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Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds

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Abstract

Three experimental diets were formulated to contain 33 g kg^{-1} seaweed meals, made of wild brown algae (kelps) *Macrocystis pyrifera* (MAC) and *Ascophyllum nodosum* (ASC) or of a cultivated green alga *Ulva clathrata* (ULVA). The diets were fed to juvenile white shrimp *Litopenaeus vannamei* (1.6 g) for 28 days. Loss of dry matter (LDM) and loss of protein (LP) after 1 hour immersion in seawater, and distilled water absorption (WA) were analyzed in the pelleted diets, as well as shrimp weight gain, feed intake, feed conversion ratio (FCR), survival, protein efficiency ratio (PER) and body pigmentation. Feed intake, FCR and PER were corrected for nutrients preprandial losses in seawater. ULVA diet resulted in lower LDM, but a higher LP and also higher WA, indicating a modification of the pellet physical quality (better hydro stability). No significant differences in feed consumption and survival were found, but ULVA diet resulted in a slightly higher final weight (4.8 for ULVA versus 4.6 and 4.3 g for ASC and MAC), and better FCR (1.7 versus 1.9 and 2.1) and PER (2.0 versus 1.7 and 1.5), the differences with MAC diet being significant (Duncan, $\alpha = 0.05$). Red color after cooking was markedly darker in the ULVA fed shrimp.

KEY WORDS: Feed evaluation, Feed ingredients, Marine Algae, Nutrition, Pacific white shrimp, pigmentation, seaweed

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Introduction

Microalgae and macroalgae have been used for years in terrestrial animal feeds to replace animal ingredients (Hansen *et al.* 2003; Leupp *et al.* 2005), as a source of pigments (Strand *et al.* 1998), to increase disease resistance (Turner *et al.* 2002), to increase egg production (Bratova & Ganovski 1982), etc. In aquatic animals, seaweeds have been used as a dietary supplement for sea bass (Valente *et al.* 2006), snakehead (Hashim & Mat-Saat 1992) and shrimp (Moss 1994; Peñaflorida & Golez 1996; Cruz-Suárez *et al.* 2000).

In some instances, the inclusion of algae in feed formulations has resulted in improved performance, including improved feed efficiency, pellet quality and animal product quality. Most nutritional studies with seaweed have investigated low dietary inclusion rates (less than 80 g kg^{-1}) to establish their possible usefulness as functional (binder effect), nutritional and nutraceutical (health protective effects) supplements. The optimum inclusion level varies depending on algae or consumer species (Peñaflorida & Golez 1996; Cruz-Suárez *et al.* 2000; Suárez-García 2006).

Brown macroalgae contain some active compounds, such as fucoidan, alginates, laminarines, etc. (polysaccharides, phycocolloids) that can improve animal resistance against bacterial and viral diseases (Takahashi *et al.* 1998; Campa-Cordova *et al.* 2002; Cruz-Suárez *et al.* 2002a; Hennequart *et al.* 2004; Hou & Chen 2005; Balasubramanian *et al.* 2006; Bansemir *et al.* 2006; Deachamag *et al.* 2006; Yeh *et al.* 2006). It has also been found that, by supplementing shrimp diets with kelp meals, such as of *Macrocystis pyrifera* (MAC), *Ascophyllum nodosum* (ASC) and *Sargassum* sp., physical properties of pellets such as integrity, water-holding capacity, texture and water stability are improved (better feed stability and lower leaching of nutrients), resulting in higher feed intake and better growth performance (Cruz-Suárez *et al.* 2000, 2002a, b, 2006; Cerecer-Cota *et al.* 2005; Suárez-García 2006).

