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Shrimp/*Ulva* co-culture: A sustainable alternative to diminish the need for artificial feed and improve shrimp quality

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ABSTRACT

Juvenile shrimp *Litopenaeus vannamei* (3.5 g initial weight) and green seaweed *Ulva clathrata* were co-cultured in outdoors tanks (2000 L) for 45 days; co-cultured *Ulva* was suspended on a mesh stretched on the water surface. Four dietary treatments were evaluated: *Ulva* alone, *U*lva + 55% feed ration, *Ulva* + 90% feed ration, and 100% feed ration (control group without co-cultured *Ulva*). The control group was fed a commercial pellet (30% protein, 8% fat) at a daily ration of 3.5% shrimp biomass. Water turbidity in co-culture tanks was lower than that in control group. Survival was similar among the experimental groups (>80%). The *Ulva* intake by shrimp improved the artificial feed conversion ratio and the growth rate: with 10 or 45% less commercial feed, growth rate improved by 60%. Additionally, *U. clathrata* intake diminished lipids content in shrimp carcass and also modified the fatty acids profile. Shrimp body carotenoids content was significantly higher in the co-culture groups, suggesting that *Ulva* carotenoids were efficiently assimilated and metabolized, and also may be involved in growth enhancement.

1. Introduction

The massive expansion of commercial shrimp culture in the last decades has begun to face some important limitations like increasing prices of raw materials, and higher quality demand of the shrimp market (Lucien-Brun and Vidal, 2006). Polyculture of several aquatic animals and seaweeds has been explored with successful results on growth rates and pollution impact (Chopin et al., 1999; Evans and Langdon, 2000; Neori, 2008; Neori et al., 1998, 2004; Petrell and Alie, 1996; Shpigel and Neori, 1996; Schuenhoff et al., 2003; Troell et al., 1997; Viera et al., 2005). Vandendriessche et al. (2007) described that some floating seaweeds may serve as refuges or feed for several fish species.

Some seaweeds, including *Ulva clathrata*, can provide specific environmental conditions in shrimp ponds, like water quality improvement by nutrient uptake and physical filtration (Copertino et al., 2009; Hamano et al., 2007; Paul and de Nys, 2008), as well as protective substrate for shrimp (Porchas-Cornejo et al., 1999). These conditions may enhance shrimp growth and health; however, the nutritional benefits that consumption of fresh macroalgae can bring to shrimp have not been reported, although some studies have shown that the consumption of fresh *Enteromorpha* sp. induced maturation of *Penaeus indicus* (Emerson 1980; Emerson et al., 1983).

Seaweed chemical composition may vary according to the species, habitat, environmental conditions and other factors (Ito and Hori, 1989; Marinho-Soriano et al., 2006). In general, green seaweeds may have two or three times more protein content than brown seaweeds (Burtin, 2003). In the case of *U. lactuca*, polyunsaturated fatty acids can represent up to 66% of total fat (Wahbeh, 1997). For *Enteromorpha* sp., Aguilera-Morales et al. (2005) reported values of 6.9% to 9.1% for linoleic acid, 3.5% to 6.4% for linolenic acid, and 2.8% to 5.7% for eicosapentaenoic acid. Carotenoids present in *U. clathrata* meal proved to be good pigmentation precursors for the white shrimp *L. vannamei* (Cruz-Suárez et al., 2009a), lutein being the main carotenoid present in this alga.

In the search of new sustainable methods to improve shrimp production with lower costs in Mexico, the green seaweed *Ulva clathrata*, known commonly also as *Enteromorpha* sp. or Ao-nori, has been cultured in shrimp ponds in the last years with patented culture technologies developed by Sinaloa Seafields; the technology was designed to maintain *Ulva clathrata* floating, which accelerates the biomass production and improves the harvesting efficiency (Moll and Deikman,

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1995; Moll, 2004; Moll, 2006). The present study was carried out to evaluate the potential nutritional benefits of co-culturing white shrimp *L. vannamei* and green seaweed *U. clathrata* in experimental tanks.

