

## Utilization of green algae *Caulerpa racemosa* as feed ingredient for tiger shrimp *Penaeus monodon*

### Pemanfaatan rumput laut *Caulerpa racemosa* sebagai bahan baku pakan udang windu *Penaeus monodon*

Widya Puspitasari<sup>1</sup>, Dedi Jusadi<sup>1</sup>, Mia Setawati<sup>1</sup>, Julie Ekasari<sup>1</sup>, Abidin Nur<sup>2</sup>, Iwan Sumantri<sup>2</sup>

<sup>1</sup>Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University (Bogor Agricultural University)

<sup>2</sup>Main Center for Brackishwater Aquaculture Development Jepara Central Java, Indonesia.

\*Corresponding author: D. Jusadi, sifounder@gmail.com

(Received August 4, 2017; Accepted May 15, 2019)

#### ABSTRACT

The study aimed to evaluate the utilization of seaweed *Caulerpa racemosa* as feed ingredient for tiger shrimp *Penaeus monodon*. This research consisted of two different stages, i.e. digestibility and growth test. Tiger shrimp with average body weight of  $5.70 \pm 0.42$  g was reared during digestibility test. The measured parameters were total protein, calcium, magnesium, and energy digestibility. The growth test was managed by using a completely randomized design consisted of four different treatments (in triplicates) of dietary *C. racemosa* meal addition levels, i.e. 0 (control), 10, 20, and 30%. Tiger shrimp with an average body weight of  $0.36 \pm 0.02$  g were cultured for 42 days in plastic containers (70×45×40 cm) with a stocking density of 15 shrimp/container. Apparent dry matter, protein, calcium, magnesium, and energy digestibilities of *C. racemosa* were 51.82, 88.67, 68.44, 16.39, 60.30%, respectively. The results presented that the growth performance of tiger shrimp fed with diet containing 10% of *C. racemosa* was not significantly different with the control ( $P>0.05$ ). However, the growth performance of the shrimp fed with diet containing more than 20% of *C. racemosa* decreased. The enzyme activity of superoxide dismutase (SOD) increased with the higher level of dietary addition of *C. racemosa*. It can be concluded that *C. racemosa* was possibly applied up to 10% in the feed formulation for tiger shrimp.

Keywords: *Caulerpa racemosa*, *Penaeus monodon*, digestibility, growth performance, shrimp

#### ABSTRAK

Penelitian ini bertujuan mengevaluasi pemanfaatan rumput laut *Caulerpa racemosa* sebagai bahan baku pakan udang windu *Penaeus monodon*. Penelitian ini dilakukan dengan dua tahap, yaitu uji pencernaan *C. racemosa* dan uji pertumbuhan udang. Udang windu yang digunakan pada uji pencernaan berbobot  $5,70 \pm 0,42$  g. Parameter uji yang diukur meliputi pencernaan total, protein, kalsium, magnesium, dan energi. Uji pertumbuhan dilakukan menggunakan rancangan acak lengkap dengan empat perlakuan dan tiga ulangan, yaitu penggunaan tepung *C. racemosa* sebesar 0 (kontrol), 10, 20, dan 30%. Udang windu dengan bobot  $0,36 \pm 0,02$  g dipelihara dalam wadah kontainer plastik ukuran 70×45×40 cm (volume air sebanyak 90 L) dengan kepadatan 15 ekor tiap wadah selama 42 hari. Hasil penelitian menunjukkan pencernaan total *C. racemosa* pada udang windu 51,82%, pencernaan protein 88,67%, pencernaan kalsium 68,44%, pencernaan magnesium 16,39%, dan pencernaan energi 60,30%. Penelitian tahap kedua pada kinerja pertumbuhan udang yang mengonsumsi pakan mengandung 10% *C. racemosa*, tidak memberikan nilai yang berbeda nyata dengan udang yang mengonsumsi pakan kontrol. Namun, kinerja pertumbuhan udang menurun setelah mengonsumsi pakan yang mengandung *C. racemosa* di atas 20%, sedangkan aktivitas enzim superoxide dismutase (SOD) meningkat. Dari penelitian ini dapat disimpulkan bahwa penambahan *C. racemosa* ke dalam formula pakan sampai 10% dapat digunakan sebagai bahan baku pakan udang windu.

Kata kunci: *Caulerpa racemosa*, *Penaeus monodon*, pencernaan, kinerja pertumbuhan, udang

## INTRODUCTION

For many years, the main source of vegetable protein for shrimp feed was obtained from terrestrial plant, such as soybean meal (Cruz-Suarez *et al.*, 2009; Suárez *et al.*, 2009; Derby *et al.*, 2016; Sharawy *et al.*, 2016; Xie *et al.*, 2016), lupin meal (Draganovic *et al.*, 2014), garden pea *Pisum sativum*, concentrated rice protein (Oujifard *et al.*, 2012; Chen *et al.*, 2017), and canola meal *Brassica* sp. (Kou *et al.*, 2015; Suárez *et al.*, 2009; Singh *et al.*, 2014). The ingredient selection is managed according to the nutrition and the digestibility (Cruz-Suarez *et al.*, 2009; Samuelson *et al.*, 2014). However, those main sources contend with the livestock needs, thus the sustainability is uncertainty (Dolomatov *et al.*, 2014; Schader *et al.*, 2015). Moreover, the existence of antinutrition, such as phytate acid, tannin, and anti trypsin, causes downturn in nutrition absorption (Zhou *et al.*, 2015; Difo *et al.*, 2015). Therefore, further study about the other source of vegetable protein with low anti nutrition level is necessary.