Enteromorpha seaweed (recently renamed *Ulva*), a green alga in the family of Ulvaceae, contains a sulphated

polysaccharide (ulvan) that has been reported as antiviral and gelling agent (Lahaye & Axelos 1993; Hirayama *et al.* 2002) but, in contrast to brown seaweeds, *Ulva* presents higher crude protein content and its main carotenoid is lutein instead of fucoxanthin. This green seaweed can be easily cultivated and represents an alternative seaweed meal to be included in shrimp diets. To our knowledge, *Ulva clathrata* has not previously been studied as an ingredient in shrimp feeds. The main objective of the present study is to show a preliminary indication of the effects obtained by the inclusion of cultivated *U. clathrata* in white shrimp *Litopenaeus vannamei* diets, in terms of pellet physical characteristics, growth performance and body pigmentation, in comparison with the inclusion of *M. pyrifera* and *A. nodosum* seaweed meals.

Materials and methods

Experimental seaweeds meals and diets

Kelp meals used in the present study were made of wild algae, sun-dried and ground (see brand names and companies in Table 1). In contrast, *Ulva* meal was made of algae cultivated in ponds such as shrimp ponds, under a patented technology, from laboratory stocks of an *U. clathrata* known strain; the algae biomass was washed in freshwater to lower the ash content, dried with hot air at a temperature under 55 °C to preserve xanthophylls and vitamins, and milled.

The proximate composition of the algae meals was determined using the following methods: Kjeldhal for protein (Tecator 1987), Soxhlet for lipids (Tecator 1983) and AOAC (1990) for ash (942.05), crude fibre (962.09) and moisture (920.36). Nitrogen-free extract (NFE) was calculated by difference. Amino acid composition of the kelp samples MAC and ASC was determined at Degussa, Hanau, Germany according to Llames & Fontaine (1994; AOAC 1997), and that of ULVA at our laboratory using an adaptation of the method described by Umagat *et al.* (1982). Carotenoid pigments of ULVA were analysed by high-performance liquid chromatography (HPLC) at Vepinsa, México.

The experimental diets were designed (Table 2) to meet the nutritional requirements of shrimp according to Akiyama *et al.* (1999), and to contain 320 g kg^{-1} of crude protein and 90 g kg^{-1} of crude lipids (as is). Each experimental diet was supplemented with 33.3 g kg^{-1} of MAC or ASC or ULVA meal. The addition of algae meal was made without consideration of the differences in composition of the different algae meals. Diets were prepared according to Cruz-Suárez *et al.* (2007).

Chemical composition of the experimental diets was determined as described before for the algae meals. The loss of dry matter (LDM) and loss of protein (LP) from the diets after a 1 h immersion in seawater (28 °C and 35 g L^{-1}) was determined for the three diets using the methodology described by Ricque-Marie *et al.* (2006). Each diet was evaluated in three replicates of each measurement. LDM and LP were calculated using the following formula (Cruz-Suárez

Table 1 Proximal composition of the experimental algae meals (g kg^{-1} as is)

	Macrocystis[1] (MAC)	*Ascophyllum*[2] (ASC)	*Ulva*[3] (ULVA)
Moisture	74	146	142
Protein[4]	61 (N × 6.25) 53 (N × 5.38)	79 (N × 6.25) 68 (N × 5.38)	234 (N × 6.25) 192 (N × 5.13)
Lipid	7	27	10
Ash	311	212	160
Fibre	105	35	46
NFE	442	501	408

[1] KELPROPAC®, produced by Productos del Pacífico, Mexico.
[2] ASL™ kelp meal, produced by Acadian Seaplants, Canada.
[3] ULVA meal, produced by Algalimentos S.A. de C.V., Mexico.
[4] According to Lourenço *et al.* (2002), protein should be calculated by N × 5.38 for brown algae and N × 5.13 for green algae, N being the analysed nitrogen concentration in the sample.

