Use of seaweed *Ulva lactuca* for water bioremediation and as feed additive for white shrimp *Litopenaeus vannamei*

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Two experimental feeding trials were conducted during 4 weeks to evaluate the use of *Ulva lactuca* in shrimp culture: 1) for wastewater bioremediation in a water integrated system with *U. lactuca*, and 2) using different inclusion levels of *U. lactuca* meal in shrimp feed. In feeding trial 1, shrimp reared under integrated system with *U. lactuca* (SWE) resulted in similar growth and feed utilization as shrimp reared with normal water exchange (CWE). Shrimp under no water exchange (NWE) resulted in significant lower growth and higher feed conversion rate (FCR) compared to the other treatments ($p < 0.05$). Nitrogen compounds and phosphate in water from SWE and CWE treatments did not present significant differences during the experimental trial ($p > 0.05$). In feeding trial 2, seaweed biomass produced by wastewater bioremediation in SWE treatment were dried and ground to formulate diets containing 0, 1, 2, and 3% *U. lactuca* meal (0UL, 1UL, 2UL, and 3UL). Shrimp fed the 3UL diet resulted in a significant ($p < 0.05$) improvement of shrimp growth and FCR, and enhanced whole body lipid and carotenoid content by 30 and 60%, respectively, compared to control diet. Seaweed *U. lactuca* is suggested as a desirable species for wastewater bioremediation in integrated aquaculture systems, and its meal as good feed additive for farmed shrimp.

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Abstract

Two experimental feeding trials were conducted during 4 weeks to evaluate the use of *Ulva lactuca* in shrimp culture: 1) for wastewater bioremediation in a water integrated system with *U. lactuca*, and 2) using different inclusion levels of *U. lactuca* meal in shrimp feed. In feeding trial 1, shrimp reared under integrated system with *U. lactuca* (SWE) resulted in similar growth and feed utilization as shrimp reared with normal water exchange (CWE). Shrimp under no water exchange (NWE) resulted in significant lower growth and higher feed conversion rate (FCR) compared to the other treatments ($p < 0.05$). Nitrogen compounds and phosphate in water from SWE and CWE treatments did not present significant differences during the experimental trial ($p > 0.05$). In feeding trial 2, seaweed biomass produced by wastewater bioremediation in SWE treatment were dried and ground to formulate diets containing 0, 1, 2, and 3% *U. lactuca* meal (0UL, 1UL, 2UL, and 3UL). Shrimp fed the 3UL diet resulted in a significant ($p < 0.05$) improvement of shrimp growth and FCR, and enhanced whole body lipid and carotenoid content by 30 and 60%, respectively, compared to control diet. Seaweed *U. lactuca* is suggested as a desirable species for wastewater bioremediation in integrated aquaculture systems, and its meal as good feed additive for farmed shrimp.

1. Introduction

In the last decades, aquaculture has been one of the fastest growing industries of food production. By 2015, farmed shrimp represented an estimate production of 4.8 million metric tons, with a value of US$24.96 billion (FAO, 2017). Some of the challenges for this growing activity are the reduction of coastal water pollution impact (Herbeck et al. 2013) and the search for non-conventional ingredients to produce high quality feeds (Little et al. 2016). The use of seaweeds in integrated multi-trophic aquaculture (IMTA) has been proposed as an alternative for environmental-sustainable expansion of aquaculture, serving as primary food source and also for water bioremediation due to their high capability of removing inorganic nutrients from wastewater (Neori et al. 2004; Neori 2008; Fleurence et al. 2012). Benefits of integrated aquaculture of shrimp and green seaweeds has been documented for *Ulva clathrata* that showed high efficiency in removing the inorganic nutrients from water effluents (Copertino et al. 2009), in addition as an improvement of feed utilization in white shrimp *Litopenaeus vannamei* (Cruz-Suárez et al. 2010) and in brown shrimp *Farfantepenaeus californiensis* (Peña-Rodríguez et al. 2016, 2017). *Ulva lactuca* improved water quality when cultured with western king prawn *Penaeus latisulcatus* (Van Khoi and Fotedar 2011) and with *L. vannamei* (Brito et al. 2014); *Caulerpa sertularioides* presence resulted in *F. californiensis* growth enhancement (Portillo-Clark et al. 2012).