*Caulerpa racemosa* is one of the marine algae species which is potentially developed as the shrimp feed ingredient. Paul *et al.* (2013) stated that the application of *Caulerpa racemosa* was able to boost high growth rate (5–7%/day) (Paul *et al.*, 2013). *Caulerpa racemosa* has a high nutrient content, i.e. protein 12.88–23.42%, carbohydrate 27.2–48.10%, and fat 0.3–2.64% (Kumar *et al.*, 2011; Murugaiyan & Narasimman, 2013). The calcium and magnesium content is higher as well compared to the terrestrial plant (MacArtain *et al.*, 2007; Kalaivanan *et al.*, 2012; Paul *et al.*, 2013). *Caulerpa* sp. contains calcium and magnesium around 5.97% and 0.4–4.1%, respectively (Santoso *et al.*, 2006; Kumar *et al.*, 2011; Gaillande *et al.*, 2016). According to Matanjun *et al.* (2009), *Caulerpa lentillifera* contains approximately 329.69 mg/100 g of calcium and 271.33 mg/100 g of magnesium. *C. racemosa* has 8.958–11.31 mg/g of carotenoids which also acts as antioxidant, regulates cell growth, modulates gene expression, and induces immunity. Vitamin C (10.10–34.70 mg/100 g of wet weight) and vitamin E (1.1–9.4 mg/100 g of wet weight) in *C. racemosa* are considered as vigorous antioxidant to increase the immunity against disease and oxidative stress (Gaillande *et al.*, 2016). In spite of delivering high nutrition, *C. racemosa* also produces secondary metabolite

named caulerpin (Felline *et al.*, 2012; Nagappan & Vairappan, 2014). The effect of caulerpin towards aquatic species, especially shrimp, has not been reported. Hence, the study was held to evaluate *C. racemosa* as feed ingredient for tiger shrimp *Penaeus monodon*.

## MATERIALS AND METHODS

### Experiment I: Digestibility test

#### Experimental feed preparation

*C. racemosa* was obtained from the coastal area of Kartini Beach, Jepara. *C. racemosa* was dried using an oven in temperature of 60–80°C for 48 hours. The dry weight was used in proximate analysis. It contained 30.03% of protein, 1.76% of crude fat, 3.29% of crude fiber, 22.22% of ash, and 42.70% of nitrogen-free extract (NFE). The commercial feed was used as base feed which is destructed then repelleting. The experimental feed is the combination of base feed and *C. racemosa* meal in ration 7:3 (NRC, 2011). The detail is shown below in Table 1.

Table 1. The composition of experimental feed for digestibility test of *C. racemosa*.

Ingredients (%)	Base feed	Experimental feed
Commercial feed	97.50	67.50
<i>C. racemosa</i> meal	0.00	30.00
Cr <sub>2</sub> O <sub>3</sub>	0.50	0.50
Carboxymethyl cellulose (CMC)	2.00	2.00
Total	100.00	100.00

#### Shrimp rearing and feces collection

The shrimp was acclimated in fiber tank for 7 days. The tanks in digestibility test were 6 plastic tank sized in 77×54×45 cm filled with sea water (salinity 25–28 g/L) and equipped with aeration. The average weight of experimental shrimp was 5.73 ± 0.38 g and the stocking density was 12 individuals/tank (29 individuals/m<sup>2</sup>). The shrimp was fed 8% of total biomass which adjusted to daily food requirement. The uneaten feed was collected. The feces was also collected three days after the experimental feed administration. The feces collection was conducted 2–3 hours after feeding. The feces was put into a film bottle and stored at -20°C until the amount sufficient for analysis.

## Experiment II: Growth test

### Experimental feed preparation

The experimental feed was crumble with three different level of *C. racemosa* meal addition (0, 10, 20, and 30%) according to the previous study by Putri *et al.* (2017). The experimental feed contained 42% of protein and 450 kcal/100 g of energy. The formulation and composition of the feed is presented in Table 2.

### Shrimp rearing and observation

The shrimp was adapted for 7 days and fed using commercial feed. Before the study was begun, the shrimp was fasted for 24 hours. The rearing container was filled with seawater in salinity of 25–28 g/L and equipped with aerations system. The container was covered using dark tarp to prevent the shrimp escape. As many of 100 individuals were collected to measure initial nutrition content. The average weight was  $0.36 \pm 0.02$  g and the stocking density was 15 individuals/container (48 individuals/m<sup>2</sup>). The shrimp was fed

three times a day (07.00, 12.00, and 17.00) and reared for 42 days. The amount of the feed was 8% of total biomass and it was adjusted to daily feed consumption. The body weight measurement was managed each two weeks. The uneaten feed and feces were collected using siphon, then water discharged was done approximately 30–50%.

### Chemical analysis

The chemical analysis consisted of chromium analysis (feed and feces), proximate analysis (the experimental feed, initial body weight, and final body weight), and also calcium and magnesium mineral analysis (*C. racemosa* meal, feed digestibility, and feces). All the procedure was referred to AOAC (1990).

### Antioxidant activity test

*C. racemosa* meal presented biological activity as antioxidant (Gaillande *et al.*, 2016) and antibacterial because it contains phenol (Michalak & Chojnacka, 2015). Superoxide

Table 2. The formulation of experimental feed for tiger shrimp with different level of *C. racemosa* meal.