Table 2 Amino acid composition of the experimental algae meals (g kg^{-1} crude protein, mean ± SD) compared with recommended dietary levels for shrimp[1]

	MAC (*n* = 3)	ASC (*n* = 1)	ULVA (*n* = 2)	Recommended dietary levels
Methionine	16 ± 1.7	19	13 ± 0.8	24
Methionine + cystine	37 ± 2.3	37		36
Lysine	45 ± 0.9	43	37	53
Threonine	42 ± 4.0	43		36
Tryptophan				
Arginine	38 ± 4.4	40	32 ± 0.0	58
Isoleucine	35 ± 4.3	34	22 ± 0.2	35
Leucine	60 ± 7.7	60	50 ± 7.4	54
Valine	46 ± 4.4	44	45 ± 1.5	40
Histidine	14 ± 1.9	15	23 ± 1.0	21
Phenylalanine	40 ± 5.6	40	38 ± 0.1	40
Phenylalanine + tyrosine			62 ± 1.2	71
Glycine	49 ± 3.0	45	59 ± 3.1	
Serine	40 ± 4.4	40	46 ± 0.6	
Proline	37 ± 6.9	34		
Alanin	81	54	55 ± 0.3	
Aspartic acid	94 ± 6.1	98	73 ± 0.0	
Glutamic acid	126 ± 17.7	131	155 ± 3.1	

[1] Levels recommended by Akiyama *et al.* (1999).
n is the number of analysed replicate samples.
MAC, *Macrocystis*; ASC, *Ascophyllum*; ULVA, *Ulva*.

et al. 2001): g kg^{-1} LDM = [(weight of feed on a DM basis before leaching – weight of feed on a DM basis after leaching)/weight of feed on a DM basis before leaching] * 1000 and g kg^{-1} LP = [(g kg^{-1} protein in feed DM * 1000) – (g kg^{-1} protein in leached feed DM * (1000 – g kg^{-1} LDM))]/ g kg^{-1} protein in feed DM.

The water absorption (WA) for the experimental diets (g kg^{-1}) was determined according to Cruz-Suárez *et al*. (2006): WA = 1000 * [(weight of the sample after submersion in distilled water – weight of the sample before submersion)/weight of the sample before submersion].

Feeding trial

A feeding trial was run at Programa Maricultura, Faculty of Biological Sciences, UANL in Monterrey N.L., Mexico, in a closed recirculation artificial seawater system. The experimental facility consisted of 60-L fibre glass tanks, each continuously fed with synthetic marine water (Fritz, Dallas, TX, USA) at a flow-through rate of 350 mL min^{-1}. Each tank is equipped with a double bottom covered with black screening and an air–water lift system for internal recirculation. The facility is designed so that possible water quality variations affect all tanks simultaneously. Water quality parameters of salinity (27–30 g L^{-1}) and temperature (24–29 °C) were measured daily; pH (8.0–8.2) and concentrations of NH_3^+ NH_4^+ (0–0.2 mg L^{-1}), NO_2^- (0–0.4 mg L^{-1}) and NO_3^- (10 mg L^{-1}) were recorded weekly. The parameters remained well within the optimum for *L. vannamei* throughout the trial.

Litopenaeus vannamei juveniles were obtained from Pecis Industries, Yucatan, Mexico, and acclimated to the conditions of the bioassay facilities in 500 L holding tanks, prior to the growth trial. Sixty-three shrimp were used in a 28 days growth trial, with a mean initial weight of 1.6 g.

Seven animals per tank were distributed into nine 60 × 30 × 35 cm fibre glass tanks by weighing individually on a digital balance after blotting off excess water on a moist cloth. Care was taken to distribute animals of the same size distribution pattern in each tank. Dietary treatments were then randomly assigned to the tanks using a three block design (three replicates). The day after distributing the animals, any dead shrimp was replaced, and feeding on the respective diets was initiated. Remaining feed and any mortality was recorded every morning and the tanks cleaned off the remaining feed and feces. The photoperiod was 12 h light : 12 h dark.

