Nutrient Content of Seaweed and Its Digestibility in
Osteochilus hasseltii

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Abstract. Seaweed is an abundant biological resource in Indonesian waters and has complete nutrients, so its utilization is very diverse. This study aims to determine the nutrient content of four seaweeds Gracilaria sp., Palmaria sp., Sargassum sp., Ulva sp.; and evaluate the digestibility in Osteochilus hasseltii. Seaweeds were taken at Pulang Sawal coast, Special Region of Yogyakarta. The digestibility measurement is carried out by providing a test diet which consists of 70% reference diet and 30% seaweed meal. Chromium oxide was used as a marker and added 0.6% to both reference and test diet. The average weight of experimental fish was 11.24±0.21 g and stocked 15 fish per aquarium, which is filled with 90L of water. The results showed that carbohydrate (NFE) is the largest component in seaweed. Ulva sp. had the highest digestibility in O. hasseltii (68.13%), while Palmaria sp., Sargassum sp., and Gracilaria sp. were 66.96%, 66.04%, and 65.23%, respectively. The four seaweeds used in this study were digestible by O. hasseltii and potential be used as an alternative ingredient in O. hasseltii diet.

1. Introduction
Feed is still the biggest obstacle in aquaculture because most of the ingredients contained in feed are imported. Efforts to utilize terrestrial plants as fish feed ingredient have been carried out, such as the use of rice bran [1], wheat bran [2], palm kernel meal [3] and cassava leaf [4]. However, the use of terrestrial plants is limited by anti-nutritional factors such as phytic acid and crude fiber, so the other kinds of plants are needed to be used as alternative ingredient in fish diet.

Macro algae or seaweed has the potential to be used as an alternative ingredient because it contains complete nutrients and easy to cultivate [5]. Several studies reported that the protein content ranges 6.3-26.6%; lipid 0.3-7.9%; ash 17.5-44.1%; crude fiber 5.74% and NFE 41.47–59.10%, in DM (dry matter) [6,7,8]. Seaweed has a high growth rate, supported by the potential of Indonesia's natural resources that meet its availability. The diversity of seaweed in Indonesia encourages efforts to use it as an economic product, such as a fish feed ingredient. Seaweed Gracilaria sp., Palmaria sp., Sargassum sp., Ulva sp. are types of seaweed which is commonly found in tropical regions. It is hoped that the use of seaweed will reduce the demand for imported ingredients which has been causing the high price of fish feed.

An important aspect in utilizing alternative ingredient is their digestibility. Feed ingredients which have high digestibility will improve growth performance and reduce aquaculture waste that pollutes the environment. Thus, information about digestibility is needed in the use of unconventional ingredients.

This research aims to determine the nutrient composition of the four types seaweed Gracilaria sp., Palmaria sp., Sargassum sp., Ulva sp.; which were taken at Pulang Sawal coast, Yogyakarta Special Region and evaluating their digestibility in O. hasseltii.

2. Material and Method
The research was conducted at the Laboratory of Nutrition and Fish Feed Technology, Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Bogor, West Java, Indonesia.

2.1. Preparation of seaweed meal
Seaweed is taken from its natural habitat on Pulang Sawal coast, Yogyakarta Special Region, Indonesia. Seaweed is cleaned with tap water until there is no more sand and dirt attached. Then dried
using an oven at 60°C for 48 hours and mashed using a grinder machine. Then the seaweed meal stored for the next stage of use.

2.2. Feed formulation
The diet for the digestibility measurement was prepared based on the Watanabe method [9]. The test diet was prepared from 70% reference diet and 30% seaweed meal. Chromic oxide (Cr$_2$O$_3$) was used as a marker and added 0.6% both to the reference and test diet. The composition of the reference and test diet was shown in table 1.

Table1. Formulation of reference and test diet (%)

| Ingredients  | Reference diet | Test diet |
|--------------|----------------|----------|
| Basal diet   | 95.4           | 65.4     |
| Seaweed meal | 0.0            | 30.0     |
| Cassava meal | 4.0            | 4.0      |
| Cr$_2$O$_3$  | 0.6            | 0.6      |
| Total        | 100.0          | 100.0    |

2.3. Fish rearing and feeding regime
Experimental fish O. hasseltii used in this study obtained from RIFAFE, Bogor, Indonesia. The average weight of experimental fish was 11.24±0.21 g and stocked 15 fish per aquarium, which is filled with 90L of water, completed with aeration system. Fish were acclimatized for seven days prior to starting treatment. Water quality parameters are maintained at optimum conditions.

2.4. Feces collection
The experimental fish fed three times a day (08.00, 12.00, 16.00) at satiation level. Feces collection started on the seventh day after the fish were fed. Feces is collected by siphoning. The collected feces then dried using an oven at 40°C for 24 hours. The collection process is carried out until the amount of feces is sufficient for analysis purposes.