On the other hand, seaweeds are an excellent source of protein, carotenoids, minerals, polysaccharides, and vitamins making their utilization as feed additives attractive (Kumar et al. 2011; Peña-Rodríguez et al. 2011; Syad et al. 2013). Some seaweeds have been suggested as a partial feed substitute (Marinho-Soriano et al. 2007) for shrimp diet, and considered a good source of protein (da Silva and Barbosa 2009), which represents the most expensive fraction of feed cost. In some cases, shrimp composition is modified when fed seaweeds, these changes may include lipid content and carotenoids (Cruz-Suárez et al. 2010; Subhra Bikash 2015), or total cholesterol (Casas-Valdez et al. 2006). The optimal level of inclusion of seaweed meal in shrimp feed varies among seaweed species, but, in most cases, studies reflect benefits when included not higher than 5% (Cruz-Suárez et al. 2009; Rodríguez-González et al. 2014; Cárdenas et al. 2015; Yu et al. 2016; Schleder et al. 2017).

The aim of this study is to evaluate *U. lactuca* as a valuable tool for wastewater bioremediation and its feasibility to be included as feed additive for shrimp. In the present work, we evaluated shrimp growth and water quality of an integrated culture system with *U. lactuca* and, on the other side, the effect of *U. lactuca* meal as feed additive at different inclusion levels on shrimp performance, lipid and carotenoid content.

2. Materials and Methods

2.1 Collection and maintenance of seaweed

Seaweed *U. lactuca* was collected from the La Paz bay in Baja California Sur, Mexico (Collection permit Conapesca #PRMN/DGOPA-019/2015). The seaweed was washed with sterilized marine water to remove epiphytes, then placed in laboratory conditions, in 5-L marine water tanks, at 25°C, with a photoperiod of 12h:12h light:dark with fluorescent light tubes of 75 W, and using Provasoli medium at a constant concentration of 0.5 ppm of nitrogen in water. Seaweed was kept under laboratory conditions during 2 weeks prior to the feeding trial.

2.2 Feeding trials

Two feeding trials were conducted to evaluate: 1) use of *U. lactuca* for water remediation and its effect on shrimp performance and 2) use of seaweed meal produced by water bioremediation as a feed additive for shrimp. For the first experimental trial, three different treatments during 28 days were evaluated: daily water exchange (CWE), daily seaweed remediated water exchange (SWE), and no water exchange (NWE). The CWE treatment consisted in 50% daily water exchange using marine water pumped from an open water intake from La Paz bay, filtered up to 1-µm mesh and sterilized by UV light. For SWE treatment, one tank of 50 L were place with 50 g of *U. lactuca* next to a shrimp tank, making a 50% water exchange between shrimp and their respective seaweed tank every day. Each seaweed tank was provided with artificial light (cool-white fluorescent lamps 70 W; Osram) with photoperiod of 12h:12h light:dark. The seaweed was partially harvested every week to maintain 50 g in each tank. The harvested seaweed was washed with distilled water and dried in a forced-air oven at 50 °C for 4 h. In the case of NWE treatment, only 5% of water was recovered in each tank per week. All treatments were evaluated in triplicate, and each replicate consisted in a 50-L fiberglass tank provided with aeration and temperature control containing 10 *L. vannamei* shrimp (initial weight 0.30 ± 0.05 g) obtained from a commercial hatchery (Acuacultura Mahr, S.A. de C.V.) and previously acclimated to laboratory conditions (28ºC and 35‰ salinity). Shrimp from all treatments were fed with a control feed of 34% crude protein and 8% lipids (see table 1, treatment 0UL), with an initial rate of 10% biomass divided in two rations distributed at 9:00 and 15:00 hours. After second day, the feeding rate was *ad libitum* by adjusting each tank according to consumed feed. The feed was manufactured in the Aquaculture nutrition laboratory at CIBNOR. All dry ingredients (≥250 μm) were mixed first, then oil-based ingredients and water were added and mixed again to obtain a homogenous mixture, and passed through a 2-mm die in a meat grinder. The pellets were dried in a forced-air oven at 45°C for 12 h, and stored at 4°C until feeding time.

During the experimental period, water temperature, pH and oxygen were monitored daily with a multiparameter YSI 556 (YSI Incorporated, USA). The total ammonia, nitrites, nitrates, and phosphate were measured every 4 days by spectrophotometric methods according to the manufacturer´s specification (LYSA, Mexico). At the end of the experimental period, shrimp performance was measured in terms of final weight, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), feed consumption, and survival.

