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Proximate composition and dry matter digestibility of *Ulva lactuca* in the black tiger shrimp *Penaeus monodon*

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Abstract. Proximate composition of both the raw dried powder and protein concentrate of *Ulva lactuca* in i*n vivo* assays of apparent digestibility of dry matter (ADMD) and apparent digestibility of ingredient (ADI) was conducted to measure the bioavailability of raw *Ulva* powder (URDP) and *Ulva* protein concentrate (UPC) as a feed ingredient in the diet of the black tiger shrimp *Penaeus monodon*. UPC contained almost three times more crude protein than RDUP but contained more than half of the ash content. Crude fat, moisture and NFE differed only slightly between the two *Ulva* forms. The major biochemical component in both UPC and RDUP was nitrogen-free extract assumed to be digestible carbohydrates. The ADMD of the test diet containing UPC was significantly higher (98.76 %) than that of the RDUP (71.53 %) while the ADI of UPC was significantly higher (99.13 %) than that of the RDUP (71.17 %). ADI is one of the good indicators of the quality of feed ingredients and can be used to select ingredients for shrimp feeds. *P. monodon* appeared to have the capacity to effectively digest *Ulva lactuca* in either raw dried powdered form or a powder protein concentrate as ingredients that might have very different nutritional qualities, which may reflect the natural preference and omnivorous generalist-type feeding behavior of this shrimp. The information obtained in this study will aid in the formulation of cost-effective diets for the black tiger shrimps.
Key Words: apparent digestibility of dry matter, bioavailability, apparent digestibility of ingredient, protein concentrate, raw *Ulva* powder.

Introduction. Juvenile black tiger shrimps (*Penaeus monodon*) consume predominantly algal material (El Hag 1984) but this consumption is reduced considerably in the adult shrimp (Marte 1980; Thomas 1972). *P. monodon* reared without supplementary feeding in extensively managed ponds shows detritus to be the most common food, followed by animal remains, diatoms, cyanobacteria and green algae (Bombeo-Tuburan et al 1993). In a pond experiment that aimed to measure the proportion of consumption of natural food to formulated diet by *P. monodon*, plant material consisted 42.3 %, 21.1 % 21.7 % after 6, 11 and 16 weeks of culture, respectively (Focken et al 1998). Thus, there are indications that *P. monodon* would feed on algal material voluntarily but perhaps at a more reduced rate as it grows bigger.

Seaweeds such as *Ulva* sp. are potential ingredients in aquafeeds which are otherwise considered as nuisance species in the Philippines (Largo et al 2004). Feeding the Nile tilapia with 5 % *Ulva* meal at either 10 % or 20 % dietary lipid levels results in improved growth, feed efficiency, nutrient utilization, and body composition (Ergun et al 2008). Snakehead fry when fed different seaweed meals favors *Ulva* sp. among brown, red, and green seaweeds tested and *Ulva* sp. gives the highest growth performance (Davies et al 1997). In carp diet, the acceptable *Ulva* meal inclusion is 5 to 15 % to replace wheat meal (Diler et al 2007). In the body of white shrimp, *Litopenaeus vannamei*, *Ulva* carotenoids is involved in growth enhancement since it is effectively assimilated and metabolized and enhances pigmentation by increasing the total carotenoid concentration (Sanchez et al 2012). As far as we are concerned, preparations of seaweeds such as *Ulva* have never been evaluated as feed ingredient in the diet of *P. monodon*.

Fish meal has been replaced considerably by soybean meal in commercial diets for *P. monodon*. However, aquaculture has become a competitor in its growing consumption of soybean meal both for human consumption as well as for farm animal feeds which has probably resulted in problems of availability and concomitant increase in production cost. It is thus necessary to search for a substitute for this protein source and seaweed such as *Ulva* is a potential choices. Thus, there is a need to evaluate this chlorophyte alga and this process should start from the measurement of its bioavailability to *P. monodon*.

In the measurement of bioavailability, the apparent dry matter digestibility (ADMD) provides a measure of the total quantity of an ingredient that is digested and absorbed. Because all components of a feedstuff are not digested equally, ADMD coefficients can provide a better estimate of the quantity of indigestible material present in a feed ingredient than does the digestibility coefficients for individual nutrients, which in some cases are minimally affected by indigestible material in the diet (Brunson et al 1997). ADMD coefficients can be quite variable among ingredients that appear to have similar proximate composition. Thus, this study primarily aims to evaluate the availability of two forms (dried powder or protein concentrate) of *Ulva lactuca* to *P. monodon* specifically to measure their apparent dry matter digestibility.

