Nutrient composition and apparent digestibility coefficient of *Ulva lactuca* meal in the Nile tilapia (*Oreochromis niloticus*)

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Abstract. This experiment was conducted to evaluate nutrien composition and apparent digestibility coefficient (ADC) of *Ulva lactuca* meal as an ingredient in the Nile tilapia (*Oreochromis niloticus*) diet. Nutrient composition and their ADC could be used to evaluate the nutritive value of a feed ingredient. The seaweed was taken from their natural habitat in Pulang Sawal Beach, Indonesia. The contents of crude protein, lipid, ash, crude fiber, carbohydrates (by difference) were 13.65%, 0.53%, 33.19%, 9.12% and 43.51%, based on dry weight, respectively. Composition of neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, cellulose, and hemicellulose were 29.73%, 14.95%, 1.43%, 11.29%, and 14.53%. In measuring the apparent nutrient digestibility of *U. lactuca*, a protein rich diet was formulated together with a test diet in which 30% by weight of the reference diet was replaced with *U. lactuca* meal. Chromic oxide was used as an inert marker and added to both reference and test diets. Fifteen fishes with mean weight of 10±0.21 g were held in 90 L aquaria and fed three times daily. Fecal samples were collected from fish. The ADC's of *U. lactuca* meal were dry matter 67.08%, crude protein 82.12%, lipid 92.34%, ash 63.59% and energy 74.25%. The result showed that nutritive value of *U. lactuca* meal in this present study was fairly digestible by Nile tilapia and it could be as an ingredient in its diet.

Key Words: nutrient digestibility, *Ulva lactuca*, feed ingredient, Nile tilapia.

Introduction. Feed has still remained a tremendous constraint in aquaculture. Since most ingredients depend on import and leading the instability of price. Some studies using local materials such as rice polish (Khan et al 2013), cassava leaf bran (Sena et al 2012) and palm kernel meal (Thongprajukaew et al 2015) have been conducted to substitute the imported ingredients. However, the use of these materials is limited due to presence of high crude fiber, which is uneasily digested, and anti nutritional factor such as phytic acid. Therefore, finding potential material as alternative ingredients has widely attracted the researchers during last two decades (Viscaino et al 2015).

Macroalgae or seaweed is reported to have great potential as alternative materials for their nutritional contents and easy-to culture that causing high availability (Pereira et al 2012; Wassef et al 2013). In general, seaweed contains complex polysaccharides as the major components, and also proteins, lipid, ash, and minerals (Wi et al 2009). Previous research showed that protein, lipid, crude fiber (based on dry basis), and nitrogen free extract of seaweed were 10.00-17.44%, 0.11-3.60%, 12.40-32.85%, 5.74-9.17%, 41.47–59.10%, respectively (Wassef et al 2005; Ergun et al 2009; Rohani-Ghadikolaei 2012). Additionally, carbohydrates contained in seaweed are more easily converted, in comparison with other terrestrial lignocellulose sources (Sanchez-Machado et al 2004; Mosier et al 2005). Seaweed has a high mineral content. Mineral that contained in *Ulva* sp. were P 160.2–479 mg/100 g, Fe 4.92-46.4 mg/100 g, Zn 1.0-1.85 mg/100 g, Mg 79.1–609 mg/100 g, Mn 1.5–316 mg/100 g, Ca 604.5–742 mg/100 g, K

1.536–6.159 mg/100 g, Na 435–4.189 mg/100 g, Cu<0,45 mg/100 g (Krishnaiah et al 2008; Benjama & Masniyom 2011; Smith et al 2010).

Seaweed *Ulva lactuca* belongs to green seaweed (Chlorophyceae) and could grow under various ranges of temperature and salinity. Its morphological characteristics are rapidly changed in accordance with environmental changes. *Ulva* sp. is found in almost shallow coastal waters of Indonesia and is able to live in sub-tropic and tropical regions, which makes it to be recognized as a cosmopolitan algae. This algae is abundant in nutrient-rich habitats, and considered as a fast growing algae. It is suggested that *U. lactuca* is feasible to be cultivated. In several tropical areas, emergence of blooming *Ulva* sp. or green tides was reported due to overgrowth of *Ulva* sp., causing the reduction of biodiversity (Buapet et al 2008).

