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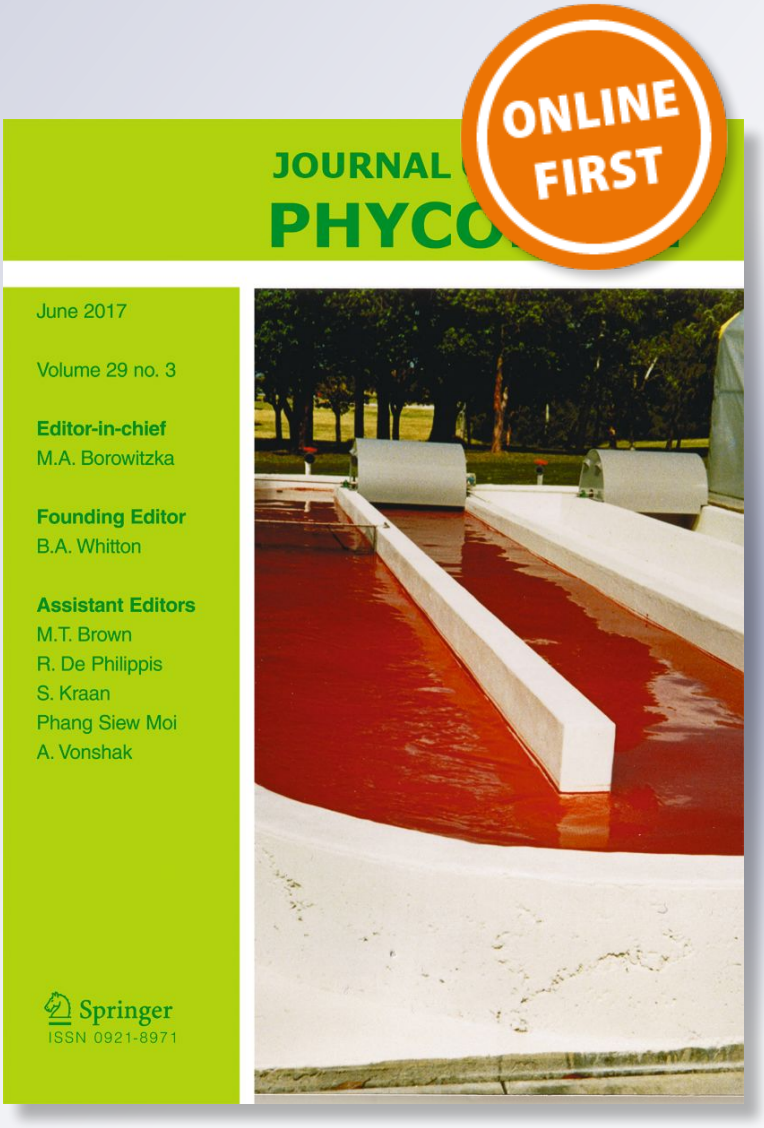

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Green seaweed *Ulva* sp. as an alternative ingredient in plant-based practical diets for Pacific white shrimp, *Litopenaeus vannamei*

X. Qiu[1] · A. Neori[2,3] · J. K. Kim[4,5] · C. Yarish[4] · M. Shpigel[6] · L. Guttman[2] · D. Ben Ezra[2] · V. Odintsov[2] · D. A. Davis[1]

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Abstract In our previous research, the supplementation of *Ulva* sp. seaweed meal in shrimp feeds as a replacement for fish meal (FM) resulted in growth depression. To understand the factors causing the growth reduction and explore the effects of the seaweed meal as a substitution for soybean meal (SBM), a series of growth trials were conducted in the present study. Shrimp (initial mean weight 0.24, 0.15, and 0.98 g in trials 1–3, respectively) were stocked at 10 shrimp per tank ($n = 4$) and offered diets for 5 to 6 weeks. In trial 1, FM level was fixed and SBM was replaced using incremental level of the second batch *Ulva* meal (UM2). Two additional diets were formulated to allow comparison of high inclusion levels of seaweed meal from three batches (UM1–3). Results confirmed reductions in performance as replacement of SBM by *Ulva* meal was increased. This data also demonstrated significant difference between batches of the *Ulva* meal with the UM2 producing the poorest results. To elucidate if digestible protein was limiting growth, in trial 2 feeds were formulated on an equal digestible protein basis. At the end of trial 2, shrimp fed with diets containing UM2 exhibited significantly reduced growth performance, survival, and lipid content of whole shrimp body as well as increased feed conversion ratio (FCR) compared to the reference diet. Although performance of shrimp was depressed in the treatments containing UM1 and UM3, this was less than that of trial 2, indicating that protein quality may be part of the problem. Given the level of protein replacement, other components of *Ulva* meal are likely to be causing poor performance. A third trial was performed to evaluate the potential of the fourth batch *Ulva* meal (UM4) containing relatively higher protein content than the first three batches. In this trial, the growth, survival, and lipid content of whole shrimp body also decreased as the level of UM4 was increased. To survey possible problems caused by high levels of minerals, the meals and select diets were analyzed for mineral content. Clearly there are shifts in mineral profiles; however, there is no obvious correlation to a mineral. Other possible reasons would include anti-nutrients present in the algae. If *Ulva* meals are to be used to their full potential, e.g., as a primary protein source, the anti-nutritional components will need to be identified, specific lines of plants with enhanced nutrient value need to be developed and of course processing technologies evaluated to produce a high quality commercial product.

Keywords Anti-nutritional components · Pacific white shrimp · Protein quality · Soybean meal replacement · *Ulva* sp.

✉ X. Qiu
xzq0006@auburn.edu

[1] School of Fisheries, Aquaculture and Aquatic Science, Auburn University, 203 Swingle Hall, Auburn, AL 36849, USA

[2] National Center for Mariculture, Israel Oceanographic and Limnological Research, 8811201 Eilat, Israel

[3] Spanish Bank of Algae, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain

[4] Department of Ecology & Evolutionary Biology and Department of Marine Sciences, University of Connecticut, 1 University Place, Stamford, CT 06901-2315, USA

[5] Department of Marine Science, School of Natural Sciences, Incheon National University, 119 Academy-ro, Yeonsu-gu, Incheon 22012, South Korea

[6] The Interuniversity Institute for Marine Sciences, Eilat, Israel

Introduction

Sustainable alternative ingredients in the shrimp feeds are necessary to support the rapid expansion of the shrimp

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industry (Qiu and Davis 2017a). Seaweed meals have emerged as candidates for utilization as a protein source in aquaculture feeds due to their good contents of amino acids, fatty acids, minerals, vitamins, carotenoid pigments, and bioactive compounds (Cruz-Suárez et al. 2010). The cultivation of seaweeds can bring some collateral benefits, such as ecosystem services, in the form of using waste nutrients produced by human activities (intensive aquaculture, agriculture, and animal operations and even municipal waste treatment) (Ryther et al. 1975; Kaushik 2010). In an integrated shrimp cultivation system, the seaweed use the metabolic residues of animals as nutrients, absorb CO_2, and produce O_2 for the environment (Marinho-Soriano et al. 2007). Meanwhile, seaweeds can also serve as a food source for shrimp. As a consequence, significant improvements in growth and survival rate have been observed when Pacific white shrimp, *Litopenaeus vannamei* (Cruz-Suárez et al. 2010; Brito et al. 2014a, b), giant tiger shrimp, *Penaeous monodon* (Tsutsui et al. 2010; Izzati 2012), and yellowleg shrimp, *Farfantepenaeus californiensis* (Portillo-Clark et al. 2012) are co-cultured with seaweeds in integrated systems.

The chemical composition of seaweeds varies, depending on species, environmental factors (water temperature, salinity, light, nutrient loading), geographical distribution and the season (Cruz-Suárez et al. 2010). Under high nutrient-enriched conditions such as the effluents of fish or shrimp farms where seaweeds are used as bio-filters, chemical compositions such as protein content, lipid content, and tissue pigmentation of seaweeds can be enhanced (Lahaye et al. 1995; Pinchetti et al. 1998). The meals used in this research were produced under experimental conditions with the intent of producing a high protein *Ulva* meal (Qiu 2017). The protein content of the fourth batch *Ulva* meal (UM4) was enhanced to 38.16% in the present study, resulting in high levels of amino acids in this batch.

In general, a number of studies demonstrated that low levels (≤ 5% of the diet) inclusion of seaweeds as protein ingredients did not result in poor performances in both freshwater fish including African catfish *Clarias gariepinus* (Abdel-Warith et al. 2016; Al-Asgah et al. 2016), Nile tilapia (Güroy et al. 2007; Marinho et al. 2013; Valente et al. 2016), red tilapia *Oreochromis* sp. (El-Tawil 2010) and rainbow trout (Soler-Vila et al. 2009; Güroy et al. 2013) and in marine fish and shrimp such as European sea bass (Valente et al. 2006), gilthead seabream *Sparus aurata* (Emre et al. 2013), and Pacific white shrimp (Rodríguez-González et al. 2014; Cárdenas et al. 2015). However, when higher levels of *Ulva* meal were evaluated most of the forementioned authors identified significant depressions in performance of the fish and shrimp.

In our previous research, dietary supplementation of *Ulva* sp. (probably *U. lactuca*) in shrimp feeds as a replacement for FM depressed their performance. As the substitution of FM caused numerous shifts in nutrients as well as possible palatability changes of the diets, we chose in this study to maintain a constant FM level and replace only the soybean meal (SBM) fraction, as an approach that attained smaller changes in nutrient content between the diets. Therefore, the purposes of this study were to evaluate the biological responses of Pacific white shrimp to the inclusion of dietary *Ulva* sp. of different meal batches of different quality and thereby explore the potential problems that this meal might possess.