Ingredients	<i>C. racemosa</i> content			
	0%	10%	20%	30%
Fish meal	30.00	30.00	30.00	30.00
Soybean meal	28.00	23.00	18.00	14.00
Squid meal	5.00	5.00	5.00	5.00
Polard	20.00	17.00	14.00	8.00
Wheat flour	10.40	7.40	4.40	3.40
<i>C. racemosa</i> meal	0.00	10.00	20.00	30.00
Coconut oil	1.00	2.00	3.00	4.00
Squid oil	2.00	2.00	2.00	2.00
Lecythin	1.00	1.00	1.00	1.00
Premix <sup>1</sup>	2.00	2.00	2.00	2.00
Vitamin C	0.10	0.10	0.10	0.10
CMC	0.50	0.50	0.50	0.50
Feed proximate (%)				
Protein	42.18	43.25	42.65	42.69
Lipid	9.34	10.61	12.18	13.47
Crude fiber	2.54	2.54	2.29	2.17
Ash	9.38	10.66	12.37	13.59
NFE	36.57	32.94	30.51	28.08
Energy (kcal/100g) <sup>2</sup>	473.93	476.99	478.41	480.78

<sup>1</sup>Premix (g/kg feed): Vit A 50 IU, vit D<sub>3</sub> 10 IU, vit B<sub>1</sub> 100 µg, vit B<sub>2</sub> 200 µg, vit B<sub>6</sub> 200 µg, vit B<sub>12</sub> 0.25 µg, vit E 600 µg, vit K<sub>3</sub> 50 µg, niacin 650 µg, panthotenic acid 300 µg, biotin 5 µg, follic acid 40 µg, inositol 1.000 µg, vit C 400 µg, phosphorus 280 µg, potassium 5.600 µg, calcium 5.600 µg, magnesium 1.820 µg, sodium 8.400 µg, iodine 196 µg, copper 1.4 µg, irone 332 µg, manganese 3.5 µg, and zinc 33.6 µg.

<sup>2</sup>Protein 5.6 kcal GE, lipid 9.5 kcal GE, carbohydrate 4.1 kcal GE (NRC, 2011). NFE = Nitrogen-free extract

dismutase (SOD) enzyme is an indicator to measure antioxidant activity. SOD enzyme is the major antioxidant enzyme to prevent reactive oxygen species (ROS) and convert superoxide free-radical to peroxide (H<sub>2</sub>O<sub>2</sub>) (Taylor & Savin, 2011). The SOD enzyme test was begun with blood collection (hemolymph) at the end of the study, precisely 24 hours after feeding.

#### Data collection

The digestibility of *C. racemosa* was calculated through base feed and experimental feed digestibility. The method referred to Bureau & Hua (2006). Growth performance was monitored through feed consumption, feed efficiency, protein efficiency ratio, feed conversion ratio (Takeuchi, 1988), specific growth rate (Halver, 1989), and survival rate.

#### Statistical analysis

All of the data were tabulated using Microsoft Excel 2013. Furthermore, the digestibility of *C. racemosa* meal was analyzed descriptively. On the contrary, growth parameter consisted of feed consumption, nutrition proximate of the shrimp, and SOD enzyme activity was analyzed statistically through parametric test (analysis of variance) using SPSS 16.0 and Duncan posthoc test in 95% of confidence level.

## RESULTS AND DISCUSSIONS

### Results

#### The digestibility of *C. racemosa*

The digestibility of *C. racemosa* meal was calculated from both base feed and experimental feed which measured through total digestibility,

protein, calcium, magnesium, and energy. Those results are presented below in Table 3.

Table 3. The digestibility of *C. racemosa* meal in tiger shrimp

Experimental parameter	Digestibility (%)
Total digestibility	51.82 ± 2.15
Protein digestibility	88.67 ± 1.27
Calcium digestibility	68.44 ± 2.51
Magnesium digestibility	16.39 ± 3.99
Energy digestibility	60.30 ± 1.29

#### Growth performance

*C. racemosa* meal addition until 10% resulted similar amount of feed consumption with the control treatment. However, *C. racemosa* meal addition exceeded 20%, decreased feed consumption. Identical case was also found in specific growth rate, protein efficiency ratio, protein retention, feed conversion, and feed efficiency which followed similar result with control. Whereas, *C. racemosa* meal addition until 30% did not significantly affect survival rate (Table 4).

#### SOD enzyme activity

SOD enzyme activity test towards hemolymph showed that there was a rising level of SOD enzyme along with the increase of *C. racemosa* addition. The 30% of *C. racemosa* meal addition delivered higher result and significantly different with other treatments (P<0.05) (Figure 1).

### Discussion

Total digestibility of *C. racemosa* is 51.82% (Table 3). The digestibility of *C. racemosa*

Table 4. Growth performance of tiger shrimp fed with different level of *C. racemosa* meal addition (control, 10%, 20%, 30%).

