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Evaluation of TARAC Technologies Acti-Meal for incorporation in aquafeeds at 20% for the omnivorous Silver Perch and 16% for the carnivorous Barramundi

David A.J. Stone and Paul Skordas

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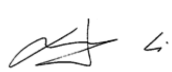
Signed: 

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EXECUTIVE SUMMARY

A 12 week growth study was undertaken to evaluate Acti-Meal (steam-distilled grape marc meal) as an ingredient for inclusion as an energy source into diets for the omnivorous Silver Perch (*Bidyanus bidyanus*) and the carnivorous Barramundi (*Lates calcarifer*). Two diet series, one series for each species, were formulated using practical ingredients to be isonitrogenous, isolipidic, isocalorific and balanced for the first two limiting essential amino acids, lysine and methionine. The Silver Perch diet series consisted of a control diet and a diet containing 20% Acti-Meal, while the Barramundi diet series consisted of a control diet and a diet containing 16% Acti-Meal. Juvenile Silver Perch (4.50 g $fish^{-1}$) and Barramundi (29.77 g $fish^{-1}$) were cultured for 12 weeks under similar conditions and fed their respective diets, to apparent satiation twice daily, in separate fresh water flow through systems at 25 ºC and 28 ºC, respectively. At the completion of the trial, there were significant differences ($P < 0.05$) in growth performance, feed utilisation, protein and energy deposition and estimated diet ingredient costs to produce one kg of fish. Both species grew more efficiently when fed the control diet, compared to fish fed their respective diet containing Acti-Meal. Results were in direct contrast to those recently reported from our laboratory at SARDI for the herbivorous Greenlip Abalone (*Haliotis laevigata*), where growth and feed utilisation were significantly improved with dietary inclusion of Acti-Meal at levels of up to 20% (Currie et al., 2019). The performance of Silver Perch was less affected by the dietary inclusion of 20% Acti-Meal than for Barramundi fed diets containing 16% Acti-Meal, which may be due to their trophic level placement. An estimation of the cost of diet ingredients required to produce one kg of fish indicated that the inclusion of Acti-Meal, at the levels tested in this study, led to a significant increase in production cost for both species, and was higher for Barramundi. Based on results from the current study, where weight gain of Silver Perch fed 20% Acti-Meal was only reduced by 15.3%, we recommend further research to investigate the suitability of lower graded dietary inclusion levels (0-15%). Further research with Acti-Meal is not recommended for Barramundi. Overall, Acti-Meal may be more suitable for inclusion in aquafeeds for herbivorous and omnivorous species, as opposed to carnivorous species.

Keywords:

Acti-Meal, grape marc meal, Silver Perch, Barramundi, growth and feed utilization.

1. INTRODUCTION

1.1. Background

Tarac Technologies collects >100,000 tonnes of grape marc each season, with a large proportion of this originating from vineyards in the Riverland region of South Australia. Grape marc in its unprocessed form is a low value co-product of the wine production industry. Tarac Technologies processes a relatively small proportion of grape marc into value added products such as ethanol and grape seed extract (GrapeEx). Both processes are costly, and yield and markets are limited. A larger proportion of the unprocessed grape marc is currently being evaluated for soil amelioration programs with a predicted price of $2 to $4 per t. Even though grape marc improves soil drainage and fertility, freight and handling costs affect the viability of this product. Tarac Technologies currently faces difficulties selling the remainder of the grape marc product, and as a result, the backlog may be so great as to overflow into the following season. This presents costly storage and other associated problems for the company. Tarac Technologies are seeking to find alternative markets to sell grape marc meal.

In an attempt to find new and more valuable markets for their grape marc, Tarac Technologies have employed processing to create a range of new products, including Acti-Meal from grape marc, and GrapeEx, a grape seed extract from grape seeds. Acti-Meal and GrapeEx have been trialled in a range of feed applications for terrestrial and marine animal production with varying results. With regard to marine animal production applications, SARDI has a proven collaborative research and development track record with Tarac Technologies. This research has focused on investigating nutritional applications of a range of grape derived products, including Acti-Meal and GrapeEx, with abalone and Yellowtail Kingfish, S*eriola lalandi* (Bellgrove et al., 2012; Lange et al., 2014; Duong et al., 2016; Stone et al., 2018; Currie et al., 2019). GrapEx improved the feed intake, antioxidant status, immune response and ultimately survival of Greenlip Abalone (*Haliotis laevigata*) exposed to high water temperature in a laboratory setting (Lange et al., 2014; Duong et al., 2016), while Acti-Meal enhanced the growth and feed efficiency of Greenlip Abalone in a laboratory setting (Currie et al., 2019).

Acti-Meal has a nutritional profile (~12% crude protein, ~10% lipid and ~60% carbohydrate) (Currie et al., 2019) that lends itself as a potential new sustainable energy source for inclusion in aquafeeds for abalone and a range of other aquacultured species, including two of the main cultured omnivorous fish species in Asia, carp and tilapia.

Recent, laboratory scale research (Currie et al., 2019) at SARDI in collaboration with Tarac Technologies, the Australian Abalone Growers Association and Australian abalone feed manufacturers, funded as part of the recently completed Thriving Abalone project, within the South Australian Government Functional Food Program, indicated that Acti-Meal is a useful ingredient and nutrient and energy source for incorporation into Greenlip Abalone (*Haliotis laevigata*) diets. Preliminary results demonstrated that Greenlip Abalone grew as well or better than abalone fed the control and commercial diets when up to a 20% inclusion level of Acti-Meal was used to replace de-hulled lupins and other more expensive dietary ingredients in abalone feeds (Currie et al., 2019). Feed efficiency was also improved when Acti-Meal was included in the diet (Currie et al., 2019).

The research of Currie et al. (2019) has led to another collaborative project with Southern Ocean Mariculture, Aquafeeds Australia, Tarac Technologies and SARDI. The project is run by Assoc. Prof. Stone and is evaluating the commercial application of Acti-Meal in a 12 month on-farm trial with Hybrid Abalone (*Haliotis laevigata x Haliotis rubra*). Nine months in, results from this trial are encouraging (Stone et al., unpublished data).

On a global scale aquaculture production is expanding rapidly (FAO, 2018). There is an ongoing and urgent need to find and identify new and novel ingredients for incorporation into aquafeeds for fish to fuel this rapid growth in aquaculture production (Gatlin et al., 2007). Positive results from research with Acti-Meal and abalone suggests this product may have potential for incorporation into aquafeeds as an energy and nutrient source for a range of commonly cultured finfish species in domestic and international markets. Bearing this in mind, and following conversations with collaborative project participants, it was decided that Acti-Meal would be evaluated in two species of fish representative of omnivorous and carnivorous species cultured globally. The chosen species were the omnivorous Silver Perch (*Bidyanus bidyanus*) and the carnivorous Barramundi (*Lates calcarifer*). Both species are routinely used in nutrition studies and commercially cultured domestically in Australia and throughout other regions of the world (Allan et al., 2000; Stone et al., 2000; Tu et al. 2011; Ahmad et al., 2013).