Material and methods

Ingredients

Four different batches *Ulva* meals (UM1–4) were obtained in the present study. UM1, UM2, and UM4 (*Ulva* sp. probably *U. lactuca* L) were obtained from National Center for Mariculture, Israel Oceanographic and Limnological Research, Eilat, Israel. UM3 (*Ulva compressa*) was obtained from Department of Ecology & Evolutionary Biology and Department of Marine Sciences, University of Connecticut, Connecticut, USA. The *Ulva* sp. and *U. compressa* meal were produced by the following three approaches: (1) UM1 and UM2: *Ulva* sp. was brought in from a neighboring algal pond, and grown on a spray-drip irrigated system, modified slightly from that in Msuya and Neori (2010). Briefly, the algae were placed on several 1 m^2 plywood boards and held by plastic 4-mm mesh netting, creating flat square mattresses 3 to 5–cm thick, inclined from the horizontal at 5.7° due West. The boards were each placed above a plastic tank unit, to which water was drained. Water from fishponds, enriched with additional nutrients to a level of 10 g ammonia-N (TAN), 15 g nitrate-N and 2 g phosphate-P m^{-2} day^{-1} (plus micronutrients, from Shefer 7–3-7 + 3, Fertilizers & Chemicals, Haifa, Israel), was dripped onto the seaweed mattresses by perforated plastic pipes at the tops of the boards. Each of the units received about 5 (±5%) m^3 m^{-2} day^{-1} of water. (2) UM3: *Ulva compressa* was cultivated in indoor tank systems in a nutrient enrich environment with a commercial fertilizer (Kim and Yarish 2014). (3) UM4: this high protein *Ulva* sp. was cultured in a nutrient-enriched pond, as described in Shpigel and Neori (2007). Briefly, a 20 by 5 m pond, bottom-aerated, received over four full volume replacements day^{-1} of fishpond effluents, and was in addition enriched by N and P (at rates of 10 g TAN m^{-2} day^{-1} and 1 g phosphate-P m^{-2} day^{-1}, respectively). After a week or 10 days, the algae were harvested by net, washed with freshwater and dried in the shade for several days, before being crushed and packed for shipping.

Table 1 Proximate composition, phosphorus content, and amino acid profile of the fish meal (FM), soybean meal (SBM), and four batches *Ulva* meal (UM1, 2, 3, and 4)

Composition (% as is)	UM1	UM2	UM3	UM4	FM	SBM
Crude protein	20.64	27.24	26.80	38.16	62.78	44.89
Moisture	8.89	13.74	11.19	8.41	7.99	10.97
Crude fat	0.53	0.12	0.42	0.10	10.56	3.78
Crude fiber	5.17	2.93	4.07	5.57	0.00	3.20
Ash	46.01	22.18	20.31	13.49	18.75	6.67
Phosphorus	0.43	0.30	–	0.42	3.15	0.66
Alanine	1.64	2.03	1.89	2.68	3.91	2.04
Arginine	0.99	1.39	1.01	1.77	3.68	3.35
Aspartic acid	2.12	2.67	3.23	3.46	5.34	5.10
Cysteine	0.34	0.39	0.46	0.49	0.47	0.62
Glutamic acid	2.02	2.59	3.02	3.35	7.47	8.24
Glycine	1.17	1.59	1.29	2.00	4.88	2.04
Histidine	0.25	0.40	0.22	0.45	1.63	1.2
Hydroxylysine	0.17	0.12	0.10	0.21	0.2	0.05
Hydroxyproline	0.2	0.30	0.38	0.35	1.03	0.05
Isoleucine	0.8	1.06	0.92	1.39	2.42	2.17
Leucine	1.22	1.87	1.50	2.43	4.21	3.57
Lysine	0.95	1.22	0.82	1.51	4.67	3.06
Methionine	0.26	0.44	0.46	0.63	1.61	0.66
Phenylalanine	0.98	1.37	1.16	1.78	2.39	2.35
Proline	0.76	1.17	1.02	1.50	3.08	2.39
Serine	0.91	1.05	0.93	1.47	2.11	1.90
Taurine	0.15	0.18	0.18	0.18	0.73	0.13
Threonine	0.94	1.17	1.13	1.56	2.41	1.75
Tryptophan	0.16	0.20	0.22	0.266	0.62	0.62
Tyrosine	0.48	0.77	0.49	0.94	1.67	1.64
Valine	1.17	1.56	1.40	2.13	2.99	2.34

Ingredients were analyzed at University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA)

Experimental design and diets

Primary ingredients and pooled batches of sun dried *Ulva* meals were analyzed at University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acids profile (Table 1). Trial 1 was performed to investigate the UM2 as a replacement for SBM and determine if there was a difference in the quality of different batches of *Ulva* meals (UM1–3). All the test diets were formulated to be isonitrogenous and isolipidic (35% protein and 8% lipid) in trial 1. Nine experimental diets were formulated (Table 2). The basal diet for this and subsequent trials was designed to have 6% fish meal (FM) in all formulations to help stabilize nutrients as well as palatability. The first seven diets utilized increasing levels (0, 5, 10, 15, 20, 25, and 30%) of the second batch of *Ulva* meal (UM2) to replace SBM. In addition, the last two diets contained high incorporation levels of *Ulva* meal from the first (UM1) and third (UM3) batch, respectively, which allowed a comparison of all three meals at equivalent levels of SBM replacement. To elucidate if digestible protein was limiting growth, trial 2 was initiated for which feeds were formulated on a digestible protein basis. Four experimental diets were formulated using the first three batches of *Ulva* meals (UM1–3) replacing SBM based on the ratio of protein digestibility of SBM and *Ulva* meals (Table 3). The protein digestibility of SBM, UM1, and UM2 were 97.03, 15.17, and 43.51%, respectively (Qiu 2017; Qiu et al. 2017). The UM3 shared similar compositions as UM2, and the protein digestibility of UM3 were estimated based on that of UM2. Trial 3 was conducted to evaluate the fourth batch Ulva meal (UM4) containing a high protein content as an alternative to FM or SBM. In this trial, five experimental diets were designed, utilizing different levels of (0, 4.75, 9.5, 12, and 24%) of UM4 as a replacement for FM or SBM on equal protein and lipid basis (35% protein and 8% lipid) (Table 4).

All experimental diets were produced at the Aquatic Animal Nutrition Laboratory at the School of Fisheries,

Table 2 Formulation of test diets utilized in trial 1

Ingredient (% as is basis)	T_1D_1	T_1D_2	T_1D_3	T_1D_4	T_1D_5	T_1D_6	T_1D_7	T_1D_8	T_1D_9
Fish meal[1]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal[2]	55.55	52.55	49.60	46.60	43.75	40.80	37.80	43.75	43.75
Corn protein concentrate[3]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Ulva meal 2[11]	0.00	5.00	10.00	15.00	20.00	25.00	30.00	0.00	0.00
Ulva meal 3[11]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	23.60	0.00
Ulva meal 1[11]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	26.30
Fish oil[2]	5.84	5.88	5.92	5.96	6.00	6.04	6.08	6.00	5.89
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C[7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mono-dicalcium phosphate[8]	1.85	1.85	1.90	1.90	1.95	1.95	2.00	1.90	1.70
Lecithin[9]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Methionine[10]	0.07	0.06	0.06	0.06	0.05	0.05	0.04	0.03	0.07
Corn starch[4]	15.04	13.01	10.87	8.83	6.60	4.51	2.43	3.07	0.64

[1] Omega Protein Inc., Houston TX, USA

[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[3] Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[4] MP Biomedicals Inc., Solon, OH, USA

[5] Trace mineral premix (g $(100\ g)^{-1}$ premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664

[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCL, 4.95; Riboflavin, 3.83; Pyridoxine.HCL, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU g^{-1}), 0.32; Vitamin D3 (1,000,000 IU g^{-1}), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81

[7] Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA

[8] J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA

[9] The Solae Company, St. Louis, MO, USA

[10] Sigma-Aldrich, St. Louis, MO, USA

[11] Three batches of *Ulva* meal experimentally produced

Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL, USA) using the standard procedures for the shrimp feeds (Qiu and Davis 2016). Briefly, diets were prepared by mixing the pre-ground dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 min. Hot water was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were pressure-pelleted using a meat grinder with a 2.5-mm die. The wet pellets were then placed into a fan-ventilated oven (< 50 °C) overnight in order to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer until use. The diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA), Midwest Laboratories (Omaha, NE, USA), or Soil Laboratories (Auburn, AL, USA) for proximate composition, amino acid profile, and mineral contents (Tables 5, 6, 7, and 8).

Growth trials

The growth trials were conducted at the E.W. Shell Fisheries Research Station, Auburn University (Auburn, AL, USA). Pacific white shrimp post larvae (PL) were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system. PLs were fed a commercial feed (Zeigler Bros., Inc., Gardners, PA, USA) using an automatic feeder for ~ 1 week, and then switched to crumbled commercial shrimp feed (Zeigler Bros., Inc) for ~ 1–2 weeks.

In all trials, the recirculating system consisted of 36 aquaria (135 L) connected to a common reservoir, biological filter, bead filter, fluidized biological filter and recirculation pump. In trial 1, there were nine treatments with four replicate groups of shrimp (0.24 g initial mean weight, 10 shrimp per tank) in each treatment which were offered

Table 3 Formulation of test diets utilized in trial 2

Ingredient (% as is)	T_2D_1	T_2D_2	T_2D_3	T_2D_4
Fish meal[1]	6.00	6.00	6.00	6.00
Soybean meal[2]	53.00	49.90	46.30	46.30
Corn protein concentrate[3]	8.00	8.00	8.00	8.00
Ulva meal 1[10]		22.00		
Ulva meal 2[10]			25.00	
Ulva meal 3[10]				25.00
Fish oil[2]	5.92	5.85	5.98	5.91
Trace mineral premix[5]	0.50	0.50	0.50	0.50
Vitamin premix[6]	1.80	1.80	1.80	1.80
Choline chloride[4]	0.20	0.20	0.20	0.20
Stay C[7]	0.10	0.10	0.10	0.10
Mono-dicalcium phosphate[8]	2.50	2.50	2.50	2.50
Lecithin[9]	1.00	1.00	1.00	1.00
Cholesterol[4]	0.08	0.08	0.08	0.08
Methionine[11]	0.05	0.04	0.04	0.04
Lyisine[11]	0.00	0.00	0.07	0.11
Corn starch[4]	20.85	2.03	2.43	2.46

[1] Omega Protein Inc., Houston TX, USA

[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[3] Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[4] MP Biomedicals Inc., Solon, OH, USA

[5] Trace mineral premix (g (100 g)$^{-1}$ premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664

[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCL, 4.95; Riboflavin, 3.83; Pyridoxine.HCL, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU g^{-1}), 0.32; Vitamin D3 (1,000,000 IU g^{-1}), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81

