Aquatic weed *Ceratophyllum* sp. as a dietary protein source: its effects on growth and fillet amino acid profile of rabbitfish, *Siganus guttatus*

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Abstract. A 16-week feeding trial was conducted to determine the potential use of the aquatic weed, *Ceratophyllum* sp., meal to replace soybean cake meal (SCM) as a protein source in diets for grow-out of rabbitfish, *Siganus guttatus*. Four iso-nitrogenous diets were formulated to contain different levels of *Ceratophyllum* weed meal (CWM) at 0 (CWM0), 20 (CWM20), 30 (CWM30) and 40 (CWM40) g $100g^{-1}$ diet substituting SCM from 38 to 0 g $100g^{-1}$ diet, respectively. Fish meal was included in the diets at the same proportion in order to meet the protein requirement level for grow-out of rabbitfish of around 28%. Rabbitfish with initial body weight of 109±1.3 g were stocked into 15 cages each 1x1x2.5 m^3. Each diet was fed twice a day at a rate of 2.5-3% of biomass. Fish were measured every month to record growth and adjust the feed ration. After 16 weeks, the weight gain and specific growth rate (SGR) of fish fed the test diets containing 0-30% CWM were similar, whereas the fish fed the CWM40 diet demonstrated lower weight gain and specific growth rate (ANOVA, $p > 0.05$). Feed conversion ratio (FCR) was relatively high for all groups and the FCR of the control diet (without CWM) was similar to diets containing CWM up to 30%. Fillet protein content of fish fed CWM20 and CWM30 diets were similar and both were significantly different ($p < 0.05$) from other two treatments (CWM0 and CWM40). Fish fed the CWM20 diet had the highest total amino acid in fillet (74.44±0.28%) which was significantly different ($p < 0.05$) from fish fed CWM0 (68.53±1.17%) and CWM40 (65.69±0.09%). Fish fed 20% CWM had significantly higher total amino acid content in fillet compared to the wild rabbitfish (70.09%). Based on the biological performance and the total amino acid content in fillet, rabbitfish could utilize CWM as dietary protein source up to 30% for grow-out in floating net cages.
Key Words: plant feedstuff, artificial diet, essential amino acid, marine herbivorous species.

Introduction. For the last decade, the volume of aquaculture production in the ASEAN countries, including Indonesia, has been increasing (FAO 2014) and aquafeed production in Indonesia has been increasing in line with production. According to the Indonesian Feed Mills Association, total aquafeed production increased from 400,000 tonnes in 2006 to 1.38 million tonnes in 2013 (Tacon & Metian 2008; Laining & Kristanto 2015). To date, the major protein sources used in aquafeeds in Indonesia are fish meal and soybean meal (SBM), both of which are generally imported. Indonesian aquafeeds use a high proportion of imported ingredients, and because of international demand for feed ingredients and, more recently, the decline in the value of the Indonesian currency, the price of aquafeeds has steadily increased. The increasing cost of feed has become the most important issue faced by farmers in the country in particular for those culturing low value freshwater species (Laining & Kristanto 2015).

Utilization of plant protein sources to partially or completely replace both fishmeal and SBM has been the subject of numerous studies for various fish species (Banuelos-Vargas et al 2014; Borlongan et al 2003; Espe et al 2006; Hansen et al 2007; Hardy 1996; Kikuchi & Furuta 2009; Kissil et al 2000; Zhang et al 2014). However, similar studies dealing with rabbitfish (*Siganus* spp.) are still limited (Laining et al 2014). Rabbitfish are marine herbivorous fish widely distributed in the Indo-West Pacific region (Woodland 1983). Several species commonly found in Malaysia, the Philippines and

Indonesia are *Siganus canaliculatus, S. guttatus, S. javus* and *S. vermiculatus* which are remarkably similar to each other (Pacoli 1983; Jaikumar 2012). Rabbitfish are potential candidates for marine aquaculture because of their herbivorous/omnivorous feeding habits (Tacon et al 1990; Duray 1998), high tolerance to environmental factors (Saoud et al 2007; Saoud et al 2008) and high growth rates (Randall et al 1997).

