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# Growth and Survival of Juvenile Queen Conch *Strombus gigas* Fed Artificial Diets Containing Varying Levels of Digestible Protein and Energy

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## Abstract

Aquaculture methods for queen conch *Strombus gigas* have been established for several decades. However, there is a need to improve husbandry techniques for the grow out of juveniles. The purpose of this study was to determine the growth and survival of juvenile queen conchs fed artificial diets with increasing levels of a red alga *Agardhiella* sp. *Agardhiella*, soy protein isolate, and fish oil were increased at the expense of catfish feed, wheat flour, or both to keep the diets isonitrogenous (38% crude protein [CP]) with increasing protein to energy ratios (83–96 mg CP/kcal). Yttrium was also incorporated as an inert marker for determining the digestibility of dietary nutrients. Juvenile queen conchs were stocked at 75/m<sup>2</sup> for a total of 16 conchs per replicate (three replicates) and were fed 125 mg of diet per conch each day for 6 months. Dietary crude protein ranged from 31% to 38%. The dietary lipid, moisture, energy, ash, fiber, carbohydrate, and yttrium in each diet were also determined. Juvenile conchs were measured (shell length and wet weight) every 6 weeks. At the end of the experiment, feces were collected to determine protein, energy, and dry matter digestibility. Results indicated the highest growth rates (0.10 mm/d and 0.11 mm/d) and survival were for conchs that were fed diets containing a soy protein isolate protein substitution of 15% or less. This study highlights the importance of the protein source in the artificial diets of juvenile queen conchs.

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The queen conch *Strombus gigas* is a large gastropod and keystone herbivore in the tropical waters of Florida and the Caribbean (Randall 1964). The queen conch fishery is the second largest subsistence and commercial fishery in the Caribbean region, and conchs have been exported worldwide intensively since the 1970s (Appeldoorn 1994). This has led to severe overfishing, and in most countries queen conch populations are at dangerously low levels. In 1992, queen conch was added to Appendix II under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Theile 2005) to ensure that international commercial trade does not threaten their survival in the wild.

General aquaculture methods for queen conchs have been established for several decades as a means to grow conchs for food and stock enhancement. However, there is a need to improve husbandry techniques for the grow out of juveniles (Davis 2000; Shawl and Davis 2004; Davis and Shawl 2005). Ideal stocking densities, substrates, and calcium requirements have been examined, but the nutritional requirements are not well known. In the wild, queen conchs graze on diatoms and detritus from seagrass blades and sand grains (Stoner and Waite 1990). Some live algal species that can be used to grow juvenile queen conchs in captivity include *Enteromorpha prolifera*, *Spyridia filamentosa*, *Spirulina platensis*, *Ceramium fastigiatum*, and *Dictyota cervicornis* (Creswell 1984; Hernandez et al. 2007). Several artificial queen conch diets have produced acceptable growth rates for cultured conch, but the conchs still exhibit low growth rates relative to wild juveniles (Davis and Shawl 2005). A previous study comparing the standard conch diet used in the Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) hatchery (catfish feed mixed with the green alga *Ulva* sp.) with diets incorporating different macroalgal species suggested that juvenile queen conch fed the red alga *Agardhiella subulata* had the highest growth rates (Acosta-Salmon et al. 2010).

Acceptable growth (80  $\mu\text{m}/\text{d}$ ) and survival rates (81.3%) have been obtained for juvenile queen conchs fed the standard diet consisting of catfish feed (36%), *Ulva* sp. (16%), alginate (5%), and seawater (43%) (Davis and Shawl 2005; Spring and Davis 2005; Shawl and Davis 2006). The incorporation of macroalgae in this artificial diet has been shown to be crucial for survival, and the macroalgae *Ulva* sp. can be substituted for *A. subulata* (Acosta-Salm3n et al. 2010).

