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Dietary inclusion of Acti-Meal improves growth and feed utilisation of greenlip abalone (*Haliotis laevigata*)

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ABSTRACT

Acti-Meal is a co-product of the wine industry, consisting of a steam distilled, milled and dried grape marc meal rich in carbohydrates (55–65%). In this 90-day study, growth and feed utilisation of juvenile (1.82 g) greenlip abalone (*Haliotis laevigata*) fed diets containing graded dietary inclusion levels of Acti-Meal (0 basal, 5, 10, 15 and 20%) were examined. Four replicates were allocated per treatment with 15 abalone per tank and abalone were fed to excess daily. All experimental diets were formulated to contain 35% crude protein, 5% crude lipid and 17.5 MJ kg^{-1} gross energy. In addition, a commercial diet was also fed to abalone and compared with the 0% diet. Growth and feed utilisation of greenlip abalone fed the commercial diet and the 0% basal diet were similar. Greenlip abalone fed any Acti-Meal inclusion level (5, 10, 15 and 20%) exhibited significantly superior growth to abalone fed the 0% basal diet. Notably, in comparison to the basal diet, specific growth rate and biomass gain were improved by 5 to 6% and 10 to 12% respectively. The improvements were achieved despite greenlip abalone consuming significantly less feed (~9%) when fed the Acti-Meal diets. Greenlip abalone fed diets with Acti-Meal also exhibited superior apparent feed conversion ratios compared to those fed the 0% basal diet, on average FCRs were reduced by 13%. We recommend dietary inclusions of up to 20% Acti-Meal to improve greenlip abalone growth. We also recommend an on-farm growth trial to validate this product.

1. Introduction

The land-based production of abalone in Australia is reliant on cost effective formulated diets, which promote feed utilisation and growth. In regards to Australian abalone, previous research has focused on understanding the nutritional requirements of greenlip abalone (*Haliotis laevigata*) (Coote et al. 2000; Dunstan et al. 2000; Vandepeer et al. 2002; Vandepeer and Van Barneveld 2003; Stone et al. 2013; Bansemer et al. 2015). Hybrid abalone (*H. laevigata* × *H. rubra*) (Mateos et al. 2012; Stone et al. 2016) and blacklip abalone (*H. rubra*) (Vandepeer and Van Barneveld 2003) have also been evaluated to a lesser extent. Currently, Australian abalone feed manufacturers use a range of palatable, digestible, nutritionally balanced, cost effective and available ingredients, which includes fish oil and meals made from fish, cereal grains, oilseeds and pulses (Coote 1998; Stone et al. 2013; Bansemer et al. 2016b). Improvement in the sustainability of abalone diets can be made by utilising cheaper alternative ingredients. Products derived from food industry waste streams are both cheap and readily available.

Acti-Meal is a relatively inexpensive co-product from the Australian wine industry waste, derived from grape marc following wine fermentation. Grape marc is steam distilled to capture residual alcohol. The process also lowers microbial activity. The resulting Acti-Meal product is a low protein (10–12% crude protein), high carbohydrate (~60%) and high crude fibre (~35%) product (personal communication Tarac Technologies, Nuriootpa, SA, Australia). It also contains moderate levels of crude lipid (~9%), rich in linoleic acid (LA), which combined may limit its inclusion into greenlip abalone diets. Total dietary lipid levels of > 5% have been reported to reduce growth performance in greenlip abalone (Dunstan et al. 2000), and as a result, commercial greenlip abalone diets are formulated to contain relatively low total lipid levels of < 5% (Stone et al. 2013). A proportion of this total lipid must be supplied in the form of fish oil, to provide essential long chain omega-3 highly polyunsaturated fatty acids (n-3 LC-PUFA) (Dunstan et al. 2000).

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Grape meal products at inclusion levels between 5% and 20% have been shown to improve some aspects of ruminant production (Hadjipanayiotou and Locuca, 1976; Moate et al. 2014; Moote et al. 2014; Voicu et al. 2014; AWRI 2016). Its feeding value has been found to be comparable to hay or straw (Winkler et al. 2015). However, the dietary application of grape meal products in aquatic species is not well understood.

The aim of the present study to evaluate the effect of graded dietary inclusion levels of Acti-Meal on the growth performance and feed utilisation of juvenile greenlip abalone.

2. Methods

2.1. Experimental animals

Greenlip abalone were purchased from South Australian Mariculture (Port Lincoln, SA, Australia). Prior to stocking, abalone were held in a flowthrough seawater system at South Australia Research and Development Institute Aquatic Sciences Centre (SAASC) (West Beach, SA, Australia) and fed a commercial diet ("Abgrow premium" 5 mm chip; Eyre Peninsula Aquafeed Pty Ltd., Lonsdale, SA, Australia) *ad libitum*.