2.5. Chemical analysis and data expression
Seaweed meal, reference and test diet, collected feces were sampled for analysis of chromium and proximate composition. Chromium analysis was carried out based on the Takeuchi method [10] while proximate using the AOAC method [11]. The moisture content analysis was carried out by evaporating the water in seaweed meal using an oven at 105°C for 3-4 hours or until a constant weight was obtained. Protein content was determined by the Kjeldahl method. Lipid content was determined by extraction using petroleum benzene solvent. The ash content was determined using a furnace at a temperature of 600°C for 4 hours. Crude fiber is determined using strong acid and alkaline solvents. Analysis of each nutrient was carried out at three replicates (n=3). The results which obtained are used to determine the mean value±standard deviation.

3. Result
The largest nutrient contained in the seaweed used in this study is carbohydrate by difference/NFE (51.94-55.56%), followed by ash (22.56-26.27%), protein (11.44-15.86%), crude fiber (6.97-9.72%) and lipid (0.58-0.72%), respectively. The nutrient composition of each seaweed is shown in figure 1. Protein content of Ulva sp. in this study (15.86%) was higher than Palmaria sp. (13.53%), Sargassum sp. (12.02%) and Gracilaria sp. (11.44%). The lipid in the four types of seaweed is less than 1.00%. Ash content in Gracilaria sp. 26.27%, much higher than Palmaria sp. (25.35%), Sargassum sp. (23.47%) and Ulva sp. (22.56%). Crude fiber content in four types of seaweed is less than 10.00%.
The results of the digestibility measurement for each seaweed are shown in figure 2. The results show that the ingredient digestibility of *Ulva* sp. is the highest (68.13%), compared to *Palmaria* sp. (66.96%), *Sargassum* sp. (66.04%) and *Gracilaria* sp. (64.79%), while the protein digestibility (86.97%) and lipid digestibility (92.64%) of *Gracilaria* sp. is the highest.

4. Discussion

The largest component contained in seaweed is carbohydrate by difference/NFE. This is in line with previous research [12] which states that carbohydrates are the most nutrient found in seaweed. The second largest component is ash, which is present in greater quantities than terrestrial plants. The high ash content in seaweed is caused by its habitat which contains high levels of salt and various minerals [13]. The variation in ash content in seaweed is caused by several factors, including species, location, geography, season, environment, physiology and mineralization processes [14]. When compared with terrestrial plant commonly used as feed ingredients, for example rice bran [15], pollard and corn [16], the ash content in seaweed is much higher.

The crude fiber content of seaweed in this study less than 10.00%. Generally, the crude fiber found in seaweed is much lower than terrestrial plants. Crude fiber known as anti-nutritional factor in some monogastric animals [17]. Crude fiber indicates the amount of fiber that can be digested and affects
energy digestibility [18] and produces pellets with weak binding strength and is easily destroyed in water. The presence of high crude fiber in feed ingredients will affect the digestibility of the material. The protein content of seaweed meal used in this study ranged from 11.44-15.86%. The varies of protein content in seaweed depend on the season and environmental conditions [19]. When compared with rice bran (11.01%) [15] and maize (8.83%) [16] which are often used as an alternative ingredient in fish diet, the protein contained in seaweed relatively higher.

The lipid in the seaweeds used in this study less than 1.00%. The lipid content in seaweed varies depending on species, geographic location, season, temperature, salinity and light intensity as well as the interaction of these various factors [20].

Nutrient digestibility is the initial stage for evaluating the potential of ingredient to be used in feed. Information on the digestibility value is needed to maximize fish growth and metabolic yields removed [21]. Digestibility shows the amount of nutrient composition that is absorbed and used for growth and metabolic processes [22]. The results (figure 3) indicate that O. hasseltii could digest Ulva sp. the highest, compared with the others. This is supported by the nutrient composition of these types of seaweed. Seaweed Gracilaria sp. has the highest ash (26.27%) and crude fiber (9.72%) when compared to the others. Ash and crude fiber are included in the components that are not easily digested by fish. Diets with high crude fiber content are reported to reduce fish body weight [23]. In addition, high ash content in feed will cause a decrease in nutrient absorption which in turn lead to decreased growth [24].

Protein digestibility of Gracilaria sp., Palmaria sp., Sargassum sp., and Ulva sp. which are used in this study, within the range of values that are good for protein digestibility by fish, 75-95% [22]. The protein digestibility of seaweed meal was not much different with pollard 82.87% [23], and soybean meal 87.40% [24].