For the second experiment, a 28 days feeding trial was performed to evaluate the *U. lactuca* meal produced by water bioremediation. Based on the control diet of assay 1, three more diets were produced including 1, 2, and 3% levels of the seaweed meal (see Table 1). All experimental seaweed-feeds were produced as described previously as in the control feed. Each treatment was evaluated in triplicate as described in assay 1, using shrimps with an initial average weight of 0.59 ± 0.09 g. Feeding strategy was conducted as in the previous trial. At the end of the experimental period, five complete shrimps and five shrimps separated in cephalothorax (head) and tail from each treatment were lyophilized for total lipid and carotenoid analysis. Total lipid content was performed according to Barnes and Blackstock (1973) by using phosphosulphovanillin method and measured by spectrophotometry (Termo, Multiskan spectrum, Vantaa-Finland) at 540 nm. Total carotenoid content was analyzed according to Palacios et al. (1999), employing acetone:methanol (2:1) for extraction and measured by spectrophotometry at 495 nm.

Water quality parameters were measured as described previously. Proximate analysis of all experiment feeds and *U. lactuca* was conducted according to AOAC (2005) methods, nitrogen free extract (NFE) was calculated through difference, and gross energy was measured with an adiabatic calorimeter. Total carotenoids from *U. lactuca* meal was analyzed as described for shrimp samples. The proximate composition of experimental feeds and *U. lactuca* meal are presented in Table 1.

2.3 Data analysis

Results were reported as means ± standard deviation (SD) and group means were compared using one-way analysis of variance (ANOVA) followed, if applicable, of a Tukey´s multiple comparison test (95% confidence). All data were analyzed with the SPSS Statistics 17.0 software.

3. Results

At the end of feeding trial 1, shrimp under daily water exchange (CWE) and daily seaweed remediated water exchange (SWE) treatments resulted in significant higher ($p < 0.05$) final weight, weight gain, and SGR compared to shrimp with no water exchange (NWE) (Table 2). Feed consumption was similar among the treatments, nevertheless FCR observed in NWE treatment was significantly higher than the rest of the treatments ($p < 0.05$). Shrimp under NWE treatment showed lower percentage of survival but not significantly different compared to the rest of treatments. At the end of the experimental period, water quality parameters were significantly different among treatments (Figure 1). The NWE treatment resulted in significant increment ($p < 0.05$) of total ammonia nitrogen, nitrites, nitrates, and phosphate compared to treatments with water exchange, whereas the pH showed no significant differences among treatments. Removal of inorganic compounds in seaweed treated water was higher than 80% for nitrogenous compounds and 64% for phosphate compared to the treatment without water exchange. Total harvest of fresh *U. lactuca*, under the experimental conditions, was 225 ± 25 g per tank, with a specific growth rate of 5.37 ± 0.41 (% day^{-1}). After drying, seaweed meal resulted with a 15.5% of crude protein and 36.5% ash (Table 1), which was used to prepare experimental feeds for feeding trial 2.

In feeding trial 2, experimental feeds did no show differences in proximal composition except for ash content in feed containing 3% of seaweed meal (3UL), which resulted 1.1% higher compared to the control feed (0UL) as expected. Total carotenoids in *U. lactuca* meal resulted in 3.5 mg g^{-1} in dry basis. Results of shrimp performance after evaluation of experimental feeds with different inclusion levels of *U. lactuca* meal (Table 3) showed that shrimp fed diet with 3% seaweed meal had a significantly higher growth in terms of final weight, weight gain, and SGR ($p < 0.05$) compared to the control diet (0UL) and that of 1% of seaweed (1UL). Shrimp fed 2UL treatment showed no significant differences in growth parameters compared to the other treatments. In terms of feed utilization, the 3UL diet induced a significantly lower ($p < 0.05$) FCR compared to the rest of the treatments. Shrimp survival was higher than 95% with all treatments. Total lipid content in whole body (Figure 2A) was significantly higher in shrimp with 3UL compared to the rest of treatments ($p < 0.05$). Additionally, shrimp fed 3UL showed significantly higher concentration of total carotenoids in the head; in the muscle, 2UL and 3UL yielded significantly higher amounts of carotenoids than the rest of treatments; and considering the whole body, all *U. lactuca* meal diets resulted in significantly higher content of carotenoids compared to the control diet (Figure 2B). Water quality parameters during the second experimental period were very stable among treatments: temperature (28 ± 0.4 °C) pH (8.0 ± 0.1), NH3, NH4+ (<0.5 mg L^{-1}), NO_2 (<0.25 mg L^{-1}), and NO_3 (<5 mg L^{-1}).