2.2. Test ingredients and diets

Acti-Meal was provided by Tarac Technologies (Nuriootpa, SA, Australia). All other dietary ingredients were provided by Eyre Peninsula Aquafeeds. The proximate composition, amino acid, fatty acid profile and mineral content of all ingredients were analysed by Asure Quality (Wellington, New Zealand) prior to diet formulation and manufacture. In brief, methods employed by Asure Quality included; AOAC 930.15 (moisture), AOAC 942.05 (ash), Kjeldahl (protein), calculation by difference (carbohydrate), calculation using protein, lipid and carbohydrate content (energy), analytical biochemistry 178 (1989) (modified) (amino acid profile), AOAC 991.39 (fatty acid profile) and Asure Quality methods (ICP-OES) (mineral content).

The suitability of Acti-Meal as a bulk dietary ingredient for greenlip abalone was assessed using a series of five diets with increasing inclusion levels (basal diet at 0 [control], 5, 10, 15 or 20%). The practical inclusion of Acti meal in diets in the current study was limited to a maximum of 20%, due to the inherently high lipid levels and low protein levels of Acti-Meal. An additional commercial diet (Abgrow premium, 5 mm chip) was also included for comparison to the 0% basal diet. On an as-fed basis, test diets were formulated to contain 35% crude protein, 5% crude lipid and a gross energy content of 17.5 MJ kg^{-1}. This was based on the nutritional requirements of juvenile greenlip abalone (Stone et al. 2013; Bansemer et al. 2015). The test diets were also formulated so the ratio of each essential amino acid to lysine was equal to, or greater than, that analysed in the soft body tissue of greenlip abalone reported by Coote et al. (2000).

The 0% basal diet, used in the present study, was the same formulation as Bansemer et al. (2016a) (Table 1). Targeted dietary protein and energy levels were achieved when increasing Acti-Meal inclusion by also increasing solvent extracted soybean meal and decreasing dehulled lupin meal inclusion levels. The biochemical composition of test diets are displayed Table 2.

Experimental diets were prepared by weighing the required amount of dry ingredients, which were then mixed in a Hobart mixer (Hobart Corp., Troy, OH, USA) for 5 mins. Water (30–40% of the total ingredient weight), fish oil, sodium alginate and calcium sulphate were then added to the dry ingredient mash and mixed for a further 7 mins. The diets were manufactured to produce a flat (4 × 3 × 2 mm) sinking pellet using a TR110 pasta machine (Macchine Per Pasta SRL, Molina Di Malo, VI, Italy), and dried at 50 °C for approximately 48 h until diets were < 12% moisture. The proximate composition, amino acid and fatty acid analyses of all test diets were carried out by Asure Quality.

Table 1
Ingredient composition of the experimental basal diet.

Ingredient inclusion level (%)	Basal diet
Salmon fish meal	6.00
Soy protein concentrate	8.00
Solvent extracted soybean meal	30.50
Wheat flour	29.20
De-hulled lupins	23.96
Fish oil	1.00
EPA Vitamin/mineral premix	0.20
Vitamin E	0.01
Monosodium phosphate	0.61
Sodium alginate	0.30
Calcium sulphate	0.22
Sum	100.00

All ingredients were provided by Eyre Peninsula Aquafeed Pty Ltd., Lonsdale, SA, Australia.

Crude fibre values for Acti-Meal were provided by Tarac Technologies. Acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin and starch values for Acti-Meal and other test ingredients were obtained using book values obtained from Feedipedia (2012–2017). These values were then used to calculate dietary crude fibre, ADF, NDF, lignin and starch values for all test diets (Table 2).

2.3. Experimental system

The experiment was conducted in a temperature-controlled system previously described in Stone et al. (2013). In brief, 24 × 12.5 L rectangular blue plastic tanks (Nally IH305, Viscount Plastics Pty Ltd) were supplied with sand filtered, UV treated, flow-through seawater at a rate of 300 mL min^{-1}. Water level was set at 2.5 cm using a standpipe with a mesh screen (0.8 mm nominal mesh size) on the outlet to retain uneaten food. Water temperature was held at 22 ± 1 °C using 3 kW immersion heaters (240 V, 3 kW, A3122–1; Hotco, Williamstown, SA, Australia).

2.4. Experimental stocking

Abalone were removed from the substrate using a spatula. Fifteen animals were weighed, measured (Table 3) and dispersed into one of four replicate tanks per dietary treatment. Abalone were stocked into the experimental system at 19 °C, acclimated to the experimental system for two weeks, and fed their respective diets. After two weeks, water temperature was slowly raised (1 °C day^{-1}) to the final temperature of 22 °C. Dead abalone were measured, weighed, recorded, and replaced with tagged abalone of similar weight.