2. Material and methods

A 6 week growth trial was carried out to test the following treatments: *Ulva* and shrimp co-culture (U100), *Ulva* and shrimp co-culture plus 55% commercial feed ration (UF55), *Ulva* and shrimp co-culture plus 90% feed ration (UF90), and shrimp monoculture with 100% feed ration (F100).

2.1. Shrimp and seaweed

Juvenile *Litopenaeus vannamei* (2–5 g individual weight) were obtained from a farm in Guasave Sinaloa, Mexico. The shrimp were transported in a fiberglass tank to the "Algalimentos" company facilities in Los Mochis Sinaloa, within approximately 3 h. The cultivated *Ulva clathrata* was obtained from "Sinaloa Seafields" laboratory stocks.

2.2. Experimental procedure (system design)

The study was carried out in 12 lined tanks (2000 L, 0.75 m water height). These tanks were placed outdoors, using pre-filtered and UV-treated seawater, with zero water exchange and with air supply over night. The experimental treatments were randomly assigned in three blocks of four tanks. 50 shrimp (3.5 ± 0.1 g) were stocked per tank.

In co-culture tanks, 1.5 kg centrifuged *U. clathrata* was suspended at the beginning of the experiment on a horizontal polyethylene 2.5×2.5 cm mesh stretched on a 1.9 cm PVC tubing square floating on the water surface, covering 90% of the surface area; algae samples were taken from the tanks every 14 days for analysis. For treatments UF55, UF90 and F100, a commercial feed (30% crude protein, 8% fat, as fed) was distributed three times a day (06:00, 12:00 and 18:00 h) on 30 cm diameter feeding trays; 100% feed ration was equivalent to a daily ration of 3.5% shrimp biomass; unconsumed feed found on trays was quantified before the next feeding.

2.2.1. Environmental parameters

Dissolved oxygen concentration, temperature, pH and salinity were measured taking samples 30 cm under the water surface, using an electronic multimeter and a refractometer, with a precision of ± 0.1 mg L^{-1} O_2, ± 0.2 °C, ± 0.01 pH and ± 0.1 g L^{-1} salinity. Dissolved O_2 concentration and temperature were measured every morning (06.00 h) and afternoon (18.00 h) during the experiment, pH and salinity only in the morning. Turbidity (Secchi disk), ammonia-N and nitrates (Saltwater Master Test kit, API) were measured every 2 days. Water samples were taken frequently from each tank to determine natural production during the experiment (unicellular plankton counting).

2.2.2. Shrimp performance

Final weight (FW), weight gain (WG), survival, specific growth rate (SGR) and artificial feed conversion ratio (aFCR), were assessed at the end of experiment. Shrimp from each treatment were frozen and sent for analysis to the quality assurance laboratory of the company "Vegetales y Pigmentos Naturales" (VEPINSA) situated in Los Mochis, Sinaloa, Mexico.

2.3. Chemical analyses

Proximate composition of seaweed, commercial feed and shrimp were analyzed according to the following methods: A.O.C.S. Bc 3–49 (97) 1–1 for crude fat, A.O.C.S. Ba 6–48 (97) 1–4 for crude fiber, A.O.C.S. Ca 11–55 (97) 1–1 for ash, A.O.C.S. Ba 4a–38 (97) 1–2 for protein and A.O.C.S. Ba 2–38 (97) 1–1 for moisture. Quantification of free astaxanthine, esterified astaxanthine, and total carotenoids were performed by HPLC using a 20×150 mm carotenoid C30 column (YMC Inc., Wilmington, NC, USA). Pigmentation was measured on shrimp, previously cooked for 3 min, by a Minolta CR300 chromameter, taking reflectance values of redness (A), yellowness (B) and lightness (L) of the first abdominal segment. A quantitative fatty acid profile in feed and shrimp was determined by gas chromatography (Varian Model 3800). Feed pellet stability in water was measured in terms of% loss of dry matter and protein after 1 h immersion in seawater according to Cruz-Suárez et al. (2009b).