1.2. Objectives

In this study, the project aims to evaluate Acti-Meal incorporated into Silver Perch (Omnivore) and Barramundi (Carnivore) aquafeeds in a laboratory based experiment at SARDI. The project objectives were:

1. Evaluate if Acti-Meal is a suitable ingredient for incorporation into aquafeeds for Silver Perch (Omnivore) and Barramundi (Carnivore) by assessing the growth performance, feed efficiency, nutrient utilisation and production economics; and
2. Provide quantitative peer-reviewed information to assist Tarac Technologies to decide to target valuable finfish aquafeed markets with their Acti-Meal product.

2. MATERIALS AND METHODS

2.1. Test ingredient, experimental treatments, diets, and feeding

Three varieties of grape marc, one derived from red grapes (Acti-Meal) and two from white grapes, were evaluated for biochemical composition (Table 1). Based on the protein (Table 1) and amino acid composition (Appendix 1) and discussions with Tarac Technologies, the red grape meal variety (Acti-Meal) was selected for inclusion into the experimental diets in this study.

Table 1. The analysed composition (as fed) of the three Tarac Technologies grape marc meals evaluated prior to the growth experiments with Silver Perch and Barramundi.

Item[1,2]	Red grape marc meal (Acti-Meal)	White grape marc meal 1	White grape marc meal 2
Moisture (%)	7.7	5.4	11.1
Dry mater (%)	92.3	94.6	88.9
Crude lipid (%)	11.2	8.6	14.9
Crude protein (%)	15.4	13.8	13.5
Ash (%)	8.5	8.3	4.0
Carbohydrate (%)	57.2	63.9	56.5
Energy (MJ kg^{-1})	16.4	16.4	17.5
Lysine (g kg^{-1})	4.5	6.5	3.9
Methionine (g kg^{-1})	1.7	1.9	1.7

[1] Carbohydrate (%) = 100% - [Moisture (%) + Crude lipid (%) + Crude protein (%) + Ash (%)].

[2] Refer to Appendix 1 for complete analysed composition of the three grape marc meals.

This 12 week study investigated the performance of Silver Perch and Barramundi when fed semi commercial diets containing Acti-Meal. The study consisted of concurrently running two separate 12 week experiments, one with each species, in separate systems. The two diets used in the Silver Perch Experiment were: 1) Silver Perch control diet; and 2) 20% Acti-Meal diet (Table 2; nominal crude protein and lipid levels of 40% and 11% as fed, respectively). The two diets used in the Barramundi Experiment were: 1) Barramundi control diet; and 2) 16% Acti-Meal diet (Table 3; nominal crude protein and lipid levels of 46% and 11% as fed, respectively). The lower inclusion level (16%) of Acti-Meal was used in the Barramundi experiment as there was no room to fit more in the nutrient dense diet formulation required for this species. The two diet series, one series for each species, were formulated using practical highly palatable and digestible ingredients at realistic commercial inclusion levels to be isonitrogenous, isolipidic, isocalorific and balanced for the first two limiting essential amino acids, lysine and methionine. Diets were also formulated to ensure that the Acti-Meal test diet ingredients were equivalent or cheaper than the corresponding control diet for each species. Estimated ingredient costs for each diet were based on information provided by Aquafeeds Australia (February, 2018) and Tarac Technologies. The estimated diet ingredient costs were \$1,063 and \$1,011 tonne^{-1} for the Silver Perch control and 20% Acti-Meal diets, and \$1,128 and \$1,121 tonne^{-1} for the Barramundi control and 16% Acti-Meal diets, respectively. All diets were manufactured using cooking extrusion at Aquafeeds Australia (Mt Barker, South Australia, Australia). Diet formulations are available on request.

Fish were fed twice daily at 10:30 h and 16:00 h to apparent satiation, which involved feeding fish for a maximum of four minutes tank^{-1} or until a feed refusal response was observed. Apparent feed intake was recorded at each meal.

2.2. Experimental fish and system

Experimental work was conducted in the Nutrition Laboratory at SARDI (West Beach, South Australia). Silver Perch (initial weight 4.50 ± 1.09 g, mean ± standard deviation; n = 180) were obtained from #1Aquaponics (Lewiston, South Australia, Australia) and Barramundi (initial weight 29.77 ± 2.44 g, mean ± standard deviation; n = 180) were obtained from Robarra (West Beach, South Australia, Australia). Upon arrival at SARDI, Silver Perch and Barramundi were transferred to holding tanks supplied with flow-through (2 L min^{-1}) and aerated bore water at 26 ± 1 °C and 28 ± 1 °C, respectively. Fish were fed the same commercial diet that they were fed prior to transport for one week. Fish were then weaned onto experimental diets for one week. After this two week acclimation period, the fish were stocked into the experiment. The experimental systems were housed in a temperature (26 °C) and photoperiod (14 L: 10 D) controlled room. Each experiment was run in separate flow through systems, each comprised of a bore water supply, 1,200 L header tank (heated to required temperature) and 12 experimental tanks (200 L volume). Experiential tanks were supplied with pre-heated flow-through (2 L min^{-1}) aerated bore water. Water temperatures were maintained at the optima for growth of Silver Perch (26 ± 1 °C) and Barramundi (28 ± 1 °C). Tanks were cleaned every second day. Mortalities were weighed and recorded and replaced with fish of a similar weight fed their respective experimental diet.

Table 2. Analysed composition (as fed) of experimental diets fed to Silver Perch for 84 days.

Item[1,2]	Control diet	20% Acti-Meal diet
Moisture (%)	8.5	7.8
Dry matter (%)	91.5	92.2
Crude protein (%)	39.5	38.7
Crude lipid (%)	11.5	10.9
Ash (%)	9.5	12.6
Carbohydrate (%)	31.0	30.0
Energy (MJ kg^{-1})	16.2	15.7
Lysine (%)	1.9	1.8
Methionine (%)	1.0	0.9

[1] Carbohydrate (%) = 100% - [Moisture (%) + Crude lipid (%) + Crude protein (%) + Ash (%)].

[2] Refer to Appendix 2 for analysed composition of the Silver Perch experimental diets.

Table 3. Analysed composition (as fed) of experimental diets fed to Barramundi for 84 days.

Item[1,2]	Control diet	16% Acti-Meal diet
Moisture (%)	8.2	8.4
Dry matter (%)	91.8	91.6
Crude protein (%)	45.6	44.9
Crude lipid (%)	13.1	11.4
Ash (%)	9.7	12.9
Carbohydrate (%)	23.4	22.4
Energy (MJ kg^{-1})	16.5	15.6
Lysine (%)	2.2	2.1
Methionine (%)	1.0	1.0

[1] Carbohydrate (%) = 100% - [Moisture (%) + Crude lipid (%) + Crude protein (%) + Ash (%)].

[2] Refer to Appendix 2 for analysed composition of the Barramundi experimental diets.

2.3. Experimental stocking and weight checks

At the commencement of the study (22 March 2018), fish were removed from their tank, anaesthetised using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 10 mg L^{-1} in 50 L of bore water. Thirty fish were weighed (Silver perch: initial starting weight = 4.5 g $fish^{-1}$, initial tank biomass 0.135 kg $tank^{-1}$; Barramundi initial starting weight = 29.8 g $fish^{-1}$, initial tank biomass = 0.893 kg $tank^{-1}$) and stocked into one of the three replicate tanks per diet per species. At the four (Weight check 1, 20 April 2018) and eight week weight checks (Weight check 2, 17 May 2018), fish were anaesthetised using AQUI-S® at a concentration of 20 ppm in bore water, weighed and returned back to their respective tanks. The final harvest occurred after 12 weeks of culture (14 June 2018). At this point, individual fish were weighed and three whole fish samples per tank were collected for proximate body composition analysis.