2.5. Feeding

During the acclimation and growth phases of the experiment, abalone were fed to excess of their daily requirements based on the total tank biomass (4% biomass day^{-1}) at 16:00 h. Tanks were cleaned the following day at 08:30 h, and uneaten feed was collected by sieving the entire tank contents through a fine mesh (500 μm). Feed rates were adjusted based on tank biomass which was determined every four weeks. Collected uneaten feed was stored at −20 °C and was later dried at 105 °C for 16 h. Daily feed intake rate was estimated by the difference between feed offered and uneaten feed in dry weight (Stone et al. 2013). Feed intake rate was corrected for leaching loss. Dry matter leaching loss was determined in quadruplicate, by immersing 4 g of each diet in tanks without abalone, for 16.5 h, sieving through a fine mesh net (500 μm), and drying to constant weight (Stone et al. 2013).

2.6. Biochemical analyses

At the commencement of the experiment, the soft tissues of 100

Table 2
Biochemical composition and diet stability of the Acti-Meal ingredient and experimental diets.

Item	Acti-Meal	EPA	Acti-Meal diets and inclusion levels (%)				
	Ingredient	Diet	0	5	10	15	20
Proximate composition (g 100 g^{-1} diet as fed)							
Moisture	9.8	10.7	13.1	11.1	11.7	10.6	11.6
Crude protein	12.2	29.3	34.7	34.9	34.9	35.2	34.9
Crude lipid	9.8	4.6	4.9	5.0	4.9	5.3	5.8
Gross energy (MJ kg^{-1})	17.29	17.24	17.43	17.69	17.46	17.73	17.60
Ash	6.9	5.9	4.8	5.5	6.1	6.3	6.6
Carbohydrate[1]	61.3	49.6	42.5	43.5	42.4	42.6	41.1
Carbohydrate composition (% dry matter)[2]							
Crude Fibre	39.7	–	6.11	6.40	6.96	8.81	10.67
ADF	54.7	–	13.76	14.54	15.62	18.13	20.64
NDF	64.1	–	7.86	8.35	9.20	11.75	14.31
Lignin	33.4	–	0.83	2.34	3.87	5.49	7.10
Starch	0.6	–	21.30	20.12	18.65	15.28	11.85
Amino acids (g 100 g^{-1} diet as fed)							
Alanine	0.48	1.24	1.43	1.48	1.53	1.55	1.48
Aspartic acid	0.48	1.79	2.47	3.38	3.82	3.85	3.63
Arginine	0.59	2.77	3.62	2.47	2.30	2.35	2.24
Glutamic acid	1.67	5.56	6.39	5.86	5.86	5.79	5.50
Glycine	0.66	1.31	1.54	1.60	1.67	1.63	1.63
Histidine	0.03	0.48	0.68	0.87	0.83	0.82	0.79
Isoleucine	0.16	1.18	1.40	1.41	1.42	1.48	1.37
Leucine	0.71	2.09	2.48	2.51	2.60	2.75	2.50
Lysine	0.34	1.63	1.62	2.00	2.30	2.35	2.24
Methionine	0.16	0.41	0.40	0.42	0.43	0.46	0.42
Phenylalanine	0.59	1.47	1.75	1.69	1.71	1.78	1.66
Proline	0.36	1.83	1.78	2.11	2.23	2.14	2.25
Serine	0.42	1.25	1.61	1.59	1.59	1.61	1.56
Threonine	0.39	1.04	1.28	1.25	1.26	1.28	1.24
Tyrosine	0.27	0.96	1.14	1.17	1.15	1.17	1.10
Valine	0.56	1.36	1.58	1.62	1.86	1.93	1.80
Fatty acids (mg 100 g^{-1} diet as fed)							
14:0	18	61	38	39	41	40	41
16:0	980	760	790	800	810	840	880
18:0	450	190	280	230	210	240	260
10:1	< 10	< 10	< 10	< 10	< 10	< 10	< 10
14:1	< 10	< 10	< 10	< 10	< 10	< 10	< 10
15:1	< 10	< 10	< 10	< 10	< 10	< 10	< 10
16:1	30	110	70	76	82	83	84
17:1	< 10	< 10	< 10	< 10	< 10	< 10	< 10
18:1n-7	79	99	83	82	86	88	92
18:1n-9	1480	1340	1420	1200	1070	1130	1200
18:2n-6	6380	1310	1650	1710	1970	2270	2610
20:4n-6	< 10	19	12	11	13	13	13
18:3n-3	160	180	190	190	200	210	240
18:4n-3	< 10	16	< 10	< 10	11	10	< 10
20:4n-3	< 10	15	< 10	< 10	< 10	< 10	< 10
20:5n-3	< 10	72	30	32	38	38	37
22:5n-3	19	26	11	12	14	14	14
22:6n-3	< 10	160	100	100	120	120	120
Σ n-3 LC-PUFA[3]	179	469	331	334	383	392	411
Minerals (mg kg^{-1}as fed)							
Calcium	6100	5400	5200	5400	6000	6500	7100
Chromium	1.4	–	–	–	–	–	–
Cobalt	0.882	–	–	–	–	–	–
Copper	36	–	–	–	–	–	–
Iodine	0.22	–	–	–	–	–	–
Iron	190	–	–	–	–	–	–
Magnesium	760	–	–	–	–	–	–
Manganese	15	–	–	–	–	–	–
Molybdenum	0.33	–	–	–	–	–	–
Nickel	0.66	–	–	–	–	–	–
Phosphorus	2500	7500	7600	7900	8200	8400	8600
Potassium	28,000	–	–	–	–	–	–
Selenium	0.042	–	–	–	–	–	–
Sodium	150	–	–	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