Seaweed culture allows continuous production of macroalgae species and ensures that the nutrient content (e.g., protein, lipid, and carbohydrate) is relatively consistent (Capo et al. 1999, 2002; Barile et al. 2004) and can even be enhanced (Shipgel et al. 1999). The Rhodophytas, *A. subulata* and *Gracilaria ferox*, and the Chlorophyta *Ulva* spp., produced under controlled conditions have been used as a mixed-seaweed diet to support production of the juvenile sea hare *Aplysia californica* (Capo et al. 2002).

Limited information exists regarding the nutritional requirements of the queen conch. In diet development for marine gas-

tro pods and other species, the importance of establishing the protein level for maximizing growth and protein efficiency cannot be overstated. Moreover, animals eat to meet their energy needs and feeds with too much energy can reduce total feed intake. Therefore, protein intake is a function of both dietary protein and energy concentration. As protein is the most expensive component of aquaculture feeds, an accurate assessment of both the protein and energy requirements can increase the efficiency of protein utilization and reduce the cost of manufactured feeds. The principal objective of this study was to determine growth and survival of juvenile queen conchs fed isonitrogenous artificial diets with varying levels of digestible protein (DP) and digestible energy (DE). A secondary objective was to evaluate the ability of queen conchs to utilize carbohydrates relative to protein for energy, thereby reducing the required and more expensive protein component of the diet.

## METHODS

Hatchery-produced juvenile queen conchs were obtained from a commercial producer (Oceans, Reefs, and Aquariums, Inc., Fort Pierce, Florida) and maintained under suitable conditions in recirculating tank systems with aragonite substrate (Shawl and Davis 2005; Spring and Davis 2005). Water quality features were recorded daily (temperature), weekly (pH, salinity, and dissolved oxygen), or spot-checked (ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen). Temperature and pH were measured with a Hanna pHep pen. Salinity was measured with a portable refractometer and dissolved oxygen was measured with an OxyGuard Handy Gamma. A Hach DR/850 colorimeter was used to measure ammonia nitrogen (Hach Method 8155), nitrite-nitrogen (Hach Method 8507), and nitrate-nitrogen (Hach Method 8039).

A randomized complete-block design was employed with diet as the fixed-factor effect and treatments blocked on recirculating systems. Treatments were run with three replicates. The standard conch diet served as a positive control (diet 2) and catfish feed as a negative control (diet 1) (Table 1). Four experimental recirculating systems were used, each consisting of a trough (2.4  $\times$  0.5  $\times$  0.6 m), sump, pump, and raised sand bed containing 45 L of Florida crushed coral aragonite substrate (Caribsea, Inc., Fort Pierce, Florida) with a 2.0–5.5-mm grain size (Davis 2005). Troughs were divided into six, identical, 0.21-m<sup>2</sup> compartments, each serving as a single replicate for the control and experimental diets. One trough with six compartments was used to hold conchs for replacement of mortalities. Troughs were cleaned monthly, and water was added to the sumps once per week to replace that lost by evaporation.

Five diets were formulated to contain increasing DP:DE ratios (Table 1). Diets 3–6 incorporated increasing graded levels of the red algae *A. subulata* and decreasing levels of a commercial catfish feed. Increasing levels of soy protein isolate were incorporated to keep the diets isonitrogenous and fish oil was incorporated to replace dietary oil lost due to the replacement of the catfish feed. Diet 2 was similar to diet 3

TABLE 1. Formulation and analyzed composition of experimental diets fed to queen conch.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredient (g/kg dry diet)						
Cargill catfish chow	975.5	735.3	735.3	588.2	441.2	294.1
<i>Agardhiella subulata</i>	0.0	106.9	106.9	214.7	322.5	430.4
Wheat flour	0.0	127.5	0.0	0.0	0.0	0.0
Soy protein isolate	0.0	0.0	117.7	147.2	176.6	206.0
Vitamin premix	0.0	2.9	2.9	2.9	2.9	2.9
Mineral premix	0.0	2.9	2.9	2.9	2.9	2.9
Fish oil	0.0	0.0	9.8	19.6	29.4	39.2
Alginate	19.6	19.6	19.6	19.6	19.6	19.6
Yttrium oxide	4.9	4.9	4.9	4.9	4.9	4.9
Analyzed composition						
Crude protein (%)	31.4	28.7	36.8	37.8	38.2	37.9
Energy (KJ/g)	18.5	19.1	18.9	18.1	17.5	16.2
Lipid (%)	9.6	7.8	7.7	7.3	10.0	8.9
Ash (%)	10.5	8.8	11.2	13.5	16.1	22.8
DP : DE ratio (mg protein/kJ) <sup>a</sup>	17.9	12.0	17.6	20.2	21.6	23.7