In the wild, rabbitfish feed on different kinds of algae, seaweeds and other aquatic plants which are typically low in protein and energy (Parazo 1990; Xu et al 2011; Jaikumar 2012). The aquatic weed *Ceratophyllum* sp. (known as *gosse* in South Sulawesi) has been used as a fresh feed for milkfish and tilapia by local farmers. *Ceratophyllum* weed meal (CWM) contains relatively high crude protein of around 23%, low lipid around 4% and a moderate (around 15%) level of fiber (Laining & Kristanto 2015). In order to evaluate the potential use of this weed as a protein source in rabbitfish diets, a feeding trial was conducted to clarify its effects on growth, feed utilization and fillet quality of rabbitfish *S. guttatus* reared in floating net cages.

Material and Method

Ceratophyllum weed meal and test diets*.* The study was conducted in June-October 2014 at the Research Institute for Coastal Aquaculture, Maros, South Sulawesi, Indonesia. Samples of fresh *Ceratophyllum* weed were obtained from brackishwater ponds used to culture milkfish (*Chanos chanos*) around Marana, South Sulawesi. The *Ceratophyllum* samples were gently washed with fresh tap water to remove dirt, then dried using a conventional oven and then stored in a cool room (16^{o}C) prior to processing. The dried weed samples were ground using a hammer mill and kept at the same temperature at 16^{o}C before being used.

Four test diets were formulated to contain different levels of CWM at 0, 20, 30 and 40% (Table 1). The CWM was incorporated to replace soybean cake meal (SCM) from 38% to 0%, respectively. All diets were iso-nitrogenous using local fish meal as the basal protein source. Fish oil was used as the lipid source at the same level for all the test diets, and palm oil was adjusted to compensate for the reduction of SCM (Table 1). Other dietary compounds were carbohydrate sources and binders including tapioca flour, sorghum flour and *Gracillaria* weed meal (Table 1). All diets contained approximately 28% crude protein and 7% crude lipid (Table 2). Analyzed amino acid profile of the four experimental diets are presented in Table 3.

Table 1

Formulation of experimental diets

Ingredients (%)	*Inclusion level of dietary CWM (%)*			
	CWM0	*CWM20*	*CWM30*	*CWM40*
Local fish meal	28	28	28	28
Ceratophyllum weed meal	0	20	30	40
Soybean cake meal	38	24	17	0
Tapioca flour	12	12	12	12
Sorghum meal	12.4	6.4	3.2	1.0
Gracillaria flour	5	5	5	5
Fish oil	0.5	0.5	0.5	0.5
Palm oil	1	1	1.2	1.4
Organic mineral	0.5	0.5	0.5	0.5
Stable vitamin C	0.1	0.1	0.1	0.1
Mineral mix[1]	0.5	0.5	0.5	0.5
Vitamin mix[2]	2.0	2.0	2.0	2.0

[1]At 20 g kg^{-1} inclusion level, provided in 1 kg of final diet: vitamin A 120,000 IU; vitamin D3, 40,000 IU; vitamin K3, 48; vitamin E 300 mg; vitamin B1, 120 mg; vitamin B2, 180 mg; vitamin B6, 120 mg; vitamin B12, 120 g; vitamin C 320 mg, Ca panthothenate, 160 mg; folic acid, 60 mg; biotin, 400 mg; inositol, 500 mg; nicotinamide, 800 mg; choline chloride, 600 mg; L-lysine, 800 mg; DL-methionine 1000 mg; [2]At 5 g kg^{-1} inclusion level, provided in 1 kg of final diet: Ca 1.63 g; P, 0.5 g; Fe, 30 mg; Mn, 20 mg; I, 0.38 mg; Cu, 1.5 mg; Zn, 18.7 mg.

Table 2

Proximate composition of experimental diets

Proximate (% dry matter)	*Inclusion level of dietary CWM (%)*			
	CWM0	*CWM20*	*CWM30*	*CWM40*
Crude protein	28.0	27.6	27.0	27.9
Lipid	7.8	7.8	7.1	6.9
Ash	13.6	11.5	12.3	12.5
Fiber	9.5	8.5	10.1	10.5
NFE	41.1	44.6	43.5	42.2

Table 3

Amino acid content (% dry matter) of experimental diets containing different level of CWM. Data are presented as mean±SD