2.4. Statistical analysis

Results were reported as means ± standard deviation (SD) and group means were compared using one-way analysis of variance (ANOVA) followed, if applicable, by a Tukey's multiple comparison test ($\alpha = 0.05$).

3. Results

3.1. Ulva and commercial feed composition

Proximate compositions of artificial feed and seaweed are shown in Table 1. Feed composition was as expected; % losses of dry matter and protein from the feed pellet, after 1 h immersion in seawater, were 12 and 9.9% respectively. Average proximate composition of *Ulva* dry matter was 21% crude protein, 1.5% fat and 38% ash, with variation coefficients of 15, 20 and 14%, respectively.

Artificial feed and seaweed fatty acid compositions (percentage relative to fat dry weight) are presented in Table 2. *U. clathrata* shows a high percentage of polyunsaturated fatty acids (PUFAs), principally linolenic acid (C18:3*n*-3), which was present at a higher level (14.5%) than that described by Aguilera-Morales et al. (2005) for *Enteromorpha sp.* (3.5–6.4%); in contrast, its content of highly unsaturated fatty acids (HUFAs), especially docosahexaenoic acid (C22:6*n*-3, DHA), was lower than that of the commercial feed.

3.2. Environmental parameters

The O_2 concentration showed significant differences ($P < 0.001$) in the afternoon, being higher for the monoculture treatment (Table 3). Turbidity was significantly affected ($P = 0.001$) by the presence of *Ulva* on the water surface, being lower as a consequence of lower plankton production (78 vs 28 cells μL^{-1}) (Table 1). Almost 90% of total cells produced in the tanks were dinoflagellates and diatoms, 5% of total cells were ciliates; no significant differences were found in predominant cells proliferation among treatments. Total ammonia-N concentration, ranging from 0.1 to 0.25 mg L^{-1}, was not significantly different among treatments ($P = 0.54$). Nitrate was not detected in any treatment during the experiment. The average temperatures during the experiment were 24.5 °C (±1.3 SD) in the morning and 27.4 °C (±1.4 SD) afternoon. Salinity and pH averages were 34.2 g L^{-1} (±1.0 SD) and 9.1 (±0.2 SD), respectively.

Table 1
Proximate composition of artificial feed and average proximate composition of *U. clathrata* during the experiment (% dry basis).

	Fat	Protein	Ash	Crude fiber	NFE
AF	8.9 ± 0.2	33.3 ± 0.1	8.6 ± 0.1	1.3 ± 0.1	47.9 ± 0.1
Uc	1.5 ± 0.3	20.7 ± 3.1	38.4 ± 5.5	5.6 ± 0.5	33.3 ± 3.5

AF = artificial feed, Uc = *Ulva clathrata*, NFE = nitrogen free extract.
Values are given as mean ± SD of triplicate determinations. In the case of *U. clathrata* determinations were made from 3 samples collected at 14 days intervals ($n = 3 \times 3$).

Table 2
Fatty acid profile (% relative to total fatty acids) of artificial feed and *U. clathrata*.

Fatty acid	Artificial feed	*U. clathrata*
C14:0	3.27	ND
C14:1*n*-3	ND	1.57
C16:0	23.07	23.5
C16:1*n*-7	3.20	0.62
C18:0	3.10	ND
C18:1*n*-9	28.5	6.99
C18:2*n*-6	19.3	9.61
C18:3*n*-3	3.03	14.5
C:18:4*n*-3	0.20	5.7
C20:0	3.25	0.27
C20:1*n*-9	0.70	ND
C20:5*n*-3	5.51	2.04
C22:1*n*-9	ND	ND
C22:5*n*-3	0.93	0.74
C22:6*n*-3	5.56	1.76
C24:1*n*-9	0.04	ND
∑Saturated[a]	32.7	25.3
∑MUFA[b]	32.4	9.18
∑PUFA[c]	22.4	24.1
∑HUFA[d]	12.2	10.1

ND = not detected.
[a] Saturated fatty acid: 14:0, 16:0, 18:0, 20:0.
[b] MUFA (Monounsaturated fatty acid): 14:1*n*-3, 16:1*n*-7, 18:1*n*-9, 20:1*n*-9, 22:1*n*-9, 24:1*n*-9.
[c] PUFA (Polyunsaturated fatty acid): 18:2*n*-6, 18:3*n*-3.
[d] HUFA (highly unsaturated fatty acid): 18:4*n*-3, 20:5*n*-3, 22:5*n*-3, 22:6*n*-3.