[7] Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA

[8] J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA

[9] The Solae Company, St. Louis, MO, USA

[10] Three batches *Ulva* meal experimentally produced

[11] Aldrich-Sigma, St. Louis, MO, USA

Table 4 Formulation of test diets utilized in trial 3

Ingredient (% as is)	T_3D_1	T_3D_2	T_3D_3	T_3D_4	T_3D_5
Fish meal[1]	6.00	3.00	0.00	6.00	6.00
Soybean meal[2]	53.00	53.00	53.00	43.00	33.00
Corn protein concentrate[3]	8.00	8.00	8.00	8.00	8.00
Ulva meal 4[11]	0.00	4.75	9.50	12.00	24.00
Fish oil[2]	5.92	6.19	6.45	6.05	6.18
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	1.80	1.80	1.80	1.80	1.80
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20
Stay C[7]	0.10	0.10	0.10	0.10	0.10
Mono-dicalcium phosphate[8]	2.50	2.90	3.10	2.60	2.60
Lecithin[9]	1.00	1.00	1.00	1.00	1.00
Cholesterol[4]	0.08	0.08	0.08	0.08	0.08
Lyisine [10]	0.00	0.07	0.13	0.11	0.22
Methionine[10]	0.05	0.10	0.15	0.11	0.17
Corn starch[4]	20.85	18.31	15.99	18.45	16.15

[1] Omega Protein Inc., Houston TX, USA

[2] De-hulled solvent extract soybean meal, Bunge Limited, Decatur, AL, USA

[3] Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[4] MP Biomedicals Inc., Solon, OH, USA

[5] Trace mineral premix (g (100 g)$^{-1}$ premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664

[6] Vitamin premix (g kg^{-1} premix): Thiamin.HCL, 4.95; Riboflavin, 3.83; Pyridoxine.HCL, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU g^{-1}), 0.32; Vitamin D3 (1,000,000 IU g^{-1}), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81

[7] Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA

[8] J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA

[9] The Solae Company, St. Louis, MO, USA

[10] Fourth batch *Ulva* meal experimentally produced

[11] Aldrich-Sigma, St. Louis, MO, USA

diets for 5 weeks. The trial 2 utilized 4 treatments with 4 replicates in each treatment. Juvenile shrimp (initial weight 0.98 ± 0.01 g) were stocked into 16 tanks with 10 shrimp in each aquarium over 6 weeks. The trial 3 contained 5 treatments with 4 replicates in each treatment. Juvenile shrimp (initial weight 0.15 ± 0.01 g) were stocked into 20 tanks with 10 shrimp in each aquarium for 6 weeks. A subsample of shrimp from the initial stocking of each trial was retained for whole body analysis to be utilized for later apparent net nutrient retention analysis. During the culturing period of each trial, shrimp were offered diets using our standard feeding protocols. The formula we used to calculate the feed inputs was presented as follows: Daily feed inputs (g) = (Estimated FCR × Expected growth × Number of shrimp) / 7. Based on historic results, feed inputs were pre-programmed assuming the shrimp would double their weight weekly up to 1 g then gain 0.8–1.3 g weekly (expected growth) with an estimated feed conversion ratio (FCR) of 1.8 across three growth trials. Daily allowances of feed were adjusted based on observed feed consumption, weekly counts of the shrimp and mortality. Shrimp were fed four times daily and the time interval of feeding is at least 2 h.

Table 5 Proximate composition, phosphorus content, and amino acid profile of the test diets used in the trial 1

Composition[1] (% as is)	T_1D_1	T_1D_2	T_1D_3	T_1D_4	T_1D_5	T_1D_6	T_1D_7	T_1D_8	T_1D_9
Crude protein	36.46	36.67	35.91	36.78	36.64	36.69	37.46	37.08	36.34
Moisture	7.32	7.92	9.44	7.56	8.29	8.03	6.46	7.74	8.48
Crude fat	10.02	8.49	8.68	9.71	8.90	8.28	6.29	6.35	7.01
Crude fiber	3.54	3.43	3.65	3.64	3.79	4.10	4.09	3.86	3.86
Ash	6.49	7.61	8.47	9.02	10.17	10.47	11.48	10.56	16.77
Phosphorus	0.99	1.01	1.00	1.03	1.01	1.06	1.02	1.00	1.02
Alanine	1.86	1.90	1.91	2.00	2.11	2.05	2.18	2.03	1.99
Arginine	2.30	2.26	2.20	2.22	2.19	2.18	2.24	2.12	2.12
Aspartic acid	3.68	3.62	3.56	3.62	3.61	3.60	3.68	3.72	3.50
Cysteine	0.50	0.47	0.47	0.48	0.48	0.47	0.47	0.50	0.47
Glutamic acid	6.68	6.48	6.25	6.29	6.26	5.98	6.07	6.14	5.95
Glycine	1.64	1.68	1.67	1.69	1.84	1.77	1.86	1.73	1.62
Histidine	0.91	0.88	0.85	0.86	0.84	0.82	0.84	0.79	0.80
Hydroxylysine	0.05	0.05	0.05	0.06	0.07	0.07	0.07	0.06	0.07
Hydroxyproline	0.11	0.10	0.11	0.12	0.18	0.37	0.16	0.22	0.17
Isoleucine	1.64	1.60	1.56	1.60	1.58	1.57	1.61	1.56	1.54
Leucine	3.22	3.14	3.09	3.16	3.20	3.08	3.22	3.03	3.00
Lysine	2.04	1.99	1.95	1.95	1.91	1.92	1.94	1.86	1.86
Methionine	0.70	0.67	0.66	0.67	0.68	0.66	0.67	0.63	0.61
Phenylalanine	1.85	1.82	1.80	1.84	1.86	1.82	1.89	1.79	1.76
Proline	2.09	1.97	2.06	1.96	2.13	2.02	1.99	2.03	2.00
Serine	1.53	1.53	1.47	1.52	1.51	1.49	1.54	1.45	1.46
Taurine	0.14	0.16	0.15	0.15	0.15	0.16	0.17	0.17	0.14
Threonine	1.35	1.35	1.34	1.37	1.38	1.39	1.44	1.37	1.34
Tryptophan	0.50	0.50	0.47	0.48	0.48	0.47	0.49	0.44	0.45
Tyrosine	1.19	1.19	1.14	1.21	1.19	1.17	1.21	1.10	1.13
Valine	1.83	1.81	1.80	1.88	1.88	1.86	1.91	1.84	1.80

[1] Diets were analyzed at University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA)

Dissolved oxygen (DO), water temperature and salinity were measured twice daily by using a YSI 650 multi-parameter instrument (YSI, USA). Hydrogen potential (pH) was measured twice weekly by using a waterproof pHTestr30 (Oakton instrument, USA). Total ammonia-nitrogen (TAN) and nitrite were evaluated every week by using the methods described by Solorzano (1969) and Spotte (1979).

At the termination of each trial, shrimp were counted and group-weighed. Final mean weight, FCR, WG, biomass, and survival were determined. After obtaining the final total weight of shrimps in each aquarium, 4 shrimps from each tank were randomly selected and frozen at − 20 °C for subsequent determination of whole body composition. Amino acid compositions were tested in trial 1 and trial 2. Proximate composition and amino acid profile of whole shrimp body were analyzed by University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA). Apparent net protein retention (ANPR) and apparent amino acid retention (AAAR) was calculated as follows:

$$ANPR\ (\%) = (Final\ weight \times Final\ protein\ content) - (Initial\ weight \times Initial\ protein\ content) \times 100 \Big/ Protein\ offered.$$

$$AAAR\ (\%) = (Final\ weight \times Final\ \mathrm{AA}\ content) - (Initial\ weight \times Initial\ \mathrm{AA}\ content) \times 100 \Big/ \mathrm{AA}\ offered.$$

Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, USA). Data from growth trial and digestibility trial were

Table 6 Mineral profile of the test diets used in the trial 1

Mineral	T_1D_1	T_1D_2	T_1D_3	T_1D_4	T_1D_5	T_1D_6	T_1D_7	T_1D_8	T_1D_9
Quantity elements (g kg^{-1})									
Calcium	8.5	9.7	9.0	9.5	9.1	8.9	9.5	9.3	13.3
Potassium	12.7	13.5	13.7	14.6	14.9	14.9	15.9	18.8	15.5
Magnesium	1.9	3.4	4.7	6.2	7.5	8.7	9.6	4.8	8.3
Sodium	0.8	1.7	2.4	3.3	4.1	4.8	5.7	7.4	14.0
Phosphorus	10.3	10.8	10.2	10.7	10.3	9.9	10.4	10.5	8.3
Sulfur	3.8	5.9	7.7	10.0	11.8	13.6	16.2	10.4	12.0
Trace elements (mg kg^{-1})									
Aluminum	97.8	119.5	133.1	160.8	173.8	185.6	199.1	99.7	1175.4
Arsenic	0.7	0.6	0.4	0.6	0.8	0.9	0.4	0.4	1.0
Boron	17.6	18.6	19.3	20.5	21.5	21.5	23.8	29.7	34.5
Barium	5.1	5.3	5.7	5.1	5.0	4.5	4.6	4.4	8.2
Cadmium	4.5	0.9	13.2	1.2	14.3	12.6	6.5	1.4	13.7
Cobalt	1.1	1.3	1.2	1.2	1.2	1.0	1.1	1.0	1.7
Chromium	1.0	1.0	1.1	1.1	1.1	1.1	1.1	0.9	3.6
Copper	39.4	110.3	21.0	22.9	18.6	23.9	27.1	28.8	23.9
Iron	59.2	43.8	69.4	74.6	66.9	74.4	66.2	48.7	904.5
Manganese	34.2	35.4	34.5	33.6	33.5	32.7	34.1	33.0	61.4
Molybdenum	3.9	4.0	3.4	3.3	3.5	2.5	2.7	3.1	0.1
Nickel	3.1	3.2	3.1	2.6	2.7	2.4	2.2	2.7	4.5
Lead	1.0	1.3	1.7	0.5	1.4	0.5	0.6	0.2	4.0
Selenium	3.3	4.6	2.3	3.1	3.8	4.4	4.8	4.5	4.2
Silicon	57.9	81.0	107.0	120.8	119.9	131.7	131.4	59.9	61.8
Zinc	158.1	165.1	145.8	145.8	135.6	155.4	153.9	152.6	174.1
Zirconium	0.9	1.0	0.9	0.9	1.0	0.9	0.9	0.9	0.7

Diets were analyzed at Auburn University, Soils Laboratory (Auburn, AL, USA)

analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among treatments followed by Tukey's multiple comparison test to determine difference between treatments in each trial. Arcsine square root transformation was used prior to analysis for the proportion data. False discover rate (FDR) controlling procedures were applied to adjust the P value to control the FDR for amino acid data. Linear, second- or third-order polynomial regressions were performed to investigate the relationship between the supplemental *Ulva* meal levels and weight gain, FCR, survival, and lipid content of whole shrimp body. To identify the most appropriate regression model, we compared P value of the model components, R^2 value, adjust R^2 value, and the sum of squares for error (SSE) with different regression models.