4. Discussion

According to the water quality parameters during experiment 1, results revealed the high efficiency of *U. lactuca* in removing nitrogen compounds and phosphorus from shrimp's wastewater (80% and 64%, respectively) under the integrated recirculation system. These results are consistent with other reports describing the high efficiency of *Ulvales* in biofiltering inorganic compounds from aquaculture effluents. Copertino et al. (2009) determined that *U. clathrata* removes up to 70-82% of the total ammonia nitrogen (TAN) and 50% of phosphate. In a study of an intensive co-culture system of *U. lactuca* and *L. vannamei*, TAN and phosphate were significantly reduced in culture water by 25.9% and 24.6%, respectively, compared to a system without seaweed (Brito et al. 2014). The nitrogenous compounds removed by seaweed reflected 15.5% protein content in meal, which revealed a higher proportion than reports in wild collected seaweed (7.1 to 10.7%) (Wong and Cheung 2000; Yaich et al. 2011; Tabarsa et al. 2012), but lower than described for *U. lactuca* cultured in a controlled system (21.1%) (Ventura and Castañón 1998). An integration of a total or partial recirculating system of *U. lactuca* and shrimp may decrease the need of out coming water, improving farm biosecurity and reducing the possibility of disease outbreaks (Muniesa et al. 2015). According to the present experimental results, *U. lactuca* meets different criteria suggested by other authors to select an efficient seaweed biofilter for integrated aquaculture, which includes nutrient intake from wastewater (Kang et al. 2011), seaweed density, and water flow rate (Al-Hafedh et al. 2015).

Water bioremediation with *U. lactuca* (trial 1) did not affect shrimp growth or feed utilization, as described by Fourooghifard et al. (2017), where the water quality improved without affecting shrimp growth in a zero water exchange system of integrated culture of *L. vannamei* and *Gracilaria cortica.* No significant growth differences were observed in *L. vannamei* cultured in floating cages with red seaweed *Kappaphycus alvarezii* compared to shrimp monoculture system (Lombardi et al. 2006). On the other hand, when no water exchange was performed, shrimp growth and feed utilization was affected possibly by water quality. It has been described that exposure to high concentrations of ammonia in water increases oxygen and energy demand in shrimp (Racotta and Hernández-Herrera 2000) reflected in lower growth (Chen and Kou 1992). However, shrimp performance in low or no water exchange culture systems can also be affected by the shrimp stock densities (Hopkins et al. 1993), feed composition (Wasielesky et al. 2006), and feeding frequency (Tacon et al. 2002).

In the case of feeding trial 2, shrimp growth was improved when fed 3% *U. lactuca* meal in feed. Rodríguez-González et al. (2014) suggest that the limiting inclusion level for *U. lactuca* meal in shrimp feed should not exceed 5%, showing that levels of 10 and 15% reduced significantly shrimp growth compared to a control diet without seaweed inclusion. Serrano et al. (2015) also experimented with 15 and 30% *U. lactuca* meal inclusion in *P. monodon* shrimp, finding no growth improvement at the lower inclusion level and significant reduction of shrimp growth at the higher inclusion level. Shrimp growth improvement at low inclusion levels were found with other seaweed meals, as for example with 2 or 4% of *Macrocystis pyrifera* (Cruz-Suárez et al. 2000) or *Sargassum sp.* (Suárez-García 2006) included in shrimp feed. Yu et al. (2016) also recommends low inclusion levels (2 or 3%) of *Gracilaria lemaneiformis* meal in order to improve weight gain in *L. vannamei*. The growth promotor effect, as in the present work, is generally attributed to vitamins, minerals and lipids present in the seaweed (Cruz-suárez et al. 2008; Tabarsa et al. 2012).