3.3. Shrimp zootechnical parameters

Survival (80–92%) was not significantly different among treatments ($P=0.61$) (Table 4). The shrimp weight gain (WG) and specific growth rate (SGR) showed highly significant differences over 6 weeks ($P<0.001$ and $P=0.001$, respectively), UF55 and UF90 treatments showing a clear improvement. Conversion ratio of commercial feed (aFCR) improved significantly with the co-culture treatments ($P=0.004$). Algae consumption was observed *de facto* and confirmed by the green colour of feces for treatment U100, while feces colour was light brown for the F100 treatment. Feces coloration for UF55 and UF90 was not uniform, showing longitudinal green/brown strands, which suggested an alternation in consumption of algae and feed, but segregation in the stomach and a simultaneous intestinal transit of algae and feed.

3.4. Shrimp composition analysis

Shrimp proximate compositions for the four treatments are shown in Table 5. UF90 shrimp had the lowest moisture content (73.4%) and the highest protein content (18.7%) among treatments. Shrimp fat content was correlated ($R^2=0.997$) with the artificial feed ration, following a quadratic model (Fig. 1). *U. clathrata* as the sole food source (U100) increased significantly ($P=0.025$) the ash content in shrimp (3.6%) in comparison to that in co-cultured shrimp with artificial feed supply (2.5–2.8%).

Table 3
Oxygen concentration, turbidity and plankton production in experimental tanks in a co-culture with *U. clathrata* without artificial feed supply (U100), a co-culture with partial ration of artificial feed (UF55 and UF90), and shrimp monoculture fed artificial feed (F100).

	U100	UF55	UF90	F100	*P* values
O_2 am (mg L^{-1})	4.4 ± 1.2	4.2 ± 1.1	4.1 ± 1.6	3.9 ± 2.1	0.626
O_2 pm (mg L^{-1})	6.6 ± 1.6^{a}	6.1 ± 1.7^{a}	6.0 ± 2.1^{a}	7.9 ± 1.6^{b}	<0.001
Turbidity (Secchi cm)	72.8 ± 4.9^{b}	72.7 ± 4.8^{b}	70.9 ± 6.7^{b}	43.8 ± 11.5^{a}	0.001
Total cell (cell μL^{-1})	30 ± 20	20 ± 15	34 ± 16	78 ± 29	0.545

Values are given as mean ± SD of triplicate determinations. Superscripts in same row indicate homogeneous subsets as determined by Tukey's test ($\alpha=0.05$).

Table 4
Averages (±SD) for initial weight (IW), final weight (FW), weight gain (WG), specific growth rate (SGR), artificial feed conversion rate (aFCR) and survival of juvenile shrimp.

	IW (g)	FW (g)	WG (%)	SGR	aFCR	Survival (%)
U100	3.5 ± 0.1	5.6 ± 0.4^{a}	61 ± 13^{a}	1.13 ± 0.2^{a}	–	92 ± 11
UF55	3.5 ± 0.1	9.4 ± 0.9^{c}	165 ± 25^{c}	2.32 ± 0.2^{b}	1.24 ± 0.2^{a}	80 ± 10
UF90	3.4 ± 0.1	9.5 ± 0.6^{c}	179 ± 18^{c}	2.44 ± 0.1^{b}	1.62 ± 0.4^{a}	95 ± 4
F100	3.5 ± 0.1	7.3 ± 0.1^{b}	109 ± 4^{b}	1.30 ± 0.4^{a}	2.61 ± 0.8^{b}	89 ± 10
P value	0.985	0.008	<0.001	0.001	0.004	0.610

Values are given as mean ± SD of triplicate determinations. Superscripts in same column indicate homogeneous subsets as determined by Tukey's test ($\alpha=0.05$).
WG (%) = (final weight − initial weight) / initial weight × 100.
SGR = 100(ln average final weight − ln average initial weight) / number of days.
aFCR = dry weight of artificial feed consumed (g) / wet weight gain (g).
Survival (%) = final number of shrimp / initial number shrimp × 100.

