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Effects of dietary protein to energy ratios on growth performance of yellowfoot limpet (*Cellana sandwicensis* Pease, 1861)

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ABSTRACT

The aquaculture of yellowfoot limpets (*Cellana sandwicensis)* is a prospect industry in research and development. The effects of dietary protein to energy (PE) ratio on growth performance were evaluated for 180 days in a flow-through system. Replicate animals (5.9 ± 1.72 g and 33.9 ± 2.13 mm) were stocked randomly on individual plates, and four paste diets containing PE ratios ranging from 87.2 to 102.9 mg/kcal were offered once daily (1600 h). A significant increase in daily feed intake ($P < 0.05$) was observed to coincide with seasonal decrease in air temperature. Although dietary treatment had no significant effect on overall growth performance ($P > 0.05$), average daily gain (ADG) and feed conversion efficiency (FCE) improved both linearly and quadratically (ADG $P = 0.03$, $P = 0.08$; FCE $P = 0.05$, $P = 0.04$, respectively). These results indicate potential seasonal growth patterns, which are controlled by environmental cues (i.e. temperature, feed availability, etc.) and must be considered in future trials. Limpets offered higher PE ratio diets did not compensate for lower energy levels with increased feed intake, and specific growth rate increased up to 0.20% BW/d as the dietary PE ratio decreased. A PE ratio of 87.2 mg/kcal produced the best tissue growth and can be recommended as a suitable formulated diet for limpet production.

1. Introduction

A group of mollusks known as limpets (order Patellogastropoda) are important seafood derived from the rocky intertidal environment (Erlandso et al., 2011; McCoy 2008). These mollusks are usually wild harvested for food consumption; however, continual exploitation has pushed some populations to the brink of extinction (Espinosa et al., 2009). Furthermore, declines in wild stocks have pushed governments to intensify management efforts as well as to consider the development of limpet aquaculture (Mau and Jha, 2017). For instance, in South Africa, the government designated multiple "Marine Protected Areas" to preserve the overharvested South African limpet (*Cymbula oculus*) (Branch and Odendaal 2003). And in Portugal (1993–1998), the Regional Government of the Azores implemented a law to ban the wild harvest of two species of limpets, *Patella aspera* and *P. candei* (Ferraz et al., 2001).

In Hawaii, the native group of limpets, referred to as opihi (*Cellana* spp.), are consumed as a staple food during traditional gatherings. Despite management efforts and law prohibiting harvest of Hawaiian limpets less than 31 mm in shell length (SL), there has been a drastic reduction in market availability. Total annual catch landings of Hawaiian limpets decreased from about 68,000 kg to 5000 kg since the early 20th century (Kay and Magruder, 1977; Bird 2006); and population densities have decreased by 99.9% for the island of Oahu since western contact (Personal Communication; CE Bird, 2017). To prevent complete decimation and overcome market deficiencies, optimizing a grow-out diet is required to support aquaculture production of these socioeconomically important limpet.

For yellowfoot limpet (*Cellana sandwicensis*), the first formulated diet was developed following formula and dietary guidelines used for abalone feeds in a study by Cho (2010). Later, Hua and Ako (2016) found optimal protein and carbohydrate requirement for adult yellowfoot limpet to be 35% and 32%, respectively. Based on this study, yellowfoot limpet appears similar to abalone (*Haliotis*) with respect to feeding behaviors, metabolism and nutrition. However, the gross energy levels were not measured and requirements are still unknown.

In other studies, both the South African abalone (*Haliotis midae)* and green abalone (*H. fulgens*) were shown to consume feed based on their energy requirement with respect to dietary protein to energy (PE) ratio, which ranged from 43 to76 mg/kcal (Green et al., 2011) and from 62 to 108 mg/kcal (Gómez-Montes et al., 2003), respectively. Although direct comparisons cannot be made between different aquaculture groups, these results were similar to that of shrimp (*Penaeus monodon*) where an increase in energy with respect to a constant protein improved growth

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performance (Bautista, 1986). These findings led to the hypothesis that feeding and growth performance of yellowfoot limpet would be affected by the dietary PE ratio. To best of our knowledge, there is no such information available for yellowfoot limpet. Therefore, the objectives of this study were to develop, fabricate and implement a novel grow-out system, and to evaluate effect of varying PE ratio diets on growth performance of yellowfoot limpet.