2.4. Biochemical analyses

Diet samples were analysed for proximate composition, energy, fatty acids, amino acids and minerals by the National Measurement Institute (Melbourne, Victoria, Australia). At the commencement of the experiment 15 fish of each species (initial fish samples) were frozen and stored at -20 °C. At the completion of the experiment, three fish (final fish samples) from each tank were collected and stored at −20 °C. Whole fish were then ground and pooled for each tank (n = 3 fish per tank). Initial and final fish samples were then analysed for crude protein and crude lipid by the SARDI Environment and Analytical Laboratories (SEAL) 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% - [moisture% + 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, respectively (Glencross 2009).

2.5. Performance indices

All data reported for each treatment for animal performance were based on the mean of the replicate tanks. All calculations using fish weight and diets were based on wet or as fed values, respectively:

- Weight gain = final weight - initial weight
- Biomass gain (kg $tank^{-1}$) = (final weight + ∑mortality weight) - (initial weight + ∑replacement weight)
- Specific growth rate (SGR, % d^{-1}) = ([ln final weight - ln initial weight] / d) × 100
- Apparent feed conversion ratio (FCR) = feed consumed / fish weight gain
- Protein deposition (%) = [(final body protein – initial body protein) / protein intake] × 100
- Energy deposition (%) = [(final body energy – initial body energy) / energy intake] × 100

- An estimation of diet ingredient costs to produce one kg of fish (\$ kg^{-1}) was calculated for each species using the following formula: Estimated diet ingredient cost (\$ kg^{-1}) x apparent feed intake (kg) / weight gain (kg)
    - Where the estimated feed ingredient costs were calculated for each diet based on ingredient information provided by Aquafeeds Australia in February 2018 and \$230 $tonne^{-1}$ for Acti-Meal (Tarac Technologies).

2.6. Water quality

Water quality parameters were measured at 11:30 h daily, and maintained at appropriate levels for acceptable growth of Silver Perch (Rowland, 1995) and Barramundi (Rimmer, 2006). Water temperature was measured using an alcohol thermometer and ranged from 26 °C to 27 °C, and from 27.5 °C to 28 °C for the Silver Perch and Barramundi systems, respectively. Dissolved oxygen (% saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark) and ranged from 90% to 100%, and from 89% to 100% for the Silver Perch and Barramundi systems, respectively. The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA) and ranged from 7.5 to 9.2 in the Silver Perch and Barramundi systems. Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA) and never exceed 3 ppt in either system. Ammonia and nitrite were not measured as fish were held in flow through systems with sufficient flow rates to negate the build-up of these nitrogenous products.

2.7. Statistical analyses

The growth experiments were both designed for analysis using single-factor ANOVA. Homogeneity of variances was assessed using Levine's Test (Winer, 1971). Differences between means were considered significant at $P < 0.05$. Unless otherwise stated, all results appear as mean ± standard error (SE) of mean ($n = 3$). All statistical analysis was carried using the IBM SPSS Statistics Version 24 computer software package.

3. RESULTS

3.1. General observations

There were no significant differences in the survival within each trial for the Silver Perch (control diet, 86.7% vs. test diet, 97.8%; $P = 0.05$; Table 4) and Barramundi (control diet, 100% vs. test diet, 94.4%; $P = 0.132$; Table 5). There were several mortalities in the first two weeks after initial stocking and practically none thereafter. There were no visual signs of disease. Both species of fish appeared to feed well on their control and test diets.

3.2. Apparent feed intake

Apparent feed intake results suggested no major problems with diet and ingredient palatability for either species (Tables 4 and 5). Apparent feed intake was similar between diets for Silver Perch ($P = 0.680$, Table 4) and Barramundi ($P = 0.285$; Table 5). However, Barramundi tended to be more sensitive to the dietary inclusion of Acti-Meal than Silver Perch.

3.3. Growth performance

For all growth performance indices, both species performed significantly better ($P < 0.05$) when fed the respective control diet as opposed to their test diet containing Acti-Meal (Tables 4 and 5). The negative impact of the inclusion of Acti-Meal on growth performance was more pronounced in Barramundi, albeit at the lower 16% dietary inclusion level, compared to Silver Perch. The differences are best exemplified in terms of percent individual weight gain, where the growth performance of Silver Perch was significantly reduced by 15.3% (342% vs. 404%; $P = 0.003$) by feeding the 20% Acti-Meal diet as opposed to the control diet (Figure 1). Whereas, the growth performance of Barramundi was significantly reduced by 45.1% (351% vs. 640%; $P < 0.01$) when fed the 16% Acti-Meal diet compared to the control diet (Figure 2).

3.4. Apparent feed conversion ratios

The apparent feed conversion ratio (FCR) of Silver Perch was significantly increased by 20.51% (control diet FCR, 1.95 vs. Acti-Meal diet FCR, 2.35; P = 0.018) when fed the control diet compared to the 20% Acti-Meal diet (Table 4). For Barramundi, feed intake decreased by 9.3% in the fish fed the 16% Acti-Meal diet compared to the control diet (Table 5). However, this decrease was not considered statistically significant (P = 0.285). In turn, the apparent FCR of Barramundi was significantly increased by 66.5% (P = 0.004), from 1.40 for the control diet to 2.33 when fed the 16% Acti-Meal diet (Table 5). Interestingly, the herbivorous Greenlip Abalone exhibited improved feed intake and FCRs when fed diets containing up to 20% Acti-Meal (Currie et al., 2019).

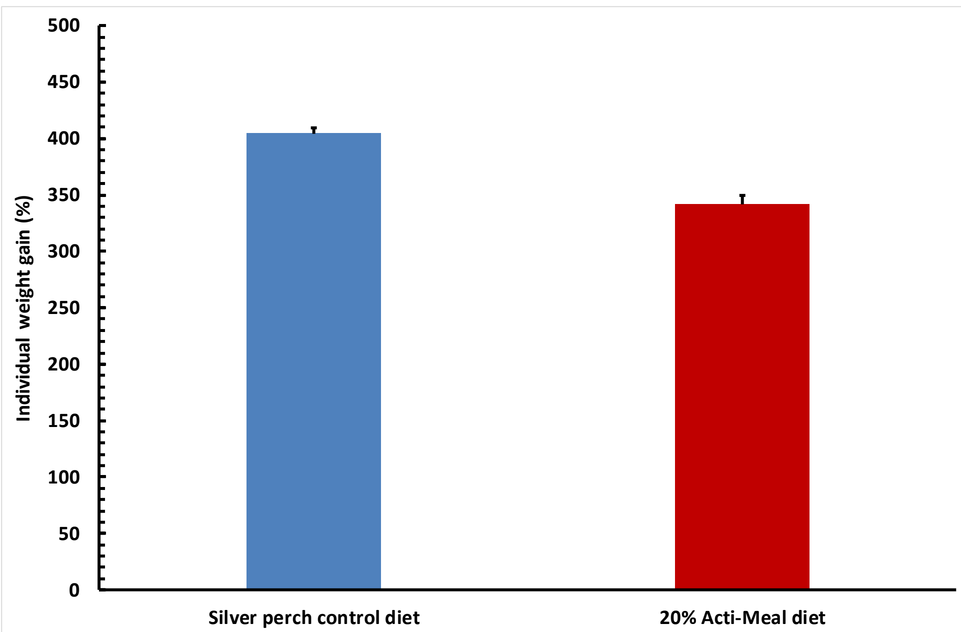

Figure 1. Individual weight gain (%) of Silver Perch fed the control diet compared to the test diet formulated to contain 20% Acti-Meal after 84 days of feeding (Initial weight 4.5 g fish^{-1}; means ± SE, n = 3; one-factor ANOVA; P = 0.003).