Shrimp whole-body fatty acid composition is presented in Table 6. Palmitic acid (C16:0) was the predominant fatty acid in all shrimp among treatments (which coincides with the data reported by Cabrera et al., 2005): however, shrimp in monoculture (F100) had significantly higher levels of palmitic acid (C16:0), and lower levels of linolenic acid (C18:3*n*-3) than those in co-culture. Levels of EPA (C20:5*n*-3) and DHA (C22:6*n*-3) were significantly higher in shrimp from the U100 treatment. The percentage of monounsaturated fatty acids in shrimp from F100 was lower but not significantly ($P=0.05$) different than for those in co-culture. Shrimp from the U100 treatment had the lowest percentage of PUFAs and highest percentage of HUFAs in contrast to the other treatments.

The average concentration of total carotenoids in *Ulva* during the experiment was 0.2% of dry weight, where trans-lutein represented 80% of total carotenoids. Quantitative analyses of carotenoids from whole shrimp body showed that *Ulva* consumption increased significantly ($P=0.02$) the total carotenoids concentration, with treatment U100 resulting in a higher pigmentation of shrimp. Astaxanthine was found as the major carotenoid constituent (76 to 89% of total carotenoids) in shrimp. Free and esterified astaxanthine mean values ranged between 12.5 to 81.9 and 22.1 to 80.3 mg kg^{-1}, respectively. Carotenoids contents in shrimp were negatively correlated with the artificial feed intake (Fig. 2).

Pigmentation of the first abdominal segment, analyzed by reflectance (chromameter), indicates that red coloration was significantly higher ($P=0.001$) for U100 and UF55 treatments (Table 7). Shrimp from U100 had the highest yellow colour ($P<0.001$) and the lowest luminosity ($P=0.02$), luminosity (or lightness) being higher in the absence of colour.

4. Discussion

The artificial feed conversion ratio (aFCR) was calculated considering the artificial feed as the sole source of nutrient. Although this expression excludes the contribution of natural production and *Ulva* to food consumed by shrimp, it is useful to visualize the feed savings and for a practical estimation of the feed cost per shrimp biomass production.

Table 5
Proximate composition of *L. vannamei* in a co-culture with *U. clathrata* without artificial feed supply (U100), a co-culture with partial ration of artificial feed (UF55 and UF90), and shrimp monoculture fed artificial feed (F100).

	Moisture	Fat	Protein	Ash	Crude fiber
U100	76.3 ± 0.2^{b}	1.4 ± 0.1^{a}	17.0 ± 0.3^{a}	3.6 ± 0.4^{b}	$1.53\pm0.03^{a,b}$
UF55	76.3 ± 1.2^{b}	2.1 ± 0.1^{b}	16.9 ± 0.7^{a}	2.5 ± 0.3^{a}	1.40 ± 0.11^{a}
UF90	73.4 ± 1.4^{a}	2.6 ± 0.1^{c}	18.7 ± 0.3^{b}	2.8 ± 0.5^{a}	$1.48\pm0.12^{a,b}$
F100	$74.4\pm1.0^{a,b}$	2.6 ± 0.1^{c}	$17.8\pm0.8^{a,b}$	$3.1\pm0.1^{a,b}$	1.63 ± 0.03^{b}
P values	0.028	<0.001	0.035	0.025	0.043

Values are given as mean ± SD of triplicate determinations. Superscripts in same column indicate homogeneous subsets as determined by Tukey's test ($\alpha=0.05$).

