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GROWTH AND NUTRIENT UTILIZATION OF GREENLIP ABALONE (*HALIOTIS LAEVIGATA*) FED *ULVA* SP. PROTEIN EXTRACT

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ABSTRACT Greenlip abalone (*Haliotis laevigata*) are commercially farmed in land-based systems in southern Australia and are fed formulated diets that typically do not contain macroalgae. In a 90-day study, the growth and nutrient utilization of juvenile greenlip abalone (1.82 g, 23.23 mm) fed diets containing *Ulva* sp. protein extract (UPE) were investigated. Animals were fed one of the four formulated test diets containing graded levels of UPE (0%, 5%, 10%, and 20%) or a commercial diet that served as a control for the 0% basal diet. Diets were formulated to contain 37% crude protein, 5% lipid, and 17.5 MJ/kg gross energy. The specific growth rate and shell growth rate (μm/day) of abalone fed the four experimental diets were similar. Dietary inclusions of UPE supported the growth of juvenile greenlip abalone and may be used to reduce dietary inclusions of solvent extracted soybean meal, dehulled lupins, and wheat flour. Currently, UPE is cost prohibitive for commercial use in greenlip abalone diet. As UPE is a by-product of the macroalgae industry, the cost of UPE would likely become more economically viable as macroalgae production increases in the future to include in diets for greenlip abalone. Once economically viable, we recommend a dietary inclusion of up to 20% UPE meal for greenlip abalone.

KEY WORDS: *Haliotis laevigata*, *Ulva* sp. protein extract, growth, nutrient utilization, nutrition

INTRODUCTION

Greenlip abalone (*Haliotis laevigata*) are native to Australia's southern waters. In the wild, they preferentially consume Rhodophyta (red) macroalgae species (Shepherd 1973). Chlorophyte (green) macroalgae species are consumed by greenlip abalone, but are typically a secondary preference to red species (Shepherd 1973). Under culture conditions, greenlip abalone are fed diets that typically contain a combination of terrestrial plant ingredients, such as wheat flour, soybean meal, and dehulled lupin meal and also fish meal and fish oil, binders, and vitamin and mineral premixes. Feed costs contribute to 30% of production costs (Stone et al. 2014a). In a bid to reduce production expenses, feed is formulated in the most cost-effective manner. In general, Australian commercial abalone feed manufactures typically do not include macroalgae meal in commercial diets because of the current high cost of this ingredient and biosecurity concerns associated with the use of imported products.

Macroalgae have recently become a widely researched dietary component for the compounded commercial diets for greenlip abalone because it has been reported to significantly improve growth, health, and feeding stimulation (Bansemer et al. 2014, Lange et al. 2014, Stone et al. 2014b, Bansemer et al. 2016a, 2016b). For example, feeding macroalgae aid in reducing mortality (summer mortality) when animals are challenged with high summer water temperatures (>23°C; Lange et al. 2014, Stone et al. 2014b). Furthermore, when macroalgae are fed to abalone in live and dried forms, abalone elicit a daylight feeding response (Bansemer et al. 2015b, Buss et al. 2015, Currie et al. 2016). This is beneficial to the industry because abalone are naturally nocturnal foragers. By inducing this daylight response, abalone will begin consuming feed during light hours, potentially before essential water soluble nutrients are leached.

Research that has investigated live and dried macroalgae for greenlip abalone has been predominantly conducted with *Gracilaria cliftonii* and *Ulva* sp. (Bansemer et al. 2016a, 2016b). Recent studies have investigated feeding live nutrient-enriched macroalgae to abalone (Naidoo et al. 2006, Viera et al. 2011, Bansemer et al. 2016a, 2016b). Protein and amino acid enrichment of macroalgae involves growing it in a nitrogen-enriched medium that increases protein and amino acid levels and may enhance abalone growth (Viera et al. 2011, Bansemer et al. 2016a). Some abalone species exhibit suboptimal growth when fed live macroalgae compared with formulated diets (Bansemer et al. 2016a). Live macroalgae may be dried and milled into a dried macroalgae meal. When investigating dried macroalgae meal inclusions in diets for greenlip abalone, the greatest growth rates in greenlip abalone have been achieved with dried and enriched *G. cliftonii* meal at 10% and 20% inclusion levels compared with a 0% basal diet (Bansemer et al. 2016a). Dried and enriched *Ulva* sp. meal, produced by Venus Shell Systems (Narrawallee, Australia), also improved greenlip abalone growth rates at a 5% inclusion level compared with a 0% basal diet (Bansemer et al. 2016a). The authors hypothesized that the superior growth observed in the greenlip abalone fed the 5% *Ulva* sp. diet was because of the upregulation of trypsin activity (Bansemer et al. 2016a).