Results

Water quality

In trial 1, DO, temperature, salinity, pH, TAN, and nitrite were maintained at 5.82 ± 0.26 mg L^{-1}, 29.7 ± 0.8 °C, 8.6 ± 0.4 ppt, 7.5 ± 0.5, 0.052 ± 0.107 mg L^{-1}, and 0.003 ± 0.004 mg L^{-1}, respectively. In trial 2, DO, temperature, salinity, pH, TAN, and nitrite were maintained at 6.20 ± 0.72 mg L^{-1}, 29.5 ± 0.9 °C, 8.4 ± 1.0 ppt, 7.5 ± 0.3, 0.092 ± 0.103 mg L^{-1}, and 0.050 ± 0.039 mg L^{-1}, respectively. In trial 3, DO, temperature, salinity, pH, TAN, and nitrite were maintained at 6.96 ± 0.31 mg L^{-1}, 28.1 ± 0.3 °C, 8.2 ± 0.6 ppt, 7.0 ± 0.3, 0.05 ± 0.04 mg L^{-1}, and 0.12 ± 0.12 mg L^{-1}, respectively. Water quality conditions in all the trials were suitable for normal growth and survival of this species (Achupallas et al. 2016).

Growth performance, feed conversion ratio (FCR), and survival

Performance of juvenile shrimp offered diets containing different levels of *Ulva* meal in trial 1 to 3 are presented in Table 9. In trial 1, final biomass was significantly reduced when more than 5% *Ulva* meal was included in the diet. Regression result indicated that there was a decreasing trend of WG as supplementation levels of UM2 were increased. Final mean weight and WG were significantly decreased

Table 7 Proximate composition, mineral composition, and amino acid profile of the test diets used in trial 2

Composition	T_2D_1	T_2D_2	T_2D_3	T_2D_4
Proximate composition (% as is)				
Crude protein	36.33	38.40	39.66	39.13
Moisture	7.15	7.59	8.93	8.34
Crude fat	9.39	9.03	9.01	8.68
Crude fiber	3.21	3.84	4.42	4.13
Ash	6.86	15.93	11.44	11.22
Quantity elements (% as is)				
Phosphorus	1.36	1.25	1.24	1.37
Sulfur	0.4	1.06	1.27	1.08
Potassium	1.33	1.73	1.65	2.13
Magnesium	0.18	0.76	0.86	0.52
Calcium	1.31	1.79	1.17	1.30
Trace elements (mg kg^{-1} as is)				
Sodium	0.1	1.16	0.51	0.77
Iron (ppm)	149	1240	286	169
Manganese (ppm)	40.1	71.6	39.1	40.1
Copper (ppm)	16.8	22.9	20.2	28.7
Zinc (ppm)	183	215	187	194
Amino acid profile (% as is)				
Alanine	1.87	2.15	2.24	2.14
Arginine	2.18	2.26	2.34	2.21
Aspartic acid	3.44	3.66	3.78	3.79
Cysteine	0.48	0.49	0.50	0.51
Glutamic acid	6.33	6.43	6.33	6.24
Glycine	1.56	1.69	1.82	1.68
Histidine	0.86	0.86	0.89	0.80
Isoleucine	1.60	1.70	1.71	1.65
Leucine	3.28	3.49	3.50	3.32
Lysine	2.01	2.03	2.16	2.05
Methionine	0.64	0.62	0.67	0.65
Phenylalanine	1.85	2.00	2.05	1.90
Proline	2.13	2.16	2.22	2.22
Serine	1.48	1.61	1.68	1.59
Taurine	0.16	0.13	0.14	0.16
Threonine	1.29	1.43	1.50	1.44
Tryptophan	0.47	0.48	0.45	0.44
Tyrosine	1.33	1.38	1.44	1.33
Valine	1.73	1.95	2.01	1.94

Proximate composition and amino acid profiles of test diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA)

Mineral composition was tested at Midwest Laboratories (Omaha, NE, USA)

Table 8 Proximate composition, mineral composition, and amino acid profile of the test diets used in trial 3

Composition	T_3D_1	T_3D_2	T_3D_3	T_3D_4	T_3D_5
Proximate composition (% as is)					
Crude protein	35.70	34.30	33.40	35.20	35.00
Moisture	8.70	9.89	10.22	9.93	10.2
Crude fat	6.71	8.03	8.21	8.37	8.65
Crude fiber	3.10	5.80	8.40	7.30	6.40
Ash	7.08	7.22	7.24	7.67	9.19
Quantity elements (% as is)					
Sulfur	0.40	0.56	0.72	0.74	1.19
Phosphorus	1.36	1.09	1.10	1.03	1.08
Potassium	1.33	1.24	1.35	1.14	1.20
Magnesium	0.18	0.29	0.40	0.40	0.66
Calcium	1.31	1.32	1.36	1.27	1.36
Sodium	0.10	0.13	0.17	0.23	0.40
Trace elements (mg kg^{-1})					
Iron	149	125	136	165	193
Manganese	40.1	54.4	59.9	50.9	54.9
Copper	16.8	16.3	16.1	16.7	15.2
Zinc	183	292	212	173	266
Amino acid profile (% as is)					
Alanine	1.87	1.86	1.88	2.03	1.85
Arginine	2.18	2.08	2.07	2.01	1.67
Aspartic acid	3.44	3.30	3.35	3.29	2.82
Cysteine	0.48	0.45	0.47	0.45	0.39
Glutamic acid	6.33	6.06	6.06	5.91	4.71
Glycine	1.56	1.46	1.39	1.56	1.42
Histidine	0.86	0.79	0.78	0.78	0.62
Hydroxylysine	0.08	0.05	0.05	0.07	0.08
Hydroxyproline	0.20	0.06	0.03	0.09	0.10
Isoleucine	1.60	1.51	1.51	1.53	1.27
Leucine	3.28	3.16	3.17	3.24	2.67
Lysine	2.01	2.01	2.00	2.02	1.78
Methionine	0.64	0.66	0.69	0.73	0.67
Phenylalanine	1.85	1.76	1.79	1.79	1.51
Proline	2.13	1.93	1.92	1.97	1.62
Serine	1.48	1.52	1.56	1.51	1.30
Threonine	1.29	1.28	1.29	1.31	1.16
Tryptophan	0.47	0.43	0.44	0.40	0.38
Tyrosine	1.33	1.27	1.25	1.25	1.02
Valine	1.73	1.74	1.72	1.81	1.54

Proximate composition and mineral composition was tested at Midwest Laboratories (Omaha, NE, USA)

Mineral composition was tested at Midwest Laboratories (Omaha, NE, USA)

when more than 10% *Ulva* meal was supplemented in the diet. FCR increased as the inclusion levels of UM2 were increased. The significant increase in FCR occurred in the diet containing 25% UM2. There was a decreasing trend of survival with the increasing incorporation levels of UM2. Shrimp fed with diets containing 25 and 30% UM2 exhibited significantly lower survival than those fed with diets supplementing with 0, 5, and 15% UM2.

Table 9 Growth performance of juvenile Pacific white shrimp (Initial weight: 0.24 g, 0.15 g, and 0.98 g in trial 1, 2, and 3, respectively) offered experimental diets for 5–6 weeks

Trial	Diet	*Ulva* levels (%)	Final biomass (g)	Final mean weight (g)	WG[3] (%)	FCR[2]	Survival (%)
Trial 1	T_1D_1	0	43.3^{a}	4.6^{a}	1734.2^{a}	1.46^{b}	95.0^{a}
$n = 4$	T_1D_2	5	36.2^{ab}	3.7^{ab}	1398.2^{ab}	1.83^{ab}	97.5^{a}
	T_1D_3	10	28.4^{bc}	3.3^{ab}	1241.5^{ab}	2.23^{ab}	87.5^{ab}
	T_1D_4	15	23.9^{cd}	2.6^{b}	948.7^{b}	2.82^{ab}	92.5^{a}
	T_1D_5	20	19.0^{cd}	2.5^{b}	990.3^{b}	2.96^{ab}	75.0^{ab}
	T_1D_6	25	16.3^{cd}	2.6^{b}	943.5^{b}	3.53^{a}	67.5^{b}
	T_1D_7	30	15.5^{d}	2.5^{b}	864.7^{b}	3.37^{ab}	65.0^{b}
	T_1D_8	23.6	26.1^{bcd}	3.0^{b}	1131.1^{b}	2.61^{ab}	87.5^{ab}
	T_1D_9	26.3	27.1^{bcd}	3.1^{b}	1226.9^{ab}	2.36^{ab}	87.5^{ab}
PSE[1]			1.2872	0.1392	61.8604	0.2020	2.6131
P value			<0.0001	0.0002	0.0008	0.0201	0.0006
Trial 2	T_2D_1	0	79.3^{a}	8.4^{a}	766.6^{a}	1.64^{b}	95.0^{a}
$n = 4$	T_2D_2	22	66.8^{a}	7.8^{a}	689.7^{a}	1.87^{b}	85.0^{ab}
	T_2D_3	25	30.6^{c}	4.9^{b}	397.3^{b}	3.58^{a}	62.5^{c}
	T_2D_4	25	57.3^{b}	7.2^{a}	618.1^{a}	2.09^{b}	80.0^{b}
PSE[1]			1.7351	2.3457	18.1043	0.1167	1.5427
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Trial 3	T_3D_1	0	42.68^{a}	4.74^{a}	3160.39^{a}	1.72^{c}	90.0^{ab}
$n = 4$	T_3D_2	4.75	34.60^{b}	3.69^{b}	2335.98^{b}	2.23^{bc}	94.0^{a}
	T_3D_3	9.5	24.84^{cd}	3.52^{bc}	2254.04^{bc}	2.51^{b}	72.0^{b}
	T_3D_4	12	27.85^{bc}	3.41^{bc}	2057.91^{bc}	2.52^{b}	82.5^{ab}
	T_3D_5	24	20.08^{d}	2.72^{c}	1718.67^{c}	3.26^{a}	74.0^{ab}
PSE[1]			0.9041	0.1135	76.79	0.0826	2.4965
P value			<0.0001	<0.0001	<0.0001	<0.0001	0.0107

Values within a column with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

[2] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight)

[3] WG: Weight gain = (Final weight − Initial weight) / Initial weight × 100%

In trial 2, in general shrimp fed UM2 exhibited poorest performance in terms of growth, FCR, and survival. Significant reductions in final biomass, final mean weight, WG, and survival were determined when UM2 was supplemented in the diet compared to other treatments. FCR was significantly increased in the treatment contained UM2 in contrast with other treatments.