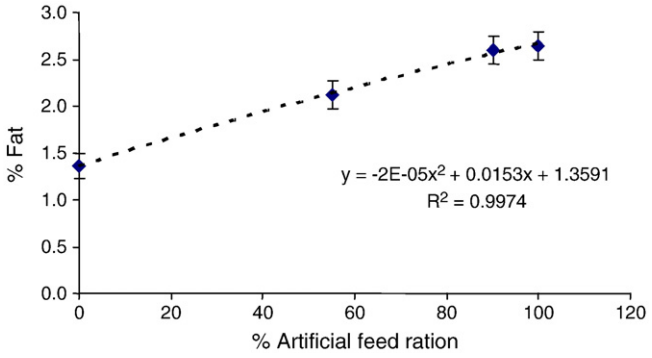

Fig. 1. Shrimp fat content (% wet basis) correlated with the artificial feed consumption.

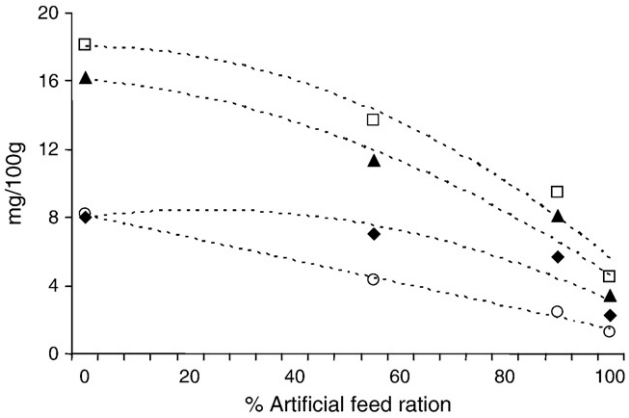

Fig. 2. Quadratic regression curves between carotenoids concentration in shrimp and artificial feed ration. ● Free astaxanthine $y = -0.067x + 8.175$, $R^2 = 0.996$; ♦ Esterified astaxanthine $y = -0.000x^2 + 0.045x + 7.952$, $R^2 = 0.879$; ▲ Total astaxanthine $y = -0.001x^2 - 0.021x + 16.09$, $R^2 = 0.960$; □ Total carotenoids $y = -0.001x^2 + 0.007x + 18.00$, $R^2 = 0.971$.

In shrimp culture ponds, unicellular organisms such as diatoms and flagellates are considered desirable due to their nutritional contribution (Bula-Meyer, 1985; Laurén-Matta et al., 1995). In the present study, the treatment F100 had an average unicellular plankton concentration of 78,000 cell mL^{-1}, which is a desirable concentration for a semi-intensive culture system (Clifford, 1992), while in the case of co-culture treatments U100, UF55 and UF90, the plankton production was lower but without an apparent negative effect on shrimp performance. Low phytoplankton production in the co-culture tanks may be due principally to the reduction of light intensity under the algal superficial layer. Also, *U. clathrata* present in the tanks may have worked as a biofilter by using the nitrogen rich wastes as a source of nutrients, as described by Hamano et al. (2007) and Copertino et al. (2009). However, the combination of co-cultured algae and artificial feed improved shrimp growth significantly, suggesting that *Ulva* sp. may act as a nutritional supplement and/or improve the utilization of nutrients from the artificial feed. Similar results reported by Porchas-Cornejo et al. (1999) showed that shrimp *Farfantepenaeus californiensis* can increase its growth rate three fold in the presence of the alga *Caulerpa sertularioides,* although in this case algae consumption was not mentioned as a possible cause of the growth improvement. In contrast, Lombardi et al. (2006) tested the feasibility of co-culturing *L. vannamei* and the Philippine seaweed *Kappaphycus alvarezii* in floating cages; but after 103 days of growth, no negative interferences between co-cultured shrimp and seaweed were found, nor significant differences between monoculture and polyculture for shrimp weight gain, survival rate, and feed conversion ratio. Van Tri and Thanh Ha (2004) reported that *L. vannamei* larvae fed fresh seaweed together with a formulated feed had higher growth rate (in length and weight) and higher survival rate (48–53%) than those fed dry seaweed and feed or dry seaweed alone.