Parameter	<i>C. racemosa</i> addition			
	0%	10%	20%	30%
W <sub>42</sub> (g)	1.25 ± 0.04 <sup>a</sup>	1.22 ± 0.03 <sup>a</sup>	1.03 ± 0.07 <sup>b</sup>	0.95 ± 0.05 <sup>b</sup>
Feed consumption (g/individual)	1.90 ± 0.06 <sup>a</sup>	1.87 ± 0.10 <sup>a</sup>	1.77 ± 0.07 <sup>ab</sup>	1.67 ± 0.08 <sup>b</sup>
SGR (%/day)	3.10 ± 0.08 <sup>a</sup>	3.04 ± 0.06 <sup>a</sup>	2.59 ± 0.13 <sup>b</sup>	2.40 ± 0.14 <sup>b</sup>
PER	1.11 ± 0.02 <sup>a</sup>	1.06 ± 0.03 <sup>a</sup>	0.88 ± 0.04 <sup>b</sup>	0.82 ± 0.04 <sup>b</sup>
PR (%)	16.74 ± 0.83 <sup>a</sup>	16.15 ± 0.35 <sup>a</sup>	12.46 ± 0.15 <sup>b</sup>	11.64 ± 1.00 <sup>b</sup>
FCR	2.14 ± 0.04 <sup>a</sup>	2.18 ± 0.07 <sup>a</sup>	2.66 ± 0.13 <sup>b</sup>	2.86 ± 0.12 <sup>b</sup>
FE (%)	46.80 ± 0.94 <sup>a</sup>	45.90 ± 1.47 <sup>a</sup>	37.63 ± 1.75 <sup>b</sup>	35.02 ± 1.55 <sup>b</sup>
SR (%)	93.33 ± 0.00 <sup>a</sup>	95.56 ± 3.85 <sup>a</sup>	93.33 ± 0.00 <sup>a</sup>	95.56 ± 3.85 <sup>a</sup>

Note: The stated value is average ± deviation standard. Different superscript letter in the same row indicates significant difference (P<0.05); W<sub>42</sub>= individual final weight (day-42); SGR= specific growth rate; PER= protein efficiency ratio; PR= protein ratio; FCR= feed conversion ratio; FE= feed efficiency; SR= survival rate.



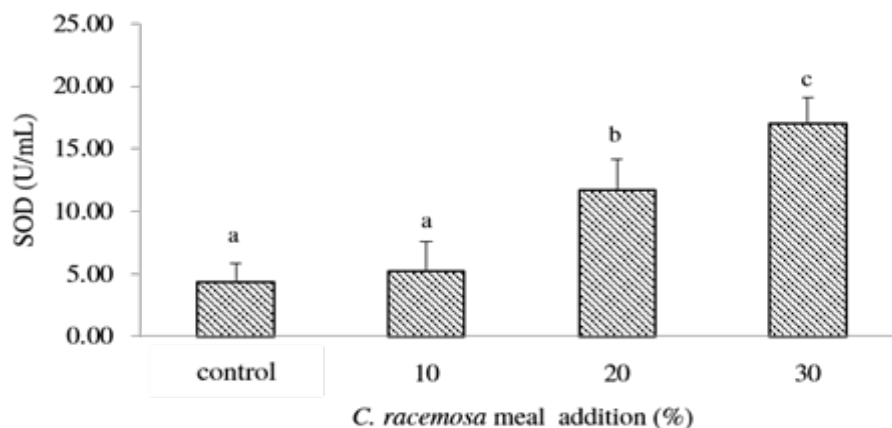


Figure 1. SOD enzyme activity in different level of *C. racemosa* meal addition (control, 10%, 20%, 30%). Different letter in the graph indicates significant difference ( $P < 0.05$ ).

was considerably lower than soybean meal (60.1–73%) and fish meal (74%) (Smith *et al.*, 2000). However, the digestibility of *C. racemosa* was noticeably higher than poultry by-product meal (PBM) (47.9%) (Luo *et al.*, 2012), meat meal (43%), canola (49%), and lupin (39%) (Smith *et al.*, 2000). Total digestibility represents total quantity of the digested material (Luo *et al.*, 2012). A material can not be entirely digested, so that there is a variable named total digestibility to represent the digested and undigested material (Sookying *et al.*, 2013)

A high protein content of *C. racemosa* (30%) was highly digested by the tiger shrimp around 87.42% (Table 3). According to NRC (2011), the protein digestibility of shrimp ranges from 75–95%. The digestibility of *C. racemosa* was noticeably higher than canola meal (80%), cottonseed meal (83%), and blood meal (66%) (Smith *et al.*, 2000; NRC, 2011). However, the digestibility of *C. racemosa* meal was lower than soybean meal (92.1%) (Hertrampf & Pascual, 2000; Smith *et al.*, 2000). The protein content of *C. racemosa* was considerably higher than the other species of green algae, such as *Enteromorpha intestinalis* (24.52%) and *Ulva lactuca* (21.06%) (Ratana-arporn & Chirapart, 2006). A decent level of digestibility is crucial in a high density aquaculture. The accumulation of uneaten feed potentially interrupts water quality, expands maintenance cost, and causes death (Lin *et al.*, 2006; Hasan *et al.*, 2012; Mohanty *et al.*, 2014).

The digestibility of calcium and magnesium in the *C. racemosa* meal was 68.44% and 16.39%, respectively (Table 3). The calcium requirement of the shrimp is 0.5–1.25%, while the shrimp requires magnesium as many of 0.1–0.3% (Hertrampf & Pascual, 2000; Hena *et al.*, 2012).

Calcium held several roles in bone formation, muscle contraction, and blood vessel. On the other hand, magnesium is necessarily needed in cell respiration, enzyme activator, and also in lipid, carbohydrate, and protein metabolism (NRC, 2011; Antony *et al.*, 2015; Bernard & Bolatito, 2016). Shrimp has the ability to directly absorb several minerals, such as magnesium and calcium, in the water through the gill and exoskeleton in a low concentration yet (Hertrampf & Pascual, 2000; NRC, 2011; Verma & Tomar, 2014). Hence, mineral addition is inevitably essential to fulfill shrimp requirement (Roy *et al.*, 2009; Pine & Boyd, 2010).

