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Proximate composition and apparent digestibility coefficient of *Sargassum* spp. meal in the Nile tilapia, *Oreochromis niloticus*

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Abstract. A study was done to estimate the apparent digestibility coefficients (ADC) of the diet in which *Sargassum* spp. meal was incorporated into the diet of the Nile tilapia (*Oreochromis niloticus*) and also the ADC of *Sargassum* meal as an ingredient. Proximate composition of *Sargassum* meal was analyzed. Both the ADC and proximate composition could be used to evaluate the nutritive value of *Sargassum* meal. Crude protein and lipid content of the seaweed meal was 10.28% and 0.41%, respectively. Carbohydrates appeared to be the major component of the seaweed at 46.61% followed by ash at 32.46%. Crude fiber was low at 10.25%. In measuring the ingredient ADC ($ADC_{ingredient}$), a protein rich, highly acceptable and palatable reference diet was formulated together with a test diet in which 30% of the reference diet was replaced with *Sargassum* meal (30%). Results showed that the addition reduced the digestible dry matter, crude protein and lipid in small but significant percentages. The ADC of the reference for dry matter was decreased significantly from 74.47 to 64.55% by the replacement by *Sargassum* meal; the resulting $ADC_{ingredient}$ for dry matter was sufficiently high at 63.9%. Similarly, the ADC of the reference diet for crude protein in the present study significantly decreased from 88.26% to 83.93% when *Sargassum* meal was added and the resulting $ADC_{ingredient}$ for crude protein was high at 84.62%. The ADC of the reference diet for crude lipid significantly decreased from 99.85% to 93.44% by the replacement and the resulting $ADC_{ingredient}$ for crude lipid was the highest among the three nutrients at 93.44%. The nutritive value of *Sargassum* meal in the present study was predicted to be favorable based on its proximate composition and apparent digestibility coefficient; it could serve as an energy source and/or possibly a mineral replacement. It is fairly digestible and its crude protein and lipid digestibility were very high.
Key Words: ingredient digestibility, seaweeds, crude protein, crude lipid, crude fiber, dry matter.

Introduction. In evaluating ingredients for use in aquaculture feeds, ingredient digestibilities is one of the important information that should be understood to fully utilize a particular ingredient in feed formulation (Glencross et al 2007; Kaushik 2001). Fish nutritionists should measure first the proximate composition and the apparent digestibility coefficient of the ingredient of interest. This is because diet design and feeding strategy have important implications on the determination of the digestible value of nutrients from any ingredient (Glencross et al 2007). Knowing the digestibility of a variety of potential fish feed ingredients may allow estimation of the digestibility of an infinite variety of diets formulated using these ingredients (Cho et al 1985).

Sargassum spp. is a largely unexploited brown seaweed genus belonging to Class Phaeophyceae, which grows wildly in enormous quantities almost all over the world. It grows in tropical and subtropical waters, growing along beaches with a rocky substrate, rolling stones and pebbles (Ganzon-Fortes et al 1993). No antinutritious elements have been detected in *Sargassum* (Carrillo Dominguez et al 2002; Casas-Valdez et al 2006). In livestock, studies have shown seaweed digestibility ranging from 28% to 67% (dry matter), and a protein degradability of 95% (Gojón et al 1998).

In measuring apparent digestibility coefficient (ADC) of a diet, the method is relatively simple and is done by adding 0.5 to 1.0% of the marker in the diets followed by collection of sufficient amount of feces. Marker is analyzed in both the diet and the fecal matter and the proportion of the marker and the nutrient of interest in the feed and feces are used to estimate the ADCs of the nutrient in a diet (Sugiura 2000). Estimating the ADC of an ingredient, in contrast to that of the diet, requires the use of a reference diet since very few feed ingredients can be fed voluntarily as the sole component of a diet to fish. The protocol involves comparison of the digestibility of a reference diet with that of a test diet, this test diet being a mixture of the reference diet and a test ingredient, generally at a 70:30 ratio (Cho & Slinger 1979). In so doing, test diets that are palatable, water-stable, and nutritionally adequate can be produced with most potential ingredients. A high feed intake and good growth rate during the digestibility trial allows the experimenter to measure the ingredient ADCs that are reliable and repeatable. The use of the reference diet has its advantages: (1) certain ingredients may not be very acceptable (palatable) to the fish as a sole component of the diet; (2) it is very difficult to produce feed particles with proper physical characteristics such as water stability, with many individual ingredients; (3) most ingredients do not contain all the essential nutrients required by fish and prolonged feeding of them as sole components may greatly affect feed intake which in turn would lead to the deterioration of the physiological status of the fish (Kaushik 2001).