The Australian macroalgae industry is positioned to expand in the near future. Venus Shell Systems has recently developed a novel potential ingredient for abalone feed, referred to as *Ulva* sp. protein extract (UPE). This meal is the product after the carbohydrate proportion of *Ulva* sp. has been extracted, which

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has a higher protein content (~42% crude protein) than *Ulva* sp. meal (~34% crude protein). Given the past success of utilizing *Ulva* sp. meal in diets for greenlip abalone, UPE may also be beneficial to improve growth and feed utilization. Given the differences in production and nutritional composition, further research is required. Therefore, the aim of this study was to investigate the effect of graded dietary inclusion levels of dried UPE (5%, 10%, and 20% inclusion levels) on growth performance and nutrient utilization for greenlip abalone.

MATERIALS AND METHODS

Test Ingredient, Diet Formulation and Manufacture

Dried UPE meal was supplied by Venus Shell Systems. The nutritional composition of the dried UPE meal and experimental diets are provided in Table 1. The UPE meal was evaluated at graded inclusion levels in a series of four formulated test diets. A basal diet (0% control diet) and three diets were formulated to contain 5%, 10%, and 20% inclusion levels of UPE meal. Inclusion of the UPE meal was achieved by reducing solvent extracted soybean meal, wheat flour, and dehulled lupin levels. The test diets were formulated based on reported nutritional requirements of greenlip abalone to contain approximately 35% crude protein, 5% crude lipid, and 17.5 MJ/kg gross energy (Stone et al. 2013, Bansemer et al. 2015a). Dietary essential amino acid levels were formulated to meet the "requirements" of greenlip abalone using the ideal amino acid ratio method and soft tissue compositions reported by Coote et al. (2000). A fifth diet, the commercial Abgrow premium diet [Eyre Peninsula Aquafeeds (EPA), Lonsdale, Australia], was included as a comparison with the 0% basal diet.

Before diet formulation and manufacture, the proximate composition of dietary ingredients was analyzed. The experimental diets were prepared by weighing and combining ingredients in a KitchenAid KPM5 bowl lift mixer (KitchenAid Pty Ltd, New South Wales, Australia) for 5 min. Fish oil, sodium alginate, calcium sulphate, and warm water (45°C; ~30% of total feed weight) were then added to the dry ingredient and mixed for a further 5 min. The pellets were manufactured using a TR110 pasta machine (Machine Per Pasta SRL, Molina Di Malo, Italy) and then dried at 50°C for 48 h. This produced flat, sinking diet chips (5 × 5 × 2 mm). Diets were stored at –20°C until used. Analyzed proximate compositions, amino acid, fatty acid, and mineral composition of the diets are displayed in Table 2.

Experimental Animals and Sytem

Juvenile greenlip abalone (6 mo old) were purchased from South Australian Mariculture (Port Lincoln, Australia) and transferred to the South Australian Research and Development Institute, South Australian Aquatic Sciences Center (West Beach, Australia). Before stocking the growth trial, abalone were housed in 180 L holding tanks and provided with flow through seawater and fed a 5-mm commercial diet Abgrow premium chip *ad libitum*.

The laboratory in which the growth trial was conducted was fitted with a temperature controlled system described previously by Stone et al. (2013). The system consisted of sand-filtered, ultraviolet-treated seawater supplied to twenty 12.5 L blue plastic rectangular culture tanks (Nally IH305; Viscount Plastics Pty Ltd.). Water was supplied to the system at a rate of 300 mL/min. A standpipe was fitted to each tank maintaining a water level of 3 cm. A mesh (2 mm) was also fixed to the standpipe to retain feed. Water temperature was maintained at 22 ± 1°C using a 3-kW immersion heater (240V, A3122-1; Hotco, Williamstown, Australia).