The shrimp were initially fed at 100 g kg^{-1} of the biomass of each tank and the ration was then adjusted to actual consumption every day, thus reducing uneaten feed to a minimum. Uneaten feed was estimated visually every morning in each tank as a percentage of the feed administered the day before. The shrimp were fed twice daily, once after cleaning in the morning and again in the afternoon; the weighed strands of feed were broken into small pieces to ensure a minimum of one pellet per shrimp at each feeding.

Zootechnical parameters and statistical analysis

Individual weights were taken at 0, 14 and 28 days. The following response variables were determined for each experimental tank. Percentage weight gain was calculated as the difference in weight from the average initial weight with respect to the initial weight; weight gain = [(average individual final weight – average individual initial weight)/ average individual initial weight] × 100. Feed consumption was estimated each day from the quantity of feed added to the tank, the feed remaining the next day, and the number of shrimp present in this tank; for each tank the feed consumption reported was the total of the consumption estimated for the 28 days period; Individual consumption = $\Sigma_{n=1}^{28}$ (consumption on the *n*th day/number of shrimp on the *n*th day). The feed conversion ratio (FCR) is the weight of feed consumed per unit of weight gain; FCR = estimated individual consumption/average individual increase in weight. Protein efficiency ratio (PER) is the weight gain per unit of protein consumed; PER = weight gain/protein intake. The survival rate was calculated as: (final number/initial number) × 100, for each tank.

Body pigmentation was assessed for each treatment on shrimp cooked for 5 min in boiling water. Individual body weights (n = 21 per diet at the beginning of the experiment) were submitted to a factorial analysis of variance (ANOVA) for the statistical comparison of mean weights between dietary treatments and tank blocks, while per tank data (n = 3 per diet for weight gain, feed consumption, FCR, PER and survival) were submitted to an one-way ANOVA between diets.

Results

Chemical composition of the experimental seaweeds and diets

The composition of the seaweeds is presented in Table 1. ULVA meal presented three to four times more protein in comparison with MAC and ASC meals (234 versus 61 and 79 g kg^{-1}, respectively). Lipid content was less for MAC and ULVA than for ASC meal (7 and 10 versus 27 g kg^{-1}). Ash and fibre contents were low for ULVA.

Compared amino acid profiles of MAC, ASC and ULVA show that algae profiles are similar and generally close to that recommended for shrimp feeds by Akiyama *et al.* (1999; Table 2).

Total carotenoids concentration in the ULVA sample used in the present work was 1.92 g kg^{-1} as is, parted in β-carotenes 7.3%, trans-luteines 82.6% and other carotenoids 10.1%, all of them in free form instead of esterified, while the absence of fucoxanthin, β-cryptoxanthin and trans-zeaxanthin was constated.

Crude protein, crude lipids and fibre content in diets were similar, as expected since alga inclusion level was fairly low; however, ash and fibre values varied according to the contents in the experimental ingredients, diet supplemented with MAC meal presenting slightly higher ash and fibre contents than those of the diets supplemented with ASC and ULVA meals (Table 3).

Table 3 Formula, chemical composition and physical properties of the experimental diets

Diet	MAC	ASC	ULVA
Ingredients (g kg^{-1} as is)			
Macrocystis meal	33.3		
Ascophyllum meal		33.3	
Ulva meal			33.3
Wheat meal	563.9	563.9	563.9
Standard Mexican fishmeal	131.4	131.4	131.4
Menhaden fishmeal	131.3	131.3	131.3
Soybean meal	34.0	34.0	34.0
Shrimp meal	40.0	40.0	40.0
Fish oil	14.2	14.2	14.2
Soybean lecithin	45.3	45.3	45.3
Vitamin C	0.5	0.5	0.5
Mineral premix	2.5	2.5	2.5
Vitamins premix	2.5	2.5	2.5
Mould inhibitor	0.5	0.5	0.5
Antioxidant	0.5	0.5	0.5
Chemical composition (g kg^{-1} dry matter)			
Protein	340	346	347
Lipid	96	97	96
Ash	81	76	75
Crude fibre	14	11	12
NFE	469	470	470
Physical characteristics			
LDM (g kg^{-1})	60^a	44^b	37^c
LP (g kg^{-1})	81^a	90^a	130^b
WA (g kg^{-1})	1120^a	1120^a	1320^b