Previous studies on *Sargassum* spp. were on its content of bioactive compounds that enables the aquatic animals to elicit first-line defense against pathogens or to adapt to unfavorable environmental conditions (Kim et al 2014; Chiu et al 2008; Cheng et al 2007). Studies on its apparent digestibility were only measured in livestocks (Gojon et al 1998) and are very scarce on fish. Studies on utilization of marine seaweed as food for aquatic animals are scarce and the nutritional value of *Sargassum* meal to aquaculture species other than mollusks are unknown. Thus the aim of the present study is to characterize the dried *Sargassum* meal by proximate analysis and measure the ADC of the ingredient in the Nile tilapia *Oreochromis niloticus*.

Material and Method. The study was done between March and May, 2015 at the Multispecies Hatchery of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas in Miagao, Iloilo, Philippines.

Diets and feeding protocols. *Sargassum* spp. meal was prepared by manually separating the leaves from the stems. The leaves were oven dried for 12 h at 60°C, ground using a miller and kept at 4°C until use. A reference and test diets (Table 1) were formulated according to Mamun et al (2007) with diatomaceous earth (analyzed to contain 85.2% acid insoluble ash, AIA) used as inert marker at a concentration of 10 g kg^{-1} diet. Test diets were composed of 70% reference diet and 30% test ingredient (*Sargassum* spp. meal) in three replicates. Feed ingredients were purchased from Southeast Asean Fisheries Development Center, Aquaculture Department (SEAFDEC-AQD) Tigbauan Iloilo, Philippines. Prior to mixing, ingredients were passed through a 250 mesh sieve. All dried ingredients were mixed thoroughly, oils added followed by the addition of gelatinized bread flour (1 part to 3 parts water). The dough was thoroughly mixed and was allowed to pass through a meat grinder with 2 mm die twice and oven dried for 24 h at 60°C. The resulting hard noodle-like diets were stored at 4°C until cutting into appropriate sizes before feeding to the Nile tilapia.

A total of 12 adult mixed-sex Nile tilapia (95.67 ± 10.14 g) were individually stocked in static 50L plastic aquaria (6 individuals per treatment) which were individually aerated. Salinity was maintained 10 ppt; all other water parameters were monitored regularly to optimum level suitable for Nile tilapia.

Table 1
Composition of the reference and test diets used for the *in vivo* digestibility experiment in Nile tilapia, *Oreochromis niloticus* (g kg^{-1})

Ingredients	*Reference diet*	*Test diets*
Sardine fish meal	280.0	
Defatted soybean meal	270.0	
Danish fish meal	20.0	
Wheat pollard	50.0	
Copra meal	50.0	
Bread flour	180.0	
Rice bran	64.8	
AIA[1]	10.0	
Soybean oil	30.0	
Vitamin mix[2]	30.0	
Mineral mix[3]	15.0	
Vitamin C	0.2	
Reference diet		700.0
Sargassum raw meal		300.0
Total	1,000.0	1,000.0
Proximate composition (%) dry weight basis		
Moisture	10.03	6.7
Crude protein	34.60	26.83
Crude fat	6.37	2.44
Crude fiber	3.81	5.19
Ash	16.81	24.06
Nitrogen free extract	28.38	34.78
Total	100.00	100.00

[1]AIA = acid insoluble ash; [2] Vitamin mix Biomax (mg kg^{-1} dry diet unless otherwise stated): Vitamin A 1 200,000 IU; Vitamin D3 200,000 IU; Vitamin E 20 000 I U; Vitamin B1 8,000; Vitamin B2 8,000; Vitamin B6 5,000; Vitamin B12 2000 mcg; Niacin 40,000; Calcium Pantothenate 20,000; Biotin 40; Folic acid 1,800; Ethoxyquin 500; Carrier q.s ad to make 1 kg; [3] Mineral premix (mg kg^{-1} dry diet unless otherwise stated): Iron 40 000; Manganese 10 000; Zinc 40 000; Copper 400; Iodine 1 800; Cobalt 20; Selenium; Carrier q.s ad to make 1 kg.

Fecal collection*.* The digestibility trial lasted for 8 weeks. Fish were fed twice a day at 9AM and 6PM at 4% body weight. Tanks were cleaned and water change at 80% was done every morning before the first feeding to prevent the growth of algae. Fecal collection started 10 days after feeding the fish with the reference or test diets. Feces were collected following the protocols described by Goddard & McLean (2001). One h after feeding in the morning, uneaten feeds and fecal matters were siphoned off. Intact fecal strands were carefully siphoned into a fine mesh plankton net and rinsed once with distilled water every 30 min for the next 6 h to reduce nutrient loss due to leaching. Feces from each tank were collected and pooled until sufficient samples were obtained. The feces were dried at 60°C for 24 h and ground to fine powder using a coffee grinder for proximate analysis.