2. Materials and methods

2.1. Animal collection

Altogether 90 wild adult yellowfoot limpets were collected from a non-disclosed intertidal location in Puna, Hawaii. The smallest, legal sized animals (approximately 31 mm SL) were selected and carefully removed using metal putty knifes. Prior to stocking, a standard 45 L cooler was filled with natural seawater, chilled to 15 °C, and supplied with aeration. Upon removal from the rocks, animals were allowed to adhere themselves to black acrylic plates, which were designed to stand vertically in the cooler. Limpets were transported within 48 h without feeding to the research facility. Upon arrival, animals were transferred into circular tubs supplied with overhead irrigation spray for quarantine and acclimation at ambient, outdoor conditions.

The conditioning of animals to a formulated feed (Hua and Ako, 2016) were initiated weeks prior to the start of the trial. Limpets were fed daily to satiation and showed no signs of acceptability or palatability issues; and animals consumed feed effectively without knocking feed off surfaces.

2.2. Diet preparation and analysis

Four experimental diets were formulated (Table 1) to make different PE ratio. To test the effect of PE ratio of diets (Diet1, 87; Diet2; 95; Diet3 97; Diet4, 103) on growth performance of limpet, crude protein was kept constant (40%) and gross energy level was graded (3.85–4.63 kcal/g). Krill meal and *Porphyra* were found to be necessary attractants in the feed and were kept constant. Alginate was used as a binder at 5% in all diets. Diatomaceous earth was used as a filler as whole wheat volume was reduced. Assuming limpets are inefficient users of fat, crude fat was kept constant at 6% for all diets. A vitamin premix (MP Biomedical LLC, Solon, OH) used for previous abalone diets were included at 1% in all diets. All diets were analyzed for their proximate nutrients, amino acid and fatty acid profile (Tables 1–3, respectively).

The four diets were moist feeds that were adhered to vertical surfaces by pressing feed to the substrate surface. To make the diets, dry starch ingredients (whole wheat and alginate) were homogenized in a food mixer for 10 min. Water was boiled and added in a 1:1 ratio (1 mL water: 1 g dry ingredient) along with oils to the mixture. Starches were homogenized for an additional 10 min. The rest of the dry ingredients were homogenized and added to the gelatinous mixture and further homogenized until reaching a dough-like consistency. To dry, the dough was rolled out into 1 cm thick sheets and air dried at room-temperature until cooled (approximately 30 min) and feeds were placed in the freezer until use.

Feed samples were analyzed for proximate composition using methods of AOAC (2006). Moisture content was determined from a 2 g sample using an air-circulated oven at 135 °C for 2 h (method 930.15) followed by ashing in a muffle furnace at 600 °C for 6 h (method 942.05). Crude protein was estimated by determining total nitrogen (N) by dry combustion using a LECO analyzer (LECO CN-2000; Leco Corp., St. Joseph, MI; method 976.05, CP = N × 6.25). Crude fat (lipid) was determined by ethyl-ether extraction (method 920.39) using an Accelerated Solvent Extractor (Dionex Corporation, Bannockburn, IL). Gross energy (GE) was determined using an oxygen bomb calorimeter (Parr Isoperibol Bomb Calorimeter 6200, Parr Instrument Co., Moline, IL) with benzoic acid as the calibration standard. Minerals were analyzed by inductively coupled plasma atomic emission spectroscopy (Thermo Jarrel Ash Corporation, Franlin, MA). Amino acid (AA) contents of diets were determined using a High Performance Liquid Chromatography system (Agilent 1200 HPLC equipped with an Agilent1200 Series diode detector, Santa Clara, CA) following procedures

Table 1
Ingredient composition and analyzed proximate nutrient profile of experimental diets.