Amino acid	*Inclusion level of dietary CWM (%)*			
	CWM0	*CWM20*	*CWM30*	*CWM40*
Aspartic acid	2.63±0.04	2.72±0.03	2.58±0.11	2.61±0.04
Glutamic acid	4.01±0.01	4.09±0.11	3.62±0.08	3.47±0.10
Serine	1.29±0.01	1.25±0.01	1.22±0.10	1.33±0.07
Histidine	0.96±0.01	0.88±0.08	0.88±0.01	0.93±0.06
Glycine	1.37±0.04	1.22±0.03	1.25±0.07	1.23±0.03
Threonine	1.31±0.01	1.28±0.04	1.28±0.10	1.35±0.07
Arginine	1.72±0.04	1.6±0.08	1.59±0.07	1.57±0.04
Alanine	1.66±0.03	1.63±0.01	1.66±0.08	1.7±0.08
Tyrosine	1.05±0.03	1.06±0.01	1.03±0.04	1.13±0.17
Methionine	0.82±0.04	0.79±0.06	0.81±0.08	0.89±0.07
Valine	1.68±0.03	1.74±0.06	1.65±0.07	1.70±0.07
Phenylalanine	1.47±0.03	1.45±0.08	1.41±0.25	1.44±0.11
I-leucine	1.52±0.03	1.55±0.06	1.46±0.06	1.48±0.08
Leucine	2.21±0.03	2.27±0.08	2.10±0.21	2.07±0.27
Lysine	1.6±0.08	1.49±0.10	1.46±0.11	1.52±0.11
Total	25.3±0.48	25.02±0.86	24.39±1.47	24.42±1.36

Feeding trial. Juvenile rabbitfish were obtained from the wild through local fishermen who collected the fish using net trap (local name *banrong*). The fish were reared in floating net cages until they were around 100 g body weight before being used for the feeding trial. During this period, the fish were maintained in 8 m^3 floating net cages and fed a formulated diet containing around 30% crude protein. For the experiment, 15 fish with initial body weight of 109.0±1.3 g were stocked into 12 cages each 1x1x2.5 m^3. Fish were fed the experimental diets daily at 08:00 and 16:00, at a rate of 2.5-3.0% of total biomass for 16 weeks. Every month, fish were individually weighed to determine their weight gain, which was used as the basis for adjustment of the feed ration.

Chemical analysis. Proximate analyses of the diet ingredients and experimental diets were carried out according to AOAC (1999) methods. Briefly, moisture was analyzed after drying the samples at 105°C for 16 hours using oven (Memmert, Germany). Crude protein was determined according to micro-Kjeldahl procedure and lipid was extracted using chloroform and methanol. Ash was analyzed using muffle furnace at 550°C (Barnstead Thermolyne, CA, USA). The amino acid profile of fish fillet was analysed using high performance liquid chromatography (HPLC) in a Shimadzu 20A HPLC (Tokyo, Japan).

Statistical analysis. Weight gain, survival, FCR, total amino acid and other parameters observed were subjected to one-way analysis of variance (SPSS version 17). Tukey's test was used to compare means.

Results and Discussion. CWM contained a medium crude protein level of 23.0% which is similar to SCM (24.2%), coconut cake meal (22.4%) and pea meal (22-24%) (Borlongan et al 2003; Laining & Kristanto 2015). The total amino acid content of the CWM was 20.5% (Table 4). All nine essential amino acids (EAA) were detected in the CWM excluding tryptophan which was not analysed in the current study. Lysine and leucine content of the CWM were moderately high at around 0.95% and 1.09%, respectively. However, the content of histidine and methionine were quite low at only 0.04% and 0.18% respectively.

Table 4

Amino acid profile of CWM

Amino acid	*% dry matter*
L-Aspartic Acid	2.59
L-Serine	1.76
L-Glutamic Acid	7.97
Glycine	0.90
L-Histidine	0.04
L-Arginine	0.70
L-Threonine	0.70
L-Alanine	0.92
L-Proline	0.64
L-Valine	0.76
L-Methionine	0.18
L-Lysin HCl	0.95
L-Isoleucine	0.70
L-Leucine	1.09
L-Phenylalanine	0.64
Total	20.54

The mean fiber content of CWM was 15.1% which is comparable with rice bran (14.3%) and seaweed *Gracillaria* (16.1%) (Laining & Kristanto 2015) but much lower than is found in other common feed ingredients such as palm oil cake (31.1%) (Laining et al 2003). In addition, its fibre content is much lower compared to other aquatic plants such as hydrophylic water hyacinth, *Eichhornia crassipes* (25.3%) (Hertrampf & Piedad-Pascual 2000). Table 5 shows the mean values of percentage weight gain (WG), specific growth rate (SGR), survival rate and food conversion ratio (FCR) of rabbitfish fed the diets containing different levels of CWM. After 16 weeks of feeding, the average weight of the rabbitfish in the experiment had increased by 96-115% (Table 5). WG and SGR values for fish fed CWM up to 30% inclusion did not significantly differ from fish fed the control diet (CWM0) (Table 5). In contrast, the diet containing 40% CWM (CWM40) demonstrated lower WG and SGR than the other treatments. No significant difference was found for survival rate between treatments.