Analyses and estimation of ADCs*.* Triplicate samples of the diets and fecal matter were homogenized and analyzed for moisture and ash contents of the samples according to standard methods (AOAC 2003). Moisture was measured by drying to a constant weight in an oven at 105°C. Ash content was determined after incineration in a muffle furnace at 550°C for 12 h. Acid insoluble ash (AIA) was determined following the method of Atkinson et al (1984). Approximately 2 g of feeds and fecal samples were incinerated in a muffle furnace for 2 h at 600°C. The resulting ash was boiled in 75 mL of 2M HCl for 5 min and was filtered through ashless filter paper. The residue was washed with boiling distilled water and allowed to dry. The filter paper with the residue was ashed for 2 h at 600°C and the resulting material was regarded as AIA.

The ADC for dry matter, protein or lipid were computed following the formula of Goddard & MacLean (2001):

$$ADC = 1 - \left[\left(\frac{F}{D}\right) * \left(\frac{Dm}{Fm}\right)\right] * 100$$

Where: F= %nutrient in the feces; D = %nutrient in the diet; Dm = % marker in the diet; Fm = % marker in the feces.

The apparent digestibility of the ingredient (ADI) was calculated using the following equation of Bureau & Hua (2006):

$$\text{ADI} = \text{ADCtest diet} + \left\{(\text{ADC test diet} - \text{ADC ref diet}) * \left(\frac{0.7 * \text{D ref diet}}{0.3 * \text{Dtest diet}}\right)\right\}$$

Where: D ref diet = % nutrient of the reference diet; D test diet = % nutrient of the test diet.

Statistical difference between 2 diets in three replicates each was compared using Studen t-test at α=0.05.

Results and Discussion. Crude protein content of *Sargassum* meal in the present study was 10.28%, lower than those obtained by Catacutan (2002) of 9.0%. This value was higher than that for *Ulva* spp. (Catacutan 2002). Other reports for seaweeds had higher crude protein (CP): 13.4% for *Ulva lactuca* (Santizo et al 2014), 14.0% in *Enteromorpha* sp. (Aquino et al 2014), 12.27% for *Enteromorpha compressa*, 13.47% for *Ulva reticulata* and 13.63% for *Padina pavonica* (Manivannan et al 2009). CP values that were lower than that in the present study were reported *Sargassum polycystum* (Matanjum et al 2009).

Seaweeds are relatively low in lipid (1–5% of dry weight) (Burtin 2003; Polat & Ozogul 2008; Jeong et al 1993; Fleurence 1999). Crude lipid (CL) content of *Sargassum* meal in the present study was 0.41%, almost similar to that reported in *Sargassum vulgare* (0.45%) (Marinho-Soriano et al 2006). The CL in the present study was lower than the value of 0.8% in the same seaweed (Catacutan 2002), 0.88% in *Ulva lactuca* meal (Santizo et al 2014), 2.0 to 3.6% *in Enteromorpha* sp (Aquino et al 2014). Rohani-Ghadikolaei et al (2012) reported crude lipid values higher than that in the present study: *Ulva lactuca* (3.6%), *Entermorpha intestinalis* (2.9%), while the lowest content was recorded in *Colpomenia sinuosa* (1.5%) and *Gracilaria corticata* (1.8%). Higher values for CL than that in the present study were reported by Chakraborty & Santra (2008) for *Enteromorpha intestinalis* (7.1%) and *Ulva lactuca* (4.4%). The only value lower than that in the present study was reported by Ortiz et al (2006) for *Ulva lactuca* (0.3%). Jeong et al (1993) observe that although the fat content of seaweeds is low, 20-50% of the total fatty acid content consists of n-3 fatty acids.

Carbohydrates appeared to be the major component in the proximate composition of *Sargassum* meal of 46.61% in the present study, a pattern also observed by Rohani-Ghadikolaei et al (2012) who found a range of 31.8% in *Hypnea valentiae* to 59.1% in *Ulva lactuca*. The carbohydrate level reported by Chakraborty & Santra (2008) for *U. lactuca* (35.3%), and by Manivannan et al (2009) for *Hypnea valentiae* (23.6%) were lower than that of the present study. However, the value in the present study was lower than that obtained by Ortiz et al (2006) for *Ulva lactuca* (61.5%).