The energy digestibility of *C. racemosa* was 60.30% (Table 3) which was lower than the energy digestibility of soybean meal (71–76%) and poultry by-product meal (PBM) (82%) (Smith *et al.*, 2000; NRC, 2011). The value showed positive correlation with the protein digestibility of soybean meal which was higher than *C. racemosa* meal. However, the energy digestibility was nearly similar with the other plant-based ingredients, such as cottonseed meal (61%) and cornmeal (60%) (NRC, 2011) and higher than canola (53%) and lupin meal (45%) (Smith *et al.*, 2000).

The diminishing level of growth performance along with the increasing level of *C. racemosa* dosage was assumed caused by the downturn of ingredients quality. The experimental feed was made isoprotein and isoenergy, thus the lower level of growth performance was supposedly caused by the low quality of the feed ingredients. According to Luis *et al.* (2010) and Sudaryono *et al.* (1999), a low quality of feed ingredients is possibly generated by low palatability which also leads to low feed consumption. Kamal and Sethuraman (2012) and Gaillande *et al.* (2016) mentioned that *C. racemosa* has a secondary metabolite

compound in the methanol fraction named caulerpin. This compound seemed unpalatable and it directly affected the feed attractiveness, especially in the higher dosage (>20%). Luis *et al.* (2010) also reported that *C. racemosa* released antifeeding chemical compound which also presumably decreased the feed consumption of *Thalassoma pavo*.

Feed palatability is essential to attract the shrimp to consume the feed. Being slow and rely on its chemoreceptor are the habits of the shrimp to discover the feed (Smith *et al.*, 2000; Aggio *et al.*, 2012; Tantikitti, 2014). Caulerpin is known to own undesirable taste for the shrimp, so that the feed consumption decreased. In addition, feed palatability is precisely affected by attractant content. Glycine and alanine amino acids are known as decent attractant for shrimp (Sudaryono *et al.*, 1999). Soybean meal contents glycine and alanine as many of 1.69% and 2.02%, respectively (Song *et al.*, 2008), compared with *C. racemosa* meal which contents glycine and alanine 1.28% dan 0.2%, respectively (Bhuiyan *et al.*, 2016). The addition of *C. racemosa* meal up to 20%, led to lower percentage of soybean meal in the feed formulation (Table 2), thus the concentration of glycine and alanine decreased as well. As a consequence, feed palatability was also declined.

The declining level of growth performance was apparently caused by caulerpin which activated after being consumed. It was shown by the increasing level of SOD enzyme (Figure 1). SOD enzyme is the major antioxidant enzyme that prevents reactive oxygen species (ROS) through counteracting free radical and phagocytosis (Matanjun *et al.*, 2010; Zhang *et al.*, 2013; Box *et al.*, 2008). The SOD enzyme increases along with the higher level of *C. racemosa* dosage. The 30% of *C. racemosa* treatment resulted in 16.97 U/mL of SOD enzyme and it was different significantly with the other treatments. The increase level of SOD enzyme was assumed to anticipate ROS as a result of detoxification process towards caulerpin. Liu *et al.* (2009) stated that caulerpin inhibits hypoxia-inducible factor-1 (HIF-1) which controls oxygen homeostasis. When the HIF-1 is inhibited, ROS will be produced and it will lead to hypoxia condition. Metabolism rate will increase to produce SOD enzyme to prevent ROS. The increase of metabolism rate is not followed by an adequate energy supply. Felling *et al.* (2012) reported that caulerpin was also found in body tissues of white seabream *Diplodus*

*sargus* fed using *C. racemosa*. As a consequence, a detoxification mechanism increased and it decelerated the growth performance.

The deficiency of feed causes the nutrient deficiency, thus the nutrient retention is declining. Protein retention (PR) presents the amount of protein retained from the digested feed. The feed is basically the energy source to fulfill the basal metabolism, daily maintenance, and growth necessity (Hu *et al.*, 2008; Kaushik & Seiliez, 2010). The protein retention of *C. racemosa* 10% treatment was 16.15% which not significantly different with control treatment (16.74%) (Table 4). On the contrary, the protein retained in the 20% and 30% treatment decreased gradually and differ significantly with the 0% and 10% treatment. It also straightly affected the declining of protein efficiency ratio (PER). The PER result of 10% *C. racemosa* treatment was 1.06 and it was not significantly different with control ( $P > 0.05$ ). The *C. racemosa* addition until 10% was assumed as a proper composition as protein source for tiger shrimp feed. On the other hand, *C. racemosa* addition more than 20% generated lower value of PER. A lower value of PER indicated that protein was used to achieve energy demand. It also implied that energy demand from non-protein energy sources, such as lipid and carbohydrates, have not fulfilled yet.

The decreased growth performance was caused by feed digestibility presumably. Feed digestibility is required to discover feed quality and show the digested nutrient composition and absorbed by the shrimp to support its growth and metabolism (Luo *et al.*, 2012). The addition of *C. racemosa* exceeded 20% caused the soybean meal substitution more than 35% (Table 2). The total digestibility of soybean meal is 73% (Smith *et al.*, 2000) and the protein digestibility ranges from 92.1–94.0% (Hertrampf & Pascual, 2000; NRC, 2011). Those values is higher than *C. racemosa* meal with total digestibility 51.82% and protein 87.42% (Table 3).

The specific growth rate (SGR) resulted by 10% of *C. racemosa* treatment was 3.04%/day which not significantly different with control (3.10%/day). In contrast, *C. racemosa* addition exceeded 20% showed lower SGR. It could be generated by inefficient feed consumption, especially protein retention, which led to low growth rate. Feed conversion ratio (FCR) is an indicator to determine feed effectivity. A low feed conversion ratio indicates high feed efficiency.