Material and Method

Experiments were carried out at the Multispecies Hatchery of the Institute of Aquaculture in the University of the Philippines Visayas, Miag-ao, Iloilo from September to December, 2013.

Seaweed collection and processing. Seaweeds (*U. lactuca*) were collected from the vicinity of Rio Hondo, Zamboanga, Philippines and were submitted for proper identification. They were washed with freshwater and air-dried as described by Tiroba (2007) where seaweeds were laid flat on the tables under a shade with constant overturning and separating the algal pieces. Samples were submitted for taxonomic verification. One kg of UPC was provided by the National Aquafeeds Program for the formulation of experimental diets for the present study. Samples of both RDUP and UPC were subjected to proximate analysis.

Reference and test diets. A reference and two test diets were formulated according to (Cho et al 1985) containing 1 % chromic oxide (Cr_2O_3) as an inert marker (Table 1). Test diets were composed of 70 % reference diet and 30 % test ingredients (RDUP or UPC). Prior to mixing, all powdered ingredients were allowed to pass through a 425 µm sieve. All dried ingredients were mixed thoroughly, the oil and lecithin were added next, and the cooked bread flour (*i.e.* the binder) was added last. The mixture was thoroughly mixed and allowed to pass through a meat grinder a few times, steamed for 15 min and dried in an oven for 8 - 12 h at 60 °C. The resulting hard noodle-like diets were stored at -20 °C prior to breaking into appropriate size and feeding to the test animals.

Experimental animal, acclimation and management. Thirty shrimps *P. monodon* (average body weight of 17.28 ± 1.67 g) were stocked randomly in nine 60 L plastic boxes. Four shrimps were assigned to each box in three replicates. The boxes were provided with continuous aeration and salinity was kept at 28 ppt. Prior to the commencement of the digestibility trial, *P. monodon* were acclimatized and fed the reference diet 3 times a day for 5 days. In the morning, uneaten feeds and fecal maters were siphoned off before feeding. Water exchange was done daily at a rate of 30 – 40 % of the total water volume. Water temperature, salinity, dissolved oxygen, pH, nitrite and total ammonia nitrogen (TAN) were monitored and exhibited ranges of 27 – 30 °C, 28 - 30 ppt, 7 - 8 mg L^{-1}, 8 - 8.4, 0.1 - 0.2 ppm and 0.1 - 0.15 ppm, respectively.

Table 1
Composition of reference and test diets used for *in vivo* digestibility experiment in giant tiger shrimp, *Penaeus monodon* (g/kg)

Ingredients	*Reference diet*	*Test diet 1 (70%Ref:30%UPC)*	*Test diet 2 (70%Ref:30%RDUP)*
Danish fish meal	320.0	224.0	224.0
Ascetes	100.0	70.0	70.0
Squid Meal	160.0	112.0	112.0
Soybean Meal	240.0	168.0	168.0
Bread flour	24.5	17.2	17.2
Rice bran	10.0	7.0	7.0
Cholesterol	5.0	3.5	3.5
Cod liver oil	97.0	67.9	67.9
Lecithin	3.0	2.1	2.1
Ligno bond	10.0	7.0	7.0
Vitamin mix	10.0	7.00	7.00
Mineral mix	10.0	7.00	7.00
BHT	0.5	0.4	0.4
Cr2O3	10.0	7.0	7.0
Ulva concentrate	-	300.0	-
Ulva raw	-	-	300.0
Total	1000.0	1000.0	1000.0

[a] Vitamin mix (unit kg^{-1} of feed): Vitamin A - 1,200,000 IU; Vitamin D3 - 200,000 IU ; Vitamin E - 20, 000; Vitamin B1 - 8000 mg; Vitamin B2 - 8000 mg ; Vitamin B6 - 5000 mg ; Vitamin B12 - 2000 mcg ; Niacin - 40,000 mg; Calcium Pantothenate - 20, 000 mg; Biotin - 40 mg; Folic Acid - 1,800 mg; Ethoxyquin - 500 mg.

Feeding and fecal collection*.* Shrimps were fed thrice a day (08:00, 12:00, and 16:00) with the test diets at 3 % body weight. They were allowed to feed for 45 min after which uneaten feeds were siphoned off. Fecal collection was done by manually by the use of a long pipette (Bautista-Teruel et al 2003; Smith & Tabrett 2004) between 1 and 2 h after feeding. Feces were collected until sufficient amount for analysis was reached, pooled, weighed and stored at -20 °C until analysis.