In trial 3, shrimp fed with diets supplementing with different levels of UM4 showed significantly reduced final biomass, final mean weight, and WG as well as increased FCR. Survival was significantly reduced when 9.5% UM4 was included in the diet to replace 6% FM.

Proximate composition and amino acid profile of whole shrimp body

Proximate composition and amino acid profile of whole shrimp body in trial 1 and trial 2 are presented in Tables 10 and 11. In trial 1, regression result indicated there was a decreasing trend of whole body lipid content as the inclusion levels of UM2 were increased. Crude lipid content was significantly reduced, while protein content was significantly increased when more than 5% UM2 was incorporated in the diets. No significant difference was detected in the moisture content (75.66 to 78.05%) across all the treatments. As a result of the enhanced protein content, several shifts in specific amino acid profile were detected. Shrimp fed with diet containing 25 and 30% of UM2 exhibited significantly higher arginine and glycine content than the one fed with reference diet. Cysteine and lysine contents in the treatment containing 15 to 30% UM2 were significantly higher than those fed with the reference diet. Histidine content in the treatment fed with diet containing 10 to 25% UM2 and 23.6% UM3 was significantly higher than the ones fed with reference diet. Shrimp fed with diet containing 30% UM2 exhibited significantly higher methionine content than those fed with diets containing 0, 5, and 10% UM2. Phenylalanine in the treatment containing 20% UM2 was significantly higher than the treatments containing

Table 10 Proximate composition and amino acid profile of whole shrimp body offered diets contain different levels of three batches *Ulva* meal (UM1, UM2, and UM3) levels in trial 1

Diet *Ulva* levels (%)	T_1D_1 0	T_1D_2 5	T_1D_3 10	T_1D_4 15	T_1D_5 20	T_1D_6 25	T_1D_7 30	T_1D_8 23.6	T_1D_9 26.3	PSE[1]	*P* value	Adjust *P* value
Moisture	75.66	78.02	76.05	77.33	76.83	77.16	78.05	77.04	76.76	0.3204	0.1925	0.3300
Protein	72.56^{d}	72.81^{cd}	74.33^{abc}	75.18^{ab}	75.50^{a}	74.77^{ab}	75.43^{a}	74.15^{abcd}	73.62^{bcd}	0.1802	<0.0001	0.0001
Lipid	7.72^{a}	6.22^{ab}	4.54^{bc}	2.75^{d}	2.90^{cd}	2.97^{cd}	2.20^{d}	4.74^{b}	5.27^{b}	0.1779	<0.0001	0.0001
Alanine	4.30	4.50	4.40	4.37	4.36	4.20	4.28	4.36	4.34	0.0256	0.0298	0.0650
Arginine	4.98^{c}	4.91^{c}	4.95^{c}	5.21^{abc}	5.22^{abc}	5.43^{ab}	5.51^{a}	5.13^{bc}	5.18^{abc}	0.0390	<0.0001	0.0003
Aspartic Acid	6.68	6.85	6.87	6.93	6.87	6.79	6.85	6.89	6.85	0.037	0.5257	0.6309
Cysteine	0.57^{c}	0.58^{c}	0.60^{bc}	0.62^{ab}	0.62^{ab}	0.62^{ab}	0.64^{a}	0.60^{abc}	0.60^{abc}	0.0037	<0.0001	0.0002
Glutamic acid	9.96	10.10	10.21	10.24	10.25	10.09	10.06	10.11	10.27	0.0694	0.7825	0.8412
Glycine	4.53^{c}	4.68^{bc}	4.85^{bc}	5.28^{abc}	5.10^{abc}	5.45^{ab}	5.68^{a}	4.98^{abc}	4.98^{abc}	0.0842	0.0011	0.0050
Histidine	1.48^{b}	1.52^{ab}	1.58^{a}	1.59^{a}	1.60^{a}	1.59^{a}	1.55^{ab}	1.60^{a}	1.56^{ab}	0.0103	0.0031	0.0105
Hydroxylysine	0.14	0.13	0.16	0.16	0.16	0.14	0.14	0.16	0.15	0.0007	0.3939	0.5252
Hydroxyproline	0.21	0.20	0.20	0.21	0.20	0.21	0.20	0.21	0.21	0.0051	0.9706	0.9706
Isoleucine	2.87	2.90	2.95	2.96	2.96	2.93	2.90	2.92	2.91	0.0129	0.2423	0.3635
Leucine	4.80	4.87	4.92	4.96	4.99	4.93	4.90	4.92	4.90	0.0227	0.2309	0.3635
Lysine	4.72^{b}	4.86^{ab}	4.92^{ab}	5.02^{a}	5.05^{a}	5.06^{a}	5.06^{a}	4.99^{ab}	4.94^{ab}	0.0312	0.0095	0.0253
Methionine	1.40^{b}	1.41^{b}	1.42^{b}	1.47^{ab}	1.46^{ab}	1.48^{ab}	1.52^{a}	1.44^{ab}	1.46^{ab}	0.0095	0.0028	0.0105
Phenylalanine	3.10^{b}	3.12^{b}	3.21^{ab}	3.24^{ab}	3.28^{a}	3.20^{ab}	3.14^{ab}	3.23^{ab}	3.12^{b}	0.0159	0.0044	0.0132
Proline	3.80	3.74	3.85	3.73	3.95	3.78	3.55	3.65	3.63	0.0639	0.5216	0.6309
Serine	2.29	2.34	2.33	2.37	2.37	2.35	2.38	2.35	2.42	0.0237	0.8062	0.8412
Threonine	2.64	2.68	2.71	2.71	2.74	2.68	2.64	2.72	2.70	0.0136	0.1540	0.2844
Tryptophan	0.84	0.87	0.88	0.90	0.89	0.89	0.89	0.89	0.88	0.0054	0.0272	0.0650
Tyrosine	2.28	2.23	2.50	2.52	2.54	2.48	2.16	2.50	2.47	0.0643	0.3161	0.4462
Valine	4.05	4.09	4.07	4.09	4.22	4.12	4.12	4.11	4.20	0.0367	0.7681	0.8412
Total	65.61	66.55	67.56	68.58	68.82	68.41	68.15	67.74	67.71	0.3262	0.0373	0.0746

Body samples were analyzed at University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA)

Values within a row with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

0 and 5% UM2 as well as 26.3% UM1. No significant differences were observed in alanine, aspartic acid, glutamic acid, hydroxylysine, hydroxyproline, isoleucine, leucine, proline, serine, threonine, tyrosine, tryptophan, valine, and total amino acid levels in whole shrimp body across all the treatments.

In trial 2, shrimp fed with diet containing UM2 exhibited significantly higher body moisture content and lower crude lipid content than those fed with the reference diet. No significant difference was detected in protein content (75.08 to 76.98%) across all the treatments. In general, amino acid profiles corresponded to the protein content. Methionine content was significantly improved when UM1, UM2, UM3 were included in the diets. No significant differences were observed in alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, hydroxylysine, hydroxyproline, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and total amino acid contents of whole shrimp body across all the treatments.

Proximate composition of whole shrimp body in shrimp in trial 3 is presented in Table 12. In trial 3, moisture contents in the treatments containing 12 and 24% UM4 were significantly higher than those fed with reference diet and diet contained 4.75% UM4. Crude protein was significantly enhanced when shrimp were fed with diet containing 24% UM4 compared to the reference diet and diet containing 4.75% UM4. Crude lipid was dramatically decreased when more than 9.5% UM4 was included in the diet. Significant enhancement in ash content was observed when the diet was incorporated with 9.5 and 24% UM4 in contrast with the reference diet.

Apparent net protein and amino acid retention

Apparent net protein and amino acid retention in trial 1 to 3 are presented in Tables 13, 14, and Table 12, respectively. In trial 1, ANPR was significantly reduced when shrimp was fed with diets supplemented with 15 to 30% UM2, 26.3% UM1, and

Table 11 Proximate composition and amino acids profile of shrimp at the conclusion of a 6-week growth trial in which shrimp were offered diets formulated to partially replace soybean meal on a digestible protein basis with three different batches of *Ulva* meal in trial 2

Diet *Ulva* meal levels %	T_2D_1 0	T_2D_2 22	T_2D_3 25	T_2D_4 25	PSE[1]	*P* value	Adjust *P* value
Moisture	75.65^{b}	76.32^{ab}	77.88^{a}	75.56^{b}	0.2029	0.0054	0.0432
Protein	75.08	76.70	76.98	75.24	0.2774	0.0675	0.1800
Lipid	6.37^{a}	5.04^{a}	2.68^{b}	5.31^{a}	0.2479	0.0015	0.0180
Alanine	4.27	4.21	4.21	4.36	0.0416	0.5419	0.5612
Arginine	5.45	5.89	5.67	5.31	0.0543	0.0126	0.0756
Aspartic acid	6.76	6.94	7.01	6.96	0.0440	0.2679	0.4559
Cysteine	0.60	0.63	0.64	0.61	0.0045	0.0387	0.1327
Glutamic acid	10.18	10.51	10.47	10.40	0.0708	0.3940	0.4728
Glycine	5.01^{b}	5.41^{ab}	5.90^{a}	5.07^{b}	0.0962	0.0246	0.0984
Histidine	1.49	1.56	1.58	1.54	0.0156	0.2442	0.4508
Hydroxylysine	0.14	0.17	0.16	0.14	0.0067	0.2203	0.4406
Hydroxyproline	0.20	0.22	0.22	0.21	0.0034	0.3229	0.4559
Isoleucine	2.95	3.00	2.95	3.01	0.0150	0.3048	0.4559
Leucine	4.95	5.08	5.00	5.03	0.0264	0.3920	0.4728
Lysine	4.92	5.20	5.13	5.10	0.0281	0.0234	0.0984
Methionine	1.46^{b}	1.58^{a}	1.53^{a}	1.53^{a}	0.0071	0.0007	0.0168
Phenylalanine	3.16	3.28	3.26	3.19	0.0314	0.5019	0.5551
Proline	4.09	4.14	3.79	4.11	0.0750	0.3737	0.4728
Serine	2.35	2.42	2.47	2.38	0.0286	0.5088	0.5551
Threonine	2.62	2.71	2.73	2.71	0.0136	0.0453	0.1359
Tryptophan	0.87	0.88	0.88	0.89	0.0046	0.5612	0.5612
Tyrosine	2.51	2.59	2.59	2.36	0.0459	0.3004	0.4559
Valine	4.10	4.21	4.36	4.23	0.0322	0.0935	0.2040
Total	68.05	70.61	70.52	69.08	0.3708	0.0884	0.2040

