proved to enhance the skin and muscle pigmentation of the targeted aquatic animals. In contrast, Tangeras and Slinde (1994) observed that diet containing algae *Phaffia rhodozyma* did not show any pigment enhancement in salmonid fish muscle. They suggested this might be due to the cell wall thickness of *Phaffia rhodozyma* restricted the pigment availability (Tangeras & Slinde 1994). In the application of *Haematocuccus pluvialis* in Pacific white shrimp diets, the enhanced pigmentation was primary due to deposition of the natural esterified astaxanthin rather than other carotenoid (lutein, β-carotene and canthaxanthin), of which derived from the *Haematocuccus pluvialis* (Ju et al. 2011). Gouveia et al. (1998) also proved that *Chlorella vulgaris* biomass is a digestible and effective source of carotenoid pigments that produce higher retention of astaxanthin in fish muscle compare to the synthetic pigments.

The market value of shrimp is often evaluated based on the visual appearance of their body colour (Parisenti et al. 2011). The shrimp appearance is associated with freshness and product quality and thus plays a significant role in maintaining the high consumer acceptance in the marketplace (Latscha 1989; Parisenti et al. 2011). Astaxanthin was found to be the main pigment responsible producing the red/orange colour of cooked or boiled shrimp (Ju et al. 2011; Ponce-Palafox et al. 2006). Mostly shrimp feed are supplemented with astaxanthin derived from synthetic source such as Carophyll Pink® to enhance the pigmentation of the shrimp before harvested (Ju et al. 2011). However, the cost of synthetic astaxanthin is high and there is consumer interest in the use of natural pigments in the seafood product (Boonyaratpalin et al. 2001). Therefore, this study has provided the opportunity for application of natural carotenoid source from the GWM in aquaculture feed industry. It is suggested that higher of inclusion GWM can be included in the pre-harvest diet to avoid the negative effect on the shrimp growth performance. However, more study should be carried out in the future to evaluate the full potential of GWM as a dietary ingredient.

CONCLUSION

Findings showed that the green water meal can serve as a high potential protein source which can be included in the diet at a level of 10% without significant negative effect on the growth performance and feed utilization of the Pacific white shrimp particularly in larger juvenile shrimp. Considering the continuous increase of fish meal price and limited supply, the findings from the present study are considered significant as it discover another high potential alternative ingredient which can be produced easily in aquaculture farms. Furthermore, the shrimp fed with the green water meal-based diets appeared to be more intense in red/orange colour and this clearly indicated that GWM is an excellent source of carotenoid that can enhance the shrimp pigmentation. Apparently, the use of GWM in the aquaculture diets will be able to reduce the dependency on imported feed ingredient and support the sustainability of aquaculture industry.

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