Ingredients	Diet (g/100 g diet)			
	1	2	3	4
Wheat flour[a]	28.50	21.55	14.50	7.55
Fish meal[b]	21.00	22.25	23.50	24.75
Soybean meal – defatted[c]	16.60	16.60	16.60	16.60
Porphyra[d]	14.00	14.00	14.00	14.00
Krill meal[e]	11.00	11.00	11.00	11.00
Alginate[f]	5.00	5.00	5.00	5.00
Diatomaceous earth[g]	1.80	7.65	13.60	19.40
Vitamin mix[h]	1.00	1.00	1.00	1.00
Menhaden fish oil[i]	0.45	0.45	0.45	0.45
Corn oil[j]	0.45	0.30	0.15	0.05
Cholesterol[k]	0.20	0.20	0.20	0.20
Analyzed composition (% dry matter basis)				
Dry matter	48.8	48.9	47.1	45.3
Crude protein	40.4	40.9	39.8	39.6
Crude fat	6.1	5.7	5.8	6.1
Ash	11.2	17.1	23.0	28.2
Gross energy (kcal/g)	4.63	4.31	4.10	3.85
Protein to energy ratio (mg/kcal)	87.2	94.9	96.9	102.9

[a] Hawaiian Flour Mill, Honolulu, HI.
[b] RMI Fishmeal, Republic of the Marshall Islands.
[c] Land-o-Lakes, Seattle, WA.
[d] Porphyra yezoensis (powder), Global Maxlink LLC, Antelope, CA.
[e] Florida Aqua Farms Inc., Dade City, FL.
[f] Sigma-Aldrich, Louis, MO.
[g] Hawaiian Hydroponics, Honolulu, HI.
[h] MP Biomedical LLC, Solon, OH. nicotinic acid (3.00 g/kg), D-calcium pantothenate (1.60 g/kg), pyridoxine HCl (0.70 g/kg), thiamine HCl (0.60 g/kg), riboflavin (0.60 g/kg), folic acid (0.20 g/kg), D-biotin (0.02 g/kg), vitamin B12 (0.1% triturated in mannitol) (2.50 g/kg), a-tocopherol powder (250 U/gm) (30.00 g/kg), vitamin A palmitate (250,000 U/gm) (1.60 g/kg), vitamin D3 (400,000 U/gm) (0.25 g/kg), phylloquinone (0.075 g/kg), and powdered Sucrose (959.655 g/kg).
[i] Virginia Prime Gold Menhaden Fishoil, Omega Protein Corporation, Houston, TX.
[j] Local supermarket, Honolulu, HI.
[k] Zeigler Brothers Inc., Gardners, PA.

Table 2
Amino acid composition of the experimental diets and pooled soft body tissue (SB) (% dry matter).

Amino Acid	Diet				SB
	1	2	3	4	
Alanine	7.40	7.84	7.83	7.84	5.70
Asparagine + Aspartate	10.10	10.05	10.32	10.06	15.34
Cystine	3.53	1.79	2.08	3.42	3.25
Glutamate + Glutamine	12.16	12.40	11.91	11.17	12.08
Glycine	6.62	6.95	6.86	7.55	8.72
Proline	5.45	5.36	5.14	5.16	4.18
Serine	4.37	4.52	4.49	4.38	3.96
Tyrosine	3.48	3.55	3.53	3.44	2.09
Taurine	1.05	1.06	1.07	1.04	3.74
Arginine	7.03	7.47	7.39	7.35	8.80
Histidine	2.71	2.73	2.85	2.74	1.30
Isoleucine	4.41	4.43	4.41	4.28	4.05
Leucine	7.26	7.32	7.33	7.10	7.22
Lysine	7.10	7.10	7.34	7.30	8.43
Methionine	3.07	2.99	3.14	3.17	3.29
Phenylalanine	4.69	4.41	4.41	4.34	3.94
Threonine	5.01	5.36	5.30	5.20	3.63
Valine	5.62	5.73	5.69	5.51	4.02