INTERNATIONAL AQUA FEED

Feature title: Application of isotopic techniques to assess the nutritional performance of macroalgae in feeding regimes for shrimp

Deadline: 30/04/2013

Application of isotopic techniques to assess the nutritional performance of macroalgae in feeding regimes for shrimp

by Julián Gamboa-Delgado PhD, research officer, Programa Maricultura, Universidad Autónoma de Nuevo León, Mexico

Juvenile Pacific white shrimp feeding on U. clathrata macroalgal biomass. Long fecal strands are frequently related to fast gut transit.

Due to their nutritional properties, several species of macroalgae have been used as dietary supplements for shrimps and other marine species. Since macroalgae represent a natural source of nutrients in the shrimp's natural environment, attempts have been done to co-culture macroalgae and shrimps.

The nutritional performance and digestibility of macroalgae-derived meals have been tested in formulated diets for shrimp. One of the aspects requiring further research is represented by the loss of nutritional properties occurring when the macroalgal biomass is dried out as compared when the algal biomass is ingested as live biomass.

Several nutritional methodologies have been used to evaluate the performance of different ingredients used or proposed for aquaculture feeds. The use of stable isotopes as tools to assess nutritional contributions of specific ingredients to growth is one of many emerging nutritional techniques applied in aquaculture.

The chemical composition of macroalgae varies among species and environmental conditions; however, most are rich in non-starch polysaccharides, vitamins, and minerals. In particular, green macroalgae (Chlorophyceae) often have higher protein content than brown seaweeds. Such nutritional properties, in conjunction with novel macroalgae production methods, have increased the interest in their use as dietary ingredients for aquaculture diets. Additionally, there are studies that have focused on their use as additives to enhance the immunological status of the farmed animals. The green macroalgae *Ulva (Enteromorpha) clathrata*, also known as aonori in Asian countries, has worldwide distribution and due to its nutritional profile, has been evaluated as a dietary supplement for aquatic species. *U. clathrata* has been mass-cultured in recent years under a patented technology developed by Aonori Aquafarms Inc. By applying this methodology, macroalgae biomass is rapidly grown in ponds without eliciting detrimental effects to the environment.

Evaluation of macroalgae in shrimp nutrition studies

Although it has been observed that use of macroalgal biomass alone as feed does not fulfil the nutritional requirements for optimal growth in marine shrimp, co-culture of *U. clathrata* and Pacific white shrimp *L. vannamei* has been conducted with positive results in terms of lower feed utilization and improvement of the shrimp nutritional quality, flesh colour and texture.

Recent nutritional studies have also shown that when dry *Ulva clathrata* meal is fed to Pacific white shrimp as an ingredient in practical diets, it has an apparent digestibility coefficient for dry matter of 83 percent, while the same value for protein is 90 percent. However, the high ash content and the relatively low protein content of this macroalgae species prevent its dietary inclusion at high levels when attempting to replace other ingredients such as fishmeal.

Table 1: Growth, survival rate and estimated consumption of formulated feed and live macroalgae biomass (dry weight) by juvenile Litopenaeus vannamei reared on five different feeding regimes for 28 days (n= 8-20, mean values ±SD).

Feeding regime	Survival (%)	Final wet weight (mg)	Weight increase (%)	Consumed formulated feed (g)	Consumed U. clathrata (g)
100A	95 ± 13[a]	995 ± 289[a]	429	0.94	-
75F/25U	93 ± 11[a]	1067 ± 364[a]	467	0.81	0.40
50F/50U	78 ± 11[ab]	768 ± 273[ab]	308	0.43	0.44
25F/75U	60 ± 21[b]	424 ± 207[b]	125	0.14	0.65
100U*	23 ± 4[c]	221 ± 49[c]	18	-	1.32

Initial wet weight = 188 ±28 mg.

Different superscripts indicate significant differences at $p<0.05$.