<sup>a</sup>Ratio of digestible protein to digestible energy as calculated from determined digestibility coefficients.

except wheat flour was substituted for soy protein isolate and fish oil.

*Agardhiella subulata* was grown in outdoor raceways (Capo et al. 1999) and after harvest was sun-dried for 48 h. The catfish feed and *A. subulata* were ground and mixed with the other dry ingredients. Yttrium oxide was incorporated as an inert marker for determining apparent digestibility of protein and energy. After the addition of alginate, oil, and seawater the diets were extruded and dried in a forced air oven. Diets were prepared by a commercial feed manufacturer (Bonney, Laramore, and Hopkins, Inc., Lesterville, Missouri).

A total of 384 juvenile queen conchs were stocked at a density of 75/m<sup>2</sup> (Spring and Davis 2005). Mean  $\pm$  SD shell length (SL) and live weight (LW) were 33.8  $\pm$  0.2 mm and 3.7  $\pm$  0.1 g, respectively. Conchs were fed at a rate of 125 mg/d per conch (Davis 2005; Davis and Shawl 2005). Mortalities were recorded and replaced to maintain stocking density. Replacement conchs were marked with acrylic paint and not included in future measurements. Replacement conchs used for each treatment were fed assigned experimental diets in the same manner, and simultaneously with the experimental conchs.

After 24 weeks, SL and LW were measured, and 10 conchs from each treatment were randomly selected for compositional analysis. Daily growth rates were calculated using the formula

$$GR = (LW_f - LW_0)/t,$$

where GR is the growth rate (g/d), LW<sub>0</sub> is the mean initial live weight (g), LW<sub>f</sub> is the mean final live weight (g), and *t* is the number of days.

Conch feces were collected for a period of 2 weeks at the end of the experiment (weeks 24–26). To collect feces, all conchs from each replicate and treatment were placed into individual

shallow plastic trays (45  $\times$  30 cm) with 1 L of seawater for approximately 8 h. The feces were collected with a pipette. Following collection, conchs were returned to their respective tanks. Feces were rinsed with a gentle stream of distilled water and collected in a drying pan. Fecal samples were dried for 24 h at 60°C in an Isotemp forced air oven (Fisher Scientific, Suwanee, Georgia).

Diets, conch meat, and feces were analyzed using standard methods (AOAC International 2003). Dietary dry matter was determined after drying to a constant weight at 105°C. Feed, fecal, and meat nitrogen (N) were determined following combustion (TruSpec N elemental analyzer, LECO-Corp, St. Joseph, Michigan) and CP was determined as N  $\times$  6.25. Dietary crude lipid was determined gravimetrically following extraction of lipid-soluble components using chloroform–methanol (Folch et al. 1957) in a Soxhlet apparatus. Dietary ash was determined following combustion of organic matter at 600°C for 2 h. Feed and fecal gross energy was determined by adiabatic bomb calorimetry (Parr 1266, Parr Instruments Co., Moline, Illinois). Meat glycogen was determined by a modified phenol–sulfuric acid colorimetric assay (Lo et al. 1970) at wave length ( $\lambda$ ) = 490 nm (DU640 spectrophotometer, Beckman-Coulter, Fullerton, California).