The FCR value of 10% *C. racemosa* treatment was 2.18 and it did not differ significantly with control treatment (2.14). It indicated that the feed efficiency of both treatments were similar (Table 4). Conversely, the 20% and 30% of *C. racemosa* treatment demonstrated higher FCR value and low feed efficiency value (Table 4). The results explained that 10% of *C. racemosa* addition was more efficient compared with 20% or above. Feed efficiency (FE) value is applied in weight added and feed consumption ratio (Watanabe, 1988; Richard *et al.*, 2010). Consumed feed will produce a value which describes feed efficient utilized by the experimental shrimp. The FE value describes the most efficient treatment in terms of energy fulfillment and growth. The FE value in this study was higher compared to the result by Widyantoko *et al.* (2015) which used 3% of *Sargassum* sp. meal in the experimental feed (33.19%).

The survival rate of tiger shrimp presented insignificant difference amongst treatments ( $P > 0.05$ ). It indicated that the addition of *C. racemosa* meal into the shrimp diet did not cause death on the experimental shrimp and it could be used as feed ingredient. A moderate toxic compound named caulerpin was able to be anticipated through SOD enzyme mechanism.

## CONCLUSION

A 10% of *Caulerpa racemosa* is potentially used as tiger shrimp diet ingredient. A higher level of *Caulerpa racemosa* addition may diminish the growth rate of tiger shrimp *Penaeus monodon*.

## ACKNOWLEDGMENT

Authors deliver sincere grateful the Marine and Fisheries Education Center, Ministry of Fisheries and Marine Affairs, Indonesia for fully funding this research with project no. 74/SJ/KP.530/III/2016.

## REFERENCES

[AOAC] Association of Official Analytical Chemists. 1990. Official methods of analysis. 15th edition. Association of Official Analytical Chemists. Washington D.C. 1094p.

Aggio JF, Tieu R, Wei A, Derby CD. 2012. Oesophageal chemoreceptors of blue crabs, *Callinectes sapidus*, sense chemical deterrents and can block ingestion of food. *Journal of Experimental Biology* 215: 1700–1710.

Antony J, Vungurala H, Saharan N, Reddy AK, Chadha NK, Lakra WS, Roy LA. 2015. Effects of salinity and  $\text{Na}^+/\text{K}^+$  ratio on osmoregulation and growth performance of black tiger prawn, *Penaeus monodon* Fabricius, 1798, juveniles reared in inland saline water. *Journal of the World Aquaculture Society* 46: 171–182.

Bernard E, Bolatito AY. 2016. Comparative study on the nutritional composition of the pink shrimp (*Penaeus notialis*) and tiger shrimp (*Penaeus monodon*) from Lagos lagoon, Southwest Nigeria. *Cogent Food & Agriculture* 2: 1–7.

Bhuiyan KA, Qureshi S, Mustafa Kamal AH, Aftabuddin S, Siddique AM. 2016. Proximate chemical composition of sea grapes *Caulerpa racemosa* (J. Agardh. 1873) collected from a sub-tropical coast. *Virology Mycology* 5: 1–6.

Box A, Sureda A, Terrados J, Pons A, Deudero S. 2008. Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae *Lophocladia lallemandii* (Montagne). *Journal of Experimental Marine Biology and Ecology* 364: 24–28.

Bureau DP, Hua K. 2006. Letter to the editor of aquaculture. *Aquaculture* 252: 103–105.

Chen J, Li X, Xu H, Sun W, Leng X. 2017. Substitute of soy protein concentrate for fish meal in diets of white shrimp (*Litopenaeus vannamei* Boone). *Aquaculture International* 25: 1303–1315.

Cruz-Suarez LE, Tapia-Salazar M, Villareal-Cavazos D, Beltran-Rocha J, Nieto-Lopez MG, Lemme A, Ricque-Marie D. 2009. Apparent dry matter, energy, protein, and amino acid digestibility of four soybean ingredients in white shrimp *Litopenaeus vannamei* juveniles. *Aquaculture* 292: 87–94.

Derby CD, Elsayed FH, Williams SA, González C, Choe M, Bharadwaj AS, Chamberlain GW. 2016. Krill meal enhances performance of feed pellets through concentration-dependent prolongation of consumption by Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 458: 13–20.

Difo VH, Onyike E, Ameh DA, Njoku GC, Ndidi US. 2015. Changes in nutrient and antinutrient composition of *Vigna racemosa* flour in open and controlled fermentation. *Journal of Food Science and Technology* 52: 6043–6048.

Dolomatov SI, Kubyshkin AV, Zukow WA, Kutya SA, Cieslicka M, Muszkieta R, Napierala M. 2014. Prospects for the replenishment of a