** Parameters in animals from feeding regime 100U were estimated on experimental day 21.*

Stable isotopes to assess the nutritional contribution of macroalgae

Over the last few decades, different isotopic methodologies have been adopted from the ecological sciences and have been applied to animal nutrition studies. Most elements in organic matter are present as two or more stable isotopes and heavier isotopes have a tendency to accumulate in animal tissue. For example, animal predators have higher isotopic values than their preys; therefore, a specific isotopic signature is conferred to each trophic level (primary producers, herbivores, carnivores).

In the case of plants and macroalgae, their carbon isotope values are strongly influenced by the type of photosynthesis they present. On the other hand, the nitrogen stable isotope values of plants and macroalgae can be easily manipulated by means of specific fertilizers, to eventually conduct nutritional studies.

By using such techniques, it can be possible to determine the proportions of available dietary nutrients that have been selected, ingested and incorporated into animal tissue (Figure 1). As the average sample size required for stable isotope analysis (carbon and nitrogen) is only 1 mg of dry tissue or test diet, the technique has been very useful in larval nutrition studies. It has been employed to quantify the proportions of nutrients incorporated from live and formulated feeds in fish and crustacean larvae.

Likewise, stable isotope analyses of different plant-derived ingredients (soy protein isolate, corn gluten and pea meal) have been carried out to explore the contribution of the dietary nitrogen supplied by these sources (as compared to fish meal) to shrimp growth. In the context of macroalgae as source of nutrients, isotopic techniques have been applied as nutritional tools to quantify the relative contributions of dietary carbon and nitrogen to the growth of Pacific white shrimp co-fed formulated feed and live macroalgal biomass of *U. clathrata*.

Experimental design

Taking advantage of the contrasting natural carbon and nitrogen stable isotope values measured in a commercial formulated feed and in live macroalgal biomass of *U. clathrata*, the study aimed to quantify the relative contribution of nutrients to the growth of Pacific white shrimp. Animals were allocated to duplicate tanks individually fitted with air lifts and connected to an artificial-seawater recirculation system.

Feeding regimes consisted of a positive isotopic control (100% formulated feed, treatment 100F), a negative isotopic control (100% macroalgae, treatment 100U) and three co-feeding regimes in which 75, 50, and 25 percent of the daily amount of consumed macroalgal biomass was substituted by formulated feed (treatments 75F/25U, 50F/50U, and 25F/75U, respectively) on a dry weight basis.

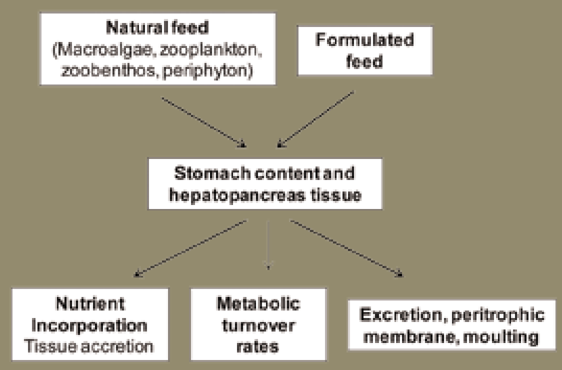

Figure 1: Carbon and nitrogen flow in shrimps produced under semi-intensive farming conditions. Bold arrows indicate components that can be isotopically analyzed to determine their origin and fate

The digestibility of both feeding sources for *L. vannamei* has been previously assessed and is similarly high (>80%). Live macroalgae was supplied to shrimp by attaching the algal biomass to plastic mesh units from which the algal filaments were constantly available and easily nibbled upon by shrimp.

Feeding rations and proportions were progressively adjusted in relation to the amount of macroalgal biomass consumed, animal survival and sampling. Shrimp samples (whole bodies and muscle tissue) and diet samples were collected and pre-treated for isotopic analysis.

Growth and survival

There was a high variability in final wet weight of shrimps under the different dietary treatments; however, a clear tendency for higher growth was observed in shrimps reared on regime 75F/25U (1,067 ±364 mg, final mean weight), followed by shrimps fed only on formulated feed (995 ±289 mg). Shrimps from both feeding regimes increased their weight more than 400 percent (Table 1).