Item	Acti-Meal	EPA	Acti-Meal diets and inclusion levels (%)				
	Ingredient	Diet	0	5	10	15	20
Zinc	15	–	–	–	–	–	–
Diet stability (%)[4]	–	72.45	73.48	74.15	72.77	70.16	72.93

ADF, acid detergent fibre; EPA, Eyre Peninsula Aquafeed Pty Ltd.; NDF, neutral detergent fibre;"-"denotes variables not analysed; diet biochemical analyses were conducted by Asure Quality (Wellington, New Zealand).

[1] Calculated by difference, carbohydrate (%) = 100% - (protein % + lipid % + ash %);

[2] Carbohydrate composition provided by Tarac Technologies for fibre content of Acti-Meal, ADF, NDF, lignin and starch were averaged based on book values (Feedipedia, 2012–2017) corroborated with values obtained by Tarac Technologies. Carbohydrate composition of experimental diets was achieved by calculation using book values (Feedipedia, 2012–2017).

[3] Σ n-3 LC-PUFA, sum of essential long chain omega-3 highly polyunsaturated fatty acids.

[4] Diet stability calculated by immersing diets in water at 22 °C in experimental tanks for 16.5 h and sieved through a fine mesh net (500 μm), dried to constant weight.

abalone were collected, shucked and stored at −20 °C, then freeze dried at −80 °C for 24 h or until a constant weight was achieved, for the initial soft tissue proximate composition. At the completion of the experiment, six abalone from each tank were collected, shucked, and stored at −20 °C. Abalone soft tissue was pooled for each tank for analysis and subsequently freeze dried for 24 h at −80 °C for 24 h or until a constant weight was achieved. Samples were then analysed for crude protein and crude lipid by the National Measurement Institute (Melbourne, VIC, Australia) using a Kjeldahl and mojonnier extraction respectively. Ash content was evaluated by ashing 0.5 g of tissue at 600 °C, until a constant weight was achieved. Carbohydrate content was calculated by difference (carbohydrate (%) = 100% - (protein % + lipid % + ash %), and energy content by calculation using the values of 17.2, 23.6 and 39.5 MJ kg^{-1} for carbohydrate, protein and lipid (Glencross 2009).

All data reported for animal performance were based on the pooled data from each tank ($n = 4$). All calculations using abalone weight were based on wet values, while feed use values were based on dry values:

$$\text{Biomass gain (g tank}^{-1}) = (\text{final weight} + \sum \text{mortality weight}) - (\text{initial weight} + \sum \text{replacement weight}).$$

Table 3
Growth performance, feed efficiency, nutrient retention, dietary dry matter apparent digestibility, proximate composition and oxygen consumption of greenlip abalone fed increasing levels of Acti-Meal for 90 days.