Body samples were analyzed at University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA)

Values within a row with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

23.6% UM3 compared to the one offered with reference diet. In general, total and individual amino acid retention corresponded to ANPR. Apparent net alanine and hydroproline retention were significantly reduced when more

Table 12 Proximate composition and apparent net protein retention (ANPR) of shrimp at the conclusion of a 6-week growth trial in which shrimp were offered diets formulated to evaluate *Ulva* meal 4 as a replacement for soybean meal and fish meal on an isonitrogen basis in juvenile shrimp (Trial 3)

Diet	UM4 levels (%)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude fiber (%)	Ash (%)	ANPR[2] (%)
T_3D_1	0	76.1^{b}	70.83^{b}	8.40^{a}	5.25	11.50^{c}	30.50^{a}
T_3D_2	4.75	76.1^{b}	70.95^{b}	6.90^{ab}	5.34	12.11^{bc}	25.11^{ab}
T_3D_3	9.5	76.9^{ab}	71.73^{ab}	6.17^{b}	5.45	12.94^{ab}	22.31^{b}
T_3D_4	12	77.9^{a}	73.02^{ab}	5.07^{bc}	4.98	12.69^{bc}	20.68^{bc}
T_3D_5	24	78.2^{a}	73.76^{a}	3.65^{c}	5.57	14.26^{a}	15.91^{c}
P value		0.0006	0.0027	< 0.0001	0.2712	0.0001	< 0.0001
PSE[1]		0.1937	0.3056	0.2541	0.0976	0.1669	0.7882

Body samples were analyzed at University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory (Columbia, MO, USA)

Values within a column with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

[2] Apparent net protein retention = (Final weight × Final protein content) − (Initial weight × Initial protein content) × 100 / Protein offered

Table 13 Apparent net protein and amino acids retention of Pacific white shrimp in trial 1

Diet *Ulva* levels (%)	T_1D_1 0	T_1D_2 5	T_1D_3 10	T_1D_4 15	T_1D_5 20	T_1D_6 25	T_1D_7 30	T_1D_8 23.6	T_1D_9 26.3	*P* value	PSE[1]	Adjust *P* value
Protein	36.4^{a}	26.5^{ab}	25.6^{ab}	19.0^{b}	18.1^{b}	17.1^{b}	15.3^{b}	21.4^{b}	22.9^{b}	1.1928	<0.0001	0.0002
Alanine	42.3^{a}	31.8^{ab}	28.5^{bc}	20.2^{bcd}	18.1^{cd}	17.2^{cd}	14.8^{d}	23.0^{bcd}	24.7^{bcd}	1.3131	<0.0001	<0.0001
Arginine	39.8^{a}	29.2^{ab}	28.1^{ab}	21.9^{b}	21.1^{b}	21.1^{b}	19.0^{b}	26.1^{b}	27.9^{ab}	1.3498	0.0003	0.0005
Aspartic acid	33.2^{a}	25.3^{ab}	23.9^{abc}	17.7^{bc}	16.7^{bc}	15.9^{bc}	14.1^{c}	19.9^{bc}	22.1^{bc}	1.1108	<0.0001	0.0002
Cysteine	20.8^{a}	16.5^{ab}	15.6^{ab}	11.9^{b}	11.3^{b}	11.0^{b}	10.2^{b}	12.8^{b}	14.4^{ab}	0.7296	0.0004	0.0005
Glutamic acid	27.3^{a}	20.9^{ab}	20.3^{ab}	15.1^{b}	14.4^{b}	14.3^{b}	12.6^{b}	17.7^{b}	19.6^{ab}	0.9825	0.0004	0.0005
Glycine	50.5^{a}	37.3^{ab}	36.1^{ab}	29.1^{b}	24.4^{b}	25.5^{b}	23.4^{b}	31.0^{b}	34.9^{ab}	1.6381	<0.0001	0.0002
Histidine	29.7^{a}	23.0^{ab}	23.0^{ab}	17.1^{b}	16.7^{b}	16.3^{b}	13.9^{b}	21.7^{ab}	21.9^{ab}	1.1148	0.0010	0.0010
Hydroxylysine	50.3^{a}	31.6^{abc}	38.6^{ab}	25.2^{bc}	20.3^{bc}	16.6^{bc}	14.2^{c}	29.3^{abc}	23.1^{bc}	2.4299	0.0004	0.0005
Hydroxyproline	34.0^{a}	26.6^{ab}	22.7^{bc}	15.9^{cd}	9.8^{de}	4.9^{e}	9.6^{de}	10.4^{de}	14.0^{d}	0.8258	<0.0001	<0.0001
Isoleucine	32.1^{a}	24.3^{ab}	23.4^{abc}	17.2^{bc}	16.5^{bc}	15.8^{bc}	13.7^{c}	20.1^{bc}	21.3^{bc}	1.1065	0.0001	0.0002
Leucine	27.3^{a}	20.7^{ab}	19.8^{abc}	14.5^{bc}	13.7^{bc}	13.5^{bc}	11.5^{c}	17.4^{bc}	18.5^{abc}	0.9449	<0.0001	0.0002
Lysine	42.2^{a}	32.7^{ab}	31.2^{ab}	23.8^{b}	23.2^{b}	22.2^{b}	19.8^{b}	28.7^{ab}	30.0^{ab}	1.5286	0.0006	0.0007
Methionine	36.4^{a}	28.2^{ab}	26.8^{ab}	20.4^{b}	18.9^{b}	18.9^{b}	17.2^{b}	24.5^{ab}	27.0^{ab}	1.3455	0.0005	0.0006
Phenylalanine	30.6^{a}	22.9^{ab}	22.1^{abc}	16.3^{bc}	15.5^{bc}	14.8^{bc}	12.6^{c}	19.3^{bc}	20.0^{bc}	1.0502	<0.0001	0.0002
Proline	33.3^{a}	25.5^{ab}	23.3^{abc}	17.8^{bc}	16.4^{bc}	16.4^{bc}	13.6^{c}	19.2^{bc}	20.5^{bc}	1.2344	0.0002	0.0004
Serine	27.4^{a}	20.4^{ab}	19.6^{ab}	14.4^{b}	13.8^{b}	13.3^{b}	11.8^{b}	17.3^{b}	18.8^{ab}	0.9469	0.0001	0.0002
Threonine	35.7^{a}	26.5^{ab}	25.0^{ab}	18.3^{bc}	17.4^{bc}	16.3^{bc}	13.9^{c}	21.3^{bc}	22.8^{bc}	1.1486	<0.0001	0.0001
Tryptophan	30.7^{a}	23.0^{ab}	23.1^{ab}	17.3^{b}	16.2^{b}	15.9^{b}	13.8^{b}	21.7^{ab}	21.9^{ab}	1.1080	0.0004	0.0005
Tyrosine	35.2^{a}	24.7^{abc}	27.1^{ab}	19.4^{bc}	18.7^{bc}	17.9^{bc}	13.1^{c}	24.4^{abc}	24.6^{abc}	1.4386	0.0008	0.0008
Valine	40.4^{a}	30.1^{ab}	28.0^{abc}	20.2^{bc}	19.7^{bc}	18.7^{bc}	16.4^{c}	24.0^{bc}	26.5^{bc}	1.3655	<0.0001	0.0002
Total	33.5^{a}	25.3^{ab}	24.2^{ab}	18.1^{b}	17.0^{b}	16.5^{b}	14.5^{b}	21.0^{b}	22.7^{ab}	1.1483	0.0001	0.0002

Apparent net protein retention = (Final weight × Final protein content) − (Initial weight × Initial protein content) × 100 / Protein offered

Apparent net amino acids (AA) retention = (Final weight × Final AA content) - (Initial weight × Initial AA content) × 100 / AA offered

Values within a row with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

than 10% of UM2 were supplemented in the diets. Arginine, aspartic acid, isoleucine, phenylalanine, proline, threonine, and valine retention were significantly lower in the treatments containing 15 to 30% UM2, 26.3% UM1, and 23.6% UM3 than the reference diet. Total amino acids, cysteine, glutamic acid, glycine, leucine, and serine retention were significantly reduced in the treatments containing 15 to 30% UM2 and 23.6% UM3. Shrimp fed with diets containing 15 to 30% UM2 exhibited significantly higher histidine, hydroxylysine, lysine, methionine, tryptophan, and tyrosine retention than those fed with reference diet.

In trial 2, ANPR was significantly reduced when UM2 and UM3 were supplemented in the diets. In general, total and individual amino acid retention corresponded to ANPR. Arginine and hydroxyproline retention were significantly lower in treatments incorporated with UM1–3. Total amino acids, cysteine, serine, threonine, and valine retention in treatments supplemented with UM2 and UM3 were significantly reduced compared to the treatments fed with reference diet. Alanine, aspartic acid, glycine, histidine, hydroxylysine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, tryptophan, and tyrosine retention were significantly depressed when UM2 was supplemented in the diet.

In trial 3, ANPR was significantly depressed when more than 9.5% UM4 was included in the diet (Table 12).