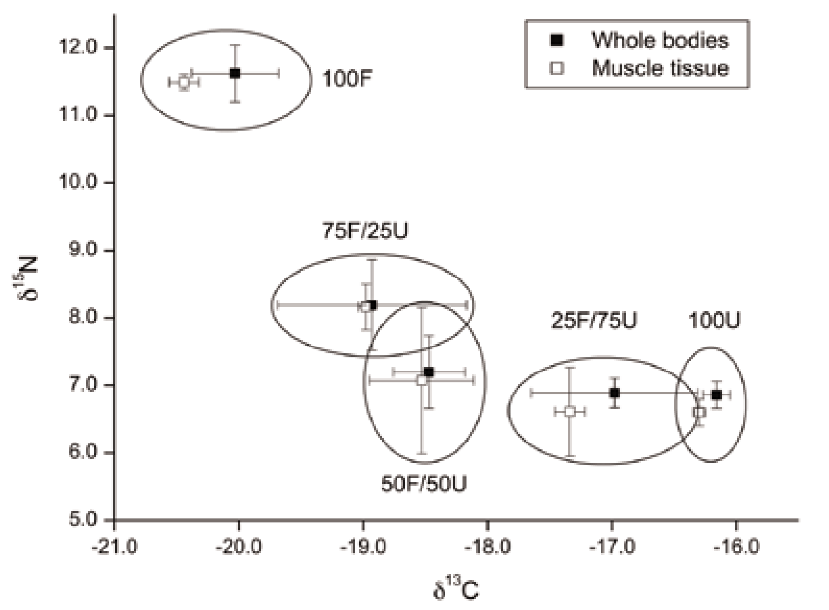

Figure 2: Carbon and nitrogen dual isotope (‰) plot of whole bodies and muscle tissue of white shrimp L. vannamei reared on feeding regimes consisting of different proportions of formulated feed and live U. clathrata biomass. Muscle tissue values for treatment 100U were estimated for day 28 from values in whole bodies. n= 2-4, mean values ±SD

Animals fed only on *U. clathrata* biomass showed very low growth (221 ±49 mg) and only 23 percent of the animals in this treatment survived by day 21. Higher survival rates (93-95%) were observed in shrimps reared on feeding regimes 100F and 75F/25U, while shrimps in dietary treatments 50F/50U and 25F/75U had respective mean survival rates of 78 and 60 percent. The positive effect of supplying both, live feeds and formulated diets has been recurrently observed in previous crustacean studies.

Dietary contributions from macroalgae and formulated feed

At the end of the experiment, isotopic values of shrimp tissue reared on co-feeding treatments were strongly biased towards the isotopic values of *U. clathrata* biomass.

Figure 2 combines carbon and nitrogen stable isotope values measured in shrimps and provides a graphic indication of the total organic matter contributed by both, the formulated feed and macroalgae. Results from an isotopic mixing model indicated that shrimps in the three co-feeding regimes incorporated significantly higher amounts of dietary carbon and nitrogen from *U. clathrata* biomass than from the formulated feed (Table 2).

At the end of the experiment, shrimps in treatment 75F/25U incorporated 68 percent of carbon from the formulated feed and 32 percent from the macroalgae. Shrimps under feeding regimes 50F/50U and 25F/75U incorporated significantly higher amounts of dietary carbon from *U. clathrata* (49 and 80%, respectively) when compared to the expected dietary carbon proportions supplied by these the co-feeding regimes (34 and 70%, respectively). Shrimp grown in co-feeding regime 75F/25U incorporated 27 percent of nitrogen form the formulated feed and the remaining 73 percent from the macroalgal biomass, while animals reared on regimes 25F/75U and 50F/50U incorporated the majority of their dietary nitrogen (98 and 96%, respectively) from the macroalgae.

Table 2: Estimated contribution of dietary nitrogen supplied from formulated feed and live biomass of Ulva clathrata and incorporated in tissue of postlarval Pacific white shrimp L. vannamei as indicated by stable isotope analysis.