NFE, nitrogen-free extract; LDM, LP, loss of dry matter, or protein, at 1 h immersion in seawater; WA, water absorption at 1 h immersion in distilled water; MAC, *Macrocystis*; ASC, *Ascophyllum*; ULVA, *Ulva*.

ANOVA probabilities for LDM, LP and WA were $P < 0.001$, $P = 0.046$ and $P = 0.026$, respectively; pooled standard errors were 0.34, 0.94 and 5.13, respectively ($n = 3$); letters in superscript indicate homogeneous subsets (in a raw), as defined by Duncan's test ($\alpha = 0.05$).

Diet stability in water was significantly different between treatments ($P < 0.001$), diet supplemented with ULVA meal presenting a lower DM leaching than diets supplemented with ASC and MAC meals (37 versus 44 and 60 g LDM kg^{-1} diet). LP was also significantly affected by the inclusion of different seaweeds ($P = 0.046$), being significantly higher for the ULVA than for MAC and ASC diets (130 versus 81 and 90 g kg^{-1}). Diet supplemented with ULVA meal absorbed significantly more water (1320 g kg^{-1}) than diets containing MAC and ASC meals, the WA for these diets being 1120 g kg^{-1} ($P = 0.026$).

Growth and pigmentation

Shrimp fed ULVA diet presented higher final weights and better weight gain at the end of the experiment (203% weight gain for ULVA versus 169% and 187% for MAC and ASC, respectively); however, differences were at the limit of statistical significance (Table 4). Feed consumption and feed consumption corrected by LDM were not affected by the experimental diets. FCR was significantly better in shrimp fed ULVA diet (1.8), while MAC and ASC meal supplementation resulted in higher FCR (≈2.1); the same tendency was observed when FCR values were corrected by LDM (1.7 versus 2.0). In terms of PER, inclusion of ULVA meal resulted again in significantly better PER (1.7 versus 1.5) and PER corrected for LP (2.0 versus 1.5 and 1.7 for MAC and ASC). At the end of the experiment, survival was over 90% for all the treatments.

Shrimp fed ULVA meal resulted in darker pink pigmentation than shrimp fed ASC and MAC diets (Fig. 1). Expanded chromatophores are clearly visible under the cuticle of the ULVA fed shrimp as well a higher diffuse pigmentation of the exoskeleton epidermal layer, compared with the MAC fed shrimp.

Discussion

Seaweeds composition

Previous studies reported that the content of crude protein, crude lipid, ash and fibre in MAC seaweed meals range from 50 to 140, 5 to 20, 310 to 450 and 45 to 89 g kg^{-1}, respectively (Castro-González *et al.* 1991, 1994; Rodríguez-Montesinos & Hernández-Carmona 1991; Cruz-Suárez *et al.* 2000), while, for ASC seaweed meals these values varied from 50 to 100, 20 to 70, 150 to 205 and ≈80 g kg^{-1}, respectively (Sharp 1987) and for green seaweed meals from 70 to 290, 5.0 to 37.0, 130 to 360 and from 28.0 to 55.0 g kg^{-1} respectively

Table 4 Feeding trial results (0–28 days) for shrimp fed diets supplemented with *Macrocystis* (MAC), *Ascophyllum* (ASC) and *Ulva* (ULVA) meals

