

Physical properties of *Rhizoclonium* meal as feed ingredient in aquafeeds

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Abstract. The present study aimed to characterize four physical properties of a meal from the macroalgae *Rhizoclonium* sp., in terms of particle size (PS), bulk density (BD), water holding capacity (WHC) and specific gravity (SG). The relative distribution of particles in the unmodified meal (0.89 mm) was compared with those of the modified seaweed meal (i.e. coarse-, medium- and fine-sized particles). The most ideal particle size distribution was produced from the medium-sized particle; the majority was in the 0.71 mm. The WHC at the coarse-particle was significantly the lowest while the fine-sized meal exhibited significantly the highest WHC. SG of the seaweed meal was not affected at all by the PS. As for the correlation of the properties of the meal, PS did not exhibit correlation with SG; BD and WHC did not exhibit correlation with SG. As a conclusion, grinding of the microalgal meal should aim for the medium-sized modification of the crudely prepared meal from *Rhizoclonium* spp. Further reduction in particle size linearly reduced bulk density while linearly increased water holding capacity. These information's on the physical properties of the seaweed meal will be useful in the production of various types of pellets whether sinking or floating, depending on the amount of inclusion of the meal.

Key Words: *Rhizoclonium* spp., particle size, water holding capacity, bulk density, specific gravity.

Introduction. Macroalgae could be a potential low-cost source of protein for fish. Various studies on the relationship between the nutritive value of feeds and culture organisms have demonstrated that the content of essential amino acids and fatty acids is the principal factor in their dietary value. All red and green seaweed seemed to be able to complement other dietary proteins to enhance the balance of essential amino acids. *Rhizoclonium* sp. is a cosmopolitan filamentous alga which occurs in a variety of habitats in Taiwan (Chao et al 2005) and in the Philippines, including semi-enclosed intertidal zones, estuaries and abandoned aquaculture ponds. It is considered a nuisance species because it removes dissolved oxygen at times, reduces space for cultured animals and hinders their growth (Caffrey 1992). Aquaculturists in other countries continue to make various attempts to suppress growth of this algae (Lin & Lin 1981; Caffrey & Monahan 1999). If this algae could be incorporated to replace some imported ingredients in the diets of shrimp and tilapia, it could reduce the price of feed production as well as create a demand for this otherwise nuisance species in fishponds.

Rhizoclonium sp. has been evaluated as a supplementary diet for *Oreochromis mossambicus* as part of a composite algal mix as supplement. Three isonitrogenous diets containing 0%, 35% and 100% algal supplementation were tested in combination with other conventional fish feed ingredients for 12 weeks (Roy et al 2011). The algal species used were *Phormidium valderianum*, *Spirulina subsalsa*, *Navicula minima*, *Chlorococcum infusionum* and *Rhizoclonium riparium* in a ratio of 35:35:12:12:6 for experimental feed formulation. The results suggested that 35% supplementation of conventional feed with composite algal mix can be used in Mossambique tilapia diet.

The aim of the present study is to measure some physical properties of *Rhizoclonium* meal. Particle size of ingredients affects the diet's utilization by animals. Feed ingredients are subjected to some form of particle size reduction before they are

incorporated into aquafeeds. Continued reduction increases both the number of particles and the amount of surface area per unit of volume, this allows increased access to digestive enzymes. Also, particle size interacts with water stability of pellets. It has been commonly accepted that in pelleting, finer grind results in a more water stable pellet. Optimal particle size enables modification of the physical characteristics of ingredients resulting in improved mixing, conditioning, pelleting, feed handling and transport and in improved animal performance (Axe & Behnke 1995). Little work has been done on the effects of PS in various ingredients for aquafeeds and this project aims to study this aspect in the *Rhizoclonium* meal.

An aquatic feed's density is a critical component as it impacts many things including plant capacity, floating and sinking properties, product appearance, and absorption of external coatings (Rokey & Plattner 2004). The pellets' floating or sinking properties are critical as the feed buoyancy impacts both the aquatic animal's nutrition as well as the aquaculture environment. The buoyancy of aquatic feed is easily correlated with bulk density.

Water holding capacity (WHC) is a good predictor of feed intake in pigs compared to feed digestibility and dietary fiber content (Kyriazakis & Ennnans 1995). This major effect of WHC on digestion is due to the ability of the non-starch polysaccharides (NSF) in the feedstuff to hold considerable quantities of water that could increase the bulk and passage rate of digesta (Smits & Annison 1996). Specific gravity (SG) of the feedstuff, on the other hand, has an important role in the transit of digesta particles through the gastrointestinal tract of animals as a measure of the biophysical quality of the feed, especially in ruminants (Bhatti & Firkins 1995).