Ash contents in most marine seaweeds are much higher than those in terrestrial plants (5–10% dry weight - DW; USDA 2001). The differences in ash contents depend on seaweed species, physiological factors, environmental changes, methods of mineralization and type of processing (Nisizawa et al 1987; Rupérez 2002). Ash content of the *Sargassum* meal in the present study (32.46%) was higher than that reported in *Sargassum ilicifolium* (29.9%) and lower than that for *Ulva lactuca* (12.4%, Rohani-Ghadikolaei et al 2012). According to Cho et al (1985) marine seaweeds or any ingredient with high ash or crude fiber content should be used sparingly in the diet because no fish can utilize very much ash or fiber in natural ingredients, including grass carp. Ash content could be a manifestation of an ingredient that has amounts of minerals necessary in the diet of aquaculture species; microalgae such as *Chaetocentrus calcitrans* which contains high ash could replace the whole mineral component of the diet for *Penaeus monodon* postlarvae (Naorbe et al 2015).

Crude fiber of the seaweed in the present study was much lower (10.25%) than those in terrestrial plants such as whole wheat (44.5% DW), beans (36.5% DW) and onions (16.9% DW) (Prosky et al 1992; Wong & Cheung 2000). Although insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were not measured in the present study, seaweeds have been reported to contain good amount of soluble dietary fiber. These fiber is in the form of sulphated polysaccharide which differ chemically and physicochemically from those of land plants, thus, they may have different physiological effects on human and animals (Jimenez-Escrig & Sanchez-Muniz 2000; Lahaye 1991). These SDFs are very influential in slowing digestion and absorption of nutrients, reduction of glucose (Scheneeman 1987; Wong & Cheung 2000). In contrast, IDF increases fecal bulk and decreases intestinal transit time (Potty 1996; Elleuch et al 2011).

The reference diet in the present study was well digested; its estimated ADCs for dry matter, crude protein and crude lipid were 74.47, 88.26 and 99.85%, respectively (Table 2). Replacement of this reference diet with *Sargassum* meal did not overly affect the digestibility of dietary protein or lipid which demonstrated that adding high levels (30%) of the seaweed meal to the protein-rich reference diet reduced the digestible dry matter, crude protein and lipid in small but significant percentages. This was manifested in the ADC of the reference for dry matter (74.47%) being decreased significantly from 74.47 to 64.55% by 30% replacement by *Sargassum* meal (Table 2); the resulting $ADC_{ingredient}$ for dry matter was sufficiently high at 63.9%. In addition to its high digestibility as an ingredient, addition of *Sargassum* meal to the fish diet likely would cause a significant bulking effect and higher amount of water in the guts of animals since algae are more likely to show a high *in vitro* water holding capacity (Bach Knudsen & Hansen 1991; Urriola & Stein 2010).

Table 2

Proximate analysis of *Sargassum* spp. raw meal ingredient used in the experimental diet (dry weight basis)

Chemical composition	*%*
Moisture	6.52
Crude protein	10.28
Crude lipid	0.41
Crude fiber	10.25
Ash	32.46
Nitrogen-free extract	40.08
Total	100.0

The ADC of the reference diet for crude protein in the present study significantly decreased from 88.26% to 83.93% when *Sargassum* meal was added. The resulting $ADC_{ingredient}$ for crude protein was high at 84.62%. The decrease in the crude protein ADC could be caused by the increase in the carbohydrate content in the feed: the higher the carbohydrate content, the lower the protein digestibility (Tunison et al 1944). The investigators explained that the undigested portion of the carbohydrates passes more rapidly through the alimentary canal, carrying with it some of the proteins. Another interesting aspect was the difference in the dietary protein between the reference diet (34.60%) and that of the test diet (26.83%). It should be expected that crude protein ADC would increase with increase in protein content (Nose 1967). In the feces, both the reference diet and test diet exhibited crude protein content of 16.51% and 11.85%, respectively (Table 3). These values were lower than that obtained by Mabrouk & Nour

(2011) in gilthead sea bream which meant that the Nile tilapia in the present study did not lose much dietary protein to fecal matter.