Item	Inclusion level (%)					ANOVA
	0	5	10	15	20	*P*-value
Growth performance and mortality						
Initial individual weight (g)	1.82 ± 0.00	1.83 ± 0.01	1.83 ± 0.01	1.82 ± 0.00	1.82 ± 0.00	0.534
Final individual weight (g)	7.57 ± 0.21[b]	8.15 ± 0.12[a]	8.29 ± 0.19[a]	8.28 ± 0.16[a]	8.20 ± 0.05[a]	0.028
Biomass gain (g $tank^{-1}$)	86.26 ± 3.09[b]	94.86 ± 1.85[a]	96.90 ± 2.81[a]	96.95 ± 2.38[a]	95.66 ± 0.72[a]	0.027
SGR (% day^{-1})	1.58 ± 0.03[b]	1.66 ± 0.01[a]	1.68 ± 0.02[a]	1.68 ± 0.02[a]	1.67 ± 0.01[a]	0.024
Mortality (%)	1.67 ± 1.67	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.483
Somatic growth parameters						
Initial shell length (mm)	23.22 ± 0.05	23.23 ± 0.07	23.28 ± 0.13	23.32 ± 0.09	23.13 ± 0.04	0.547
Final shell length (mm)	38.83 ± 0.36[b]	39.93 ± 0.19[a]	40.38 ± 0.46[a]	40.45 ± 0.14[a]	39.89 ± 0.15[a]	0.009
Shell growth rate (μm day^{-1})	173.35 ± 3.90[b]	185.47 ± 2.72[ab]	190.00 ± 5.71[a]	190.34 ± 2.44[a]	186.27 ± 1.83[ab]	0.027
Feed utilisation						
Feed intake rate (g as fed kg $abalone^{-1}$ day^{-1})	11.03 ± 0.24[a]	10.02 ± 0.02[b]	10.35 ± 0.16[b]	10.26 ± 0.30[b]	9.64 ± 0.19[b]	0.004
Apparent FCR	0.80 ± 0.01[a]	0.70 ± 0.01[b]	0.72 ± 0.02[b]	0.71 ± 0.02[b]	0.67 ± 0.01[b]	< 0.001
Nutrient retention						
Apparent PD	26.15 ± 0.75[b]	30.31 ± 0.72[ab]	29.96 ± 1.80[ab]	30.16 ± 0.25[ab]	33.50 ± 1.84[a]	0.015
Apparent ED	17.68 ± 0.55	20.33 ± 0.61	19.83 ± 1.13	18.79 ± 0.20	20.95 ± 1.18	0.088
Dietary DM ADC (%)	45.92 ± 3.74	55.83 ± 5.56	55.21 ± 2.76	61.73 ± 2.78	57.05 ± 2.82	0.089
Proximate composition						
Moisture (%)	81.57 ± 0.40	81.21 ± 0.37	81.31 ± 0.31	81.85 ± 0.20	81.61 ± 0.59	0.797
Protein (% wet)	11.35 ± 0.24	11.41 ± 0.11	11.56 ± 0.22	11.76 ± 0.10	11.95 ± 0.40	0.407
Lipid (% wet)	1.08 ± 0.02	1.03 ± 0.05	1.04 ± 0.04	1.00 ± 0.02	1.05 ± 0.03	0.520
Ash (% wet)	2.44 ± 0.07	2.58 ± 0.05	2.66 ± 0.05	2.63 ± 0.01	2.58 ± 0.07	0.115
Energy (MJ kg^{-1} wet)	5.61 ± 0.58	5.49 ± 1.26	5.51 ± 0.85	5.42 ± 0.46	5.56 ± 1.06	0.648
Protein (% dry)	61.60 ± 0.20[b]	60.75 ± 1.06[b]	61.85 ± 0.78[b]	64.78 ± 0.37[a]	64.95 ± 0.29[a]	0.001
Lipid (% dry)	5.88 ± 0.08	5.45 ± 0.18	5.55 ± 0.18	5.50 ± 0.07	5.73 ± 0.05	0.161
Ash (% dry)	13.27 ± 0.42	13.75 ± 0.41	14.22 ± 0.44	14.48 ± 0.09	14.05 ± 0.24	0.176
Energy (MJ kg^{-1} dry)	20.17 ± 0.07[a]	19.94 ± 0.03[b]	19.95 ± 0.06[b]	20.08 ± 0.01[ab]	20.22 ± 0.03[a]	0.002
Oxygen consumption (mg kg^{-1} abalone h^{-1})	10.89 ± 0.89	11.45 ± 1.39	10.90 ± 3.94	8.72 ± 0.87	11.99 ± 1.43	0.533

SGR, specific growth rate; FCR, feed conversion ratio; PD, protein deposition; ED, energy deposition; DM ADC, dry matter apparent digestibility coefficient. Initial soft tissue content of greenlip abalone (dry): moisture (77.02%), protein (69.20%) lipid (5.30%), ash (11.71%) and energy (20.08 MJ kg^{-1}). Data presented as mean ± SE, $n = 4$. Values without a common lower uppercase letter are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

Specific growth rate (SGR,%day^{-1})
= ([ln final individual weight − ln initial individual weight]/days)
× 100.

Shell growth rate (μm day^{-1}) = (final shell length − initial shell length)
/days.

Apparent feed intake rate = (((feed offered − uneaten feed collected
− ((uneaten feed collected
×[%leaching loss without animals x 0.5]))
/tank biomass.

Apparent feed conversion ratio (FCR) = feed consumed
/abalone weight gain.

Apparent protein deposition
= ([final soft body protein − initial soft body protein]/protein intake)
× 100.

Apparent energy deposition
= ([final soft body energy − initial soft body energy]/energy intake)
× 100.

2.7. Dietary dry matter apparent digestibility coefficient

Faeces were collected daily 6 h after tanks were cleaned and stored at −20 °C until analysis. Samples were freeze dried for 48 h to a constant mass. Samples were analysed for dietary dry matter apparent digestibility coefficient (DM ADC) using the ash insoluble acid (AIA) method of Van Keulen and Young (1977) modified by Montaño-Vargas et al. (2002). Due to small sample weights, two replicate samples comprised of equal faecal samples from two replicate tanks, were used per treatment. The following equation was used to calculate dietary DM ADC:

Dietary DM ADC (%) = ([AIA in faeces–AIA in feed]/AIA in faeces)
× 100.