Dietary and fecal yttrium was determined by inductively coupled plasma spectroscopy (Perkin-Elmer, Waltham, Massachusetts). Apparent digestibility (AD) of protein and energy was calculated according to Ward et al. (2005), that is,

$$AD(\%) = 1 - [(V_{\text{feed}}/N_{\text{feces}}) \times (Y_{\text{feces}}/N_{\text{feed}})] \times 100,$$

where *Y* is yttrium concentration (%) and *N* is nutrient concentration (%).

TABLE 2. Mean (SE)<sup>a</sup> growth, tissue composition, and apparent digestibility coefficients for queen conch fed the experimental diets during a 24-week trial. Means in the same row followed by different letters are significantly different ( $P < 0.05$ ).

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
<b>Growth</b>						
Final live wet weight (g)	6.8 (0.9) z	6.5 (0.3) yz	7.2 (0.4) yz	7.0 (0.3) yz	6.0 (0.6) yz	4.6 (0.1) y
Final shell length (mm)	40.7 (1.1) z	39.9 (0.5) yz	41.8 (0.6) yz	40.8 (0.7) yz	39.9 (1.6) yz	36.2 (0.2) y
Growth rate ( $\mu\text{m}/\text{d}$ )	42.2 (11.9) z	40.2 (4.5) z	47.5 (6.6) z	47.7 (9.7) z	35.8 (17.1) z	16.0 (4.0) z
Survival (%)	47.9 (25.6) z	70.8 (7.5) z	72.9 (7.5) z	72.9 (5.5) z	60.5 (15.0) z	25.0 (13.0) z
<b>Tissue composition</b>						
Meat protein (% DM)	65.3 (3.5) z	61.3 (0.9) yz	62.6 (0.7) yz	62.8 (1.1) yz	66.2 (0.5) yz	57.3 (1.4) y
Meat glycogen (% DM)	1.8 (0.5) xyz	2.6 (0.3) xz	2.1 (0.2) yz	0.9 (0.2) xyz	0.0 (0.2) xy	-0.4 (0.1) xy
<b>Apparent digestibility</b>						
Crude protein (%)	77.0 (0.1) z	51.0 (7.8) z	61.2 (11.5) z	73.4 (16.9) z	65.4 (2.5) z	87.4 (4.8) z
Energy (%)	73.0 (3.5) z	63.6 (6.9) z	67.6 (9.5) z	76.0 (14.6) z	66.1 (2.9) z	86.3 (4.2) z

<sup>a</sup>Diets 1 and 6,  $n = 2$ ; diets 2-5,  $n = 3$ .

Complete mortality within one replicate each for two of the treatments, diet 1 and diet 6, necessitated data analysis as an unbalanced design. Homogeneity of variances was tested using Levene's test. Growth, tissue response variables, and digestibility coefficients were evaluated as a one-way analysis of variance (ANOVA) using the general linear model and least-squares marginal means, and formulated diets were tested against the catfish feed using Dunnett's test adjusted to control the type I error rate. To evaluate the ability of dietary carbohydrate to spare protein, diets 2 and 3 containing equivalent concentrations of *A. subulata* were tested against each other using an orthogonal contrast. Significant differences were reported at the  $P < 0.05$  level. Stepwise multiple linear regression analyses were performed on the formulated diets to evaluate the effects of DP, DE, and the DP:DE ratio and dietary *A. subulata*, soy protein isolate, lipid, and ash on response variables. Relationships were considered significant at the  $P < 0.05$  level. Multiple linear regression and ANOVA were conducted using SAS software version 9.1.3 (SAS, Cary, North Carolina). Where curve fitting suggested the application of nonlinear regression, analysis was performed using Prism 4.03 (GraphPad Software Inc., San Diego, California).

## RESULTS

Water quality conditions remained within an ideal range for queen conchs throughout the experiment (Davis 2005). Mean temperature was 27.4°C, salinity was 35‰, dissolved oxygen was 8.6 mg/L, and pH was 8.0. Ammonia nitrogen, nitrite-nitrogen, and nitrate-nitrogen levels were undetectable. Final LW and SL were significantly lower in conchs fed diet 6 relative to those fed diet 1 (Table 2). No other differences in weight, shell length, or growth rate were observed relative to the conchs fed the catfish feed. Although poor survival was observed among the treatments fed the catfish feed and diet 6, no significant dif-

ferences were observed relative to the treatments fed the catfish feed. Similarly no differences were detected between conchs fed the two treatments with the same level of *A. subulata* incorporation (diets 2 and 3).