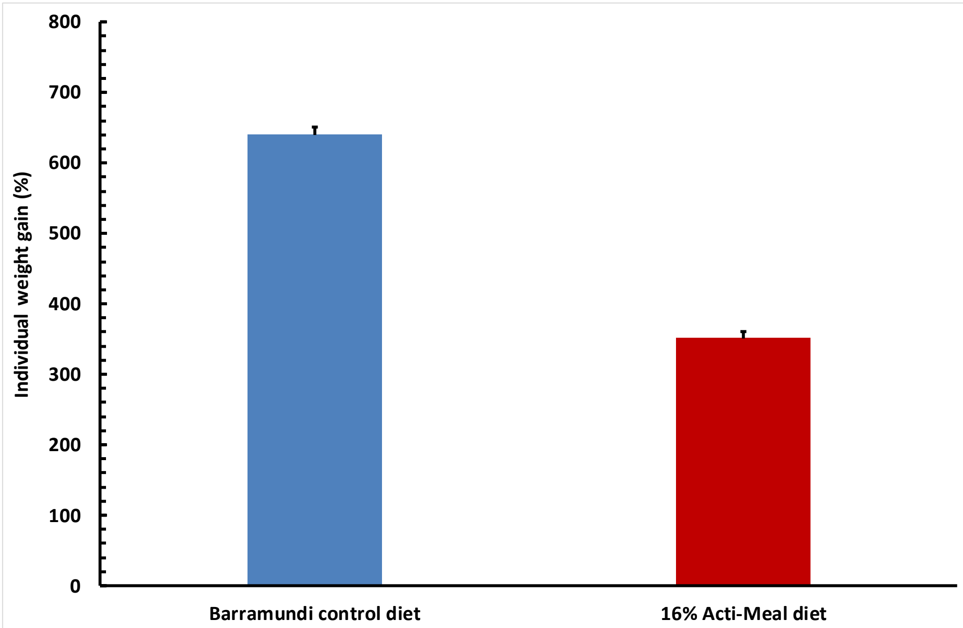

Figure 2. Individual weight gain (%) of Barramundi fed the control diet compared to the test diet formulated to contain 16% Acti-Meal after 84 days of feeding (Initial weight 29.8 g fish^{-1}; means ± SE, n = 3; one-factor ANOVA; $P < 0.001$).

3.5. Body composition

Results for whole fish body composition for Silver Perch and Barramundi are reported in Tables 4 and 5, respectively. The whole body composition of Silver Perch fed either diet were similar (Table 4; $P > 0.05$). The moisture, dry matter, ash and energy content of Barramundi were not significantly affected by diet ($P > 0.05$; Table 5), but the lipid content was significantly reduced by 31.2% (6.4% vs. 9.3%; $P = 0.018$; Table 5). While not significant ($P = 0.081$), there was also a 12.3% reduction in energy content (6.4% vs. 7.3%) of Barramundi fed the test diet containing 16% Acti-Meal compared to those fed the control diet (Table 5). The reduction in lipid and energy contents may be indicative of limitations in energy availability from Acti-Meal.

Table 4. Growth performance, feed intake, fish body composition (as fed basis), protein and energy deposition and diet ingredient input cost estimates for Silver Perch fed the control diet or the test diet formulated to contain 20% Acti-Meal after 84 days.

Item[1]	Control diet	20% Acti-Meal diet	ANOVA *P* value
Growth performance			
Survival (%)	86.7 ± 3.3	97.8 ± 2.2	0.050
Initial individual weight (g fish^{-1})	4.5	4.5	na
Final individual weight (g fish^{-1})	22.7 ± 0.3	19.9 ± 0.4	**0.003**
Final individual weight gain (g fish^{-1})	18.2 ± 0.3	15.4 ± 0.4	**0.003**
Specific growth rate (SGR, % d^{-1})	1.93 ± 0.01	1.77 ± 0.02	**0.004**
Initial biomass (g tank^{-1})	135.0	135.0	na
Final biomass (g tank^{-1})	681 ± 7	596 ± 11	**0.003**
Biomass gain (g tank^{-1})	546.0 ± 7.4	461.4 ± 10.8	**0.003**
Feed utilisation			
Feed intake (g tank^{-1})	1067 ± 33	1082 ± 8	0.680
Apparent feed conversion ratio (FCR)	1.95 ± 0.1	2.35 ± 0.1	**0.018**
Whole fish body composition[2]			
Moisture (%)	65.6 ± 1.4	65.4 ± 0.9	0.930
Dry matter (%)	34.4 ± 1.4	34.6 ± 1.4	0.937
Crude protein (%)	15.0 ± 0.7	16.2 ± 0.6	0.232
Crude lipid (%)	14.5 ± 0.8	13.4 ± 0.1	0.254
Ash (%)	3.5 ± 0.3	4.1 ± 0.3	0.251
Energy (MJ kg^{-1})	9.5 ± 0.4	9.4 ± 0.3	0.833
Nutrient deposition			
Protein deposition (%)	24.14 ± 0.83	22.92 ± 0.58	0.295
Energy deposition (%)	32.05 ± 2.60	27.34 ± 1.29	0.179
Estimate of diet ingredient costs to produce one kg of Silver Perch[3]			
Ingredient cost kg of fish^{-1} ($)	2.08 ± 0.07	2.37 ± 0.07	**0.048**
Difference over control diet (%)	-	13.9	na

[1] Values, mean ± SE, *n* = 3. Where appropriate, one-factor ANOVA was used to compare means within each category and differences between means were considered significant at $P < 0.05$.

[2] Initial Silver Perch whole body composition (mean ± standard deviation, *n* = 3): moisture, 71.2 ± 0.2%; dry matter, 28.8 ± 0.2%; crude protein, 14.8 ± 0.5%; crude lipid, 9.3 ± 0.5; energy, 7.2 ± 0.1 MJ kg^{-1}; and ash 3.7 ± 0.1%.

[3] The estimated diet ingredient costs for the Silver Perch control and Acti-Meal diets were $1,063 and $1,011 tonne^{-1}, respectively (Aquafeeds Australia, February 2018).

Table 5. Growth performance, feed intake, fish body composition (as fed basis), protein and energy deposition and diet ingredient input cost estimates for Barramundi fed the control diet or the test diet formulated to contain 16% Acti-Meal after 84 days.