The overall objective of this study is to determine some physical properties of the *Rhizoclonium* spp., namely particle size, density, water holding capacity and specific gravity.

Material and Method

Collection and preparation of the *Rhizoclonium* sp. meal. *Rhizoclonium* sp. was collected from two different sites: Leganes, Iloilo and Villa Arevalo, Iloilo. These were immediately transported to the laboratory for preparation. The collected algae were air dried for 48 h, oven dried for 24 h at 60°C and were pulverized using a mechanical grinder and then passed through three sieve sizes, namely 2360 µm, 710 µm and 150 µm mesh sieve to collect coarse, medium and fine particles, respectively. The powdered *Rhizoclonium* sp. (from here on referred to as *Rhizoclonium* meal) was then stored at -20°C.

Four physical indices were determined: particle size (PS), bulk density (BD), water holding capacity (WHC), and specific gravity (SG). The steps in measuring each of the physical characteristics were replicated thrice and the mean and the standard deviation calculated.

Particle size (PS). The particle size of the *Rhizoclonium* meal was determined using sieve analysis (ASAE 2003). The unmodified (*i.e.* unsieved) and modified (coarse-, medium-, and fine-sized particles) samples of the seaweed meal were subjected to sieve analysis. The samples were further subjected to bulk density (BD), water-holding capacity (WHC) and specific gravity (SG) measurements in 3 replicates.

One hundred grams of each size category were passed through 6-stacked sieves and manually shaken for 10 min. The weight of the particles not filtering through each screen and the weight of the particles that filtered through all screens were collected and weighed. The size of the screen openings used were 2360, 1400, 710, 250, 180, and 150 microns. The weight values of the collected particles on each screen were entered in appropriate columns of spreadsheet to determine the diameter of the i^{th} sieve in stack (i^{th} = individual measurements), average particle size or geometric mean diameter (GMD) and geometric standard deviation (GSD) according to the following equations (1), (2), and (3):

$$(1) \quad d_i = (d_u \times d_o)^{0.5}$$

Where: d_i = diameter of the i^{th} sieve in the stack
 d_u = nominal sieve aperture size next larger than i^{th} sieve (just above in set) mm
 d_o = normal sieve aperture size of the i^{th} sieve

The average particle size (also known as the geometric mean diameter, GMD) was calculated on a weight basis using the following equation:

$$(2) \quad d_{gw} = \log^{-1}[\sum W_i \log d_i / \sum W_i]$$

where d_{gw} = geometric mean diameter (μm)
 W_i = mass on i^{th} sieve (g)

The standard deviation (GSD) was calculated from the following equation:

$$(3) \quad S_{gw} = \log^{-1}[\sum W_i (\log d_i - \log d_{gw})^2 / \sum W_i]^{0.5}$$

Where: S_{gw} = geometric standard deviation

Bulk density (BD). BD was determined as described by Makinde & Sonaiya (2007). A glass funnel of known volume was weighed, the meal sample poured into the funnel and leveled off to the brim without pressing. The funnel and its content were weighed again and the initial weight was subtracted from the final weight to obtain the weight of the sample, which was then divided by the known volume of the funnel.

Water holding capacity (WHC). WHC of the seaweed meal was determined using the method described by Makinde & Sonaiya (2007). A glass funnel of known volume lined inside with filter paper was weighed. A sample of the meal was poured into the funnel and leveled off to the brim without pressing. Another filter paper was placed on top of the sample. The funnel with its contents was weighed again and the difference obtained was the dry weight of the sample. The funnel and its content were set-up below a burette filled with distilled water. Water was allowed to drop from the burette at 70 drops min^{-1} through the known volume of sample in the glass funnel. At the first drop of water from the funnel, the burette was stopped and the wet sample weighed. The volume of water absorbed by the sample was read-off from the burette. The final wet weight was subtracted from the initial weight of the funnel and its content to obtain the weight of water absorbed by the sample. The weight of water held by the sample to the weight of the dry feed was considered as the water holding capacity of the sample expressed as g water g^{-1} dry feed.

Specific gravity (SG). The steps in determining BD were repeated to determine BD of one kg of sample. This BD value was used to determine specific gravity (SG) of the *Rhizoclonium* meal. Specific gravity was expressed as the ratio of the bulk density of known mass of the experimental sample to the density of water.