Significant differences were detected in tissue composition (Table 2). Meat protein was significantly lower in conchs fed diet 6 than those fed the catfish feed. Similarly, meat glycogen was significantly lower in conchs fed diet 5 and diet 6 relative to those fed the catfish feed. There were no significant differences detected in meat protein of the conchs fed the two diets containing similar levels of *A. subulata*; however, there was significantly higher glycogen levels in the conchs fed the diet containing wheat flour (diet 2) relative to the diet without (diet 3).

Apparent digestibility of crude protein ranged from 51.0% to 87.4% and energy digestibility ranged from 63.6% to 86.3%. No differences were detected among either the protein or energy digestibility coefficients. Application of the coefficients to dietary protein and energy resulted in a linear increase of DP:DE from 12.0 to 23.7 mg protein/kJ with increasing soy protein isolate and *A. subulata*. The increase in DP:DE from diet 2 to diet 6 was a function of increasing digestible protein since DE was constant across all the treatments (Figure 1). Multivariable regression suggested the increase in DP:DE across diets 2 through 6 (Table 1) was due to soy protein isolate (partial  $R^2 = 0.99$ ) because of its effect on the digestible protein ( $R^2 = 0.88$ ) component of the ratio.

Multiple linear regression analyses of the five formulated diets suggested a relationship between *A. subulata* concentration and final LW, although only half the variation was explained by this variable (Table 3). Conversely, glycogen concentration was inversely related to *A. subulata* and soy protein isolate levels, although the analysis suggests the decrease was strongly related to increasing *A. subulata* ( $R^2 = 0.876$ ) (Figure 2). Meat protein was related to digestible energy although the relationship

TABLE 3. Partitioned ANOVA table and parameter estimates for the multivariable regression analyses of selected growth and tissue responses (as the dependent variables) and dietary ingredients or composition (as the independent variables) in queen conch fed five formulated diets containing *Agardhiella subulata*.

Dependent variable	Independent variable	F-value	P	Partial R <sup>2</sup>	Model R <sup>2</sup>
Final live wet weight	<i>Agardhiella</i>	12.56	0.004	0.512	0.512
	Soy protein isolate	3.95	0.072	0.129	0.641
Glycogen	<i>Agardhiella</i>	84.75	<0.001	0.876	0.876
	Soy protein isolate	4.89	0.049	0.038	0.914
Meat protein	Digestible energy	7.34	0.019	0.379	0.379
Glycogen	DP : DE <sup>a</sup>	53.23	<0.001	0.816	0.816

<sup>a</sup>Digestible protein : digestible energy ratio.

was weak. The analysis also suggested a relationship between glycogen depletion and an increasing DP:DE ratio ( $R^2 = 0.816$ ). No other relationships were detected.

## DISCUSSION

Although temperature, water quality, and density were within optimum limits previously reported for rearing queen conch (Spring and Davis 2005; Shawl and Davis 2006), overall growth was relatively slow. Previous studies with juvenile queen conchs fed macroalgal containing artificial diets report growth rates of approximately 80  $\mu\text{m}/\text{d}$  (Spring and Davis 2005; Shawl and Davis 2006). The reason for the slow growth observed during the present study is unclear. However, a genetic component cannot be overlooked as a previous study using conchs from the same stock resulted in similar growth rates (Acosta-Salmón et al. 2010).