Item[1]	Control diet	16% Acti-Meal diet	ANOVA *P* value
Growth performance			
Survival (%)	100 ± 0.0	94.4 ± 2.9	0.132
Initial individual weight (g fish^{-1})	29.8	29.8	na
Final individual weight (g fish^{-1})	220.3 ± 3.3	134.3 ± 2.8	**< 0.001**
Final individual weight gain (g fish^{-1})	190.5 ± 3.3	104.5 ± 2.8	**< 0.001**
Specific growth rate (SGR, % d^{-1})	2.38± 0.02	1.79 ± 0.03	**< 0.001**
Initial biomass (g tank^{-1})	893	893	na
Final biomass (g tank^{-1})	6609 ± 99	4029 ± 85	**< 0.001**
Biomass gain (g tank^{-1})	5715.4 ± 98.6	3136.1 ± 84.6	**< 0.001**
Feed utilisation			
Feed intake (g tank^{-1})	8032 ± 521	7290 ± 303	0.285
Apparent feed conversion ratio (FCR)	1.40 ± 0.1	2.33± 0.1	**0.004**
Whole fish body composition[2]			
Moisture (%)	68.4 ± 0.7	72.1 ± 1.4	0.069
Dry matter (%)	31.6 ± 0.7	27.9 ± 1.4	0.069
Crude protein (%)	18.0 ± 1.0	18.0 ± 1.5	0.989
Crude lipid (%)	9.3 ± 0.5	6.0 ± 0.7	**0.018**
Ash (%)	3.8 ± 0.3	4.0 ± 0.3	0.644
Energy (MJ kg^{-1})	7.3 ± 0.1	6.4 ± 0.4	0.081
Nutrient deposition			
Protein deposition (%)	32.42 ± 0.85	21.89 ± 0.77	**0.001**
Energy deposition (%)	32.31 ± 2.40	17.74 ± 1.78	**0.008**
Estimate of diet ingredient costs to produce one kg of Barramundi[3]			
Ingredient cost kg of fish^{-1} ($)	1.58 ± 0.09	2.61 ± 0.15	**0.004**
Difference over control diet (%)	-	65.2	na

[1] Values, mean ± SE, *n* = 3. Where appropriate, one-factor ANOVA was used to compare means within each category and differences between means were considered significant at $P < 0.05$.

[2] Initial Barramundi whole body composition (mean ± standard deviation, *n* = 3): moisture, 72.0 ± 3.3%; dry matter, 28.0 ± 3.3%; crude protein, 16.3 ± 1.9%; crude lipid, 7.1 ± 1.2; energy, 6.6 ± 0.7 MJ kg^{-1}; and ash 3.7 ± 0.1%.

[3] The estimated diet ingredient costs for the Barramundi control and Acti-Meal diets were $1,128 and $1,121 tonne^{-1}, respectively (Aquafeeds Australia, February 2018).

3.1. Nutrient deposition

Protein and energy deposition were both slightly lower for Silver Perch fed the 20% Acti-Meal diet compared to the control diet (Table 4). However, these differences were not statistically significant ($P > 0.05$). In contrast, there were large and significant differences between the corresponding protein (21.89% vs. 32.42%; $P = 0.001$) and energy (17.74% vs. 32.31%; $P = 0.008$) deposition indices for Barramundi fed the 16% Acti-Meal diet compared to the control diet (Table 5).

3.2. Estimated ingredient costs to produce one kg of each species of fish

In both cases, in terms of diet ingredient cost, it was more expensive to produce each species of fish when Acti-Meal was included in the diet (Tables 4 and 5). Feeding Silver Perch the 20% Acti-Meal diet resulted in a significant 13.9% cost premium over the control fed fish to produce one kg of fish ($P = 0.048$; Table 4). Whereas, when using the 16% Acti-Meal diet a significantly higher diet ingredient cost premium of 65.2% was required to produce the equivalent amount of Barramundi fed the control diet ($P = 0.004$; Table 5).

4. DISCUSSION

The main objective of this study was to evaluate if Acti-Meal was a suitable ingredient for incorporation into aquafeeds at 20% for the omnivorous Silver Perch, or at 16% for the carnivorous Barramundi. Growth performance, feed and nutrient utilisation and ingredient inputs in relation to production costs were impacted when Acti-Meal was included in diets for both species, however, more so for Barramundi than Silver Perch (Tables 4 and 5; Figures 1 and 2).

Diets containing Acti-Meal were accepted well by both species and feed intake was not significantly impacted (Tables 4 and 5). This suggests that Acti-Meal did not present any palatability issues at levels tested in the current study. This is in line with results reported for the herbivorous Greenlip Abalone where improved feed intake and FCRs were observed when abalone were fed diets containing up to 20% Acti-Meal (Currie et al., 2019).

Silver Perch (1.93% d^{-1}) and Barramundi (2.38% d^{-1}) fed the control diets grew well in this study (Tables 4 and 5). To put the growth performance of both species of fish in the current study in context, Stone et al. (2003a) reported specific weight gains of 1.19% d^{-1} for slightly larger (26 g $fish^{-1}$) juvenile silver perch fed a fishmeal control diet, while Nankervis et al. (2000) reported specific growth rates of 1.00 to 1.04% d^{-1} for slightly larger (45 g $fish^{-1}$) juvenile barramundi fed diets with comparable protein, lipid and energy levels. Results for protein and energy deposition for Silver Perch fed the control diet were comparable with those reported by Stone et al. (2003a) for similar sized Silver Perch fed fishmeal based diets. While protein deposition results for barramundi fed the control diet were similar to those reported by Nankervis et al. (2000) for juvenile barramundi fed low energy diets.

The negative impacts of Acti-Meal inclusions on growth performance, body composition, feed and nutrient utilisation for both species (Tables 4 and 5) may be associated with limitations in dietary energy digestibility and availability due to the complex carbohydrate component of Acti-Meal. The Acti-Meal tested in this study contained a high level (57.2%) of total carbohydrate (Table 1). This carbohydrate component is predominantly composed of the following classes and types of non-starch polysaccharides (NSP) and starch: crude fibre (40% dry weight); acid detergent fibre (55% dry weight); neutral detergent fibre (64% dry weight); lignin (33% dry weight); and starch (< 1% dry weight) (Currie et al., 2019). Both test diets contained moderate levels of total carbohydrate ranging between 30% and 31% for Silver Perch, and 22% and 23% for Barramundi (Tables 2 and 3). Wheat flour and lupins in the control diet were substituted with the Acti-Meal ingredient in the Acti-Meal test diets for both species. Wheat contains high levels of starch (~60%), while only trace amounts of starch (< 3%) are present in lupins (Stone et al., 2003b). The NSP, β-(1, 4)-galactan, is the predominant energy storage carbohydrate in lupins (Stone et al., 2003b). Therefore, the substitution of wheat flour with Acti-Meal in the test diets resulted in a higher starch component in the control diets (~17% and ~10% starch) as opposed to the Acti-Meal test diets (~8% and ~6% starch) for Silver Perch and Barramundi, respectively. Both Silver Perch and Barramundi have been reported to lack the required enzymes, such as cellulase, β-glucanase and β-xylanase, to efficiently digest the complex non-starch polysaccharides, such as those present in Acti-Meal and lupins (Stone, 2003; Stone et al., 2003b). However, with regard to energy availability from wheat starch, Allan et al. (2003) reported Silver Perch were capable of digesting 83.4% (14.1 MJ kg^{-1}) of energy from wheat starch when it was included in the diet at 30% compared to 7.6% (1.3 MJ kg^{-1}) for Barramundi.