2.8. Oxygen consumption rate

Oxygen consumption rate was evaluated as per Duong et al. (2016), with some modifications. In brief, at the end of the growth experiment, five abalone from each treatment tank were placed into 5 L chambers supplied with their original temperature-controlled seawater for three days to allow their metabolism to recover from handling (Harris et al. 1997; Harris et al. 1999). Flow rates were maintained at the same rate as used during the experimental period. Abalone were fed 4.0% bw d^{-1} for two days then starved on the third day. At 09:00 h on the fourth day, initial water samples were taken. Immediately after that, the chambers were sealed and the water flow was halted for 45 min. After 45 min incubation period, the chamber lids were opened and the dissolved oxygen (% saturation and mg L^{-1}) was determined. Abalone were weighed and measured in each chamber. Oxygen consumption rate was calculated as the difference between levels of oxygen in the seawater in the chamber, before and after incubation, accounting for the volume of the chamber, the incubation time and the biomass to give final measurements in mg O_2 kg^{-1} h^{-1}. Possible interference due to significant bacterial oxygen consumption was standardised by a blank determination of oxygen consumption in an identical chamber with no abalone.

2.9. Water quality

Water quality parameters, in ten randomly selected tanks, were evaluated daily. Water temperature was measured using a thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark) calibrated daily in water-saturated air. The pH was measured using a meter, which was calibrated monthly, in pH 4 and 7 buffer solutions (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (ppt) was measured using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA) calibrated in deionised water daily.

2.10. Statistical analyses

IBM SPSS (Version 23 for Windows; IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and a Shapiro Wilk test, respectively. Two-tailed *t*-tests were used to determine differences between abalone fed the 0% basal diet and commercial diet. Data, for the 0% basal diet, 5, 10, 15 and 20% Acti-Meal diets, were analysed using one-factor ANOVA to determine the effects of diet. The Student Newman–Keuls (SNK) test was used to identify significant differences among multiple treatment means. A significance level of $P < 0.05$ with 95% confidence was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

3. Results

3.1. General observations

There were no significant differences for abalone initial individual weight and shell length between treatments ($P > 0.05$). The average initial individual weight and shell length was 1.82 ± 0.01 g and 23.28 ± 0.19 mm, respectively (Table 3). Water quality parameters were monitored daily and maintained at levels appropriate for greenlip abalone (mean ± standard deviation, range): water temperature (21.6 ± 0.2, 21.0–22.2 °C), dissolved oxygen (95.9 ± 1.8, 89–100% saturation), (8.6 ± 0.2, 7.8–9.5 mg L^{-1}), pH (8.15 ± 0.14, 7.89–8.38) and salinity (36.4 ± 0.8, 35–38 ppt). Throughout the study, abalone exhibited normal signs of feeding and fed actively on all diets. There were no visual signs of disease observed in experimental animals. Mortalities across all dietary treatments were low, on average 0.21%, and primarily occurred during the first two weeks, likely due to handling during stocking (Table 3).

3.2. Growth performance

Abalone fed the commercial diet and 0% basal diet had statistically similar final individual weight (7.85 and 7.57 g; $P = 0.254$; Two-tailed *t*-test), biomass gain (90.30 and 86.23 g tank^{-1} $P = 0.257$), SGR (1.62 and 1.58% day^{-1} $P = 0.271$), final shell length (39.57 and 38.83 mm $P = 0.211$) and shell growth rate (182.50 and 173.35 μm day^{-1} $P = 0.131$).

There was a significant effect of Acti-Meal inclusion level on growth performance, including final individual weight ($P = 0.028$; one-factor ANOVA; Table 3), biomass gain ($P = 0.027$), SGR ($P = 0.024$), final shell length ($P = 0.009$) and shell growth rate ($P = 0.027$). Abalone fed all inclusion levels of Acti-Meal (5–20%) exhibited superior growth performance to those fed the 0% basal diet, and there were no significant differences between abalone fed diets with Acti-Meal (Table 3).

3.3. Feed utilisation

Abalone fed the commercial diet and 0% basal diet had similar feed intake rates (11.29 and 11.03 as fed g kg abalone^{-1} day^{-1}; $P = 0.501$;

Two-tailed *t*-test) and apparent FCRs (0.81 and 0.80; $P = 0.790$).