Artificial diets containing algae have been used successfully to grow other gastropod species. Juvenile abalone *Haliotis asi-*

*nine* exhibited faster growth on an artificial diet mixed with the red macroalgae *Gracilariopsis heteroclada* (Capinpin and Corre 1996). Similarly, *H. midae* exhibited good growth when fed a commercial diet containing fish meal and *Spirulina* (Naidoo et al. 2006), and a diet containing gelatin mixed with fish chow proved effective for the apple snail *Pomacea patula* (Espinosa-Chávez and Martínex-Jerónimo 2005). Experiments with the Florida apple snail *P. paludosa* also indicate that high growth occurs with a mixed diet of catfish feed and the green macroalgae *Ulva* sp. (Garr et al. 2011).

The nutritional value of a mixed diet depends on nutrient composition, digestibility, and palatability. There were no differences detected in the apparent protein and energy digestibility values among the treatments. The values ranged from 51% to 87% and from 64% to 86% for protein and energy, respectively. To our knowledge, digestibility values for queen conch have not been reported before, but these values are similar or slightly higher than those reported elsewhere for abalone (Maguire et al. 1993; Coote et al. 2000).

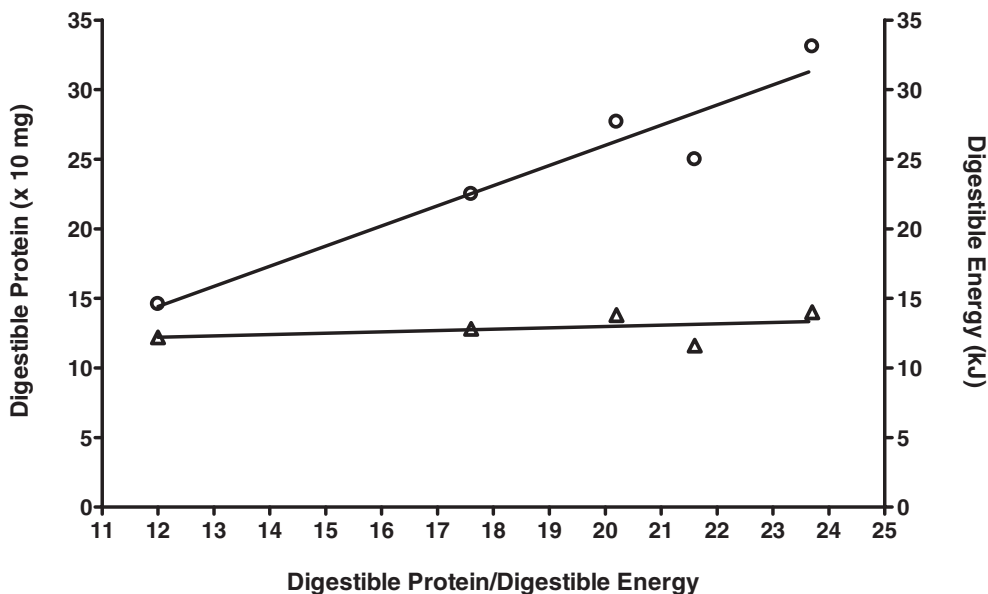


FIGURE 1. Digestible protein : digestible energy ratios of diets containing *Agardhiella* as a function of digestible protein (circles) and digestible energy (triangles).

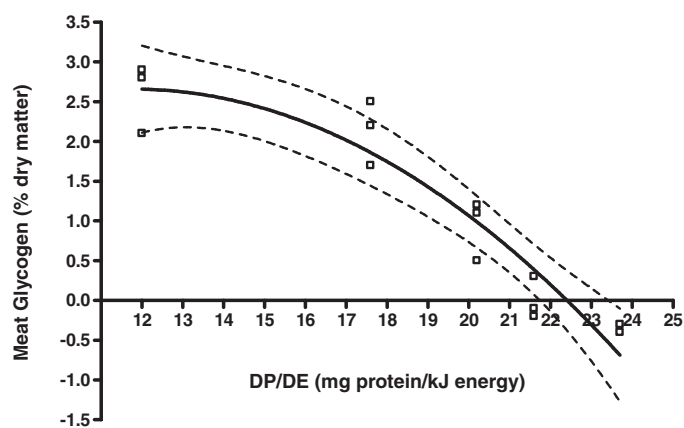


FIGURE 2. Tissue glycogen levels in queen conch fed experimental diets with increasing digestible protein : digestible energy (DP/DE) ratios for 160 d. The dotted lines represent the 95% confidence interval.