Stocking and Feeding

Abalone were gently prised from the tank substrate using a spatula. Animals were randomly selected, weighed (1.82 ± 0.01 g), and the shell length (23.23 ± 0.08 mm) was measured. Fifteen abalone were then assigned to each of four replicate culture tanks per dietary treatment ($n = 300$ animals total). At stocking, the initial water temperature was 18°C. A 2-wk water temperature acclimation period was used. In the first week of acclimation, water temperature was maintained at 18°C. During the second week, water temperature was increased

TABLE 1.

Dietary composition (g/100 g diet as fed) of experimental diets.

Item	UPE meal inclusion level (%)			
	0	5	10	20
UPE meal	0.00	5.00	10.00	20.00
Salmon fish meal (65% protein)	6.00	6.00	6.00	6.00
Soy protein concentrate	8.00	8.00	8.00	8.00
Solvent extracted soybean meal (48% protein)	35.75	34.22	31.30	24.90
Wheat flour	23.05	23.43	23.46	23.36
Fish oil	1.00	1.00	1.00	1.00
EPA vitamin/mineral premix	0.20	0.20	0.20	0.20
Sodium alginate	1.20	1.20	1.20	1.20
Vitamin E (adsorbate)	0.01	0.01	0.01	0.01
Dehulled lupins (*Lupinus angustifolius*, Gungaru)	23.96	20.11	18.00	14.50
Calcium sulphate ($CaSO_4$)	0.22	0.22	0.22	0.22
Monosodium phosphate	0.61	0.61	0.61	0.61
Total (g)	100.00	100.00	100.00	100.00

TABLE 2.

Nutritional composition of the dried protein enriched *Ulva* sp. meal (PEU), dried UPE meal ingredient, and the EPA and the UPE meal diets.

Item	PEU* meal	UPE meal	EPA diet	UPE meal diets (%)			
				0	5	10	20
Proximate composition (g/100 g diet as fed)							
Moisture	10.60	4.60	10.70	12.20	11.20	11.20	11.80
Crude protein	33.60	42.10	29.25	37.31	36.38	36.50	36.19
Lipid	4.70	5.30	4.60	5.30	5.20	5.30	5.50
Gross energy (MJ/kg)	15.67	17.40	17.24	17.71	17.75	17.76	17.61
Ash	16.90	16.90	5.90	5.60	5.90	6.00	6.40
Carbohydrate†	34.20	31.10	49.55	39.59	41.32	41.00	40.11
Amino acids (g/100 g diet as fed)							
Alanine	2.55	3.29	1.24	1.49	1.58	1.68	1.85
Aspartic acid	4.19	5.06	2.77	3.66	3.65	3.59	3.65
Arginine	1.73	2.19	1.79	2.60	2.50	2.41	2.35
Glutamic acid	3.74	5.23	5.56	6.71	6.35	6.18	6.10
Glycine	1.68	2.11	1.30	1.62	1.64	1.73	1.72
Histidine	0.22	0.54	0.48	0.71	0.74	0.63	0.58
Isoleucine	1.11	1.81	1.18	1.49	1.43	1.42	1.42
Leucine	1.99	3.33	2.09	2.62	2.58	2.58	2.61
Lysine	1.50	1.28	1.63	1.84	1.83	1.90	1.72
Methionine	0.46	0.74	0.41	0.38	0.42	0.44	0.49
Phenylalanine	1.56	2.57	1.47	1.83	1.84	1.86	1.93
Proline	1.38	1.63	1.83	1.76	1.76	1.87	1.73
Serine	1.57	1.97	1.25	1.65	1.68	1.74	1.76
Threonine	1.43	2.08	1.04	1.32	1.34	1.37	1.46
Tyrosine	0.75	1.35	0.96	1.23	1.19	1.17	1.19
Valine	2.03	2.55	1.36	1.67	1.61	1.64	1.72
Fatty acids (mg/100 g diet as fed)							
14:0	95.0	30.00	61.00	39.00	41.00	45.00	44.00
16:0	960.0	1,210.00	760.00	840.00	900.00	980.00	1,060.00
18:0	480.0	530.0	190.00	300.00	280.00	280.00	250.00
10:1	56.0	<10.0	<10.00	<10.00	<10.00	<10.00	11.00
14:1	10.0	<10.0	<10.00	<10.00	<10.00	<10.00	<10.00
15:1	70.0	43.0	<10.00	<10.00	<10.00	<10.00	17.00
16:1	58.0	72.0	110.00	74.00	76.00	82.00	89.00
17:1	23.0	<10.0	<10.00	<10.00	<10.00	<10.00	<10.00
18:1*n* – 7	280.0	410.0	99.00	88.00	125.00	160.00	250.00
18:1*n* – 9	24.0	41.0	1,340.00	1,520.00	1,380.00	1,340.00	1,220.00
18:2*n* – 6	210.0	280.0	1,310.00	1,810.00	1,660.00	1,610.00	1,530.00
20:4*n* – 6	29.0	22.0	19.00	12.00	13.00	15.00	22.00
18:3*n* – 3	680.0	870.0	180.00	210.00	250.00	300.00	420.00
18:4*n* – 3	550.0	<10.0	16.00	<10.00	<10.00	47.00	95.00
20:4*n* – 3	39.0	<10.0	15.00	<10.00	<10.00	<10.00	13.00
20:5*n* – 3	85.0	54.0	72.00	33.00	35.00	39.00	48.00
22:5*n* – 3	79.0	110.0	26.00	13.00	21.00	29.00	49.00
22:6*n* – 3	<10.0	<10.0	<10.00	<10.00	100.00	110.00	110.00
Minerals (mg/kg as fed)							
Calcium	17,000	4,200	5,400	5,800	5,900	6,400	5,400
Phosphorus	14,000	3,500	7,500	8,600	8,400	8,100	7,200