Allan et al. (2003) also reported a reduction in relative growth rate for both Silver Perch and Barramundi as dietary wheat starch inclusion content increased from 0% to 15% to 30%. The relative growth of Silver Perch fed the diet containing 15% wheat starch was not affected, but growth when fed the 30% wheat starch diet was reduced by only ~5% compared to that observed for Silver Perch fed the control diet devoid of wheat starch (Allan et al., 2003). Whereas, ~15% and 30% reductions in relative growth of Barramundi occurred when fed the diets containing 15% or 30% wheat starch compared to the control diet (Allan et al., 2003). In contrast, the herbivorous Greenlip Abalone exhibited improved feed utilisation and growth when fed diets containing up to 20% Acti-Meal (Currie et al., 2019). Bansemer et al. (2016) reported abalone possess a range of digestive enzymes capable of digesting a range of complex carbohydrates, including NSP found in seaweeds and starch. Improved digestibility of the NSP component of Acti-Meal would result in increased liberation of carbohydrate monomers to be utilized as energy for metabolic processes.

With regard to the estimated diet ingredient cost to produce each species of fish, diets were formulated to ensure that the Acti-Meal test diet ingredient costs were equivalent or cheaper than the corresponding ingredients used in the control diet for each species. Aquafeeds Australia provided estimates of diet ingredient costs in February, 2018. The estimated diet ingredient cost were \$1,063 tonne^{-1} and \$1,011 tonne^{-1} for the Silver Perch control and 20% Acti-Meal diets, respectively, and \$1,128 tonne^{-1} and \$1,121 tonne^{-1} for Barramundi control and 16% Acti-Meal diets, respectively. Even though the Acti-Meal diet ingredient costs were lower in both cases, when combined with negative impacts in FCR, the actual ingredient costs required to produce one kg of fish were significantly higher for both species of fish using the corresponding Acti-Meal diets (Tables 4 and 5). Again, more so for Barramundi.

5. CONCLUSIONS AND RECOMENDATIONS

Based on the results for growth performance, feed and nutrient utilisation and the estimated ingredient costs to produce each species of fish in this study, Acti-Meal is not recommended to be suitable for inclusion in diets at 20% for the omnivorous Silver Perch or at 16% for the carnivorous Barramundi. The reduced performance of Acti-Meal for dietary application was more pronounced in Barramundi than Silver Perch. Disparities in performance may stem from the fact that the non-starch polysaccharide component of Acti-Meal results in limitations in energy availability for both species, more so for the carnivorous Barramundi. This outcome is in complete contrast to results reported for the herbivorous Greenlip Abalone where inclusion contents of up to 20% Acti-Meal resulted in improved growth and feed efficiency (Currie et al., 2019). Based on results from the current study, where weight gain of Silver Perch fed the 20% Acti-Meal diet was only reduced by 15.3%, we recommend further research to investigate the suitability of lower graded dietary inclusion levels of Acti-Meal (0-15%) in diets for this species. Due to the larger reduction in weight gain (45.1%) when Acti-Meal was included at a lower level of 16%, further research with Acti-Meal and Barramundi is not recommended. Overall, Acti-Meal may be more suitable for inclusion in aquafeeds for herbivorous and omnivorous species, as opposed to carnivorous species.

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APPENDIX

Appendix 1: Analysed composition of the three grape marc meals

Table A1. Analysed composition of the three grape marc meals.

Item	Method	Units	Red meal	White Meal 1	White Grape meal 2
Proximates					
Moisture	VL298	g/100g	7.7	5.4	11.1
Fat (Mojonnier extraction)	VL302	g/100g	11.2	8.6	14.9
Saturated Fat	VL289	g/100g	1.5	1.4	1.9
Protein (N x 6.25)	VL299	g/100g	15.4	13.8	13.5
Ash	VL286	g/100g	8.5	8.3	4
Carbohydrates	VL412	g/100g	57	64	57
Energy (kj)	VL412	kJ/100g	1640	1640	1750
Amino Acids					
Aspartic Acid	VL450	mg/kg	12000	10000	9900
Serine	VL450	mg/kg	6500	5900	5600
Glutamic Acid	VL450	mg/kg	24000	17000	26000
Glycine	VL450	mg/kg	7200	6900	8400
Histidine	VL450	mg/kg	2700	3100	3000
Arginine	VL450	mg/kg	5800	7600	8200
Threonine	VL450	mg/kg	5400	5500	3900
Alanine	VL450	mg/kg	6800	6100	5200
Proline	VL450	mg/kg	6800	7300	5300
Tyrosine	VL450	mg/kg	3400	3500	3300
Valine	VL450	mg/kg	6600	5500	5300
Lysine	VL450	mg/kg	4500	6500	3900
Isoleucine	VL450	mg/kg	5300	4700	4400
Leucine	VL450	mg/kg	9500	8500	7900
Phenylalanine	VL450	mg/kg	5500	5400	4900
Methionine	VL450	mg/kg	1700	1900	1700
Hydroxyproline	VL450	mg/kg	860	800	280
Taurine	VL450	mg/kg	<50	<50	<50
Aspartic Acid	VL450	% of protein	7.79	6.49	6.43
Serine	VL450	% of protein	4.22	3.83	3.64
Glutamic Acid	VL450	% of protein	15.58	11.04	16.88
Glycine	VL450	% of protein	4.68	4.48	5.45
Histidine	VL450	% of protein	1.75	2.01	1.95
Arginine	VL450	% of protein	3.77	4.94	5.32
Threonine	VL450	% of protein	3.51	3.57	2.53
Alanine	VL450	% of protein	4.42	3.96	3.38
Proline	VL450	% of protein	4.42	4.74	3.44
Tyrosine	VL450	% of protein	2.21	2.27	2.14
Valine	VL450	% of protein	4.29	3.57	3.44
Lysine	VL450	% of protein	2.92	4.22	2.53
Isoleucine	VL450	% of protein	3.44	3.05	2.86
Leucine	VL450	% of protein	6.17	5.52	5.13
Phenylalanine	VL450	% of protein	3.57	3.51	3.18
Methionine	VL450	% of protein	1.10	1.23	1.10
Hydroxyproline	VL450	% of protein	0.56	0.52	0.18
Trace Elements					
Calcium	VL247	mg/kg	7200	7600	6200
Copper	VL247	mg/kg	36	50	19
Iron	VL247	mg/kg	330	720	220
Magnesium	VL247	mg/kg	1400	1200	1700
Manganese	VL247	mg/kg	17	18	18
Phosphorus	VL247	mg/kg	3500	3000	3500
Potassium	VL247	mg/kg	29000	26000	9500
Selenium	VL247	mg/kg	0.065	0.064	0.033
Zinc	VL247	mg/kg	20	23	21

Table A1. Analysed composition of the three grape marc meals (Continued).