Evaluation of digestibility is a meaningful aspect in determining material for feed ingredients. Feed containing high digestible ingredients is associated with better growth performance and lower feed waste that potentially pollutes the environment. Therefore, evaluation of apparent digestibility of alternative ingredients was required (Glencross et al 2007), since the evaluation is useful to estimate the digestibility of formulated feed (Cho et al 1985). This study aimed to investigate the digestibility of *U. lactuca* as feed ingredient in Nile tilapia (*Oreochromis niloticus*) diet.

Material and Method. The experiment was conducted from January to March 2016 in Wet Laboratory, Department of Nutrition and Fish Feed Technology, Research and Development Center of Freshwater Fisheries, Bogor, Indonesia.

Sample collection and preparation*.* *U. lactuca* was collected from Pulang Sawal Beach, Indonesia. The seaweed was washed with freshwater to remove sand and unwanted materials. It was powdered after drying process subsequent to washing and stored in hermetic plastic bag for future use.

Fish and experimental condition. The aquaria (60x50x40 cm) with aeration system were used. These aquaria were used for reference and test diet. The experimental fish was Nile tilapia, obtained from Bogor, Indonesia. The fish with mean weight of 10±0.21 g were distributed with density of 15 fish in each aquaria. Water quality was maintained at 29-30^{o}C, pH 7, dissolved oxygen (DO) >3 mg L^{-1}, and ammonia-N (NH_3-N) < 0.05 mgL^{-1}.

Diet preparation. Reference diet (Table 1) was formulated based on nutritional requirement for tilapia. Chromic oxide (Cr_2O_3) was used as marker, and incorporated (at 0.6%) in both reference and test diets (Takeuchi 1988). The test diet consisted of reference diet (70%) and *U. lactuca* powder (30%). The mixing was carried out from the smallest proportion of feed ingredient using mixer to obtain homogenous mixture. The diet dough was then formed and dried using an oven at 60ºC for 12 hours. The proximate composition of diet was presented in Table 1.

Fecal collection*.* The experimental fish were acclimatized to rearing condition for 7 days prior to treatment. The water was replaced at 80% of total volume at morning before feeding. The fish were fed three times a day (morning, afternoon, and evening) at satiation level. In the day 5, the fecal collection was carried out after treatments of reference and test diets. Syphoning was done every day to remove feces and other debris. The feces was collected and dried at 60ºC for 48 hours. The dried feces was collected in hermetic plastic bags and stored in a refrigerator. The fecal collection was conducted every 2 hours to avoid nutrient leaching, and performed to obtain sufficient amount for analysis.

Table 1

Composition and proximate analysis of the reference and test diets (g kg^{-1})

Feed ingredients	*Reference diet*	*Test diets*
Fish meal	143.2	98.6
Soybean meal	455.5	313.8
Wheat bran	250.6	172.6
Cassava meal	74.2	51.1
Fish oil	7.2	5.0
Palm oil	16.4	11.3
Vitamin mix	16.9	11.6
U. lactuca meal	0	300.0
CMC	30.0	30.0
Cr_2O_3	6.0	6.0
Total	1000	1000
Proximate analysis (% in dry weight)		
Moisture	3.14	4.48
Crude protein	35.50	29.07
Crude lipid	5.84	4.21
Ash	9.61	16.78
Crude fiber	6.06	6.59
NFE[a]	42.98	43.25

Note: [a] Nitrogen Free Extract, calculated by difference.