Results and Discussion. Figure 1 presents the relative distribution of particles when the unmodified *Rhizoclonium* meal (0.89 mm) was compared with the modified meal (*i.e.* those processed to obtain coarse-, medium- and fine-sized particle classification; Figures 2-4, respectively) with geometric mean diameter, GMD, of 1.43 mm, 0.65 mm and 0.17 mm, respectively (Table 2). The greatest change in particle size between the unmodified (Figure 1) and those that were modified (Figures 2-4) seemed to be the reduction or altogether removal of the larger size particles. In pigs, (Wondra et al 1995) demonstrated that reducing the particle size of corn from 1200 to 400 μm in the diet improved the digestibility of dry matter, N, and energy. This observation remains to be demonstrated in aquaculture species. In the unmodified seaweed meal (Figure 1), size distribution seemed to be not ideal since the bulk of the particles (~57 to 63%) remained at 1400 to 2360 μm sieve size. In poultry nutrition, the recommended mean particle size

is between 600 to 800 μm and if this is assumed to be so in aquafeeds, then more than half of the milled *Rhizoclonium* meal in the present study would be less than ideal. Further reduction in size could be done by putting back to the mill those that did not pass the 710 μm sieve size. The ideal size seemed to be those that are retained on 710 μm sieve size (*i.e.* the medium-sized particles shown in Figure 3) with a GMD of 0.65 μm and a GSD 1.54 (Table 2); the latter index indicates the size variability. This signified that most of particles of the *Rhizoclonium* grind were in the sizes close to their median size (*i.e.* GMD) and that the uniformity of particle size was good; also its frequency of distribution almost followed a normal distribution curve. The fine-sized particle in the modified meal (Figure 4) showed that 80% of the particles were at 0.15 mm, very fine particles that might contribute to the undesirable fines in the final pellet product.

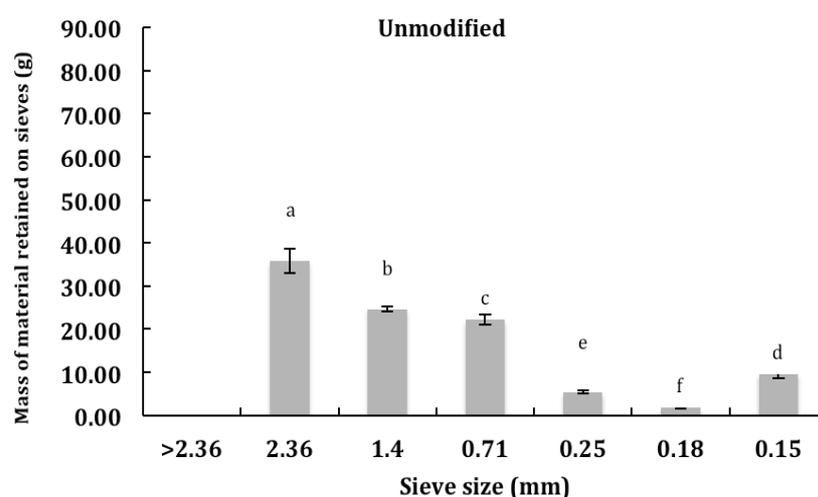


Figure 1. Particle size distribution in unmodified *Rhizoclonium* meal.

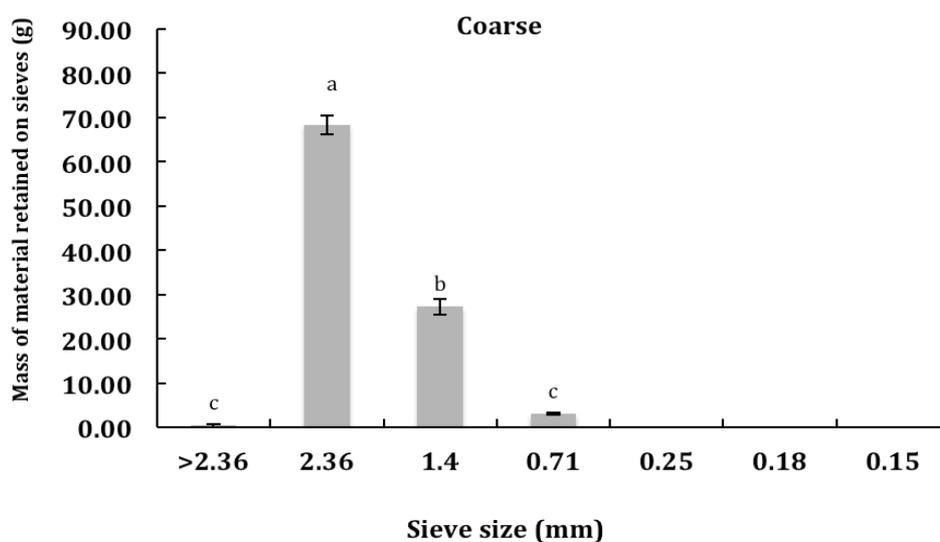


Figure 2. Particle size distribution in coarse *Rhizoclonium* meal.