Recent studies evaluating *Ceratophyllum* or CWM as a dietary ingredient for aquatic species are not readily available, and thus difficult to compare with the findings of this study. Hajra (1987) reported that the submerged aquatic weed *Ceratophyllum demersum* offered freshly *ad libitum* supported the growth of grass carp (*Ctenopharyngodon idella*), a freshwater herbivorous fish. In the present study, similar growth between fish fed SCM or control diet and the diets containing CWM up to 30% demonstrated that SCM could be replaced with CWM up to 55% in rabbitfish diet. FCRs were generally high for all groups but were significantly higher for fish fed the CWM30 and CWM40 diets (Table 5). Lower growth and higher FCR's of fish fed the highest CWM inclusion levels indicated that CWM may contain certain nutrients that cause adverse effect on the rabbitfish growth. Even though the fiber content of CWM is moderately low, its fiber type might be the indigestible derivatives such as lignin and cellulose which are commonly identified in other aquatic weeds including *Pistia stratiotes* and *Lemna perpusilla* (Chanda et al 1991).

Table 5

Growth, survival rate and feed utilization of rabbitfish, *S. guttatus*, fed different levels of CWM. Data are presented as mean±SD

Variables	*Inclusion level of dietary CWM (%)*			
	CWM0	*CWM20*	*CWM30*	*CWM40*
Initial weight (g)	108.9±2.2[a]	109.0±1.3[a]	109.0±0.9[a]	109.0±0.9[a]
Final weight (g)	233.0±5.7[b]	226.8±1.6[ab]	233.8±9.9[b]	214.0±6.7[b]
Weight gain (%)	114.1±7.5[b]	108.1±3.9[ab]	114.6±10.1[b]	96.3±4.7[a]
SGR (% day^{-1})	0.64±0.03[b]	0.61±0.02[ab]	0.64±0.04[b]	0.56±0.02[a]
Survival rate (%)	91.1±10.2[a]	95.6±7.7[a]	91.1±7.7[a]	88.9±7.7[a]
Feed intake (g $fish^{-1}$)	318±44[a]	323±18[a]	363±36[a]	364±21[a]
FCR (g g^{-1})	2.6±0.4[a]	2.7±0.1[a]	2.9±0.2[ab]	3.4±0.2[b]

Treatment means with different superscripts are significantly different ($p < 0.05$).

Feed intake of rabbitfish ranged from 318–364 g $fish^{-1}$ and no significant effects ($p > 0.05$) were found among groups for this parameter (Table 5). Similar feed intake of all diets even at the highest inclusion level revealed that CWM did not negatively influence the palatability of the pellets. The proximate composition of fish flesh is presented in Table 6. Crude protein content was highest (76%) at CWM inclusion levels of 20–30%. However, at the highest inclusion level of 40%, fillet crude protein content was significantly lower (71%), and this was not significantly different to the control (CWM0). The protein content of wild fish was similar to the higher values obtained using the CWM20 and CWM30 diets.The lipid content of fish fillets was similar across all diet treatments and was higher than that of wild fish.

Table 6

Fillet proximate composition of *S. guttatus* fed test diets with different levels of CWM. Data are presented as mean±SD

Nutrient (% dry matter)	*CWM0*	*CWM20*	*CWM30*	*CWM40*	*Wild fish*
Crude protein	70.7±1.1[a]	75.5±1.3[b]	76.0±0.6[b]	70.8±1.0[a]	75.5±0.4[b]
Lipid	15.8±0.8[b]	13.8±1.1[b]	14.2±0.3[b]	14.8±0.7[b]	7.2±0.2[a]
Ash	4.6±0.8[a]	5.6±0.8[a]	4.7±0.3[a]	4.4±0.3 [a]	4,8±0.4[a]
Fiber	0.4±0.1[a]	0.5±0.0[a]	0.4±0.1[a]	0.6±0.1[a]	0.5±0.1[a]
NFE	8.6±1.0	4.6±1.0	4.7±1.3	9.4±2.1	12.4±0.2

Treatment means with different superscripts are significant different ($p < 0.05$).