Item	Method	Units	Red meal	White Meal 1	White Grape meal 2
Fatty acid profile (% total fatty acid)					
Saturated Fatty Acids					
C4:0 Butyric	VL289	%	<0.1	<0.1	<0.1
C6:0 Caproic	VL289	%	<0.1	<0.1	<0.1
C8:0 Caprylic	VL289	%	<0.1	<0.1	<0.1
C10:0 Capric	VL289	%	0.1	0.2	0.1
C12:0 Lauric	VL289	%	<0.1	<0.1	<0.1
C14:0 Myristic	VL289	%	0.1	0.2	0.1
C15:0 Pentadecanoic	VL289	%	<0.1	<0.1	<0.1
C16:0 Palmitic	VL289	%	8.1	10.1	8
C17:0 Margaric	VL289	%	<0.1	0.1	<0.1
C18:0 Stearic	VL289	%	4.6	5.2	4.7
C20:0 Arachidic	VL289	%	0.3	0.5	0.2
C22:0 Behenic	VL289	%	0.1	0.3	<0.1
C24:0 Lignoceric	VL289	%	<0.1	0.2	<0.1
Total Saturated	VL289	%	13.7	17	13.5
Mono-unsaturated Fatty Acids					
C14:1 Myristoleic	VL289	%	<0.1	<0.1	<0.1
C16:1 Palmitoleic	VL289	%	0.3	0.6	0.2
C17:1 Heptadecenoic	VL289	%	<0.1	<0.1	<0.1
C18:1 Oleic	VL289	%	16.9	17.7	16.5
C20:1 Eicosenic	VL289	%	0.2	0.2	0.2
C22:1 Docosenoic	VL289	%	<0.1	<0.1	<0.1
C24:1 Nervonic	VL289	%	<0.1	<0.1	<0.1
Total Mono-unsaturated	VL289	%	17.4	18.4	16.9
Poly-unsaturated Fatty Acids					
C18:2w6 Linoleic	VL289	%	66.7	61.5	68
C18:3w6 gamma-Linolenic	VL289	%	<0.1	<0.1	<0.1
C18:3w3 alpha-Linolenic	VL289	%	0.9	1.5	0.5
C20:2w6 Eicosadienoic	VL289	%	<0.1	<0.1	<0.1
C20:3w6 Eicosatrienoic	VL289	%	<0.1	<0.1	<0.1
C20:3w3 Eicosatrienoic	VL289	%	<0.1	<0.1	<0.1
C20:4w6 Arachidonic	VL289	%	<0.1	<0.1	<0.1
C20:5w3 Eicosapentaenoic	VL289	%	<0.1	<0.1	<0.1
C22:2w6 Docosadienoic	VL289	%	<0.1	<0.1	<0.1
Omega 3 Fatty Acids	VL289	%	1.1	1.8	0.6
Omega 6 Fatty Acids	VL289	%	66.8	61.6	68
C22:4w6 Docosatetraenoic	VL289	%	<0.1	<0.1	<0.1
C22:5w3 Docosapentaenoic	VL289	%	<0.1	0.1	<0.1
C22:6w3 Docosahexaenoic	VL289	%	<0.1	<0.1	<0.1
Total Poly-unsaturated	VL289	%	67.8	63.4	68.6
Total Mono Trans Fatty Acids	VL289	%	<0.1	<0.1	<0.1
Total Poly Trans Fatty Acids	VL289	%	0.2	0.2	0.1
P:M:S Ratio	VL289		01:03.3	3.7:1.1:1	5.1:1.3:1

Table A1. Analysed composition of the three grape marc meals (Continued).

Item	Method	Units	Red meal	White Meal 1	White Grape meal 2
Fatty acid profile (g/100g)					
Saturated Fatty Acids					
C4:0 Butyric	VL289	g/100g	-	-	-
C6:0 Caproic	VL289	g/100g	-	-	-
C8:0 Caprylic	VL289	g/100g	-	-	-
C10:0 Capric	VL289	g/100g	0.0	0.0	0.0
C12:0 Lauric	VL289	g/100g	-	-	-
C14:0 Myristic	VL289	g/100g	0.0	0.0	0.0
C15:0 Pentadecanoic	VL289	g/100g	-	-	-
C16:0 Palmitic	VL289	g/100g	0.9	0.9	1.2
C17:0 Margaric	VL289	g/100g	-	0.0	-
C18:0 Stearic	VL289	g/100g	0.5	0.4	0.7
C20:0 Arachidic	VL289	g/100g	0.0	0.0	0.0
C22:0 Behenic	VL289	g/100g	0.0	0.0	-
C24:0 Lignoceric	VL289	g/100g	-	0.0	-
Total Saturated	VL289	g/100g	1.5	1.5	2.0
Mono-unsaturated Fatty Acids					
C14:1 Myristoleic	VL289	g/100g	-	-	-
C16:1 Palmitoleic	VL289	g/100g	0.0	0.1	0.0
C17:1 Heptadecenoic	VL289	g/100g	-	-	-
C18:1 Oleic	VL289	g/100g	1.9	1.5	2.5
C20:1 Eicosenic	VL289	g/100g	0.0	0.0	0.0
C22:1 Docosenoic	VL289	g/100g	-	-	-
C24:1 Nervonic	VL289	g/100g	-	-	-
Total Mono-unsaturated	VL289	g/100g	1.9	1.6	2.5
Poly-unsaturated Fatty Acids					
C18:2w6 Linoleic	VL289	g/100g	7.5	5.3	10.1
C18:3w6 gamma-Linolenic	VL289	g/100g	-	-	-
C18:3w3 alpha-Linolenic	VL289	g/100g	0.1	0.1	0.1
C20:2w6 Eicosadienoic	VL289	g/100g	-	-	-
C20:3w6 Eicosatrienoic	VL289	g/100g	-	-	-
C20:3w3 Eicosatrienoic	VL289	g/100g	-	-	-
C20:4w6 Arachidonic	VL289	g/100g	-	-	-
C20:5w3 Eicosapentaenoic	VL289	g/100g	-	-	-
C22:2w6 Docosadienoic	VL289	g/100g	-	-	-
Omega 3 Fatty Acids	VL289	g/100g	0.1	0.2	0.1
Omega 6 Fatty Acids	VL289	g/100g	7.5	5.3	10.1
C22:4w6 Docosatetraenoic	VL289	g/100g	-	-	-
C22:5w3 Docosapentaenoic	VL289	g/100g	-	0.0	-
C22:6w3 Docosahexaenoic	VL289	g/100g	-	-	-
Total Poly-unsaturated	VL289	g/100g	7.6	5.5	10.2
Total Mono Trans Fatty Acids	VL289	g/100g	-	-	-
Total Poly Trans Fatty Acids	VL289	g/100g	0.0	0.0	0.0
P:M:S Ratio	VL289		01:03.3	3.7:1.1:1	5.1:1.3:1
Other					
Mono trans fats	VL289	g/100g	<0.1	<0.1	<0.1
Mono-unsaturated fat	VL289	g/100g	1.9	1.5	2.4
Omega 3 fats	VL289	g/100g	0.1	0.1	<0.1
Omega 6 fats	VL289	g/100g	7.2	5.1	9.7
Poly trans fats	VL289	g/100g	<0.1	<0.1	<0.1
Poly-unsaturated fat	VL289	g/100g	7.3	5.2	9.8
Trans fats	VL289	g/100g	<0.1	<0.1	<0.1

Appendix 2: Analysed composition of the Silver Perch and Barramundi test diets

Table A2. Analysed composition of Silver Perch and Barramundi diets.