* PEU data from Bansemer et al. (2016a).

† Carbohydrate (g/100 g) was calculated by difference: carbohydrate% = 100 – (moisture + protein + ash).

slowly (~1°C/day) until the experimental temperature of 22 ± 1°C was reached.

After stocking, abalone were fed their respective diets daily at 4:00 PM to excess of their daily requirements (4% biomass/day). Tanks were cleaned at 8:30 AM the following day. Uneaten feed was collected by sieving the contents of each tank through fine mesh (500 μm). Feed rates were adjusted at monthly intervals after stocking using bulk tank weights. Uneaten feed was stored at –20°C.

At the completion of the growth trial, the feed was dried at 105°C for 16 h to determine feed consumption. Feed remained in the water for 16.5 h between feeding and cleaning. Feed lost due to leaching over this period was determined by placing feed in tanks without abalone and collecting it after 16.5 h had

elapsed. The feed was then dried at 105°C for 16 h. The amount leached was determined by subtracting the remaining dried feed from the initial amount introduced to the tank (Stone et al. 2013). Mortalities that occurred during the growth trial were weighed, measured, and replaced with a tagged animal of similar weight.

Biochemical and Water Quality Analysis

Immediately before stocking the growth trial, 100 animals were weighed (2.23 ± 0.13 g) and measured (shell length; 24.68 ± 0.52 mm). These animals were stored at –20°C for initial analysis of proximate soft tissue composition. At the completion of the growth and oxygen consumption experiment, five abalone per tank were collected and were stored at –20°C for final soft tissue proximate analysis. The methods for analysis of proximate soft tissue composition for protein and lipid were conducted according to the Kjeldahl method (Chromy et al. 2015) and an in-house method at the National Measurement Institute (Melbourne, Australia), respectively. Carbohydrate and energy composition was determined via calculation (NRC 2011).

$$\begin{aligned}\text{Carbohydrate(\% dry)} &= 100 - (\text{ash} + \text{protein} + \text{lipid})\cdot \\ &\quad \text{Energy(MJ/kgdry)} \\ &= [\text{lipid(\%)} \times 39.5\ \text{MJ} + \text{protein(\%)} \\ &\quad \times 23.6\,\text{MJ} + \text{carbohydrate(\%)} \\ &\quad \times 17.2\,\text{MJ}]/100.\end{aligned}$$

Proximate composition of abalone soft tissue ash content was determined at 600°C for 8 h.

Apparent Dry Matter Digestibility Coefficient

Apparent dry matter digestibility coefficient (DM ADC) of feed was determined using the acid insoluble ash (AIA) method described by Montano-Vargas et al. (2002) and calculated using the following equation: DMD(%) = 100 – {[AIA in feed(%)/AIA in faeces(%)] × 100}.