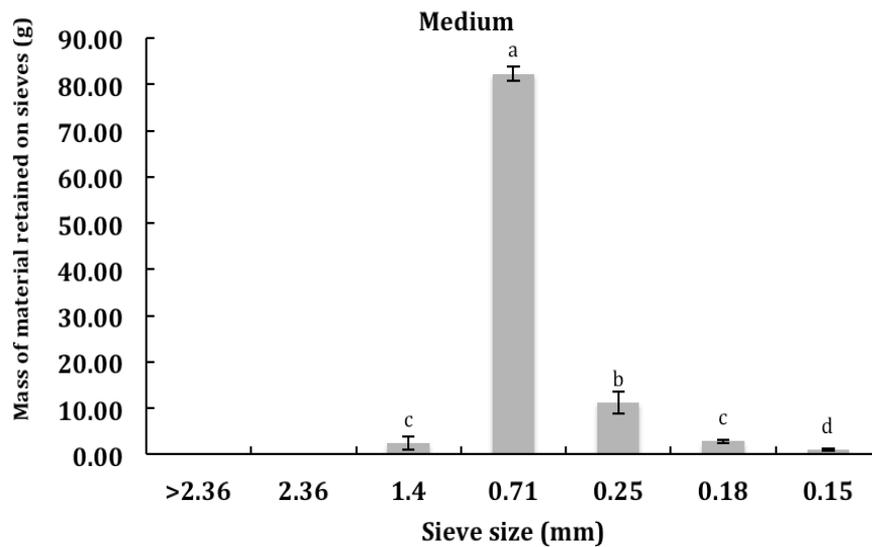


Figure 3. Particle size distribution in medium-sized *Rhizoclonium* meal.

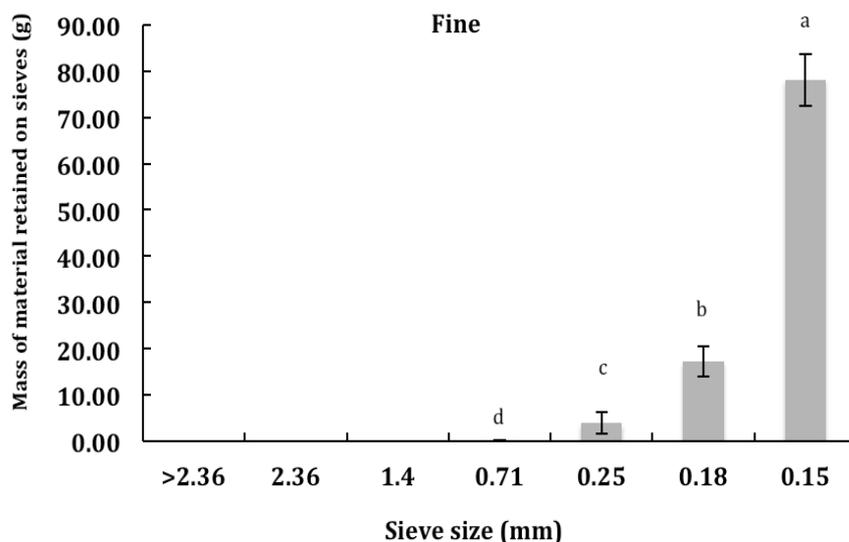


Figure 4. Particle size distribution in fine-sized *Rhizoclonium* meal.

There were particle size (PS) effects on the physical characteristics of the *Rhizoclonium* meal as shown in Table 1. The implication of this effect is that particle size could determine the physical characteristics of a feed raw material used in formulating a feed regardless of its proximate values. The final feed formulated with the same feed raw material may yield different physical characteristics because of variations in PS levels being used. The seaweed meal exhibited higher bulk density (BD) at the unmodified and at coarse PS than at the medium and fine PS. This indicates that even though the crude fiber of the materials may remain the same, their densities could be manipulated to suit the desire of the feed formulator, with possible ultimate effects on the performance of aquaculture species. The relationship of the PS (*i.e.* GMD) and BD is reflected in Table 3 showing a very high correlation (Pearson's $r=0.95$) between the two physical indices.