The lack of significant differences in performance between shrimp receiving *Ulva* + 55% feed ration or *Ulva* + 90% ration suggests that the impact of these proportions of *Ulva* to feed on growth rate seems about equal (within statistical uncertainty). So, the best mix depends primarily on the relative costs of *Ulva* and feed. *Ulva*, in the pond, without harvest or processing costs, is much cheaper than shrimp feed, and it might be therefore interesting to test a complete response curve, including intermediate levels such as *Ulva* + 70% feed, and *Ulva* + 25% feed, the later being of special interest from an economical point of view.

Shrimp fatty acid profile was clearly modified by the co-culture treatments, with a much higher DHA content in shrimp under co-culture conditions. This may be due to the lipid sources and quantity consumed, as described by various authors (Gonzalez-Felix et al., 2002, 2003; Cheng and Hardy, 2004; Zhou et al., 2007). However, since DHA concentration in *Ulva* was far lower than in the artificial feed, it seems that DHA found in shrimp may have been produced by the elongation of the linolenic acid imported in abundance through *Ulva* consumption. But, even while *U. clathrata* could be a good source of polyunsaturated lipids, these being precursors of the fatty acids considered essential to shrimp (Kanazawa et al., 1979), it appears that the macroalga alone was not able to support the maximum shrimp growth, probably due to the absence of supplemental dietary energy and protein. Similarly, Patnaik et al. (2006) observed that the replacement of menhaden fish oil by a HUFA-rich alga meal in diets did not have a significant effect on *L. vannamei* growth.

Table 6
Whole-body fatty acid profile (% relative to total fatty acids) of shrimp in a co-culture with *U. clathrata* without artificial feed supply (U100), a co-culture with partial ration of artificial feed (UF55 and UF90), and shrimp monoculture fed artificial feed (F100).

Fatty acid	U100	UF55	UF90	F100	*P* value
C14:0	0.69 ± 0.2^{a}	1.13 ± 0.1^{b}	1.08 ± 0.2^{b}	1.30 ± 0.1^{b}	0.009
C16:0	20.91 ± 1.6	20.70 ± 0.6	21.05 ± 0.9	24.51 ± 2.9	0.079
C16:1*n*-7	1.34 ± 0.8	0.70 ± 0.1	0.47 ± 0.1	0.54 ± 0.2	0.108
C18:0	11.65 ± 4.4	8.80 ± 2.1	7.12 ± 0.5	6.20 ± 0.7	0.119
C18:1*n*-9	$16.03 \pm 2.4^{a,b}$	20.92 ± 2.5^{b}	$17.94 \pm 5.4^{a,b}$	12.20 ± 0.7^{a}	0.056
C18:2*n*-6	6.19 ± 1.5^{a}	15.51 ± 0.6^{b}	16.02 ± 0.9^{b}	15.61 ± 3.0^{b}	0.001
C18:3*n*-3	2.20 ± 0.4^{c}	$1.81 \pm 0.3^{b,c}$	$1.47 \pm 0.1^{a,b}$	1.02 ± 0.1^{a}	0.004
C:18:4*n*-3	0.63 ± 0.4	1.20 ± 0.4	1.23 ± 0.2	0.98 ± 0.2	0.157
C20:1*n*-9	0.84 ± 0.1	1.28 ± 0.4	1.45 ± 0.2	1.42 ± 0.6	0.282
C20:5*n*-3	13.95 ± 1.3^{b}	10.43 ± 0.5^{a}	10.79 ± 0.9^{a}	10.32 ± 0.9^{a}	0.003
C22:1*n*-9	ND	0.16 ± 0.5	0.13 ± 0.0	0.12 ± 0.1	0.967
C22:5*n*-3	1.18 ± 0.1^{b}	$0.71 \pm 0.5^{a,b}$	0.42 ± 0.1^{a}	0.51 ± 0.2^{a}	0.005
C22:6*n*-3	8.07 ± 2.0^{b}	3.74 ± 0.4^{a}	3.78 ± 0.8^{a}	3.59 ± 0.6^{a}	0.032
C24:1*n*-9	0.24 ± 0.0	0.51 ± 0.2	0.48 ± 0.1	0.35 ± 0.3	0.266
∑Saturated[d]	33.2 ± 5.8	30.6 ± 2.7	29.2 ± 1.1	32.0 ± 3.5	0.605
∑MUFA[e]	18.5 ± 3.2	23.5 ± 1.8	20.4 ± 5.1	14.6 ± 0.6	0.050
∑PUFA[f]	8.4 ± 1.5^{a}	17.3 ± 0.4^{b}	17.5 ± 1.1^{b}	16.6 ± 3.1^{b}	0.001
∑HUFA[g]	23.8 ± 2.8^{b}	16.1 ± 1.7^{a}	16.2 ± 2.0^{a}	15.4 ± 0.8^{a}	0.003