Chromium analysis*.* Feeds, feces and corresponding blanks were done in 3 replicates. Fecal samples were thawed, dried, ground using mortar and pestle and thoroughly mixed prior to analysis. One g of each sample set aside for moisture and ash determination prior to digestion. One mL distilled water was added to the sample, mixed, dried at 60 - 80 °C and placed in a furnace for 1 h at 550 °C. To the resulting residue was added 5 mL of 6 M HCl, heated to dryness and the resulting residue was dissolved in warm 30 mL of 0.1N N_2O_3. The resulting solution was filtered, made up to 50 mL with distilled water and was subjected to flame atomic absorption spectrophotometry following the method of Monteiro et al (2002).

The apparent dry matter digestibility coefficient (ADMD) of the experimental diets were computed using the following formula (Maynard et al 1981):

$$ADMD = 100 - [100 \times (C_{diet}/DM_{diet}) \times (DM_{feces}/C_{feces})]$$

Where:
C = chromic oxide content
DM = dry matter

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

$$ADI = ADM_{test\ diet} + [(ADM_{test\ diet} - ADM_{ref\ diet}) \times (0.7 \times DM_{ref\ diet}) / (0.3 \times DM_{test\ ingredient})]$$

***Statistical analysis**.* Statistical analysis was performed using Statistical Analysis Software Program (SPSS) version 16. Data were presented as mean ± standard error of the mean (SEM) for each treatment. Data were analyzed for normal distribution using Kolmogorov-Smirnov test and Levene's test for homogeneity of variances before doing one-way analysis of variance (ANOVA). Data on digestibility, growth parameters, feed efficiency and nutrient utilization were subjected to ANOVA, and to determine the ranks of the means, Tukey's Test post hoc analysis was performed at α=0.05.

Results and Discussion. The proximate composition of Dried *Ulva* Powder (RDUP) and *Ulva* Protein Concentrate (UPC) are presented in table 2. UPC contained almost three times more crude protein than RDUP but contained more than half of the ash content. Crude fat, moisture and NFE differed only slightly between the two *Ulva* forms. The major biochemical component in both UPC and RDUP was nitrogen-free extract assumed to be digestible carbohydrates.

Table 2

Percent proximate composition of Raw Dried *Ulva* Powder (RDUP) and *Ulva* protein concentrate (UPC)

Composition (DM, %)	*UPC*	*RDUP*
Moisture	12.5	14.5
Crude protein	38.4	13.4
Crude fiber	2.8	4.4
Ash	14.7	31.7
Nitrogen-free extract (NFE)	31.2	34.6
TOTAL	100.0	100.0

ADMD coefficients of diets are presented in figure 1 and that of the ADI of *U. lactuca* (UPC or RDUP) in figure 2. The ADMD of the test diet containing *Ulva* protein concentrate (UPC) was significantly higher (98.76 %) than that of the RDUP (71.53 %) while the ADI of UPC was significantly higher (99.13 %) than that of the RDUP (71.17 %).

The UPC protein content (38.4 % dry basis) resulted from this study was relatively lower than that of seaweed protein concentrates (dry weight basis) measured by (Kandasamy et al 2012): 60.35 ± 2.00 % in *Enteromorpha compressa*; 53.83 ± 0.70 % in *Enteromorpha linza*; but was higher than that of (*Enteromorpha tubulosa*) 33.36 ± 1.04 %. The relatively low recovery of protein concentrate from *Ulva lactuca* may be attributed to higher amount of polysaccharides (*e.g.* xylans and cellulose) as well as phenolic compounds. Different solvent extracts of *Enteromorpha sp.* (*E. compressa, E. linza, and E. tubulosa*) possess good amounts of phenol (Ganesan et al (2011). Extraction of protein concentrates from seaweeds would be complicated by the large amounts of cell wall anionic or neutral polysaccharides and phenolic compounds (Kandasamy et al 2012; Ragan & Glombitza 1986; Wong et al 2004). The presence of oxidized phenolic compounds could have reacted with amino acids and proteins in the present study inhibiting the activity of proteolytic enzymes. The ability of phenolic compounds to form insoluble complexes with protein interferes with the utilization of dietary protein, thus lowering its nutritional value (Wong & Cheung 2001).

There are various inert markers already available commercially apart from chromic oxide. However, its use has had various criticisms special when it has been shown to be unevenly distributed in the fecal matter of crustaceans (Jones & De Silva 1997b; Leavitt 1985). Nonetheless, it remains to be the most popular inert marker among fish nutritionists (Jones & De Silva 1997b; Mu et al 2000; Sudaryono et al 1996). In crustaceans, it has been demonstrated that the chromic oxide method gives an acceptable estimate of digestibility (Jones & De Silva 1997b; Smith & Tabrett 2004).