Discussion

In our previous research, *Ulva* sp. can be included in the shrimp feeds up to 6.35% as a replacement of 2% FM without compromising the performance of shrimp, however more than 6.35% inclusion of *Ulva* sp. resulted in depressions in growth and lipid content of whole shrimp body (Qiu 2017). As the replacement of FM results in shifts in numerous nutrients as well as possible palatability changes of the diet, we chose to shift the nutrition model to replace SBM in the present study as this results in fewer shifts in nutrients. In trial 1, results from regression analysis indicated that there are clear decreasing trends of growth and survival as UM2 inclusion levels was increased (Figs. 1a, c, 2, and 3). Inclusion levels of UM2 up to

Table 14 Apparent net protein and amino acid retention of Pacific white shrimp in trial 2

Retention *Ulva* meal levels %	T_2D_1 0	T_2D_2 22	T_2D_3 25	T_2D_4 25	*P* value	PSE[1]	Adjust *P* value
Protein	34.2^{a}	29.4^{ab}	14.4^{c}	26.0^{b}	0.8054	< 0.0001	< 0.0001
Alanine	37.5^{a}	28.5^{a}	13.6^{b}	27.3^{a}	0.6848	< 0.0001	< 0.0001
Arginine	41.5^{a}	38.8^{b}	18.2^{c}	32.5^{b}	1.1379	< 0.0001	< 0.0001
Aspartic acid	32.6^{a}	28.0^{a}	13.9^{b}	24.9^{a}	0.7763	< 0.0001	< 0.0001
Cysteine	20.5^{a}	18.9^{ab}	9.6^{c}	16.2^{b}	0.5597	< 0.0001	0.0001
Glutamic acid	26.7^{a}	24.2^{a}	12.4^{b}	22.6^{a}	0.6799	< 0.0001	< 0.0001
Glycine	53.4^{a}	47.9^{a}	25.0^{b}	41.3^{a}	1.5060	< 0.0001	0.0002
Histidine	28.6^{a}	26.9^{a}	13.3^{b}	26.2^{a}	0.8115	< 0.0001	0.0001
Hydroxylysine	29.3^{ab}	25.7^{b}	11.2^{b}	44.7^{a}	2.1886	0.0015	0.0015
Hydroxyproline	16.9^{a}	13.4^{b}	7.6^{c}	11.3^{b}	0.3435	< 0.0001	< 0.0001
Isoleucine	30.5^{a}	26.1^{a}	12.9^{b}	24.8^{a}	0.6891	< 0.0001	< 0.0001
Leucine	25.0^{a}	21.6^{a}	10.7^{b}	20.6^{a}	0.5786	< 0.0001	< 0.0001
Lysine	40.7^{a}	38.1^{a}	18.0^{b}	33.9^{a}	0.9340	< 0.0001	< 0.0001
Methionine	38.1^{a}	38.1^{a}	17.3^{b}	32.2^{a}	0.9552	< 0.0001	< 0.0001
Phenylalanine	28.3^{a}	24.3^{a}	11.9^{b}	22.7^{a}	0.7167	< 0.0001	< 0.0001
Proline	32.4^{a}	28.8^{a}	13.2^{b}	25.5^{a}	0.8271	< 0.0001	< 0.0001
Serine	26.3^{a}	22.2^{ab}	11.0^{c}	20.3^{b}	0.6670	< 0.0001	< 0.0001
Threonine	33.6^{a}	28.1^{ab}	13.7^{c}	25.5^{b}	0.7495	< 0.0001	< 0.0001
Tryptophan	30.5^{a}	27.2^{a}	14.5^{b}	27.3^{a}	0.7670	< 0.0001	< 0.0001
Tyrosine	31.2^{a}	27.7^{a}	13.5^{b}	23.8^{a}	0.9244	0.0001	0.0001
Valine	39.4^{a}	32.0^{ab}	16.4^{c}	29.7^{b}	0.9798	< 0.0001	< 0.0001
Total	32.3^{a}	28.4^{ab}	14.1^{c}	25.8^{b}	0.7669	< 0.0001	< 0.0001

Apparent net protein retention = (Final weight × Final protein content) − (Initial weight × Initial protein content) × 100 / Protein offered

Apparent net amino acids (AA) retention = (Final weight × Final AA content) − (Initial weight × Initial AA content) × 100 / AA offered

Values within a row with different superscripts are significantly different based on Tukey's multiple range test

[1] Pooled standard error

10% as a replacement of ~ 6% SBM in shrimp feeds did not compromise the growth of shrimp, however significant reduction in growth was detected when shrimp fed with diets containing more than 10% UM2. Survival rate was significantly decreased when more 25% UM2 was supplemented in the diets. Shrimp fed with diets containing UM1 and UM3 replacing the same levels of SBM as UM2 exhibited higher WG and survival clearly demonstrating differences across batches of *Ulva* sp.

The poor response of increasing levels of *Ulva* meal could be due to a range of dietary problems with increasing levels as well as problems with nutrient shifts between batches of Ulva meal. One hypothesis is that due to the high ash content of the *Ulva* meals that there may be a mineral toxicity occurring (NRC 2016). Hence the mineral profiles of the first three batches of *Ulva* meal (Qiu 2017) and the diets in trial 1 were analyzed (Table 6). Clearly there are shifts in mineral profiles with a number of minerals increasing in levels. However, if one also assumes that diets made with UM1 would have higher mineral levels than UM2 and UM3, there is no obvious correlation to a mineral that could be causing a toxicity. Other possible nutritional problems could come about by limitations of protein, amino acids, or digestibility of the protein and amino acids. This can be mediated by formulating the diets on a digestibility basis and supplementing possible limiting amino acids.

In addition to the growth trial, the ingredient was included in a digestibility trial. The protein, energy, and amino acid digestibility of *Ulva* meals were significantly lower compared to the FM and SBM (Qiu 2017), which might serve as a partial explanation for the reduced growth. To elucidate if digestible protein was limiting growth, trial 2 was initiated for which feeds were formulated on a digestible protein basis. Since methionine and lysine are typically the two most limiting amino acids in shrimp feeds, they were also balanced on the digestible basis. The results indicated that growth and survival were not affected when experimental diets were supplemented with UM1, however, significant reduced growth and survival were detected when shrimp fed with diets containing UM2.

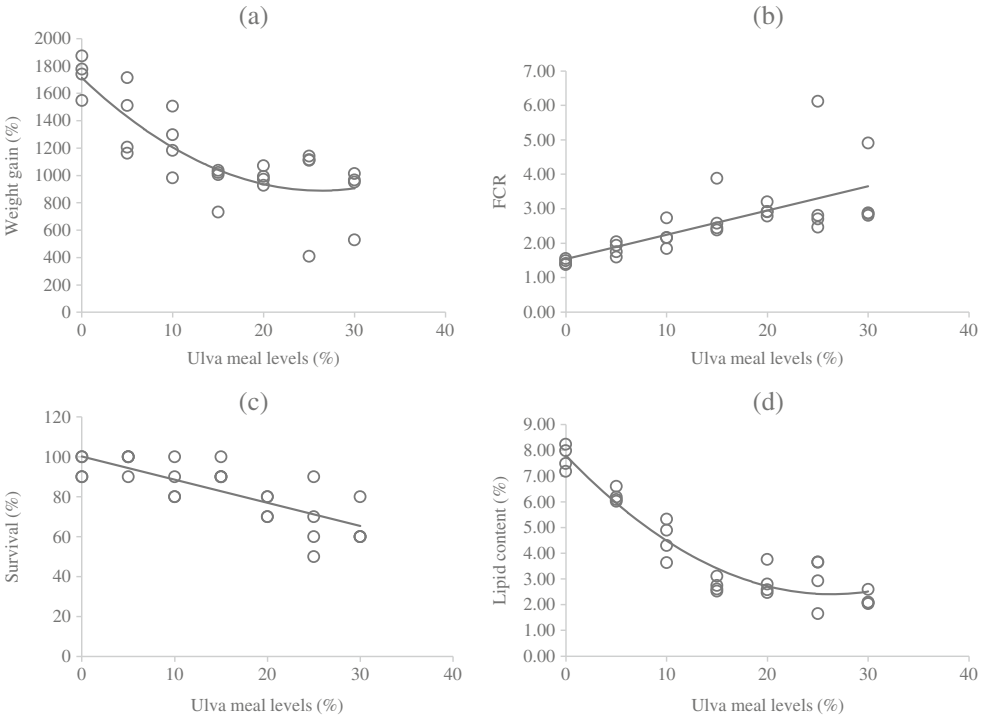

Fig. 1 **a** In trial 1, relationship between weight gain (y) and the inclusion level of *Ulva* meal 2 (x) in the diets. The regression line is described by $y = 1.1925\ x^2 – 62.699x + 1713.1$ ($R^2 = 0.6815$, $P < 0.0001$). **b** In trial 1, relationship between FCR (y) and supplemental *Ulva* meal 2 levels (x) in the diets. The regression line is described by $y = -0.0703x + 1.5451$ ($R^2 = 0.4766$, $P < 0.0001$). **c** In trial 1, relationship between survival (y) and supplemental *Ulva* meal 2 levels (x) in the diets. The regression line is described by $y = -1.1607x + 100.27$ ($R^2 = 0.6113$, $P < 0.0001$). **d** In trial 1, relationship between lipid content (y) of shrimp body and supplemental *Ulva* meal 2 levels (x) in the diets. The regression line is described by $y = 0.0078\ x^2 – 0.4095x + 7.8033$ ($R^2 = 0.9051$, $P < 0.0001$)

The same response was observed in trial 1 in which reduced growth and survival were also observed for shrimp offered diets with UM2. This indicated there might be some other factors affecting the growth and survival of shrimp. The growth performance of shrimp offered high levels of UM1 and UM3 was restored when the diets were balanced for

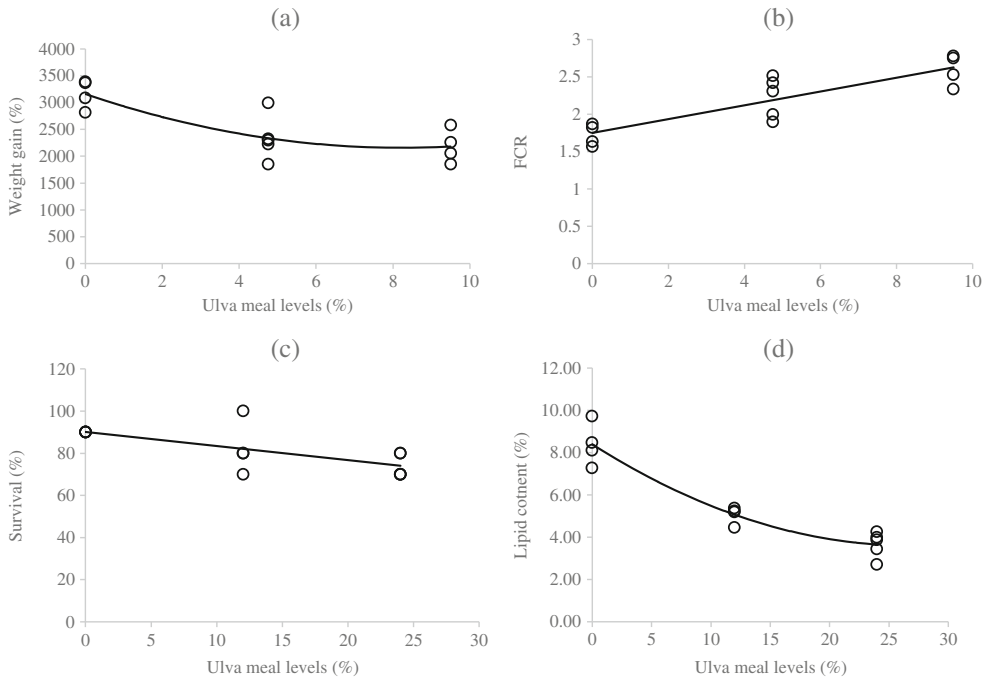

Fig. 2 **a** In trial 3, relationship between weight gain (y) of shrimp and incorporation levels of *Ulva* meal 4 levels (x) in the diets replacing soybean meal (SBM). The regression line is described by $y = 2.6501\ x^2 – 123.67x + 3160.4$ ($R^2 = 0.8872$, $P < 0.0001$). **b** In trial 3, relationship between FCR (y) and supplemental *Ulva* meal 4 levels (x) in the diets replacing SBM. The regression line is described by $y = 0.064x + 1.7319$ ($R^2 = 0.7897$, $P < 0.0001$). **c** In trial 3, relationship between survival (y) and supplemental *Ulva* meal 2 levels (x) in the diets replacing SBM. The regression line is described by $y = -0.6681x + 90.17$ ($R^2 = 0.4905$, $P = 0.0077$). **d** In trial 3, relationship between lipid content (y) of shrimp body and supplemental *Ulva* meal 4 levels (x) in the diets replacing SBM. The regression line is described by $y = 0.0067\ x^2 – 0.3577x + 8.3975$ ($R^2 = 0.9093$, $P < 0.0001$)