Total amino acid was highest (73–74%) in fish fed the CWM20 and CWM30 diets (Table 7), which reflects the pattern seen in the proximate analyses of fish flesh where protein values were highest for these two treatments. Specifically, EAA of phenylalanine, lysine and leucine in fillet of fish fed 20% and 30% CWM were significantly different from fish fed the other diets. It is likely that these EAAs that are found in moderately high levels in CWM could be efficiently utilized by rabbitfish using diets incorporating CWM.

Table 7
Amino acid profiles of fillet rabbitfish fed different levels of CWM. Data are presented as mean ±SD

Amino acid	*Amino acid (% dry matter)*				
	CWM0	*CWM20*	*CWM30*	*CWM40*	*Wild fish*
Aspartic acid	7.55 ± 0.14^{ab}	8.13 ± 0.11^{d}	8.03 ± 0.04^{cd}	7.31 ± 0.01^{a}	7.72 ± 0.06^{bc}
Glutamic acid	11.51 ± 0.07^{a}	12.47 ± 0.05^{bc}	12.73 ± 0.11^{c}	11.58 ± 0.09^{a}	12.33 ± 0.07^{b}
Serine	2.70 ± 0.04^{b}	2.88 ± 0.03^{c}	2.85 ± 0.05^{bc}	2.50 ± 0.03^{a}	2.67 ± 0.08^{ab}
Histidine	1.77 ± 0.02^{ab}	1.91 ± 0.02^{ab}	1.93 ± 0.02^{b}	1.70 ± 0.11^{a}	1.81 ± 0.03^{ab}
Glycine	3.59 ± 0.10^{b}	3.90 ± 0.04^{c}	3.63 ± 0.03^{b}	3.01 ± 0.01^{a}	3.57 ± 0.04^{b}
Threonine	3.26 ± 0.08^{ab}	3.52 ± 0.04^{b}	3.35 ± 0.04^{b}	3.01 ± 0.04^{a}	3.20 ± 0.14^{ab}
Arginine	4.50 ± 0.71^{a}	4.93 ± 0.04^{a}	4.90 ± 0.04^{a}	4.38 ± 0.04^{a}	4.90 ± 0.04^{a}
Alanine	4.31 ± 0.20^{ab}	4.75 ± 0.05^{a}	4.69 ± 0.12^{b}	4.24 ± 0.04^{a}	4.71 ± 0.04^{b}
Tyrosine	2.72 ± 0.03^{ab}	2.93 ± 0.04^{b}	2.89 ± 0.12^{b}	2.61 ± 0.06^{a}	2.73 ± 0.04^{ab}
Methionine	2.30 ± 0.13^{a}	2.51 ± 0.08^{a}	2.48 ± 0.03^{a}	2.23 ± 0.04^{a}	2.35 ± 0.11^{a}
Valine	4.00 ± 0.71^{a}	4.41 ± 0.01^{a}	4.39 ± 0.16^{a}	4.03 ± 0.03^{a}	4.24 ± 0.16^{a}
Phenylalanine	3.14 ± 0.06^{a}	3.49 ± 0.02^{c}	3.42 ± 0.06^{bc}	3.12 ± 0.04^{a}	3.28 ± 0.04^{ab}
I-leucine	3.93 ± 0.04^{ab}	4.21 ± 0.08^{b}	4.18 ± 0.11^{ab}	3.85 ± 0.09^{ab}	3.98 ± 0.11^{ab}
Leucine	6.17 ± 0.02^{ab}	6.57 ± 0.04^{c}	6.52 ± 0.09^{bc}	5.98 ± 0.13^{a}	6.29 ± 0.04^{ab}
Lysine	7.09 ± 0.13^{b}	7.83 ± 0.02^{d}	7.45 ± 0.09^{c}	6.14 ± 0.03^{a}	6.31 ± 0.04^{a}
Total amino acid	68.53 ± 1.17^{ab}	74.44 ± 0.28^{d}	73.44 ± 1.04^{cd}	65.69 ± 0.09^{a}	70.09 ± 0.86^{bc}

Treatment means with different superscripts are significant different ($p < 0.05$).

Conclusions. Based on biological performances and total amino acid content in fish fillet, CWM could be incorporated up to 30% replacing 55% of SCM in grow-out diets for rabbitfish reared in floating net cages.

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