Oxygen Consumption

Oxygen consumption was measured at the completion of the growth trial using the methods previously described in Duong et al. (2016). The oxygen consumption rate was determined by the difference between oxygen concentrations in final and initial water samples, multiplied by chamber volume, divided by incubation time, and adjusted for biomass. This produced a final value in mg oxygen/kg abalone/h.

Performance Indices

Calculation for all performance indices (abalone wet weight was used for all calculations regarding weight, and dry values were used for all calculations regarding feed) are described as follows:

$$\begin{aligned}\text{Biomass gain(g/tank)} &= \left(\text{final weight} + \sum \text{mortality weight}\right) \\ &\quad - \left(\text{initial weight} + \sum \text{replacement weight}\right)\end{aligned}$$

$$\text{Specific growth weight(SGR, \%/day)} = [(\ln \text{final weight} - \text{initial weight})/\text{days}] \times 100$$

$$\text{Shell growth rate(}\mu\text{m/day)} = (\text{final shell length} - \text{initial shell length})/\text{days}.$$

$$\begin{aligned}\text{Apparent feed consumption} &= [\text{feed offered} - \text{uneaten feed collected} \\ &\quad - (\text{uneaten feed collected})/\times \%\ \text{leaching loss without animals}]/\text{tank biomass}\end{aligned}$$

$$\text{Apparent feed conversion ratio(FCR)} = \text{feed consumed/abalone weight gain}$$

$$\text{Apparent protein deposition(PD)} = [(\text{final soft tissue protein} - \text{initial soft tissue protein})/\text{protein intake}] \times 100$$

$$\text{Apparent energy deposition(ED)} = [(\text{final soft tissue energy} - \text{initial soft tissue energy})/\text{energy intake}] \times 100$$

Water Quality

Daily water quality parameters were monitored and recorded (Table 3). Salinity (g/L) was measured using a portable salinity refractometer (model RF20; Extech Instruments, Nashua, NH). Water temperature was measured using a thermometer. Dissolved oxygen (% saturation and mg/L) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerod, Denmark). The pH was measured using a pH meter (Oakton pHtester 20; Oakton Instruments, Vernon Hills, IL).

Statistical Analysis

All statistical analyses were completed using IBM SPSS statistics software (IBM SPSS Statistics for Windows, Version 23.0; IMB Corp., Armonk, NY). Homogeneity of variance was verified

TABLE 3.

Summary of water quality parameters monitored daily for the duration of the growth study.

	Salinity (g/L)	Temperature* (°C)	Dissolved oxygen (mg/L)	Dissolved oxygen (% saturation)	pH
Average	36.80 ± 0.80	21.60 ± 0.20	8.60 ± 0.20	95.90 ± 1.80	8.15 ± 0.14
Range	35.00–38.00	21.00–22.20	7.80–9.50	89.00–100.00	7.89–8.38

* Temperature is the average after acclimatization period. All other parameters are the average for the entirety of the experimental period.

through the Levene's test by determining equality of variances. An independent sample *t*-test was conducted to compare each variable for the 0% basal and EPA commercial diet. One-way analysis of variance (ANOVA) was used to determine differences between growth and nutrient utilization parameters for abalone fed diets only in the experimental diet series (UPE; 0%, 5%, 10%, and 20%). Where significant differences were observed, the Student–Newman–Keuls post hoc test was utilized to determine the differences between the mean treatment values. The significance level implemented for all statistical analyses was $P < 0.05$ with 95% confidence interval. All data are presented as mean ± SEM of four replicate tanks, except where indicated.

RESULTS

General Observations

There was no significant difference between treatments in the average initial weight (1.82 ± 0.01 g) or shell length (23.22 ± 0.09 mm) of abalone at the commencement of the trial (Table 4). Water quality parameters were monitored daily and maintained at appropriate levels for greenlip abalone (Table 3). Abalone exhibited normal feeding behaviors and activities throughout the 90-day study period. Mortality rates during the study were low (1.33%). The animals displayed no visual signs of disease throughout the study.