Item	Method	Units	Silver Perch Diet 1 Control	Silver Perch Diet 2 20% Acti-Meal	Barramundi Diet 3 Control	Barramundi Diet 4 16% Acti-Meal
Proximate composition						
Moisture	VL298	g/100g	8.5	7.8	8.2	8.4
Fat (Mojonnier extraction)	VL302	g/100g	11.5	10.9	13.1	11.4
Protein (N x 6.25)	VL299	g/100g	39.5	38.7	45.6	44.9
Ash	VL286	g/100g	9.5	12.6	9.7	12.9
Carbohydrates	VL412	g/100g	31	30	23	22
Energy (kj)	VL412	kJ/100g	1620	1570	1650	1560
Amino Acids						
Aspartic Acid	VL450	mg/kg	25000	24000	30000	29000
Serine	VL450	mg/kg	16000	16000	19000	19000
Glutamic Acid	VL450	mg/kg	56000	52000	60000	56000
Glycine	VL450	mg/kg	22000	22000	27000	28000
Histidine	VL450	mg/kg	8800	7300	9300	8900
Arginine	VL450	mg/kg	22000	21000	27000	25000
Threonine	VL450	mg/kg	14000	13000	17000	17000
Alanine	VL450	mg/kg	15000	14000	18000	18000
Proline	VL450	mg/kg	19000	18000	21000	21000
Tyrosine	VL450	mg/kg	9300	8800	11000	11000
Valine	VL450	mg/kg	13000	13000	16000	16000
Lysine	VL450	mg/kg	19000	18000	22000	21000
Isoleucine	VL450	mg/kg	12000	11000	14000	14000
Leucine	VL450	mg/kg	23000	22000	28000	27000
Phenylalanine	VL450	mg/kg	14000	13000	17000	17000
Methionine	VL450	mg/kg	9600	8700	10000	9700
Hydroxyproline	VL450	mg/kg	3900	3900	4300	5800
Taurine	VL450	mg/kg	7800	7200	7700	7600
Minerals						
Calcium	VL247	mg/kg	22000	29000	22000	31000
Copper	VL247	mg/kg	9.5	16	7.4	14
Iron	VL247	mg/kg	170	270	190	280
Magnesium	VL247	mg/kg	1300	1700	1500	1800
Manganese	VL247	mg/kg	280	270	360	130
Phosphorus	VL247	mg/kg	14000	18000	16000	19000
Potassium	VL247	mg/kg	9100	15000	9500	13000
Selenium	VL247	mg/kg	1.6	1.1	1.2	1.1
Sodium	VL247	mg/kg	300	250	320	270
Zinc	VL247	mg/kg	100	92	98	100
Iodine	VL345	mg/kg	0.94	1	1.3	1.4

Table A2. Analysed composition of Silver Perch and Barramundi diets (Continued).

Item	Method	Units	Silver Perch	Silver Perch	Barramundi	Barramundi
			Diet 1	Diet 2	Diet 3	Diet 4
			Control	20% Acti-Meal	Control	16% Acti-Meal
Saturated Fatty Acids						
C4:0 Butyric	VL289	%	<0.1	<0.1	<0.1	<0.1
C6:0 Caproic	VL289	%	<0.1	<0.1	<0.1	<0.1
C8:0 Caprylic	VL289	%	<0.1	<0.1	<0.1	<0.1
C10:0 Capric	VL289	%	0.3	0.3	0.3	0.3
C12:0 Lauric	VL289	%	0.1	0.1	0.1	0.1
C14:0 Myristic	VL289	%	2.1	2.2	2.1	2.1
C15:0 Pentadecanoic	VL289	%	0.4	0.4	0.4	0.4
C16:0 Palmitic	VL289	%	16	16.6	17.2	18.7
C17:0 Margaric	VL289	%	0.5	0.3	0.4	0.3
C18:0 Stearic	VL289	%	4.1	3.7	4.2	4.3
C20:0 Arachidic	VL289	%	0.5	0.5	0.5	0.4
C22:0 Behenic	VL289	%	0.5	0.6	0.6	0.3
C24:0 Lignoceric	VL289	%	0.2	0.3	0.2	<0.1
Total Saturated	VL289	%	24.8	25	26	27
Mono-unsaturated Fatty Acids						
C14:1 Myristoleic	VL289	%	<0.1	<0.1	<0.1	0.1
C16:1 Palmitoleic	VL289	%	3.6	4	4.1	5
C17:1 Heptadecenoic	VL289	%	<0.1	<0.1	<0.1	<0.1
C18:1 Oleic	VL289	%	26	28.8	29.2	31
C18:1 Vaccenic	VL289	%	2.4	2.7	2.5	2.7
C20:1 Eicosenic	VL289	%	3.8	5	3.8	4.3
C22:1 Cetoleic	VL289	%	1.4	2	1.4	1.8
C22:1 Docosenoic	VL289	%	0.7	1	0.1	0.8
C24:1 Nervonic	VL289	%	0.6	0.7	0.6	0.7
Total Mono-unsaturated	VL289	%	38.6	44.3	42.3	46.4
Poly-unsaturated Fatty Acids						
C18:2w6 Linoleic	VL289	%	22.3	16.3	19.7	13.7
C18:3w6 gamma-Linolenic	VL289	%	<0.1	<0.1	<0.1	<0.1
C18:3w3 alpha-Linolenic	VL289	%	1.8	2.3	2	1.7
C20:2w6 Eicosadienoic	VL289	%	0.2	0.2	0.2	0.2
C20:3w6 Eicosatrienoic	VL289	%	<0.1	<0.1	<0.1	0.1
C20:3w3 Eicosatrienoic	VL289	%	0.1	0.2	0.1	0.1
C20:4w6 Arachidonic	VL289	%	0.5	0.5	0.4	0.5
C20:5w3 Eicosapentaenoic	VL289	%	2.6	2.9	2.1	2.6
C22:2w6 Docosadienoic	VL289	%	<0.1	<0.1	<0.1	<0.1
Omega 3 Fatty Acids	VL289	%	12.1	12.2	9.9	10.7
Omega 6 Fatty Acids	VL289	%	23.3	17.3	20.6	14.7
C22:4w6 Docosatetraenoic	VL289	%	0.1	0.1	<0.1	0.1
C22:5w3 Docosapentaenoic	VL289	%	0.9	1.1	0.8	0.9
C22:6w3 Docosahexaenoic	VL289	%	6.3	5.5	4.6	4.9
Total Poly-unsaturated	VL289	%	35.7	29.8	30.8	25.8
Total Mono Trans Fatty Acids	VL289	%	0.2	0.2	0.2	0.2
Total Poly Trans Fatty Acids	VL289	%	0.8	0.6	0.8	0.6
P:M:S Ratio	VL289		1.4:1.6:1	1.2:1.8:1	1.2:1.6:1	1.0:1.7:1
Others						
Saturated Fat	VL289	g/100g	2.9	2.7	3.4	3.1
Mono trans fats	VL289	g/100g	<0.1	<0.1	<0.1	<0.1
Mono-unsaturated fat	VL289	g/100g	4.4	4.8	5.5	5.3
Omega 3 fats	VL289	g/100g	1.4	1.3	1.3	1.2
Omega 6 fats	VL289	g/100g	2.7	1.9	2.7	1.7
Poly trans fats	VL289	g/100g	<0.1	<0.1	0.1	<0.1
Poly-unsaturated fat	VL289	g/100g	4.1	3.2	4	2.9
Trans fats	VL289	g/100g	0.1	<0.1	0.1	<0.1