Feeding regime	Expected*	Observed	
		Whole bodies	Muscle tissue
75F/25U			
Formulated feed	79.6a**	15.9 b	20.5 b
Ulva biomass	20.4	84.1	79.5
50F/50U			
Formulated feed	66.1a	2.2 b	6.9 b
Ulva biomass	33.9	97.8	93.1
25F/75U			
Formulated feed	30.1a	1.0 b	3.2 b
Ulva biomass	69.9	99.0	96.8

**Expected proportions are estimated from the actual proportions of formulated feed and macroalgal biomass offered (on a dry weight basis).*

***Superscripts indicate significant differences between expected and observed dietary contributions.*

The lower growth attained by these animals indicated that a very high proportion of the isotopic change was due to high nitrogen metabolic turnover and not to tissue accretion. Due to its lower carbon and nitrogen contents, the macroalgal biomass had to be consumed at higher amounts in order to supply the observed elemental contributions to shrimp whole bodies and muscle tissue.

The availability and incorporation of nutrients from formulated and live feeds

The higher than expected contributions of macroalgal carbon and nitrogen to shrimp growth are possibly related to the high digestibility of *U. clathrata* and its continuous availability for shrimp. Chemical analyses of *U. clathrata* have shown that it typically contains low to medium protein levels (20 - 30%) and very low lipid levels. The cell wall polysaccharides in macroalgae might represent more than half of dry algal matter, but a tentative role of the latter as energy source is unlikely as specific enzymatic activities for these polysaccharides (ulvanase, fucoidanase) have not been reported for Penaeid shrimps. Despite their lower nutrient concentration, live feed contains higher water content which contributes to higher digestibility.

In contrast, formulated feed can contribute nutrients that are scarce or absent in live feed, but the incorporation of such nutrients is limited by low feed digestibility or unsuitable formulation. Previous co-feeding experiments conducted on postlarval shrimp and larval fish have shown that the supplied live feed frequently contributes higher proportions of nutrients to the growth of the consuming animals than those supplied by formulated feeds in co-feeding regimes.

Conclusion

Although the live macroalgae by itself was not nutritionally complete for Pacific white shrimp, it supplied a very significant proportion of structural carbon and nitrogen when co-fed with formulated feed.

However, the high amount of nutrients derived from the live macroalgae biomass in co-feeding regimes supplying more than 50 percent of macroalgae, was not reflected in a fast growth increase. This was possibly due to the restriction of other nutrients in this macroalgae species. Interestingly, shrimp under the co-feeding regime supplying 75 percent of formulated feed and 25 percent of live macroalgae biomass showed higher growth rates than animals reared only on commercial formulated feed, although the difference was not statistically significant.

The low levels of energy, amino acids and fatty acids in the macroalgae biomass available to shrimp, were compensated through high ingestion rates, which caused a higher incorporation of nutrients in shrimp tissue. On the other hand, it is very likely that the carbohydrates and lipids supplied by the formulated feed significantly contributed to the energy requirements of shrimp under the three co-feeding regimes.

The importance of the natural productivity to shrimp grown in semi-intensively managed ponds has been widely documented. The systematic use of macroalgae in production ponds not only provides a significant nutritional supply to cultured organisms, but also offers substrate for periphyton growth and refuge for moulting shrimps. In addition, it has been demonstrated that *Ulva clathrata* and other macroalgae species are efficient removers of the main dissolved inorganic nutrients, hence maintaining good water quality levels in aquaculture ponds and effluents.

Diverse isotopic techniques can be applied to elucidate the transfer of nutrients at the level of amino acids and fatty acids; therefore, future experimental assays might reveal what specific nutrients are contributed from the macroalgal biomass (or any other component of the natural biota) and from the supplied formulated feeds. The loss of some nutritional properties that occurs in dietary ingredients that undergo drying (or freeze drying) has not been thoroughly explained and future studies applying stable isotopes might shed some light on the differences observed when aquatic animals consume moist or dry dietary components.

References

Burtin, P. 2003. Nutritional value of seaweeds. Electron. J. Environ. Agric. Food Chem. 2:498–503.