Analytical methods. Seaweed (*U. lactuca*), diet and fecal sample were pulverized for analysis. The proximate analysis was carried out in Chemical Laboratory, Department of Nutrition and Fish Feed Technology, Research and Development Center of Freshwater Fisheries, Bogor, Indonesia. Moisture content was determined by drying at 105°C to constant weight, while ash content was determined using furnace (Furnace THERMOLYNE 47900; at 600°C for 4 hours). Protein content (Nx6.25) was conducted based on Kjeldahl method (destruction by FOSS Tecator™; distillation by Kjeltec FOSS 2100; titration by JENCONS Digitrate Pro). Lipid content was determined by extracting petroleum eter (FOSS Soxtec™ 2055), while crude fiber was determined using gravimetric method by reacting sample to acid and alkaline to separate fiber fraction and other components. Crude fiber fraction was determined according to previous method of Van Soest et al (1991). Wet destruction method was done to determine Cr_2O_3 content in the diet and feces, and the absorbance was detected using spectrophotometer (Takeuchi 1988). Diet digestibility was determined as:

$$ADC(\%) = 100 - (100 \times \frac{a}{a'} \times \frac{b'}{b})$$

where a: Cr_2O_3 in diet (%), a': Cr_2O_3 in feces (%), b: nutrient in diet (%), b': nutrient in feces (%) (Goddard & McLean 2001).

The ingredient digestibility was determined as:

$$ADC_{ing}(\%) = \frac{(ADT - 0.7\ AD)}{0.3}$$

where ADC_{ing} is ingredient digestibility, ADT: digestibility of test diet (%), AD: digestibility of reference (%) (Bureau & Hua 2006).

Data expression. The data were tabulated using Microsoft excel, and expressed as mean±standard deviation. The experiment was conducted at triplicates (n = 3).

Results and Discussion. Table 2 presents proximate composition of *U. lactuca* meal. The results showed that *U. lactuca* meal had low lipid and crude fiber content, but high ash content and NFE (Nitrogen Free Extract).

Table 2

Nutrient composition of *U. lactuca* meal

Parameters	*% in dry weight*
Moisture	3.06±0.05
Crude protein	13.65±0.09
Lipid	0.53±0.05
Ash	33.19±0.56
Crude fiber	9.12±0.27
Nitrogen Free Extract (NFE)[a]	43.51±0.87
Gross Energy (GE)[b]	259.78±3.37

Note: [a] Calculated by difference; [b] Expressed in kcal g^{-1}, calculated according to energy values of 1 g protein = 5.6 kcal, 1 g lipid = 9.4 kcal, 1 g carbohydrate = 4.1 kcal (Watanabe 1988).

Based on the results, we found that protein content of *U. lactuca* meal was 13.65 %DW, higher than other seaweeds such as *Sargassum* sp. (5.40 %DW), *Eucheuma cottonii* (9.76 %DW) and *Caulerpa lentillifera* (10.41 %DW) (Matanjun et al 2009). The protein content of seaweed used was also higher than *U. lactuca* from Saudi Arabia (11.50 %DW) (Abdel-Warith et al 2016) and Turkey (9.91 %DW) (Ergun et al 2009), but lower than *U. lactuca* from Chile (27.2 %DW) (Ortiz et al 2006) and Egypt (17.44 %DW) (Wassef et al 2013). This finding was augmented by Dawczynski et al (2007) and De Oliveira et al (2009) that protein content of seaweed was highly different, depending on climate and environmental conditions. Protein content of *U. lactuca* was also higher compared with rice bran (11.01 %DW) (Sirikul et al 2009) and corn (8.83 %DW) (Huang et al 2015).

We found that lipid content of *U. lactuca* was 0.53±0.05 %DW, lower than previous research (1-5%DW) by Polat & Ozogul (2008). The discrepancy of lipid content may result from species, geographical location, climate, temperature, salinity, light intensity, and interacted factors (Miyashita et al 2013; Yaich et al 2011). Seaweed is not lipid source, but contains unsaturated fatty acids that may be equal to terrestrial material. Although it has low lipid content, 20-50% of fatty acids were categorized as n-3 groups (Jeong et al 1993). The concentration dissimilarity is a result of temperature of habitat. Narayan et al (2004) reported that seaweed originated from low temperature sea had higher poly unsaturated fatty acid (PUFA) content than seaweed from tropical area. Seaweed from tropical area is rich in saturated fatty acids. Additionally, Nelson et al (2002) found that this discrepant content was associated with PUFA properties that were more sensitive to environmental conditions, contributing to macro algae physiology.