	MAC	ASC	ULVA	*n*	Pooled standard error	ANOVA probability
Body weight (g)						
Initial	1.58	1.59	1.59	21	0.02	0.98
Final	4.28[a]	4.56[ab]	4.79[b]	20[1]	0.10	0.09[2]
Weight gain (%)	169	187	203	3	7.2	0.17
Feed consumption (g/shrimp)	5.96	5.97	5.7 1	3	0.19	0.85
Feed consumption, Lixcor (g/shrimp)	5.61	5.71	5.50	3	0.18	0.92
Feed conversion ratio	2.23[a]	2.01[ab]	1.78[b]	3	0.08	0.04
Feed conversion ratio, Lixcor	2.10[a]	1.92[ab]	1.71[b]	3	0.07	0.06
Protein efficiency ratio	1.41[a]	1.55[ab]	1.73[b]	3	0.06	0.04
Protein efficiency ratio, Lixcor	1.54[a]	1.70[a]	1.99[b]	3	0.08	0.01
Survival (%)	90	100	95	3	3.37	0.58

Superscript letters indicate homogeneous subsets (in a raw) as defined by Duncan's test ($\alpha = 0.05$).

Lixcor, values were corrected for the preprandial nutrient losses by leaching in seawater.

[1] *n* values for final weight were 19 for MAC, 21 for ASC and 20 for ULVA.

[2] Probability given by two-way ANOVA for diets, blocks and diets * block interaction were $P = 0.088$, $P = 0.261$ and $P = 0.467$, respectively.

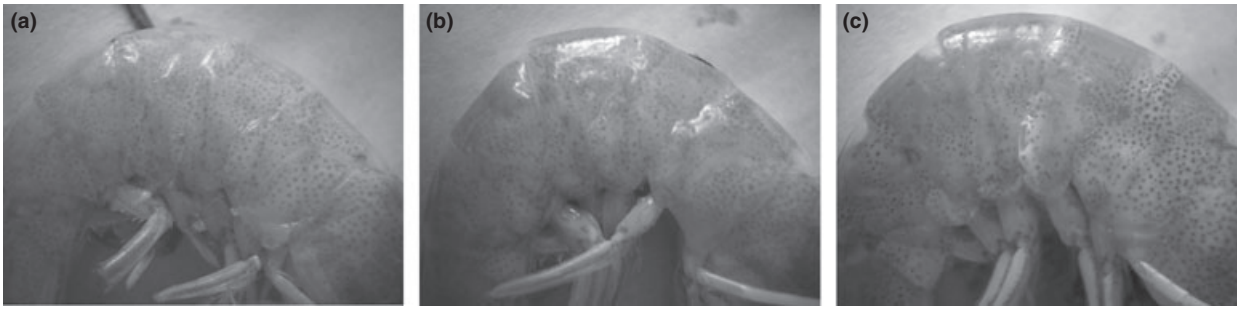

Figure 1 Pigmentation in shrimp fed diets containing *Macrocystis* meal (a), *Ascophyllum* meal (b) or *Ulva* meal (c).

(Hashim & Mat-Saat 1992; Wahbeh 1997; Ventura & Castañon 1998; Wong & Cheung 2000, 2001; Aguilera-Morales *et al*. 2005; Marsham *et al*. 2007). The proximal compositions of the three experimental seaweed meals in the present study were close to those reported previously, with the actual ULVA sample situated in the high for protein and in the low for lipids and ash, inside the green algae range.

Concerning the amino acid composition of *U. clathrata*, the lack of previous reference does not allow assessing the analytical results proposed in the present paper. New data given on pigment composition in *Ulva* confirm the difference between brown and green algae: brown algae contain mainly fucoxanthin, and some chlorophyll-α and -χ, β-carotene and xanthophylls (Barrett & Anderson 1980; Strand *et al*. 1998; Dhargalkar & Kavlekar 2004), while green algae, such as *Ulva* sp., *Enteromorpha clathrata* and *Chaetoniorpha torta,* contain chlorophyll-α and -β, β-carotene, lutein and zeaxanthin (Nadakal 1960; Burtin 2003; Dhargalkar & Kavlekar 2004). The *Ulva* sample tested in the present study contained mostly lutein and some β-carotene, and lacked fucoxanthin and trans-zeaxanthin.