Growth Performance and Somatic Growth Parameters

Abalone fed the EPA diet and the 0% basal diet had similar biomass gain (90.30 and 91.48 g/tank, $P = 0.754$; independent samples *t*-test), final weight (7.85 and 7.91 g, $P = 0.997$) and shell length (39.57 and 39.57 mm, $P = 0.814$), shell growth rate (182.50 and 181.70 µm/day, $P = 0.870$), and specific growth rate (SGR) (1.62% and 1.63%/day, $P = 0.785$), respectively. Biomass gain per tank ($P = 0.882$; one-way ANOVA), final weight ($P = 0.879$) and shell length ($P = 0.878$), shell growth rate ($P = 0.921$), and SGR ($P = 0.901$) were not significantly influenced by the dietary inclusion level of UPE meal (Table 4).

TABLE 4.

Growth performance, feed efficiency, nutrient retention, soft tissue composition, and DM ADC of greenlip abalone fed the EPA diet and diets containing graded levels of UPE meal.*†

Diet	EPA diet	UPE meal (%)				Statistical analysis	
		0	5	10	20	Independent samples *t*-test‡	One-way ANOVA§
Growth performance							
Initial weight (g)	1.83 ± 0.01	1.82 ± 0.01	1.82 ± 0.01	1.82 ± 0.01	1.82 ± 0.01	0.452	0.991
Final weight (g)	7.85 ± 0.07	7.91 ± 0.23	7.84 ± 0.14	8.07 ± 0.32	8.05 ± 0.23	0.814	0.878
Biomass gain (g/tank)	90.30 ± 0.99	91.48 ± 3.45	90.20 ± 2.05	93.75 ± 4.94	93.51 ± 3.48	0.754	0.882
SGR (%/day)	1.62 ± 0.01	1.63 ± 0.03	1.62 ± 0.02	1.65 ± 0.05	1.65 ± 0.03	0.785	0.901
Somatic growth parameters							
Initial shell length (mm)	23.17 ± 0.14	23.22 ± 0.08	23.22 ± 0.08	23.21 ± 0.12	23.27 ± 0.03	0.592	0.896
Final shell length (mm)	39.57 ± 0.39	39.57 ± 0.31	39.49 ± 0.22	39.71 ± 0.65	39.95 ± 0.40	0.997	0.879
Shell growth rate (µm/day)	182.5 ± 3.49	181.70 ± 3.19	180.81 ± 1.79	183.29 ± 8.08	185.29 ± 4.21	0.870	0.921
Feed utilization							
Apparent feed consumption rate (g as fed/kg abalone/day)	11.29 ± 0.28	10.68 ± 0.20	10.09 ± 0.21	10.44 ± 0.16	10.20 ± 0.05	0.013	0.111
Apparent FCR	0.81 ± 0.02	0.76 ± 0.02	0.72 ± 0.01	0.74 ± 0.02	0.72 ± 0.01	0.143	0.358
Nutrient retention							
Apparent PD	31.18 ± 0.87	25.98 ± 0.77	28.49 ± 0.82	26.40 ± 1.57	28.26 ± 0.78	0.004	0.264
Apparent ED	20.43 ± 0.34	22.21 ± 0.76	23.59 ± 0.29‖	22.25 ± 0.86	23.36 ± 0.59	0.076	0.350
Soft tissue proximate composition							
Moisture (%)	80.85 ± 0.14	80.99 ± 0.31	80.51 ± 0.28	81.27 ± 0.48	80.85 ± 0.38	0.701	0.558
Protein (% dry)	60.93 ± 0.92	60.65 ± 0.43	60.38 ± 1.24	59.13 ± 0.91	60.38 ± 0.96	0.796	0.665
Lipid (% dry)	5.88 ± 0.17	5.78 ± 0.19	5.75 ± 0.15	5.40 ± 0.20	5.43 ± 0.14	0.704	0.285
Ash (% dry)	14.29 ± 0.28	13.26 ± 0.09	13.65 ± 0.32	13.80 ± 0.30	13.56 ± 0.20	0.013	0.475
Carbohydrate (% dry)	18.91 ± 1.09	20.32 ± 0.62	20.22 ± 1.04	21.67 ± 0.78	20.64 ± 0.88	0.292	0.602
Energy (MJ/kg dry)	19.95 ± 0.09	20.09 ± 0.08	20.00 ± 0.15	19.81 ± 0.08	19.94 ± 0.10	0.300	0.362
Oxygen consumption rate (mg/kg/h)	10.37 ± 0.52	14.29 ± 0.35	11.43 ± 0.51	12.01 ± 0.71	9.88 ± 0.33	0.020	0.056
DM ADC (%)	79.21	90.67	91.53	80.93	87.25	–	–