U. lactuca showed high content of ash (36.5%) similar to the value reported by Rodríguez-González et al. (2014) (41.7%), which could explain the limiting inclusion level of seaweed meal in the feed. High inclusion levels of seaweed meal in feed reflects higher contents of ash, which has been related with decrement of feed digestibility (Brunson et al. 1997; Yang et al. 2009). In a study in black tiger shrimp *Penaeus monodon*, apparent digestibility of *U. lactuca* meal was significantly lower (71%) than for protein concentrate from *U. lactuca* (99%) (Santizo et al. 2014). This limitation on *Ulva* meal in feed is not present when fresh seaweed is used as food, like when shrimp are fed *U. lactuca* (Pallaoro et al. 2016) or *U. clathrata* (Cruz-Suárez et al. 2010), which, in both cases, could be save at around 50% of pelleted feed without negative effects on shrimp growth.

The increase of 30% in whole body lipid content of shrimps fed 3% *U. lactuca* meal diet, respect to control feed, was also described in *L. vannamei* co-cultured with *U. clathrata,* where a combination of pelleted feed and seaweed increased up to 50% total lipid content in shrimp (Cruz-Suárez et al. 2010). This increase in shrimp lipid content could be partially attributed to carotenoids content in the algae. Total carotenoids present in the *U. lactuca* meal in the present study was in the range described for the same species and others *Ulvales* (240 to 500 ug g^{-1} fresh weight) (Xia et al. 2004; Kumar et al. 2010; Peña-Rodríguez et al. 2011). Shrimp fed diets with *U. lactuca* meal significantly increased whole body carotenoid content, with the highest concentration in the head. Penaeid shrimp effectively use carotenoids from *Ulvales* to increase body pigmentation. Shrimp fed fresh *U. clathrata* increase carotenoid content as the use of pelleted food decreased (Cruz-Suárez et al. 2010). In another study, feeds with 3.3% of seaweed (*U. clathrata*) meal inclusion diet resulted in higher shrimp pigmentation after cooking respect to *Ascophilllum nodosum* and *Macrocystis pyrifera* diets (Cruz-Suárez et al. 2009). A diet containing 5% of *Enteromorpha intestinalis* meal increased significantly the astaxanthin content in *P. monodon* muscle compared to a control diet after 30 days of feeding trial (Subhra Bikash 2015).

5. Conclusions

In conclusion, the results of the present study demonstrated the potential of *U. lactuca* seaweed for integrated aquaculture systems in terms of nitrogen and phosphate water bioremediation, and the benefits of using the seaweed biomass produced as feed additive for shrimp at 3% of inclusion level, as revealed by the improvement in growth, feed conversion rate, and body carotenoid content.

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Figure 1

Water quality parameters during experiment 1.

CWE: 50% daily water exchange; SWE: 50% daily exchange with water bioremediation by *Ulva lactuca*; NWE: 0% water exchange. A) Water variations of total ammonia nitrogen (N-NH_3); B) Water variations of nitrate (NO_3); C) Water variations of nitrite (NO_2); D) Water variations of phosphate (PO_4); E) Water variations of pH. (*) are significantly different ($p<0.05$).