Table 3

Apparent digestibility coefficients of dry matter of the reference, test diets and test ingredient (dried *Sargassum* meal)

		F	*D*	*Dm*	*Fm*	*ADC*
Dry matter	Reference diet	91.66±0.05	89.67±0.18	2.16±0.03	8.64±0.21	74.47±0.83*
	Test diet	89.09±0.00	92.59±0.41	3.06±0.08	8.30±0.16	64.55±1.42
	Dried *Sarg.* meal					63.9±1.45
Crude protein	Reference diet	16.51±0.28	35.08±0.28	2.50±0.04	10.03±0.24	88.26±0.19*
	Test diet	11.85±0.00	27.20±0.21	3.59±0.10	9.74±0.20	83.93±0.80
	Dried *Sarg.* meal					84.62±0.80
Crude fat	Reference diet	0.04±0.02	6.44±0.04	2.50±0.04	10.03±0.24	99.85±0.12*
	Test diet	0.40±0.0	2.26±0.11	3.59±0.10	9.74±0.19	93.40±0.22
	Dried *Sarg.* meal					93.44±0.19

*significantly different ($p < 0.5$) when Student t-test was performed; F = % nutrient in feces; D = % nutrient in the diet; Dm = % marker in the diet; Fm = % marker in feces; ADC = apparent digestibility coefficient of nutrient or ingredient.

The ADC of the reference diet for crude lipid significantly decreased from 99.85% to 93.44% by replacing with *Sargassum* meal 30% of the reference diet and the resulting $ADC_{ingredient}$ for crude lipid was the highest among the three nutrients at 93.44%. Although lipid content of the seaweeds are generally low, digestibility of the crude lipid depends on their composition and saturation level (Nose 1967); it decreased with increase in number of carbon atoms in the fatty acid chain, and increases with the number of double bonds. Fatty acid profile was not done in the present study, but the decrease in the ADC of crude lipid could be the result of the presence of long chain fatty acid more than the increase in the double bonds. This aspect needs further investigation.

The dry matter ADC of the reference diet in the present study (74.47%) was lower than that for the black tiger shrimp *Penaeus monodon* (85.13%, Santizo et al 2014) but within the range of 66-77% reported by Salim et al (2004) in *Labeo rohita*, the adult of which naturally feed on phytoplankton and macrovegetation (Khan & Siddiqui 1973), the food habit of which is similar to the Nile tilapia. This could be because the latter consisted of highly digestible protein sourced from the marine environment such as fish meal (32%), *Ascetes* meal (10%), squid meal (16%) and soybean meal (24%). In the present study, since the Nile tilapia is an omnivorous fish, the reference diet consisted of marine animal and plant protein sources such as sardine fish meal (28%), Danish fish meal (2%), defatted soybean meal (27%), wheat pollard (5%), copra meal (5%), and rice bran (6.5%). Foods of plant origin are usually digested to a lesser degree than foods of animal origin (Hepher 1988). Plants have thicker and more resistant cell walls which are more difficult for the digestive enzymes to penetrate.

In measuring ADC of water hyacinth (*Eichhornia crassipes*) protein concentrate, Hontiveros & Serrano (2015) used a formulation similar to that in the present study. Their ADC for dry matter was high at 98.8% which was reduced to 92.1% when the plant protein concentrate replaced 30% of the reference diet. Santizo et al (2014) measured the dry matter ADC when *Ulva* meal was added to the reference diet and it decreased from 85.13 to 71.53%, a reduction of 13.6% while that in the present study the reduction was only 9.92%. However, the dry matter $ADC_{ingredient}$ of *Ulva* was higher (71.17%) than that in the present study (63.9%). Thus, *Ulva* meal was more digestible to the shrimp in the study of Santizo et al (2014) than the *Sargassum* meal to the Nile tilapia in the present study. The powder seaweeds were equally digestible with that of aquatic plants such as water hyacinth (Hontiveros & Serrano 2015). The water hyacinth had $ADC_{ingredient}$ of 74.2% for dry matter. Concentrating *Ulva* protein, in contrast, resulted in a more digestible ingredient (99.13%, Santizo et al 2014). This could be the result of an increase in the protein content concomitant with a decrease in the carbohydrate content.

Conclusions. Proximate composition of the *Sargassum* meal contained less than 7% water and crude lipid content was low at 0.41%. Crude protein was low to be considered as protein supplement but because of its high carbohydrate content, it could be an energy supplement to the diet of the Nile tilapia. Crude fiber content was minimal but its high ash content was at a level observable in other macroalgae. Adding 30% *Sargassum* meal to the reference diet decreased ADC for dry matter, crude protein and crude lipid by small percentages albeit significant. Ingredient ADC of the *Sargassum* meal for dry matter was relatively high at 63.9%, quite high for both crude protein and lipid. Proximate composition and values of apparent digestibility coefficients for nutrients showed that the *Sargassum* meal had potential to be an ingredient in the diet of the Nile tilapia.

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