Cruz-Suárez, L.E., A. León, A. Peña-Rodríguez, G. Rodríguez-Peña, B. Moll, D. Ricque-Marie. 2010. Shrimp/Ulva co-culture: a sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture 301: 64–68.

Gamboa-Delgado, J. 2013. Nutritional role of natural productivity and formulated feed in semi-intensive shrimp farming as indicated by natural stable isotopes. Reviews in Aquaculture 5: 1-12. In press.

Gamboa-Delgado, J., M.G. Rojas-Casas, M.G. Nieto-López, L.E. Cruz-Suárez 2013. Simultaneous estimation of the nutritional contribution of fishmeal, soy protein isolate and corn gluten to the growth of Pacific white shrimp (*Litopenaeus vannamei*) using dual stable isotope analysis. Aquaculture 380-383: 33-40.

Gamboa-Delgado, J., A. Peña-Rodríguez, L.E. Cruz-Suárez, D. Ricque D. 2011. Assessment of nutrient allocation and metabolic turnover rate in Pacific white shrimp *Litopenaeus vannamei* co-fed live macroalgae *Ulva clathrata* and inert feed: dual stable isotope analysis. J. Shellfish Res. 30: 1–10.

Moll, B. (Sinaloa Seafields International). 2004. Aquatic surface barriers and methods for culturing seaweed. International patent (PCT) no. WO 2004/093525 A2. November 4, 2004.

Villarreal-Cavazos D.A. 2011. Determinación de la digestibilidad aparente de aminoácidos

de ingredientes utilizados en alimentos comerciales para camarón blanco (*Litopenaeus vannamei*) en México. PhD Thesis. Universidad Autónoma de Nuevo León, Mexico.

http://eprints.uanl.mx/2537

More Information:

Julián Gamboa-Delgado PhD

Tel: +52 8352 6380

Email: julian.gamboad@uanl.mx

March-April 2013 - Advertising promotion:

Display advertising:			
Advert size	UK£	€	US$
Full page	2,999	3,675	4,824
Mini page	2,216	2,716	3,564
Half page	1,678	2,056	2,699
Third page	1,137	1,393	1,829
Quarter page	920	1,127	1,479
Eighth page	672	823	1,081

Advertise within this issue and your advert will appear in:

Our printed magazine

Our online edition magazine

Two issues of the E-Newsletter

*The Features Archive of www.docstoc.com, www.scribd.com & www.slideshare.net

Classified advert *(appears in six issues)*			
Advert size	UK£	€	US$
4 cm deep	621	751	962
5 cm deep	776	939	1,203
6 cm deep	931	1,127	1,443
7 cm deep	1086	1,314	1,683
8 cm deep	1,242	1,503	1,925

Classified advert

Classified adverts placed in the magazine appear for one year. The adverts will appear in the print & online magazine. The advert will also appear in our online 'Market Place' on www.docstoc.com & www.scribd.com.

Please note that invoices are raised in UK£'s and so exchange rates may vary slightly

Contact the Marketing team on - Tel: +44 1242 267706
Lee Bastin - Email: leeb@aquafeed.co.uk
Darren Parris - Email: darrenp@aquafeed.co.uk
Tom Blacker - tomb@aquafeed.co.uk

Deadline: 30/04/2013

We guarantee

- To expose your advertisement to more people, more often and for much longer than anyone else
- That your advertisement will be found and redistributed by more of the right people
- That your advertisement will be put in front of more of your target market than that achieved by any one of our competitors
- To deliver the industry's maximum ROI (return on investment) based on your advertising spend with us

Digital Circulation

As well as appearing in the paper version of the magazine, this feature and the advertisements placed within it will be included in a digital re-print and circulated electronically with fully functioning links. The full magazine will also appear as a digital version on our website and in the magazine's online archive.

Article Reprints

We can produce either four or eight page versions of any specific feature that has appeared in the magazine (including back issues up to 2006). These features have been used by our customers as point of sale materials, promotional materials for shows and exhibitions etc.
For full details please visit www.aquafeed.co.uk/reprints