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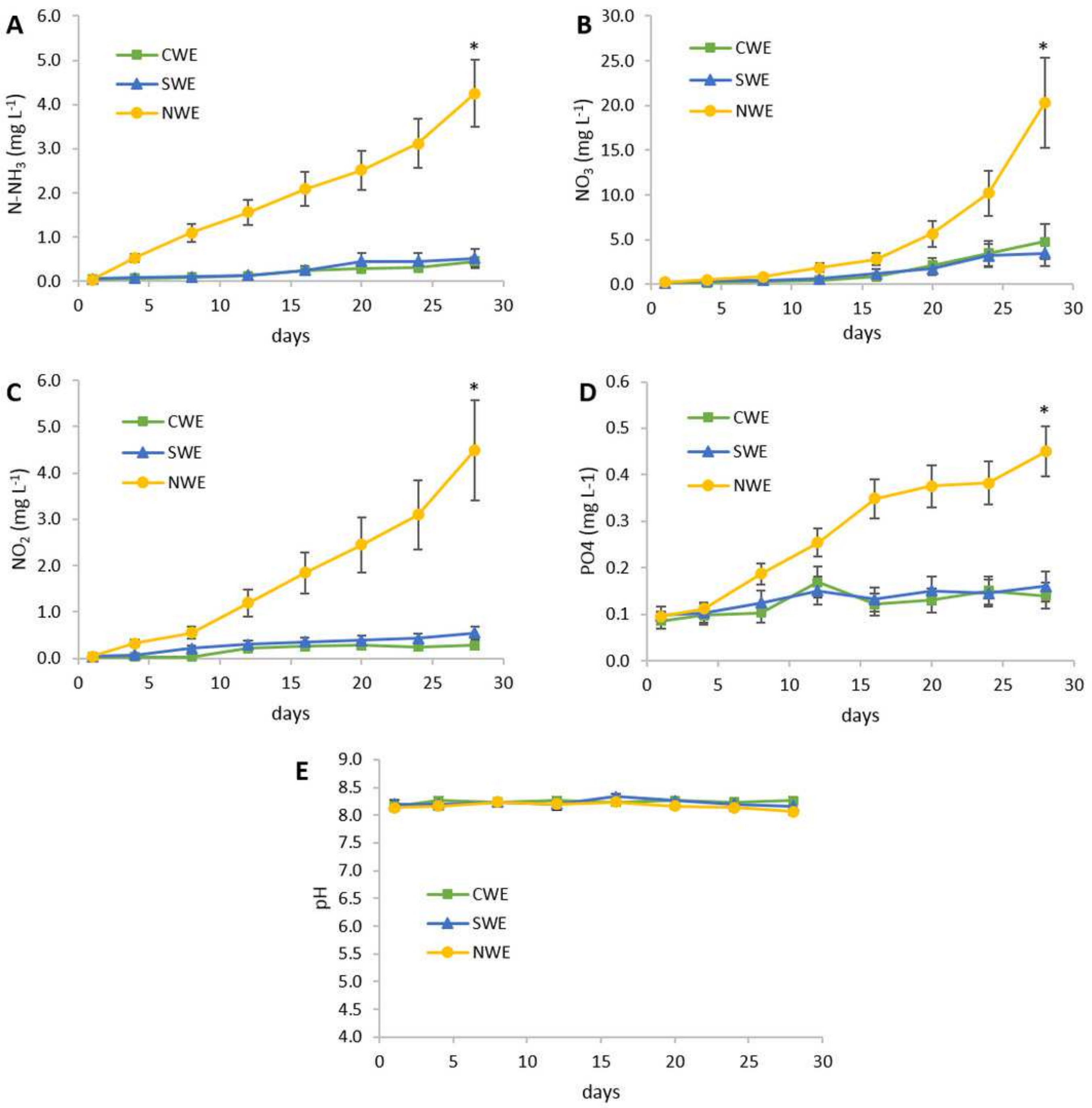
A
N-NH$_3$ (mg L^{-1})
CWE
SWE
NWE
*
days
B
NO$_3$ (mg L^{-1})
CWE
SWE
NWE
*
days
C
NO$_2$ (mg L^{-1})
CWE
SWE
NWE
*
days
D
PO4 (mg L-1)
CWE
SWE
NWE
*
days
E
pH
CWE
SWE
NWE
days

Figure 2

Total lipids and total carotenoid in shrimp fed experimental diets containing different inclusion levels of U. lactuca meal.

A) Total lipids and B) Total carotenoids in shrimp fed experimental diets containing 0% (0UL), 1% (1UL), 2% (2UL) and 3% (3UL) of *U. lactuca* meal. Values are given as mean ± SD of multiple determinations (n=5). Different superscripts denotes statistical differences among treatments ($p<0.05$).