The highest component of *U. lactuca* is carbohydrate (NFE), which reaches 43.51±0.88 %DW. This result is in accordance with Rohani-Ghadikolaei et al (2012), reported that carbohydrate is the major component (59.1 %DW) in *U. lactuca* isolated from Persia gulf. NFE (nitrogen free extract) is a carbohydrate which is digested by fish. The main fraction of NFE is non-structural carbohydrate, starch, an intracellular energy storage (Lahaye & Robic 2007).

The second highest component in *U. lactuca* is ash (33.19±0.56 %DW), which is much higher than terrestrial ingredient. Ash content represents the mineral. However, presence of ash must be limited since it leads to limited use of diet by fish (Cho et al 1985). High content of ash in *U. lactuca* was caused by habitat condition that is rich in salt and various minerals (MacArtain et al 2007). Mabeau & Fleurence (1993) reported that ash content of seaweed was 8-40%. This variation resulted from such factors as species, location, geographical condition, climate, environment, physiology, and mineralization (Ruperez 2002; Sanchez-Machado et al 2004; Siddique et al 2013). Plausible mechanism of high mineral content in seaweed is absorption of minerals, macro elements, and trace elements in sea water, yielding high mineral content compared to terrestrial plants. Ash content of seaweed was higher compared to other terrestrial sources such as rice bran (14.75 %DW) (Sirikul et al 2009), wheat bran (5.80 %DW) and corn (1.07 %DW) (Huang et al 2015). It is noteworthy that high mineral content of seaweed provides desirable effects as mineral sources in diet.

Crude fiber content of *U. lactuca* is 9.12±0.26 %DW. This is commonly lower than terrestrial plants (Wong & Cheung 2000), such as rice bran (18.30 %DW) (Sirikul et al 2009). Crude fiber was reported as anti-nutritional factor on some monogastric animals (Sundu et al 2009). Crude fiber represents non digestible fibers, and affects the energy digestibility (Jung & Allen 1995), as well as results in low binding and water soluble pellet (Webster et al 2002). High crude fiber content alters the digestibility of ingredient. However, seaweed-derived polysaccharides showed physicochemical properties as binding agent in feed (Hashim & Saat 1992). Our data showed that crude fiber (9.12%) and fiber fraction of *U. lactuca* had much lower in comparison with wheat bran (NDF 42.90 %DW and hemicellulose 30.30 %DW) (Huang et al 2015). The highest crude fiber fraction was hemicellulose (14.53%) meanwhile the lowest one was lignin (1.43%). These results were in accordance with previous results of fiber fraction in *U. lactuca* originated from Tunisia (Yaich et al 2011). The comparative result is presented in Figure 1.