Feed intake rate was significantly affected by Acti-Meal inclusion level. Abalone fed diets containing Acti-Meal (5–20%) consumed less than those fed the 0% basal diet ($P < 0.01$; Table 3). There was also a significant effect of Acti-Meal inclusion on the apparent FCR of abalone. Abalone fed diets containing Acti-Meal (5–20%) had significantly better FCRs than those fed the 0% basal diet ($P < 0.001$; Table 3) and there was no significant difference in FCR between abalone fed the diets that contained Acti-Meal.

3.4. Soft tissue composition

There was no significant difference in soft tissue composition between abalone fed the commercial diet and the 0% basal diet regarding moisture (80.85 and 81.56%; $P = 0.143$; Two-tailed *t*-test), protein (60.93 and 61.60% dry; $P = 0.501$), lipid (5.88 and 5.87% dry; $P = 1.000$), ash (14.29 and 13.27% dry; $P = 0.888$) or energy (19.95 and 20.17 MJ kg^{-1} dry; $P = 0.106$).

On a wet basis, there was no significant influence of Acti-Meal inclusion level on abalone soft tissue composition for moisture (%), protein (%wet), lipid (% wet), ash (% wet) or energy (MJ kg^{-1} wet) ($P > 0.05$; Table 3; one-factor ANOVA).

On a dry basis, there was a significant influence of Acti-Meal inclusion level on protein (% dry) soft tissue composition, with abalone fed 15 or 20% Acti-Meal diets being higher in tissue protein than abalone fed all the other diets ($P < 0.05$; Table 3). There was a significant influence of Acti-Meal inclusion level on energy soft tissue composition (MJ kg^{-1} dry). Abalone fed diets containing 0 and 20% Acti-Meal inclusion levels had higher soft tissue energy levels than abalone fed all other diets ($P < 0.05$; Table 3). There was no significant influence of Acti-Meal inclusion level on abalone soft tissue composition for lipid (% dry) or ash (% dry) ($P > 0.05$; Table 3; one-factor ANOVA).

3.5. Nutrient utilisation

Abalone fed the commercial diet had significantly higher protein deposition (PD) (31.18%) than those fed the 0% basal diet (26.16%) ($P = 0.005$; Two-tailed *t*-test). There was no significant difference between abalone fed the commercial diet and 0% basal diet for energy deposition (ED) (17.38% and 17.90% respectively; $P = 0.742$).

There was a significant effect of Acti-Meal inclusion level on PD. Abalone fed the 20% Acti-Meal diet had significantly higher PD values than those fed the 0% basal diet ($P = 0.015$; Table 3). In addition, there was a general trend for PD values to be numerically higher for abalone fed diets containing Acti-Meal than for those fed the 0% basal diet. There was no significant effect of Acti-Meal inclusion level on ED ($P = 0.088$).

3.6. Dietary dry matter apparent digestibility coefficient

Abalone fed the commercial diet and 0% basal diet had similar dietary DM ADCs (48.67 vs 45.92% respectively).

Acti-Meal inclusion had no significant effect on apparent dietary DM ADC ($P = 0.089$; one-factor ANOVA; Table 3), but there was a trend for abalone fed diets containing Acti-Meal had to have higher dietary DM ADCs than those fed the 0% basal diet.

3.7. Oxygen consumption rate

Abalone fed the commercial diet and 0% basal diet had statistically similar oxygen consumption rates (10.37 vs 10.85 mg kg abalone $^{-1}$ h^{-1} respectively; $P = 0.717$; Two-tailed *t*-test). There was no significant effect of Acti-Meal inclusion level on the oxygen consumption rate of abalone ($P = 0.553$; one-factor ANOVA; Table 3).

4. Discussion

The Australian abalone aquaculture industry is highly reliant on cost effective formulated diets that promote good growth. The growth and feed conversion ratio of greenlip abalone fed the commercial and 0% basal diets were similar. Specific growth rates (1.58 to 1.68% day^{-1}) and shell growth rates (166.48 to 190.34 μm day^{-1}) in the present study were comparable to results for similar sized greenlip abalone grown at 22 °C (1.5% day^{-1}, 167–175 μm day^{-1}, Stone et al., 2013; 1.49% day^{-1}, 239 μm day^{-1}, Bansemer et al., 2016a; 1.63% day^{-1}, 182 μm day^{-1}, Bates et al., 2017) and exceeded the commercial industry standard of ~100 μm day^{-1} for greenlip abalone (Stone et al. 2013). Even better growth was achieved for greenlip abalone fed diets containing 5 to 20% Acti-Meal than the 0% basal diet (Table 3).

