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Effect of dietary protein and carbohydrate levels on weight gain and gonad production in the sea urchin *Lytechinus variegatus*

Laura E. Heflin [a,*], Victoria K. Gibbs [a], Mickie L. Powell [a], Robert Makowsky [a], John M. Lawrence [b], Addison L. Lawrence [c], Stephen A. Watts [a]

[a] *University of Alabama at Birmingham, Department of Biology, 1300 University Blvd., Birmingham, AL 35294, United States*
[b] *University of South Florida, Department of Biology, 4202 East Fowler Avenue, Tampa, FL 33620, United States*
[c] *Texas A&M University, Texas AgriLife Research Mariculture Laboratory, 1300 Port Street, Port Aransas, TX 78373, United States*

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ABSTRACT

Adult *Lytechinus variegatus* were fed eight formulated diets with different protein (ranging from 12 to 36%) and carbohydrate (ranging from 21 to 39%) levels. Each sea urchin (n = 8 per treatment) was fed a daily sub-satiation ration of 1.5% of average body weight for 9 weeks. Akaike information criterion analysis was used to compare six different hypothesized dietary composition models across eight growth measurements. Dietary protein level and protein: energy ratio were the best models for prediction of total weight gain. Diets with the highest (≥68.6 mg P kcal^{-1}) protein:energy ratios produced the most wet weight gain after 9 weeks. Dietary carbohydrate level was a poor predictor for most growth parameters examined in this study. However, the model containing a protein × carbohydrate interaction effect was the best model for protein efficiency ratio (PER). PER decreased with increasing dietary protein level, more so at higher carbohydrate levels. Food conversion ratio (FCR) was best modeled by total dietary energy levels: Higher energy diets produced lower FCRs. Dietary protein level was the best model of gonad wet weight gain. These data suggest that variations in dietary nutrients and energy differentially affect organismal growth and growth of body components.

1. Introduction

Protein is one of the most necessary and costly nutrients of most aquatic animal diets. Adequate provision of dietary protein decreases feed intake (Agatsuma, 2000; Daggett et al., 2005; Fernandez and Boudouresque, 1998; Fernandez and Boudouresque, 2000; Frantzis and Grémare, 1992; Hammer et al., 2004; Hammer et al., 2006a, 2006b; McBride et al., 1998; Meidel and Scheibling, 1999) and increases growth (Agatsuma, 2000; Akiyama et al., 2001; Cook et al., 1998; Fernandez, 1997; Fernandez and Boudouresque, 1998; Fernandez and Pergent, 1998; Hammer et al., 2004; Hammer et al., 2006a; Meidel and Scheibling, 1999; Taylor, 2006) and roe production (de Jong-Westman et al., 1995a; Fernandez, 1997; Barker et al., 1998; Cook et al., 1998; Meidel and Scheibling, 1999; Pearce et al., 2002b; Hammer et al., 2004; Chang et al., 2005; Schlosser et al., 2005; Hammer et al., 2006a; Marsh and Watts, 2007; Woods et al., 2008) in a number of sea urchin species. However, several studies have hypothesized that there is a level of protein level at which growth is maximized (Hammer et al., 2006a; Kennedy et al., 2005; Marsh and Watts, 2007; McBride et al., 1998; Senaratna et al., 2005).

Despite its value as a nitrogen source, metabolism of protein as an energy source is energetically inefficient (Marsh and Watts, 2007) and nitrogenous waste is a water pollutant (Basuyaux and Mathieu, 1999). Furthermore, high protein levels (Pearce et al., 2002b; Woods et al., 2008) and possibly protein sources or the presence of specific amino acids (Hirano et al., 1978; Hoshikawa et al., 1998; Komata et al., 1962; Murata et al., 2001, 2002; Osako et al., 2007; Pearce et al., 2002a; Robinson et al., 2002; Woods et al., 2008) have been suggested to have an adverse effect on the quality of sea urchin roe. Therefore, a formulated diet should provide individuals with adequate protein for maximal growth and production, but excess protein should be avoided. Exact dietary protein requirements (amino acid requirements) for sea urchins have not been established but, as with other animals, requirements may vary among species and age classes.

In addition to a source of amino nitrogen, urchins require energy for production. Soluble carbohydrates are easily digested by sea urchins, and numerous carbohydrases have been identified in the sea urchin gut (Lawrence et al., 2007), indicating that sea urchins can most likely utilize carbohydrates from a wide array of sources. Carbohydrates are also a much more efficient energy source than protein (Marsh and Watts, 2007).

Recent studies indicate that sea urchins may adjust feed intake to satisfy energy requirements regardless of other nutrient levels (Hammer, 2006; Lawrence et al., 2009; Otero-Villanueva et al.,

* Corresponding author at: University of Alabama at Birmingham, 1300 University Blvd., CH 374, Birmingham, AL 35294, United States. Tel.: +1 205 934 8313; fax: +1 205 975 6097.
E-mail address: leheflin@uab.edu (L.E. Heflin).

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2004; Taylor, 2006). In some cases, decreased protein intake resulting from energy satiation led to decreased somatic growth and organ production in adult sea urchins (Fernandez and Pergent, 1998; Hammer, 2006) and decreased growth in juvenile sea urchins (Taylor, 2006). Consequently, feed intake must be measured to accurately determine dietary requirements for these nutrients. Other studies reported compensation for an imbalance in calorie:protein ratio by selective nutrient absorption (reviewed in Lawrence and Lane, 1982). In cases where dietary carbohydrate levels are limited, sea urchins may use dietary protein as an additional energy source, thus, decreasing growth and production (Hammer et al., 2006a; Schlosser et al., 2005).