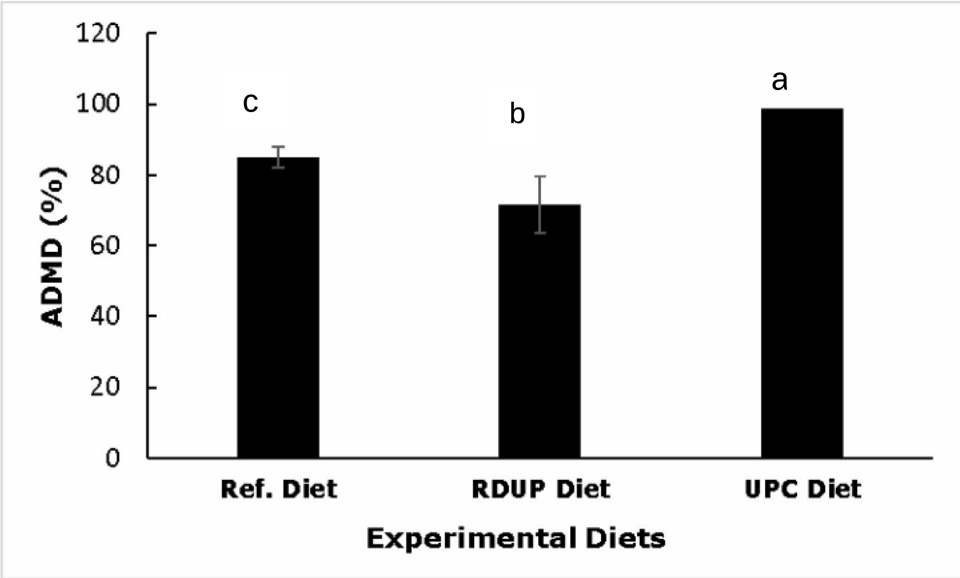

Figure 1. Apparent Digestibility Coefficients of dry matter of diets (ADMD). Bars with the same letters are significantly different (P<0.05). UPC, *Ulva* protein concentrate; RDUP, raw dried *Ulva* powder.

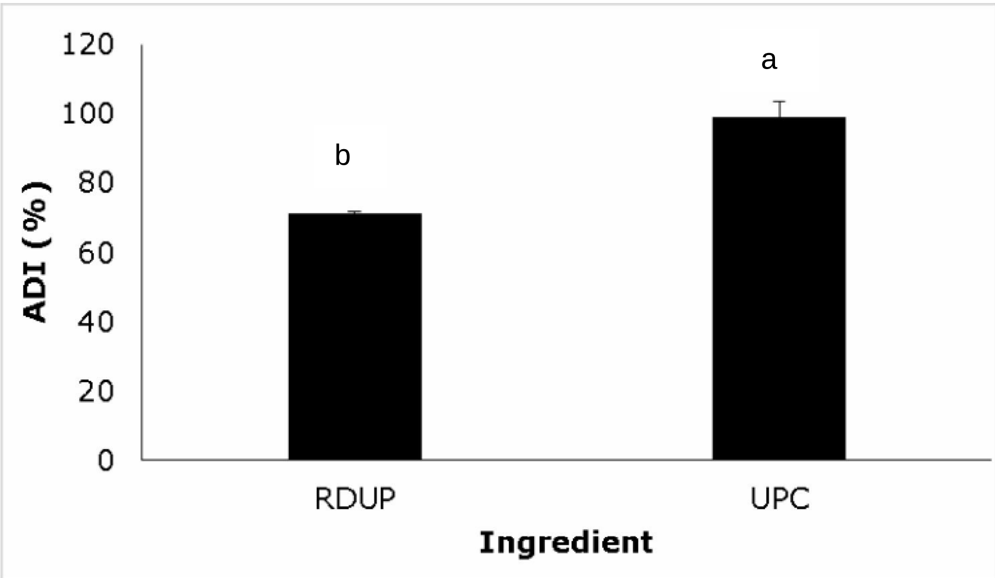

Figure 2. Apparent digestibility coefficients for dry matter of test ingredients (*i.e.* ADI of *Ulva* protein concentrate and raw dried *Ulva* powder). Bars with same letters are not significantly different ($P \geq 0.05$). ADI, apparent digestibility coefficient of test ingredient; UPC, *Ulva* Protein Concentrate; RDUP, Raw *Ulva* Dried Powder.