Lipid digestibility of Gracilaria sp., Palmaria sp., Sargassum sp., and Ulva sp. in this study is in line with previous studies which stated that lipid digestibility ranged 72.0-97.5% [25]. Although the lipid content in seaweed is low, the digestibility of lipid depends on the fatty acid composition. In addition, high lipid digestibility is associated with high lipase activity in fish [26].

5. Conclusion
Carbohydrate by difference (NFE) was the largest nutrient content in seaweed, followed by ash. Ulva sp. had the highest ingredient digestibility 68.13%, while Gracilaria sp. had the highest protein and lipid digestibility, 86.97% and 92.64%, respectively. The four types of seaweed used in this study digested well by O. hasseltii.

6. References
[1] Singh R, Singh P, Nayak S K, Reang D and Tripathi G 2017 Int. J. Fish. Aquatic Stu. 25(5) 135-139
[2] Khanom M, Golder J, Chisty M A H, Debnath S, Arafat S T and Parvez M S 2017 Int. J. Res. Stu. Biosci. 5(4) 31-36
[3] Ilham Zulfahmi I, Herjayanto M, Batubara A S and Affandi R 2019 Pak. J. Nutr. 8(8) 753-760
[4] Diarra S S, Koroiagilagi M, Tamani S, Maluhola L, Isiolo S, Batibasila J, Vaea T, Rota V and Lupea U 2017 J. Agr. Rural Develop. Trop. Subtrop. 118(1) 105–112
[5] Overland M, Mydland L T and Skrede A 2018 J. Sci. Food Agric. 1-12
[6] Penalver R, Lorenzo J M, Ros G, Amaroowicz R, Pateiro M and Nieto G 2020 Mar. Drugs 18(301) 1-27
[7] Garcia JS, Palacios V and Roldan A 2016 J. Nutr. Food Sci. 6(505) 1-7
[8] Rosemary T, Arulkumar A, Paramasivam S, Mondragon-Portocarrero A and Miranda J M 2019 Mol. 24(2225) 2-14
[9] Watanabe T 1988 Fish Nutrition and Mariculture (Tokyo: JICA Tokyo University of Fisheries)
[10] Takeuchi T 1988 Laboratory work chemical evaluation of dietary nutrients Fish Nutrition and Mariculture ed Watanabe T (Tokyo: JICA Textbook the General Aquaculture Course) pp 179-23
[11] Association of Official Analytical Chemists 2005 Official Methods of Analysis (Washington: Benjamin Franklin Station)
[12] Mwalugha H M, Wakibia J G, Kenji G M and Mwasaru M A 2015 J. Food Res. 4(6) 28-38
[13] Morais T, Inacio A, Coutinho T, Ministro M, Cotas J, Pereira L and Bahcevandziev 2020 J. Mar. Sci. Eng. 8 559-583
[14] Siddique M A M, Khan M S K and Bhuiyan M K A 2013 Int. Food Res. J. 20(5) 2287-2292
[15] Sirikul A, Moongngarm A and Khaengkhan P 2009 Asian J. Food Agro-Ind. 2(4) 731-743
[16] Huang Q, Su Y B, Li F, Liu L, Huang C F, Zhu Z P and Lai C H 2015 Asian Aust. J. Anim. Sci. 28(6) 847-854
[17] Jha R, Fouhse J M, Tiwari U P, Li L and Willing B P 2019 Anim. Front. Vet. Sci. 6(48) 1-12
[18] Hervik A K and Svihus B 2019 J. Nutr. Met. 1-7
[19] Aroyehun A Q, Palaniveloo K, Ghazali F, Rizman-Idid M and Razak S A 2019 Mol. 24(3298) 1-23
[20] Susanto E, Fahmi A S, Hosokawa M and Miyashita K 2019 Mar. Drugs. 17(630) 1-21
[21] Small B C, Hardy R W and Tucker C S 2016 Anim. Front. 6(4) 42-49
[22] National Research Council 2011 Nutrient Requirements of Fishes (Washington DC: National Academy of Sciences
[23] Sun Y, Zhao X, Liu H and Yang Z 2019 J. Aquacult. Res Dev. 10(577) 1-7
[24] Small B C, Hardy R W and Tucker C S 2016 Anim. Front. 6(4) 42-49
[25] Agyekum A K, Nyachoti M 2017 Eng. 3(5) 716-725
[26] Ribeiro F B, Lanna E A T, Bomfim M A D, Donzele J L, Moises Q and Cunha P S L 2011 Rev. Bras. Zoo. 40(5) 939-946
[27] Kopruçu K and Ozdemir Y 2005 Aquacult. 250 308-316
[28] Sklan D, Prag T and Lupatsch I 2004 Aquacult. Res. 35 358-364
[29] Adhami B, Amirkolaie A K, Oraji H and Kenari R E 2017 Aquacult. Nutr. 23(5) 153-159