Based on results, there are several benefits of using Acti-meal in greenlip abalone diets. Greenlip abalone fed dietary inclusions of up to 20% Acti-Meal exhibited superior growth compared to abalone fed the 0% basal diet. Notably, SGR was significantly improved by 5 to 6% and the daily shell growth rate by 6.5 to 9% for greenlip abalone fed diets containing up to 20% Acti-Meal. These contributed to significant biomass gains over the basal diet which ranged from 10 to 12%. The improvements were achieved despite greenlip abalone consuming significantly less feed (~9%) when fed the Acti-Meal diets. This led to improvements in FCRs of between 10 and 16%, compared to abalone fed to the 0% basal diet (Table 3). Protein deposition was higher when Acti-Meal was included at 20%. Soft body tissue protein and energy levels tended to be higher in abalone fed inclusions of Acti-Meal. As a result protein and energy deposition tended to improve with the addition of Acti-Meal. These factors had no effect on the oxygen consumption rate of greenlip abalone.

Several confounding factors that may have contributed to the improvement in feed utilisation and growth of greenlip abalone fed increasing levels of Acti-Meal were considered and discounted. One factor may be the progressive reduction in de-hulled lupin meal with increasing Acti-Meal inclusion. This is unlikely as previous studies with greenlip abalone have also progressively removed dietary de-hulled lupin meal by substitution with either dried *Ulva* sp. meal (Bansemer et al., 2016a), dried *Ulva* sp. protein extract meal (Bates et al. 2017) or peanut meal (Currie et al., unpublished data) without improving feed utilisation or growth performance. Another factor may have been the increase in solvent extracted soybean meal with decreasing dehulled lupin meal inclusion. This is also unlikely as soybean meal and dehulled lupin meals have been shown to have similar digestibility coefficients for greenlip abalone and other abalone species (Vandepeer 2002; Sales and Britz 2003).

Nutrient digestibility is one area that may have contributed to the observed improvements in feed utilisation and growth performance of greenlip abalone fed up to 20% Acti-Meal. Differences in dietary nutrient digestibility are likely due to the influence of the different carbohydrate composition of Acti-Meal compared to the basal diet. Acti-Meal is rich in carbohydrates (Table 2) and the progressive addition of Acti-Meal in diets resulted in a concomitant increase in a crude fibre content (ADF, NDF and lignin) while starch levels decreased (Table 2). Previous research on the effect of carbohydrates on the nutrient digestibility in abalone is limited and has mainly focused on high cellulose diets (Vandepeer 2002; Sales and Britz 2002). While there were no significant differences, the dietary DM ADCs of diets for greenlip abalone fed dietary inclusions of Acti-Meal were 17 to 29% higher in comparison to abalone fed the 0% basal diet (Table 3). An increase in replication from two to four replicates would have improved the statistical power to detect significant difference between dietary treatments.

Previous research relating to the effects of dietary fibre content on growth performance in abalone species has focused on cellulose content and results have been unequivocal. Lee et al. (2017) demonstrated Japanese abalone (*Haliotis discus hannai*) fed a diet containing 15% cellulose exhibited improved growth performance in comparison to diets containing a 15% inclusion of other more digestible carbohydrate

sources, such as corn starch and wheat flour. Maguire et al. (1997) reported growth performance was not affected in greenlip abalone when fed diets containing up to 15% ground rice husks (high in dietary fibre, typically cellulose). In contrast, Uki et al. (1985) reported growth rate reductions in Japanese abalone fed increasing levels of cellulose up to 20%. It is clear that more research focussed on evaluating the effects of different carbohydrate sources in abalone species is required.

Although dietary protein is considered the first limiting macronutrient for abalone growth (Fleming and Hone 1996; Britz and Hecht 1997), carbohydrate composition has been suggested to be equally as important for greenlip abalone (Bansemer et al., 2016b). Currently, formulated diets contain 30% to 60% starch rich cereal derived carbohydrates (Fleming et al. 1996). These carbohydrate sources are used to satisfy energy requirements (Fleming et al. 1996), reduce feed costs (Dunstan 2010) and improve the binding (Fleming et al. 1996; Sales and Britz 2002). Starch is often considered to be highly digestible. However, in two separate studies Vandepeer (2002) found that greenlip abalone fed increasing inclusions of pre-gelatinised waxy starch resulted in significantly reduced energy and protein digestibility coefficients. Interestingly, dietary inclusions of Acti-Meal, which is high in insoluble carbohydrates, resulted in improved growth. The positive results of using Acti-Meal, suggest that current Australian commercial diets may have an over-reliance on highly refined cereal-based ingredients for the provision of energy. It would be beneficial in future studies to investigate other less refined ingredients, with consideration of the carbohydrate composition.

In conclusion, there were significant improvements in feed and nutrient utilisation and growth performance of greenlip abalone fed diets containing Acti-Meal. Based on these results we recommend the testing of dietary inclusion of up to 20% Acti-Meal for commercially manufactured diets for greenlip abalone and other abalone species. Pilot scale farm trials are required to validate laboratory results before the ingredient can be used in commercial diet formulations. Further research involving the use of grape meal products for abalone aquafeeds should be ideally determined on a case-by-case basis due to variations between grape species, cultivation methods, drying, milling, storage and other treatment processes.

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