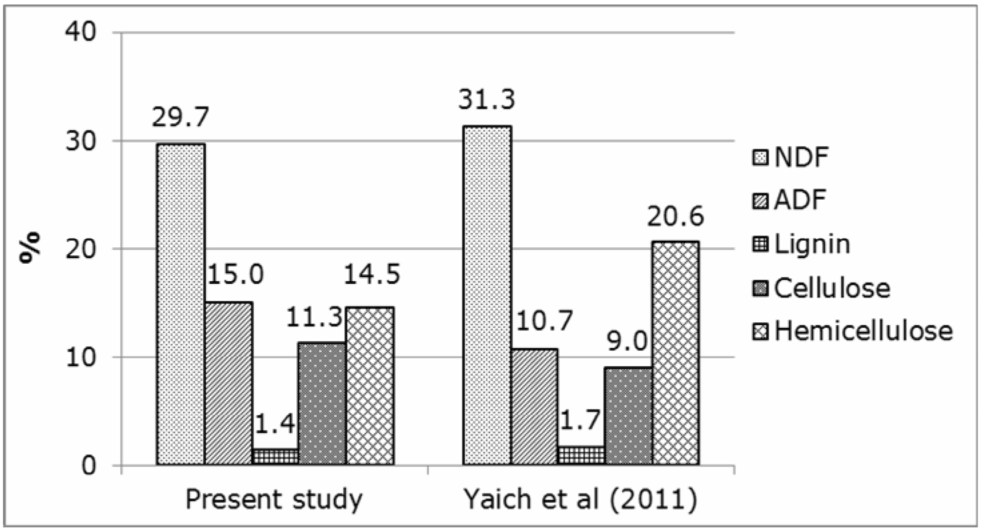

Figure 1. Crude fiber fraction of *U. lactuca*.

Digestibility of an ingredient could exhibit the nutrient digestibility by fish (Jimoh et al 2010). Hence the digestibility of an ingredient is an upmost factor for feed formulation since efficiency of nutrient digestibility highly contributes to growth performance of fish. In addition, the performance is also affected by nutritional composition and fish capability to digest and absorb nutrients (Rust 2003). Nutrient composition and feeding management are the fundamental aspect in designing feed formulation and feeding treatment. Our results revealed that *U. lactuca* had acceptable nutritional composition as an ingredient for tilapia feed. The digestibility coefficient of dry matter, protein, lipid, ash, and energy is exhibited in Figure 2. Lipid showed the highest ADC (92.34%), meanwhile protein, energy, dry matter, and ash were 82.12%, 74.25%, 67.08%, 63.59%, respectively.

Digestibility of dry matter is commonly used to understand the digestibility of all nutrients that are digested by fish. High digestibility of dry matter represents the quality of feed ingredient. Dry matter also constitutes amount of carbohydrate contained in a feed ingredient, since carbohydrate accounts for 50-80% of dry matter. In proximate analysis, cell wall components such as hemicellulose, cellulose, and lignin are recognized as carbohydrate (crude fiber and NFE). Other factors possibly affecting dry matter digestibility are percentage in feed formulation, nutritional composition, and presence of minerals. In addition, differences in dry matter digestibility are contributed by dissimilarities in properties of feed components which include suitability to enzymatic hydrolysis and other substance activities. Compared with other terrestrial plants such as corn (52.30%), wheat bran (45.0%) (Guimaraes et al 2012) and cocoa bran (38.1%) (Ramos et al 2012), the digestibility of *U. lactuca* is high (67.08±2.81%). The digestibility of dry matter positively correlated with carbohydrate digestibility (Lee &

Pham 2011). Starch is digested in anterior section of fish digestive tract, and it highly depends on solubility in digestive liquid. Low environmental temperature is associated with lower digestibility of the starch. The carbohydrate digestibility is also linked with activity of carbohydrase enzyme. Stone et al (2003) found that higher water temperature associated with higher activity of carbohydrase enzyme, thus increasing carbohydrate digestion. In addition, seaweed was reported to have high water holding capacity (Urriola & Stein 2010). Hence seaweed incorporation must be limited since it leads to production of bulky properties and increases moisture content in fish digestive tract.