SNK, Student–Newman–Keuls. Initial soft tissue composition of greenlip abalone (dry): moisture (77.02%), protein (69.20%), lipid (5.30%), ash (12.50%), carbohydrate (12.44%), and energy (20.56 MJ/kg).

* Mean ± SE of the mean, $n = 4$.

† A significance level of $P < 0.05$ was used for all statistical tests.

‡ Indicates a significant difference between EPA and basal formulated diet, independent samples *t*-test $P \leq 0.05$, $n = 4$.

§ No significant difference ($P \leq 0.05$) was determined using a one-factor ANOVA, SNK, $n = 4$, between the mean values of the four formulated diets (UPE diets 0%, 5%, 10%, and 20%).

Feed Utilization

Abalone fed the EPA diet and the 0% basal diet had similar apparent feed consumption rates (11.29 and 10.68 g as fed/kg abalone/day, $P = 0.132$; independent samples t-test) and apparent FCR (0.81 and 0.76, $P = 0.143$). Apparent feed consumption rates ($P = 0.111$) and apparent FCR ($P = 0.358$) were not significantly influenced by the dietary inclusion level of UPE meal (Table 4; one-way ANOVA).

Nutrient Retention

Abalone fed the EPA diet and the 0% basal diet had similar ED ($P = 0.076$; independent samples t-test). Abalone fed the EPA diet and 0% basal diet had significantly different PD ($P = 0.004$). Both ED ($P = 0.350$) and PD ($P = 0.264$) were not significantly influenced by the dietary inclusion of UPE meal (Table 4; one-way ANOVA).

Soft Tissue Proximate Composition

Abalone fed the EPA diet and the 0% basal diet had similar soft tissue levels of moisture (80.85% and 80.99%, $P = 0.701$; independent samples t-test), protein (60.93% and 60.67% dry, $P = 0.796$), lipid (5.88% and 5.67% dry, $P = 0.704$), carbohydrate (17.50% and 18.90% dry, $P = 0.292$), and energy (19.71 and 19.81 MJ/kg dry, $P = 0.300$; Table 4). Abalone fed the EPA diet had higher final soft tissue ash levels ($P = 0.013$; independent samples t-test) than abalone fed the 0% basal diet.

Soft tissue levels of moisture ($P = 0.558$), protein ($P = 0.665$), lipid ($P = 0.285$), carbohydrate ($P = 0.602$), energy ($P = 0.362$), or ash ($P = 0.475$) were not significantly influenced by the dietary inclusion level of UPE meal (Table 4; one-way ANOVA).

Oxygen Consumption Rates

Abalone fed the EPA diet had lower oxygen consumption rates compared with those fed the 0% basal diet (10.37 versus 14.29 mg/kg/h, $P = 0.020$; independent samples t-test). There was no significant difference ($P = 0.056$; one-way ANOVA) in the oxygen consumption rates of abalone fed the four test diets (0%, 5%, 10% and 20% UPE meal inclusion) (Table 4).

Apparent Dry Matter Digestibility Coefficients (DM ADC)

Because of insufficient samples sizes, fecal material from each replicate tank for each treatment was pooled for apparent DM ADC determinations. As a result, replication and statistical analysis could not be performed. The results for DM ADC of the five diets are presented in Table 4. The EPA diet had the numerically lowest DM ADC, whereas the 5% UPE diet exhibited the highest.

DISCUSSION

The UPE investigated in the current study was produced by Venus Shell Systems as a co-product of nutraceutical and cosmetic production. The growth rate of abalone in the current study was comparable to previous studies on greenlip abalone, ranging from 1.62% to 1.65%/day (Stone et al. 2013, Bansemer et al. 2015a). Shell growth rates (>180 μm/day) were also well above the industry standard of ~100 μm/day (Stone et al. 2013). In addition, as the growth and FCR of the EPA and 0% basal diets were similar, experimental results derived from the UPE diet series can be interpreted with confidence.