An additional issue against *in vivo* measurement of apparent digestibility is nutrient loss which could stem from two sources: the reference diet and the test diets. A higher nutrient loss value for the reference diet with respect to the test diets leads to an underestimate of the nutrients loss in the test ingredients. Another reason for overestimation of ADCs could be the leaching of nutrients from the feces, which has been estimated as not being significant by Fenucci et al (1982). In contrast, data from Smith & Tabrett (2004) show that a one hour delay in collecting the feces can result in an overestimation of 1.7 and 0.7 percent units for crude protein and dry matter ADCs, respectively. Cruz-Suárez et al (2009) argue that leaching of nutrients from feces is assumed to be of somewhat smaller importance when using the difference method to obtain ingredients ADCs, because the difference should be independent of fecal nutrient lost by leaching. In the present study, feces were collected by patiently waiting for the first sign of egestion and immediately sucked into a pipette to keep leaching to a minimum. The maximum exposure time of feces in the rearing water was less than 40 min suggesting that nutrient leaching from feces was likely to have caused insignificant errors in digestibility determinations. This precaution was done despite the observation that crustaceans pass fecal material enclosed in a peritrophic membrane (Dall & Moriarty 1983) which contributes significantly to the reduction of leaching. Thus, leaching loss from feces was considered to be a minimal source of error and the use of chromic oxide as an inert marker appeared to be reliable for *P. monodon.*

It has been shown that dry matter digestibility provides a better estimate of the quantity of indigestible material that are in the ingredients than nutrient digestibility (Brunson et al 1997). In the present study, for the first time, dry matter digestibility coefficients were measured for the raw dried powder form and the protein concentrate of *U. lactuca* in the black tiger shrimp. The ADI for the protein concentrate was far higher than that of the raw dried form. This finding could be similar to the observation (Akiyama et al 1989; Cruz-Suárez et al 2009; Siccardi 2006) that the dry matter digestibility of the soybean protein isolate is higher than that of its counterpart soybean meal in the Pacific white shrimp *L. vannamei*. Although not measured in the present study, protein hydrolysate might have been produced during the process of concentrating protein leading to a higher ADI for the UPC. The inclusion of a protein hydrolysate is recommended for the spiny lobster, *Jasus edwardsii* for the purpose of increasing dry matter digestibility (Simon 2009).

Considering that the ingredient was a plant material, it indicated that *P. monodon* had somewhat adaptable digestive system which could be attributed to the feeding habit of the shrimp being omnivorous. The juveniles feed mainly on algal materials (El Hag 1984) but the adults are opportunistic feeders, prefer animal protein but consume a small amount of algal material (Marte 1980; Thomas 1972). The raw dried form of *U. lactuca* containing almost a third of what the UPC contained but almost double the crude fiber content, was consistent with the observation of Luo et al (2008) in the Chinese mitten crab. These authors together with other authors (Akiyama et al 1989; Brunson et al 1997; Bureau & Cho 1999; Reigh et al 1990) observe that ADMD of feed ingredients were negatively related to their fiber content. However, no apparent trend in relation to the ash content of the diets was observed (Catacutan et al 2003; Irvin & Williams 2007; Jones & De Silva 1997a). When fiber and ash content of the ingredients increase, the value for ADMD decrease (Yang et al 2009).

With only the dry matter digestibility data available in the present study, it is interesting whether a trend exists on the relationship between dry matter digestibility and nutrient digestibility of ingredients. Several authors find that the higher the ADMD of an ingredient, the higher is the crude protein digestibility in crustaceans, Luo et al (2008) in the Chinese mitten crab *Eriocheir sinensis*, Akiyama et al (1989), Cruz-Suárez et al (2009) and Siccardi (2006) in the Pacific white shrimp *L. vannamei*. Observation in the present study leaned towards this trend although it could be considered provisional at best since only one ingredient in two forms was measured.

Conclusions. *In vivo* assays of apparent digestibility of dry matter is one of the good indicators of the quality of feed ingredients and can be used to select ingredients for shrimp feeds. *P. monodon* appeared to have the capacity to effectively digest *U. lactuca* in either raw dried powdered form or a powder protein concentrate as ingredients that might have very different nutritional qualities, which may reflect the natural preference and omnivorous generalist-type feeding behavior of this shrimp. The information obtained in this study will aid in the formulation of cost-effective diets for black tiger shrimps. Growth trials are needed to further examine the nutritive value of these feedstuffs.

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