Dietary lipid ranged from 7% to 10%, which could be a contributing factor to the lower than anticipated growth rate. The lipid requirement of herbivorous molluscs is low (Mai et al. 1995a). *Haliotis tuberculata* and *H. discus hannai* fed 25% crude protein diets exhibited highest weight gain when dietary lipid was 3%, and weight gain was significantly suppressed when lipid content was greater than 7% (Mai et al. 1995a). An inverse effect on growth performance of *H. asinina* was observed as dietary lipid increased from 1.3% to 19.0% (Thongrod et al. 2003). Growth significantly declined at dietary lipid levels greater than 5.8% accompanied by an intake reduction of the high lipid diets (Thongrod et al. 2003). Britz and Hecht (1997) reported no difference in consumption among *H. midae* fed diets with 2, 6, or 10% lipid, but noted it was probably an artifact of high variability in their daily consumption values. However, Van Barneveld et al. (1998) demonstrated a linear decrease in digestibility of nitrogen, energy, and amino acids with increasing dietary oil and recommended that lipid levels in manufactured feeds for abalone not exceed 3% of the diet. It seems reasonable to speculate that queen conchs are similar to other herbivorous marine gastropods, and that it would be prudent to maintain dietary lipid levels less than 7% for this species until dietary requirements for essential fatty acids are established. The lipid level used on the present diets was based on the amount of lipid contained in the standard diet previously used in our hatchery.

Because of the relatively low lipid tolerance of marine gastropods, carbohydrates are thought to be the preferred source of energy. There were no differences detected in growth or survival of queen conchs fed diets 2 and 3. The two diets were similar except diet 2 had wheat flour substituted at 127.5 g/kg for the soy protein isolate and fish oil in diet 3. The substitution resulted in a reduction in dietary protein from 37% to 29% and the DP:DE ratio from 17.6 to 12.0 mg protein/kJ. Conversely, dietary carbohydrates were increased in the diet containing wheat flour by an

additional 104 g/kg of the dry diet suggesting queen conchs are similar to other herbivorous marine gastropods that efficiently utilize carbohydrates as an energy source.

Moreover, there were no differences detected in meat protein between conchs fed the 29% and 37% CP diets, although those consuming the diet containing wheat flour had significantly more tissue glycogen (Table 2). Queen conchs were able to meet their protein accretion and tissue storage needs on the higher carbohydrate, lower protein diet. Activity of digestive enzymes have not been evaluated in queen conch, but other marine gastropods exhibit high activity of proteinases, amylases, and other carbohydrases, and low activity of lipases (Mai et al. 1995a; Knauer et al. 1996). Therefore, it is likely that queen conchs are similar to other herbivorous gastropods that digest protein and carbohydrates well, but not lipid.

Lack of greater weight gain and protein accretion in conchs fed the 37% CP diets relative to the 29% CP diet would indicate that the additional dietary protein was catabolized. This suggests the protein requirement is not more than 29% of the dry diet, which provides sufficient DE. This is similar to the requirement reported for *H. laevigata* (27% CP) and *H. discus hannai* (25–30%), but lower than for *H. tuberculata* (30–40%) (Mai et al. 1995b; Bautista-Teruel and Millamena 1999; Coote et al. 2000). However, requirements are highly dependent on protein quality as well as digestibility, which can vary widely even among closely related species (Vandepeer et al. 2003).

Due to dilution ascribed to increasing ash, dietary energy decreased with increasing incorporation of *Agardhiella*. However, digestible energy remained constant. This was probably a function of both the increasing levels of soy protein isolate and *Agardhiella* at the expense of catfish feed. *Agardhiella* contains substantial levels of carrageenans and floridean starch (Chopin et al. 1990), both of which are readily digestible by other marine gastropods (Foster and Hodgson 1998; Vandepeer and Van Barneveld 2003). Although the digestibility of soy protein isolate has not been determined in queen conch, it has been reported as highly digestible in fish (Tibbetts et al. 2004; Glencross et al. 2005; Riche and Williams 2009). Conversely, no dietary ingredients met the level of significance to suggest a relationship to the DE component of the ratio.