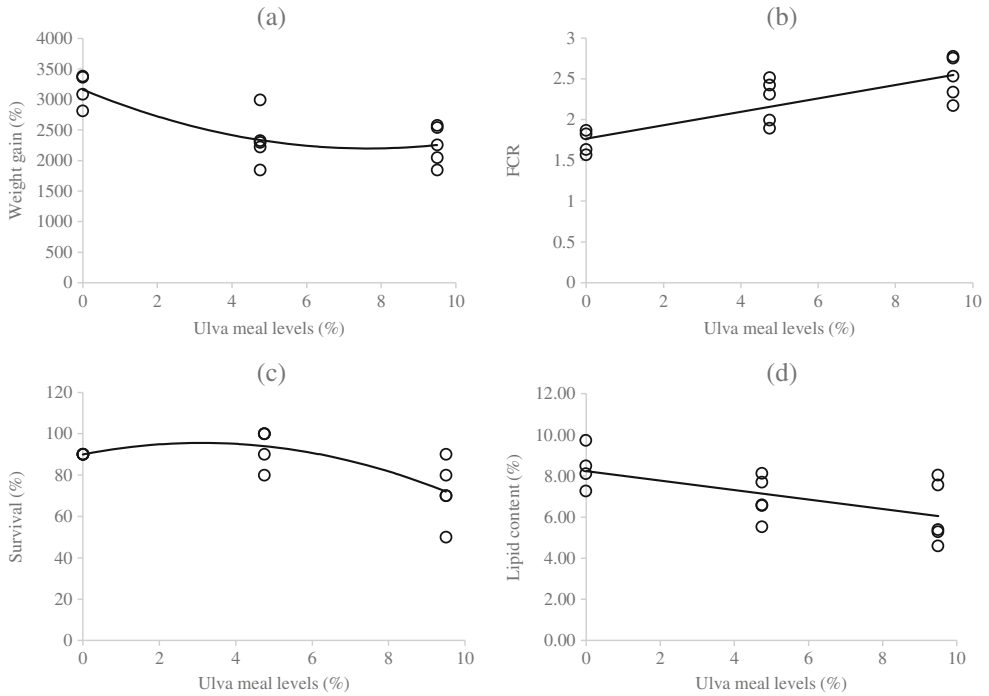

Fig. 3 **a** In trial 3, relationship between weight gain (y) of shrimp and incorporation levels of *Ulva* meal 4 levels (x) in the diets replacing fish meal (FM). The regression line is described by $y = 16.45\ x^2 - 251.71x + 3160.4$ ($R^2 = 0.6241$, $P = 0.0049$). **b** In trial 3, relationship between FCR (y) and supplemental *Ulva* meal 4 levels (x) in the diets replacing FM. The regression line is described by $y = 0.0823x + 1.7662$ ($R^2 = 0.6721$, $P = 0.0003$). **c** In trial 3, relationship between survival (y) and supplemental *Ulva* meal 2 levels (x) in the diets replacing FM. The regression line is described by $y = -0.5762\ x^2 + 3.5789x + 90$ ($R^2 = 0.5294$, $P = 0.0359$). **d** In trial 3, relationship between lipid content (y) of shrimp body and supplemental *Ulva* meal 4 levels (x) in the diets replacing FM. The regression line is described by $y = -0.2132x + 8.2424$ ($R^2 = 0.3866$, $P = 0.0176$)

digestible protein, indicating that part of the problem is probably due to low nutrients availability in *Ulva* meal. However, this did not solve the problem for UM2 which had both poor survival and poor growth in both trial 1 and 2.

Additionally, trial 3 evaluated the potential of UM4 which contained relatively higher protein content (~ 38%) than first three batches as a feed ingredient in shrimp diet. In general, growth was significantly reduced in all treatments when FM or SBM were replaced by UM4. As the inclusion of UM4 at 4.75% as a replacement for 3% FM would only result in minor shifts in nutrient availability, this clearly demonstrated there should be other factors in the *Ulva* meal other than nutrient digestibility decrease the growth of shrimp.

There are relatively few studies looking at the efficacy of *Ulva* meal in aquatic animal feeds particularly with regard to shrimp. In general, a number of studies demonstrated that low levels (≤ 5% of the diet) did not result in poor performances in both freshwater fish (e.g., African catfish *Clarias gariepinus* (Abdel-Warith et al. 2016; Al-Asgah et al. 2016), Nile tilapia (Güroy et al. 2007; Marinho et al. 2013; Valente et al. 2016), red tilapia *Oreochromis* sp. (El-Tawil 2010) and rainbow trout (Soler-Vila et al. 2009; Güroy et al. 2013)) and in marine fish and shrimp such as European sea bass (Valente et al. 2006), gilthead seabream *Sparus aurata* (Emre et al. 2013), and Pacific white shrimp (Rodríguez-González et al. 2014; Cárdenas et al. 2015). However, when higher levels of *Ulva* meal were evaluated most of the forementioned authors identified significant depressions in performance of the fish and shrimp. These findings are consistent with our results in the present study.

To further investigate the impacts of *Ulva* sp. on the whole shrimp body composition, shrimp in trial 1 and trial 2 were analyzed for proximate composition and amino acid profile (Tables 10 and 11), and proximate composition of shrimp body was presented in Table 12. There were consistently decreasing trends of whole body lipid content as inclusion rates of UM2 or UM4 were increased in trial 1 and 3, which may due to the significant lower energy availability of this ingredient compared to the ingredients it replaced (Qiu 2017). In both trial 1 and trial 3, shrimp fed with diets containing UM2 exhibited significantly lower lipid content than those fed with diets containing UM1 and UM3 replacing the same levels of SBM on a iso-nitrogenous or digestible protein basis, confirming the differences among various batches of *Ulva* meal. The reductions in lipid contents indirectly resulted in improvements in moisture or protein contents of whole shrimp body across the trials. In trial 1, improvement in protein content also translated to enhancements in several amino acids (arginine, cysteine, glycine, histidine, lysine, methionine, and phenylalanine) levels.

Similarly, Al-Asgah et al. (2016) recorded lipid content of carcass was significantly reduced, while protein and moisture content of carcass were significantly increased when 30% *Gracilaria arcuata* meal was supplemented in the diets for African catfish. However, other authors did not report differences in lipid or protein content of whole body of fish and shrimp species including African catfish, European sea bass gilthead sea bream, giant freshwater prawn, Nile tilapia, Pacific white shrimp, and rainbow trout. (Valente et al. 2006, 2016; Güroy et al. 2007, 2013; Soler-Vila et al. 2009; Yildirim et al. 2009; Emre et al. 2013; Marinho et al. 2013; Felix and Brindo 2014; Rodríguez-González et al. 2014; Abdel-Warith et al. 2016). Variations among these studies could be attributed to different experimental animals, seaweeds species, and inclusion levels of seaweeds.

In terms of apparent net protein and amino acid retention, there was a decreasing trend of ANPR as supplementation levels of *Ulva* meal was increased in trial 1 and trial 3. In trial 1, apparent individual/total amino acid retention decreased as *Ulva* meal inclusion levels was increased, which corresponded to ANPR. The shrimp fed with diet containing UM2 replacing the same levels of SBM as UM1 and UM3 exhibited lower ANPR and AAAR in both trial 1 and trial 2, demonstrating the second batch *Ulva* meal produced the poorest result. Similarly, a number of studies have documented negative effects of seaweed meals on the protein retention in Nile tilapia (Marinho et al. 2013), rainbow trout (Güroy et al. 2013; Soler-Vila et al. 2009; Yildirim et al. 2009) and European sea bass (Valente et al. 2006). ANPR was influenced by a number of factors including dietary protein levels, feed intake, final weight, and initial weight of animals as well as the final and initial protein content of animals (Qiu and Davis 2017b). As feed input was calculated based assumed FCR and expected growth, the exact feed intake was not measured. Given the high FCR in the treatments containing moderate and high levels of *Ulva* meal, the accurate ANPR and AAAR would be masked.

If one looks at this from a feed manufacture side, an ingredient is only going to be used if it can be included in a feed formulation at a significant rate or it brings special properties to the diets. Looking across the diets that have been evaluated there is no indication of a benefit of *Ulva* meal supplementation at low levels and there is major reduction in performance when 20–25% of *Ulva* meal is included in the diets. On an isonitrogenous basis the high levels of inclusion evaluated in this study we are only bringing ~ 5.5% protein or 3% protein on a digestible protein basis. Given the reduction of growth that is occurring across the growth trials, one would have to conclude that it is not protein quality but some other component the meal causing problems. One theory has been advanced but is beyond the scope of this research is that *Ulva* sp. is producing a chemical defense against herbivory.

Conclusions

Under the conditions of this research, moderate and high inclusion levels (> 5%) of *Ulva* meal as a replacement of soybean meal resulted in depressions in performance of shrimp. There were significant differences among different batches of *Ulva* meal with the second batch producing the poorest results. Protein quality is the part of the problem, however some other factors such as anti-nutritional components presented in *Ulva* would have a major negative impact. If *Ulva* meals are to be used to their full potential, e.g., as a primary protein source, further researches about identification of the anti-nutritional components, development of specific lines of *Ulva* with enhanced nutrient value, processing technologies evaluated to produce a high quality commercial product are warranted.

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