- feed protein deficit in aquaculture. *Russian Journal of Marine Biology* 40: 233–240.
- Draganovic V, Boom RM, Jonkers J, van der Goot AJ. 2014. Lupine and rapeseed protein concentrate in fish feed: A comparative assessment of the techno-functional properties using a shear cell device and an extruder. *Journal of Food Engineering* 126:178–189.
- Felline S, Caricato R, Cutignano A, Gorbi S, Lionetto MG, Mollo E, Regolli F, Terlizzi A. 2012. Subtle effects of biological invasions: cellular and physiological responses of fish eating the exotic pest *Caulerpa racemosa*. *Plos One* 7: 1–10.
- Gaillande C, Payri C, Remissenet G, Zubia M. 2016. Caulerpa consumption, nutritional value, and farming in the Indo-Pacific region. *Journal of Applied Phycology* 29: 1–18.
- Halver JE. 1989. *Fish Nutrition*. New York: Academic Pr.
- Hasan BMA, Guha B, Datta S. 2012. Optimization of feeding efficiency for cost effective production of *Penaeus monodon* Fabricius in semi-intensive pond culture system. *Journal of Aquaculture Research & Development* 3: 1–7.
- Hena MA, Idris MH, Wong SK. 2012. Chemical composition of water from tiger shrimp *Penaeus monodon* culture ponds at Malacca, Malaysia. *International Journal of Agriculture and Biology* 14: 395–400.
- Hertrampf JW, Piedad-Pascual F. 2000. Fish Protein Concentrate (Hydrolysed). In *Handbook on Ingredients for Aquaculture Feeds*. Springer. Dordrecht. pp. 192–197.
- Hu Y, Tan B, Mai K, Ai Q, Zheng S, Cheng K. 2008. Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. *Aquaculture Nutrition* 14: 499–506.
- Kalaivanan C, Chandrasekaran M, Venkatesalu V. 2012. Effect of seaweed liquid extract of *Caulerpa scalpelliformis* on growth and biochemical constituents of black gram (*Vigna mungo* (L.) Hepper). *Phykos* 42: 46–53.
- Kamal C, Sethuraman MG. 2012. Caulerpin–A bis-indole alkaloid as a green inhibitor for the corrosion of mild steel in 1 M HCl solution from the marine alga *Caulerpa racemosa*. *Industrial & Engineering Chemistry Research* 51: 10399–10407.
- Kaushik SJ, Seiliez I. 2010. Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. *Aquaculture Research* 41: 322–332.
- Kou H, Xu S, Wang AL. 2015. Effect of replacing canola meal for fish meal on the growth, digestive enzyme activity, and amino acids, of ovate pompano, *Trachinotus ovatus*. *The Israeli Journal of Aquaculture-Bamidgeh* 67: 1–10.
- Kumar M, Kumari P, Trivedi N, Shukla MK, Gupta V, Reddy CRK, Jha B. 2011. Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *Journal of Applied Phycology* 23: 797–810.
- Lin HZ, Li ZJ, Chen YQ, Zheng WH, Yang K. 2006. Effect of dietary traditional Chinese medicines on apparent digestibility coefficients of nutrients for white shrimp *Litopenaeus vannamei*. *Boone. Aquaculture* 253: 495–501.
- Liu Y, Morgan JB, Coothankandaswamy V, Liu R, Jekabsons MB, Mahdi F, Nagle DG, Zhou YD. 2009. The caulerpa pigment caulerpin inhibits HIF-1 activation and mitochondrial respiration. *Journal of Natural Products* 72: 2104–2109.
- Luis MV, Jerez PS, Sempere JTB. 2010. Effects of *Caulerpa racemosa* var. *cylindracea* on prey availability: an experimental approach to predation of amphipods by *Thalassoma pavo* (Labridae). *Hydrobiologia* 654: 147–154.
- Luo L, Wang J, Pan Q, Xue M, Wang Y, Wu X, Li P. 2012. Apparent digestibility coefficient of poultry by-product meal (PBM) in diets of *Penaeus monodon* (Fabricius) and *Litopenaeus vannamei* (Boone). and replacement of fishmeal with PBM in diets of *P. monodon*. *Aquaculture Research* 43: 1223–1231.
- MacArtain P, Gill CI, Brooks M, Campbell R, Rowland IR. 2007. Nutritional value of edible seaweeds. *Nutrition Reviews* 65: 535–543.
- Matanjun P, Mohamed S, Mustapha NM, Muhammad K. 2009. Nutrient content of tropical edible seaweeds. *Eucheuma cottonii*, *Caulerpa lentillifera*, and *Sargassum polycystum*. *Journal of Applied Phycology* 21: 75–80.
- Matanjun P, Mohamed S, Muhammad K, Mustapha NM. 2010. Comparison of cardiovascular protective effects of tropical seaweeds, *Kappaphycus alvarezii*, *Caulerpa lentillifera*, and *Sargassum polycystum*, on high-cholesterol/high-fat diet in rats. *Journal of Medicinal food* 13: 792–800.