The data suggest that a DP:DE ratio of 12 mg protein/kJ of energy in manufactured feeds for queen conchs will support growth and survival and maximize tissue energy storage (Figure 2). This is similar to the optimum range of 10.9–14.4 mg protein/kJ reported for *H. laevigata* (Coote et al. 2000). Reducing the level of dietary protein with a lower cost energy source like carbohydrates will reduce feed costs.

The complete depletion of tissue glycogen in conchs fed diets 5 and 6 is consistent with the signs of starvation (Carefoot et al. 1993). Abalone fed macroalgae exhibited tissue glycogen stores of 2.33%, similar to the values observed in queen conchs fed diets 2 and 3, but was five times lower in abalone fasted for 46 d (Carefoot et al. 1993). Multivariable regression with dietary

ingredients as independent variables suggested increasing levels of *A. subulata* ( $R^2 = 0.88$ ) were principally responsible for the observed decrease in meat glycogen (Table 3). However, both the increase in *A. subulata* and soy protein isolate were confounded in the experimental design as both were increased in graded increments. Application of nonlinear regression supports this confounding effect where glycogen depletion can be explained as a function of *A. subulata* ( $y = 2.3 \times 10^{-5} x^2 - 0.021x + 4.3$ ,  $R^2 = 0.92$ ) or soy protein isolate ( $y = 1.0 \times 10^{-3} x^2 + 0.005 x + 2.64$ ,  $R^2 = 0.89$ ) concentrations.

For queen conchs, a constant DE across the dietary treatments would logically imply a comparable intake of all the diets to meet their energy requirements, barring other circumstances. Feed consumption was not measured in the present study; therefore, a reduction in feed intake could not be verified. However, significantly lower survival, growth, and depleted glycogen stores are clear evidence of reduced intake, probably as a result of poor palatability.

Marine gastropods exhibit chemosensory preferences (Fleming 1995). Abalone were observed rejecting feed containing 20% gelatin (Coote et al. 2000). Additionally, consumption and growth were lower among *H. midae* fed sole protein diets containing soya oil cake relative to fish meal or casein, which was attributed to poor palatability (Britz 1996). Williams (2008) observed reductions in growth, poor survival, and depletion of tissue energy stores in Florida pompano *Trachinotus carolinus* in a dose-dependent manner when dietary soy protein isolate surpassed 10% of the dry diet. Further study using a cross-over design indicated pompano clearly rejected pellets containing a threshold level of soy protein isolate (T. Williams, personal communication). Significantly lower conch meat protein in the highest soy protein isolate treatment supports the hypothesis the animals were reluctant to eat the diet. The depressed meat protein in conch suggests tissue protein was being catabolized to provide gluconeogenic substrates as observed in *H. kamschatkana* (Carefoot et al. 1993).

Many aquatic and terrestrial plants contain polyphenolic compounds that impart an astringent taste. Sakata et al. (1991) reported that marine gastropods exhibit an aversion to polyphenolic compounds. Similarly, high levels of phenolic compounds led to growth reduction and deterred grazing in abalone (Winter and Estes 1992; Fleming 1995). Under proper conditions phenolic compounds can also bind methionine and lysine making them unavailable (Sosulski 1979), reduce total nitrogen availability (Tugwell and Branch 1992; Fleming 1995), and work in a dose-dependent manner (Winter and Estes 1992). Soy products and other legumes contain various phenolic compounds. This suggests queen conch, like other marine gastropods, may have an aversion to a threshold level of phenolic or some other compound associated with the soy protein isolate. This aversion to increasing levels of soy protein isolate is probably related to an unidentified chemical cue or amino acid imbalance as reported in other animals (Gietzen 1993).

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