Juvenile greenlip abalone exhibited similar growth and nutrient utilization when fed diets that contained UPE, which suggests that this ingredient may be included into a commercial formulated diet for greenlip abalone up to 20% and reduce dietary inclusions of solvent extracted soybean meal, de-hulled lupins, and wheat flour, without compromising growth and nutrient utilization. In contrast to previous studies, UPE did not improve abalone growth and nutrient utilization. Improvements in abalone growth achieved in previous studies have been attributed to the carbohydrate, protein (amino acid) and fatty acid composition of the diet. For example, Bansemer et al. (2016a) reported that growth was significantly improved with the dietary inclusion of 5% enriched, dried *Ulva* sp. compared with the 0% basal diet. This superior growth, in combination with other factors, was hypothesized to be due to the upregulation of the digestive enzyme trypsin or changes in the microbiome community (Bansemer et al. 2016a). Trypsin level is typically influenced by dietary protein level; however, the diets in the Bansemer et al. (2016a) and current study were iso-nitrogenous.

In addition, Bansemer et al. (2016a) reported significantly improved growth rates in juvenile greenlip abalone when fed diets that contained 5%, 10%, and 20% *Gracilaria* sp. meal. The major structural carbohydrate found in *Gracilaria* sp. is agar, whereas cellulose, ulvan, and xyloglucan are abundant in *Ulva* sp. (Lahaye & Robic 2007). It was hypothesized that carbohydrates specific to *Gracilaria* sp. increased β-galactosidase and α-amylase activities. These enzymes are responsible for agar and α-linked glucose polymer digestion, respectively (Bansemer et al. 2016a). This response in β-galactosidase and α-amylase activities were not observed in abalone fed *Ulva* sp. meal diet series (Bansemer et al. 2016a). The carbohydrate differences between red macroalgae species and green macroalgae species may be one of the reasons why abalone fed *Gracilaria* sp. inclusions exhibited improved growth compared with the 0% basal diet, whereas those fed UPE in the current study did not. An area of future research may be to investigate the effect a combination of UPE and enriched *Gracilaria* sp. meal formulated into diets may have on juvenile greenlip abalone growth rates and nutrient utilization.

In the current study, apparent protein deposition was not influenced by dietary UPE inclusions. This suggests that greenlip abalone were able to utilize protein from the UPE as efficiently as currently used commercial dietary ingredients. Interestingly, differences in the nutrient utilization were apparent between abalone fed the 0% basal diet and the commercial formulated diet. The significantly greater apparent protein deposition in abalone fed the EPA diet compared with the 0% basal diet suggests that the EPA diet had sufficient dietary protein, whereas abalone utilized excess protein in the 0% basal diet as an energy source (Stone et al. 2013, Bansemer et al. 2015a). It may be beneficial in future studies to investigate the energy budget of greenlip abalone fed different dietary protein levels to improve the nutritional knowledge of this species.

Dietary UPE inclusions for juvenile greenlip abalone were successful. The growth rates and nutrient utilization for juvenile greenlip abalone fed the 0% basal diet were the same as those fed up to a 20% dietary inclusions of UPE. These results suggest

that UPE can be incorporated into formulated diets for greenlip abalone by reducing the inclusion level of solvent extracted soybean meal, de-hulled lupin meal, and wheat flour without impacting growth or nutrient utilization. These findings are important for future research into the optimization of abalone nutrition. Currently, UPE is not an economically viable ingredient for commercial abalone feed manufacture. Enriched *Ulva* sp. meal is estimated to cost in excess of $20/kg to produce in Australia by Venus Shell Systems (Bansemer et al. 2016a). As UPE is a more highly refined product, the costs of production are predicted to be even greater (Personal communication, Dr. Pia Winberg, Venus Shell Systems Pty. Ltd, Bomaderry, Australia). The cost of this product would likely become more economically viable to include in diets for greenlip abalone as macroalgae production increases and the cost of manufacture decreases in the future (Pia Winberg, Venus Shell Systems Pty. Ltd, personal communication). Once economically viable, we recommend a dietary inclusion of up to 20% UPE meal for greenlip abalone.

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