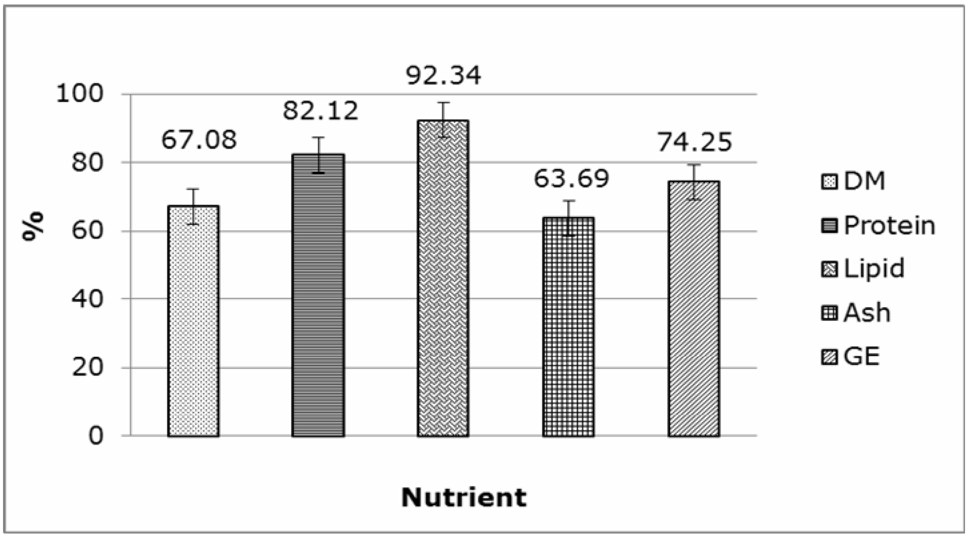

Figure 2. Nutrient digestibility coefficient of *U. lactuca* meal in the Nile tilapia (DM = dry matter; GE = gross energy).

The ADC of protein in *U. lactuca* was 82.12±2.43%. This result was in accordance with previous studies that protein digestibility of various plant sources in Nile tilapia was 75-95% (Koprucu & Ozdemir 2005). Our study revealed that coefficient of protein digestibility was higher than previous results (63.4%) reported by Pereira et al (2012). Additionally, Ramos et al (2012) reported that protein digestibility of rice bran, cassava leaf, and cocoa bran were 51.6%, 49.8% and 38.5%, respectively. Noreen & Salim (2008) stated that coefficient of protein digestibility was affected by such factors as drying, temperature, and storage duration. Protein quality of feed ingredients determines growth performance; hence protein digestibility is the upmost parameter to consider. The quality of protein associated with composition and digestion of amino acids. Therefore protein digestibility indicated the digestibility of amino acids (Koprucu & Ozdemir 2005; De-Oliveira et al 2012), and was influenced by proportion of amino acids (Carter et al 2001). Deficiency in essential amino acids promotes improper utilization of protein, negatively affecting on fish growth and feed efficiency.

Our study showed that lipid digestibility of *U. lactuca* was 92.34±0.36%. This finding was augmented by preceding studies that showed similar result of lipid digestibility in Nile tilapia (72-97.5%) (Sklan et al 2004). Although lipid content of seaweed is quite low, its digestion is dependent on composition of fatty acids and degree of saturation. Higher carbon chain in fatty acid accounts for lower lipid digestion, but higher presence of double bond positively affects lipid digestion. Previous study reported that 20-50% of fatty acids in seaweed were n-3 group that had double bonds (Jeong et al 1993). Furthermore, high digestibility of lipid was attributed to lipase action in Nile tilapia (Sargent et al 1989).

Energy digestibility of *U. lactuca* in Nile tilapia was 74.25±3.99%, which was similar with Sklan et al (2004) (39–89%). Our finding was lower in comparison with other terrestrial plant such as wheat bran (91.3%) and corn (83.9%) (Pezzato et al 2002), but higher than cocoa bran (27.10%) (Ramos et al 2012). The ADC of energy correlated negatively with crude fiber content. Agbo et al (2014) found that energy digestibility was specifically affected by crude fiber, lipid, and carbohydrate contained in diet, as well as species and environmental temperatures.

Our study found that ash digestibility of *U. lactuca* in Nile tilapia was 63.59±4.91%. Compared with other nutrient (dry matter, protein, lipid, and energy), ADC for ash was the lowest. This result was similar to research that reported by Koprucu & Ozdemir (2005). This is attributed to fundamental characteristics of ash which was not easily digested by fish, consequence of its low solubility and characteristics of fish physiology.

Conclusions. High ash and carbohydrate content in *U. lactuca* suggested that it was considerable as a mineral and energy source in fish diet. *U. lactuca* had low crude fiber content comparing with terrestrial plants. Regarding to ADC nutrient of *U. lactuca* meal, it suggested that Nile tilapia was capable to utilize the nutrient. Nutritional components of *U. lactuca* and its ADC values indicated that *U. lactuca* was a desirable candidate as feed ingredient for Nile tilapia.

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