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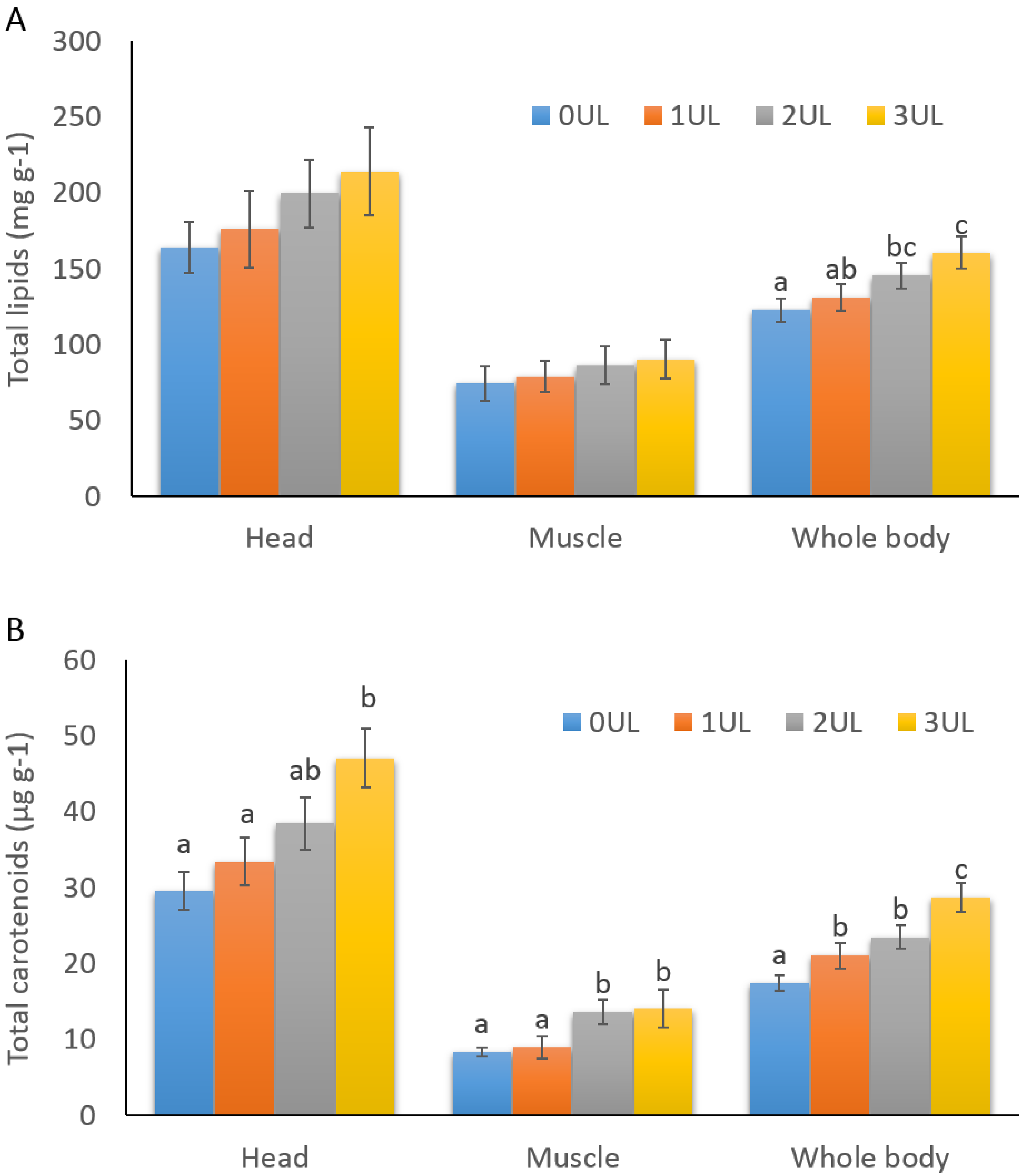
A
Total lipids (mg g-1)
300
250
200
150
100
50
0
0UL 1UL 2UL 3UL
a ab bc c
Head
Muscle
Whole body
B
Total carotenoids (µg g-1)
60
50
40
30
20
10
0
0UL 1UL 2UL 3UL
a a ab b
a a b b
a b b c
Head
Muscle
Whole body

Table 1 (on next page)

Ingredients, proximate composition, and gross energy of experimental diets and *Ulva lactuca* meal.

Table 1. Ingredients, proximate composition, and gross energy of experimental diets and *Ulva lactuca* meal.

	0UL	**1UL**	**2UL**	**3UL**	***U. lactuca***
Ingredients					
Fish meal [a]	240	240	240	240	
Soybean meal [b]	230	230	230	230	
Wheat meal [c]	401	391	381	371	
Soy lecithin [d]	41	41	41	41	
Corn gluten [e]	30	30	30	30	
Fish oil [f]	24	24	24	24	
Vitamin premix [g]	18	18	18	18	
Grenetin [h]	10	10	10	10	
Mineral premix [i]	5	5	5	5	
Vitamin C [j]	1	1	1	1	
Ulva lactuca meal	0	10	20	30	
Proximate composition (g 100 g^{-1} dry matter)					
Moisture	8.3±0.1	8.2±0.1	8.7±0.2	8.5±0.1	8.7±0.1
Protein	33.9±0.24	33.9±0.06	33.7±0.10	33.5±0.08	15.5±0.1
Lipids	7.9±0.08	7.9±0.03	7.9±0.06	7.9±0.12	0.3±0.01
Crude Fiber	0.87±0.01	0.86±0.06	0.86±0.03	0.87±0.06	3.3±0.1
Ash	6.6±0.03	6.9±0.03	7.3±0.01	7.7±0.03	36.5±0.1
NFE	50.6	50.3	50.2	50.0	44.5
Gross energy (MJ kg^{-1})	18.09±0.47	17.93±0.22	17.77±0.35	17.60±0.28	9.46±0.14