Few studies have examined the relationship between dietary protein and dietary energy requirements in sea urchins. Understanding this relationship may be an important step in the formulation of a feed suitable for sustainable sea urchin aquaculture. The purpose of this study is to examine the effect of combinatorial variations in dietary protein and carbohydrate level, presented in a defined daily ration, on organismal growth and roe production in the sea urchin *Lytechinus variegatus*.

2. Materials and methods

2.1. Collection and initial measurements

Adult *L. variegatus* (ca. 19.5 ± 2.01 g wet weight) were collected from St. Joseph Bay (30°N, 85.5°W), FL and transported to Texas AgriLIFE Mariculture Research Laboratory in Port Aransas, Texas. Nineteen individuals were randomly selected for initial evaluation. Individuals were weighed (to the nearest mg) and dissected by a circular incision around the peristomial membrane. The gut (esophagus, stomach, and intestine combined), and gonads were removed. The gut was cleaned in seawater to remove remaining food pellets. The organs were blotted on a clean paper towel to remove excess water and weighed. Organs were dried at 60 °C for 48 h to constant weight, and dry weights were recorded. Mean dry organ and total dry weights (the sum of the organ dry weights) were calculated for the initial sub-sample and used as estimated initial dry organ and total dry weights for the remaining 64 urchins. The remaining urchins, were weighed and assigned randomly to one of eight dietary treatments (n = 8 per diet). Initial wet weights did not vary significantly among dietary treatments ($P < 0.05$).

2.2. Culture conditions

Sea urchins were held in a semi-recirculating system with both mechanical and biological filtrations and UV sterilization. The culture system (2400 L) was composed of 16 interconnected 20 L fiberglass tanks containing water distributed from a central sump. Each tank held four cylindrical plastic mesh cages (12 cm dia., 30 cm height, constructed of 4 mm open mesh). Each plastic cage was inserted into a PVC coupling (11.5 cm I.D.) and elevated with PVC spacers to allow unimpeded water circulation throughout the cage. Each cage housed one individual. A 12:12 light:dark photoperiod was maintained.

Water volume in each tank was maintained by a central standpipe, and natural seawater was supplied to each mesh enclosure at a ca. rate of 25 L h^{-1} (water exchange rate of 3000% per day). Fresh seawater was passed through a sand filter and a stratified Diamond water filter (5 µ, Diamond Water Conditioning, Horton, WI). Water in the entire culture system was exchanged in the system at a rate of 10% per day. Water quality parameters were determined by color metric analysis.

2.3. Feed and feed preparation

Eight semi-purified diets were formulated and produced using both purified and practical ingredients. Levels of dietary protein and carbohydrate (Tables 1 and 2) ranged from 12 to 36% protein (using purified plant and animal protein sources) and 21 to 39% carbohydrate (using a purified starch source). Total levels of protein and carbohydrate were adjusted with acid washed diatomaceous earth which has no effect on sea urchins at the levels used (unpublished data). All other nutrients were constant among treatments. The proximate components are shown in Table 2. Dry ingredients were mixed with a PK twin shell® blender (Patterson-Kelley Co., East Stroudsburg, PA) for 10 min. Dry ingredients were then transferred to a Hobart stand mixer (Model A-200, Hobart Corporation, Troy, OH) and blended for 40 min. Liquid ingredients were added, and the mixture was blended for an additional 10 min to a mash-like consistency. The diets were extruded using a meat chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 4.8 mm die. Feed strands were separated and dried on wire trays in a forced air oven (35 °C) for 48 h. Final moisture content of all feed treatments was 8–10%. Feed was stored in air-tight storage bags at 4 °C until used.

2.4. Feeding rate

Each sea urchin was proffered a limited daily ration equal to 1.5% (sub-satiation) of the initial average wet body weight. Feeding at sub-satiation ensured that urchins consumed all their food in a 24 hour period and allowed for direct measure of feed intake. A sub-satiation feeding regime also prevented individuals from compensating for a dietary deficiency by increasing consumption. Individuals were weighed every three weeks and feed rations were adjusted to be equivalent to 1.5% of the average body weight (Table 3). Feed intake of the presented diet was confirmed by direct observation. Feces were removed by siphoning immediately prior to feeding each day.

Daily feeding rate was calculated as:

$$(1)\ \text{Average wet weight of individuals (g)} \times 0.015.$$

Protein:energy ratio of each feed was calculated as:

$$(2)\ \text{Protein (mg)/energy content (kcal)}.$$

Total energy content of each feed (per g) was calculated based on the methods of Phillips (1972):

$$(3)\ \%\text{protein}/100 \times 5650(\text{cal g}^{-1}) + \%\text{carbohydrate}/100 \times 4000\left(\text{cal g}^{-1}\right) + \%\text{lipid}/100 \times 9450\left(\text{cal g}^{-1}\right).$$

2.5. Weight gain and production

Individuals were weighed every three weeks. Wet weight gain over the 9-week period was calculated as:

$$(4)\ \text{Final wet weight (g)} - \text{initial wet weight (g)}$$

Table 1
Calculated protein and carbohydrate levels (as fed), total energy, protein: energy, and protein: carbohydrate ratios in each of the eight diets tested.

Protein (%)	Carbohydrate (%)	Total energy (cal g^{-1})	Protein:energy ratio (mg P $kcal^{-1}$)	Protein:carbohydrate caloric ratio
36	21	3749	95	1.7
28	30	3299	76	0.93
19	21	2783	68	0.90
19	30	3130	60	0.63
19	39	3478	54	0.49
12	21	2380	50	0.57
12	30	2727	44	0.40
12	39	3075	39	0.31