- Michalak I, Chojnacka K. 2015. Algae as production systems of bioactive compounds. *Engineering in Life Sciences* 15: 160–176.
- Mohanty RK, Mishra A, Patil DU. 2014. Water budgeting in black tiger shrimp *Penaeus monodon* culture using different water and feed management systems. *Turkish Journal of Fisheries and Aquatic Sciences* 14: 487–496.
- Murugaiyan K, Narasimman S. 2013. Biochemical and mineral contents of selected green seaweeds from Gulf of Mannar coastal region, Tamil Nadu, India. *International Journal of Research in Plant Science* 3: 96–100.
- Nagappan T, Vairappan CS. 2014. Nutritional and bioactive properties of three edible species of green algae, genus *Caulerpa* (Caulerpaceae). *Journal of Applied Phycology* 26: 1019–1027.
- [NRC] National Research Council. 2011. Nutrient requirements of fishes. Washington DC (US): National Academy of Sciences.
- Oujifard A, Seyfabadi J, Kenari AA, Rezaei M. 2012. Fish meal replacement with rice protein concentrate in a practical diet for the Pacific white shrimp, *Litopenaeus vannamei* Boone. 1931. *Aquaculture International* 20: 117–129.
- Paul NA, Neveux N, Magnusson M, de Nys R. 2013. Comparative production and nutritional value of “sea grapes” the tropical green seaweeds *Caulerpa lentillifera* and *C. racemosa*. *Journal of Applied Phycology* 26: 1833–1844.
- Pine HJ, Boyd CE. 2010. Adsorption of magnesium by bottom soils in inland brackish water shrimp ponds in Alabama. *Journal of the World Aquaculture Society* 41: 603–609.
- Putri NT, Jusadi D, Setiawati M, Sunarno MTD. 2017. Potential use of green algae *Caulerpa lentillifera* as feed ingredient in the diet of Nile tilapia *Oreochromis niloticus*. *Jurnal Akuakultur Indonesia* 16: 184–192.
- Ratana-arporn P, Chirapart A. 2006. Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. *Kasetsart Journal* 40: 75–83.
- Richard L, Blanc PP, Rigolet V, Kaushik SJ, Geurden I. 2010. Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black tiger shrimp, *Penaeus monodon*, using a factorial approach. *British journal of nutrition* 103: 984–995.
- Roy LA, Davis DA, Nguyen TN, Saoud IP. 2009. Supplementation of chelated magnesium to diets of the pacific white shrimp, *Litopenaeus vannamei*, reared in low-salinity waters of west Alabama. *Journal of The World Aquaculture Society* 40: 248–254.
- Samuelsen TA, Mjos SA, Oterhals A. 2014. Influence of type of raw material on fishmeal physicochemical properties, the extrusion process, starch gelatinization and physical quality of fish feed. *Aquaculture Nutrition* 20: 410–420.
- Santoso J, Gunji S, Yoshi-Strak Y, Suzuki T. 2006. Mineral contents of Indonesian seaweeds and mineral solubility affected by basic cooking. *Food Science and Technology Research* 12: 59–66.
- Schader C, Muller A, Scialabba NEH, Hecht J, Isensee A, Erb KH, Schwegler P. 2015. Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. *Journal of the Royal Society Interface* 12: 1–12.
- Sharawy Z, Goda AMS, Hassaan MS. 2016. Partial or total replacement of fish meal by solid state fermented soybean meal with *Saccharomyces cerevisiae* in diets for Indian prawn shrimp, *Fenneropenaeus indicus*, postlarvae. *Animal Feed Science and Technology* 212: 90–99.
- Singh, R, Chatli MK, Biswas AK, Sahoo J. 2014. Quality of  $\omega$ -3 fatty acid enriched low-fat chicken meat patties incorporated with selected levels of linseed flour/oil and canola flour/oil. *Journal of Food Science and Technology* 51: 353–358.
- Smith DM, Allan GL, Williams KC, Barlow C. 2000. Fishmeal replacement research for shrimp feed in Australia. In: Cruz-Suarez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MA, Civera-Cerecedo R. editor. *Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola*. 19-22 November. 2000. Merida. Yucatan. Mexico.
- Song YS, Frias J, Villaluenga CM, Valdeverde CV, Mejia EG. 2008. Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. *Food Chemistry* 108: 571–581.
- Sookying D, Davis DA, Soller Dias Da Silva F. 2013. A review of the development and application of soybean-based diets for Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition* 19: 441–448.

- Suárez JA, Gaxiola G, Mendoza R, Cadavid S, Garcia G, Alanis G, Suárez A, Faillace J, Cuzon G. 2009. Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931). *Aquaculture* 289: 118–123.
- Sudaryono A, Tsvetnenko E, Evans LH. 1999. Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. *Aquaculture nutrition* 5: 277–285.
- Takeuchi T. 1988. Laboratory work chemical evaluation of dietary nutrition. In: [Fish nutrition and mariculture]. JICA textbook the general aquaculture course. Watanabe T. (Ed). Tokyo. Kanagawa International Fish Training Center. 224p.
- Tantikitti C. 2014. Feed palatability and the alternative protein sources in shrimp feed. *Songklanakarin Journal of Science and Technology* 36: 51–55.
- Taylor DPP, Savin TZ. 2011. Antioxidant enzyme activities in Pacific white shrimp *Litopenaeus vannamei* in response to environmental hypoxia and reoxygenation. *Aquaculture* 318: 379–383.
- Verma D, Tomar V. 2014. An investigation into environment dependent nanomechanical properties of shallow water shrimp (*Pandalus platyceros*) exoskeleton. *Materials Science and Engineering C* 44: 371–379.
- Watanabe. 1988. *Fish Nutrition Ana Mariculture*. Japan: Kanagawa International Fisheries Training Centre Japan International Cooperation Agency (JICA).
- Widyantoko W, Pinandoyo, Herawati VE. 2015. Optimization addition of flour brown seaweed (*Sargassum* sp.) which different in feed on growth and survival rate of juvenile tiger shrimp *Penaeus monodon*. *Journal of Aquaculture Management and Technology* 4: 9–17.
- Xie SW, Liu YJ, Zeng S, Niu J, Tian LX. 2016. Partial replacement of fish-meal by soy protein concentrate and soybean meal based protein blend for juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 464: 296–302.
- Zhang J, Liu YJ, Tian LX, Yang HJ, Liang GY, Yue YR, Xu DH. 2013. Effects of dietary astaxanthin on growth, antioxidant capacity and gene expression in Pacific white Shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition* 19: 917–927.
- Zhou YG, Davis DA, Buentello A. 2015. Use of new soybean varieties in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition* 21: 635–643.