The WHC at the unmodified and medium PS were similar in values while those at the coarse and fine PS were significantly the lowest (almost three times of the WHC of the unmodified) and highest WHC (almost double the WHC of the unmodified), respectively (Table 2). The negative relationship between PS (*i.e.* GMD) and WHC of the

Rhizoclonium meal was very marked as reflected in Table 2 where the Pearson's *r* was -0.997. Kyriazakis & Ennans (1995) noted that WHC is a good predictor of feed intake in pigs compared to feed digestibility and dietary fiber content. This could also be the case with aquaculture species. This major effect of WHC on digestion is due to the ability of the non-starch polysaccharides (NSPs) in the feedstuff to hold considerable quantities of water that could increase the bulk and passage rate of digesta (Smits & Annison 1996). The changes in WHC of the *Rhizoclonium* meal as a function of PS emphasizes the improvement in its utilization that may not be captured by proximate analysis as have observed by Omede (2010).

SG of the seaweed meal was not affected at all by the PS (Table 1). This is in contrast with the observation of Omede et al (2011) in poultry in which the novel feedstuff like the rumen digesta, leaf meal of *Microdesmis puberula* and poultry dung which exhibited similar PS effects trend as BD. But the SG of these novel feedstuffs were very low (0.02–0.36) compared to the uniform SG value of 10.45 in the present study. This was way above the minimum feed particle SG of 1.2 suggested by Kaske et al (1992) and Bhatti & Firkins (1995).

Table 1
Physical characteristics of the *Rhizoclonium* meal

Category	Geometric mean diam. (mm)	Geometric Standard dev.	Bulk density (g cm ⁻³)	Water-holding cap. (g water g ⁻¹ sample)	Specific gravity
Unmodified	0.89±0.04	2.08±0.05	0.50±0.00	1.42±0.01	10.45±0.00
Coarse	1.43± 0.01	1.29±0.00	0.54±0.01	0.56±0.18	10.45±0.00
Medium	0.65±0.02	1.54±0.02	0.39±0.00	1.68±0.12	10.45±0.00
Fine	0.17±0.00	2.81±0.02	0.33±0.00	2.58±0.04	10.45±0.00

Table 2
Pearson product-moment correlation coefficient of the biophysical characteristics

Index	Pearson's <i>r</i>
GMD & BD	0.950
GMD & WHC	-0.997
GMD & SG	0.079
BD & WHC	-0.936
BD & SG	0.331
WHC & SG	-0.084

As for the correlation of the physical properties of the seaweed meal, PS (*i.e.* GMD) did not exhibit correlation with SG. BD and WHC did not exhibit correlation with SG.

In many feed raw materials, what determines physical characteristics such as BD, WHC and SG is the nature or physical structure of the material (fibrous nature and the kind of nonstarch polysaccharides (NSPs) they are made of which may be soluble or non-soluble) among other factors listed by De Lange (2000). This means that for a proper ranking of the nutritional value of novel feedstuffs to be developed, there is the need for further physical quality characterization. At present however, research attention is focused more on biochemical and replacement values of novel feedstuffs for conventional feed raw materials (Omede 2010).

Conclusions. Assuming that the optimum mean PS is between 600 to 800 µm found in poultry nutrition, the ideal size were those retained on 710 µm sieve size (*i.e.* the medium-sized particles) with a GMD of 0.65 µm and a GSD 1.54. In this modified meal, most particles were in the sizes close to their median size (*i.e.* GMD) and that the uniformity of particle size was good and its frequency of distribution resembled a normal

distribution curve. There were PS effects on the physical characteristics of the *Rhizoclonium* meal which indicated that even though the crude fiber remained the same, their densities could be manipulated to suit the desire of the feed formulator. The negative relationship between particle size (*i.e.* GMD) and WHC of the *Rhizoclonium* meal was very marked. The change in WHC of the *Rhizoclonium* meal as a function of particle size emphasizes the improvement in its utilization that may not be captured by proximate analysis. SG of the seaweed meal was not affected at all by the PS. The four biophysical factors measured in the present study give a clue on the nature or physical structure of the material (fibrous nature and the kind of NSPs they are made of which may be soluble or non-soluble) among other factors.

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