Values are given as mean ± SD of triplicate determinations. Superscripts in same row indicate homogeneous subsets as determined by Tukey's test ($\alpha = 0.05$). ND = not detected.

[d] Saturated fatty acid: 14:0, 16:0, 18:0.

[e] MUFA (Monounsaturated fatty acid): 16:1*n*-7, 18:1*n*-9, 20:1*n*-9, 22:1*n*-9, 24:1*n*-9.

[f] PUFA (Polyunsaturated fatty acid): 18:2*n*-6, 18:3*n*-3.

[g] HUFA (highly unsaturated fatty acid): 18:4*n*-3, 20:5*n*-3, 22:5*n*-3, 22:6*n*-3.

Table 7
Shrimp colour reflectance after 3 min cooking in boiling water.

	Redness	Yellowness	Lightness
U100	16.8 ± 3.9^{b}	34.0 ± 4.3^{c}	65.2 ± 2.3^{a}
UF55	16.2 ± 4.5^{b}	30.7 ± 6.1^{b}	69.1 ± 3.2^{b}
UF90	11.5 ± 2.9^{a}	26.3 ± 2.8^{a}	73.7 ± 2.5^{c}
F100	12.1 ± 4.0^{a}	29.1 ± 4.3^{ab}	71.6 ± 4.0^{d}
P values	0.001	<0.001	0.020

Values are given as mean ± SD from triplicate determinations. Superscripts in same column indicate homogeneous subsets as determined by Tukey's test ($\alpha = 0.05$).

The higher pigmentation of shrimp in co-culture groups confirms that carotenoids present in *Ulva*, luteins principally, are efficiently assimilated, metabolized and deposited by the shrimp (Cruz-Suárez et al., 2009a). Vernon-Carter et al. (1996) and Arredondo-Figueroa et al. (1999) found that extracts rich in lutein from *Tagetes erecta* included in the shrimp diet were possibly metabolized into astaxanthine and deposited by the Pacific white shrimp. Though the values from shrimp colorimetry by reflectance showed significant differences, it is necessary to make a complete study to standardize the values according to commercial needs, taking into account the tendency for an increase of artificial feed supply to diminish or dilute the pigmentation effect of *Ulva* intake.

5. Conclusion

Ulva co-cultured with shrimp in the present experiment contained 21% crude protein, 1.5% fat and 38% ash (dry basis); its presence in co-culture tanks modified the water quality, decreasing water turbidity and total unicellular plankton cell number. The joint use of co-cultured *Ulva* and artificial feed in the experimental tanks allowed a 60% shrimp growth increase and an improvement of shrimp carcass quality: lipids diminished, while total carotenoids, as well as total and esterified astaxanthine increased following quadratic models. Shrimp/*Ulva* co-culture appears therefore to be a beneficial alternative to shrimp monoculture since it may diminish the need for commercial feed at the same time as it increases the economical value of the final product through greater size, better fatty acid profile and higher pigmentation. However, while the potential nutritional benefit was clearly shown, further studies have to be carried out to establish best practices for shrimp and *Ulva* co-culture.

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