[a,f] Proteinas Marinas y Agropecuarias SA de CV, Jalisco, MX.
[b] Promotora industrial acuasistemas SA de CV (PIASA), Baja California Sur, MX.
[c] Molino San Cristobal, Sonora, MX.
[d] Suministros AZ, Baja California Sur, MX.
[e] Agro Insumos Basicos, SA de CV, MX.
[g] Vitamins: Vit. A, (20,000 UI/g) 90 mg/kg; Vit. B1, 9 mg/kg; Vit. B2, 54 mg/kg; Vit. B5, 90 mg/kg; Vit. B6, 18 mg/kg; Vit. B12, 0.04 mg/kg; Vit. K3, 36 mg/kg; Vit. D3, (850,000 UI/g) 144 mg/kg; Vit. H, 1 mg/kg; folic acid, 3.24 mg/kg; Inositol, 90mg/kg. Sigma aldrich, Missouri, US.
[h] Knox, Estado de Mexico, MX.
[i] Minerals: $CoCl_2$, 20 mg/kg; H_2MnO_5S, 3.3 g/kg; $H_{14}O_{11}SZn$, 66 g/kg; $CuH_{10}O_9S$, 1.3 g/kg; $FeSO_4$, 20 g/kg; Na_2SeO_3, 50 mg/kg; KI, 330 mg/kg. Sigma Aldrich, Missouri, US.
[j] Rovimix Stay C 35%, DSM, Heerlen, NL.

Table 2 (on next page)

Growth, feed utilization and survival after 4-week experimental trial with *L. vannamei* reared under regular water exchange (CWE), water recirculation with *U. lactuca* (SWE) and no water exchange (NWE).

Table 2. Growth, feed utilization and survival after 4-week experimental trial with *L. vannamei* reared under regular water exchange (CWE), water recirculation with *U. lactuca* (SWE) and no water exchange (NWE)

	CWE	SWE	NWE	*P* value
Final weight (g)	2.15±0.06 b	2.08±0.04 b	1.82±0.05 a	0.000
Weight gain (%)	613±19 b	593±12 b	503±16 a	0.000
SGR (% day^{-1})	7.02±0.10 b	6.91±0.06 b	6.42±0.10 a	0.000
FC (g)	2.20±0.08	2.11±0.02	2.14±0.05	0.214
FCR	1.19±0.01 a	1.18±0.02 a	1.41±0.06 b	0.000
Survival (%)	90±10	96±6	83±6	0.171

Values are given as mean ± SD of triplicate determinations. Means with different superscripts in same row are significantly different ($p < 0.05$).

Weight gain (%) = (final weight−initial weight)/ initial weight × 100.

SGR (% day-1) =100 (ln(average final weight)−ln(average initial weight)) /number of days.

FC (g) = pelleted feed consumed per shrimp

FCR = pelleted feed consumed (g) /wet weight gain (g).

Survival (%) = final number of shrimp/ initial number of shrimp × 100.

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Table 3 (on next page)

Growth performance, feed utilization, and survival after 4-week experimental trial with L. vannamei juveniles fed diets containing different levels of U. lactuca meal.

Table 3. Growth performance, feed utilization, and survival after 4-week experimental trial with *L. vannamei* juveniles fed diets containing different levels of *U. lactuca* meal.

	0UL	1UL	2UL	3UL	*P* value
Final weight (g)	2.54±0.08 a	2.55±0.08 a	2.58±0.11 ab	2.78±0.06 b	0.026
Weight gain (%)	330±13 a	332±13 a	337±19 ab	371±10 b	0.028
SGR (% day^{-1})	5.21±0.11 a	5.23±0.11 a	5.27±0.15 ab	5.54±0.08 b	0.030
FC	2.47±0.06	2.44±0.04	2.51±0.06	2.53±0.03	0.163
FCR	1.27±0.03 b	1.25±0.05 b	1.26±0.05 b	1.15±0.03 a	0.028
Survival (%)	100	96±6	100	100	0.441

Values are given as mean ± SD of triplicate determinations. Means with different superscripts in same row are significantly different ($p<0.05$).