Pellet stability and WA capacity

Several studies reported that seaweed meals can be excellent binders for aquatic feeds. Hashim & Mat-Saat (1992) reported that *Ulva* spp. resulted in the best feed stability (1.5 g kg^{-1} LDM after 1 h) for snakehead feeds followed by *Sargassum* sp. and *Polycavernosa* sp. meals (18 g kg^{-1} LDM), and *Gracilaria* spp. meal (20 g kg^{-1} LDM). Peñaflorida & Golez (1996) reported diet stabilities about 930–940 and 880 g kg^{-1} (60–70 and 120 g kg^{-1} LDM) in shrimp diets supplemented with *K. alvarezii* or *G. heteroclada* meals after one and four hour immersion in seawater. Cruz-Suárez *et al*. (2000) observed that the inclusion of 32 g kg^{-1} of kelp meal resulted in better shrimp pellet feed stability

(50 g kg^{-1} LDM) than for diets supplemented with a synthetic binder (90 g kg^{-1} LDM).

In the present study, pellet stability values were similar to those reported previously for shrimp feeds; however the diet supplemented with ULVA meal had the best stability (963 g kg^{-1}), followed by the ASC meal (956 g kg^{-1}) and MAC meal (940 g kg^{-1}), which suggest that polysaccharides from ULVA meal were better binding agents than those from MAC and ASC. Lahaye & Axelos (1993) reported that water-soluble polysaccharides from *Ulva* spp. exhibit excellent thermally reversible gelling properties. Fucoidan concentration in MAC meal generally is lower than in ASC meal (44 versus 170 g kg^{-1}, unpublished data); this may partially explain why the ASC diet presented better diet stability than MAC diet. Ulvan concentration in ULVA meal grown under similar conditions to that used in the present experiment can reach 220 g kg^{-1} (unpublished data), which may also explain the superior binding properties of ULVA meal. In contrast, higher loss of protein for the ULVA diet was unexpected and remains unexplained.

Pellet WA capacity may be affected by the type of ingredients, presence of natural or artificial binders, feed manufacture process, etc. (Cruz-Suárez *et al*. 2006). Phospholipids (lecithin) (Pomeranz 1991), polysaccharides (Fleury & Lahaye 1991; Pomeranz 1991), wheat meal (Cerecer-Cota *et al*. 2005), gluten meal (Cerecer-Cota *et al*. 2005), kelp meal (Cerecer-Cota *et al*. 2005) and extrusion process (Hilton *et al*. 1981) tend to increase WA, while artificial binders (Cruz-Suárez *et al*. 2000; Cerecer-Cota *et al*. 2005) and pelleting process (Hilton *et al*. 1981) significantly reduce this parameter. Seaweed's WA capacity is modulated by the type and quantity of polysaccharides present (Percival 1968; Sharp 1987; Rodríguez-Montesinos & Hernández-Carmona 1991; Ray & Lahaye 1995a,b; Suzuki *et al*. 1996; Paradossi *et al*. 1999; Marais & Joseleau 2001; McHugh 2003). Cruz-Suárez *et al*. (2000, p. 239) observed higher WA capacity (1800 g kg^{-1}) in diets supplemented with 30 g kg^{-1} of alginic acid than in diets supplemented with 20 (1300 g kg^{-1}) and 40 g kg^{-1} (1500 g kg^{-1}) of kelp meal.

In the present study, WA was higher for the ULVA diet and lower for MAC and ASC diets (1320 g kg^{-1} versus 1120). There is an indication that the presence of sulphate groups on the polysaccharidic fractions and/or the synergic effect with saccharidic moieties present in ulvan resulted in a better water-binding capacity than other polysaccharides (Paradossi *et al*. 1999). These characteristics may explain why ULVA diet presented the best water stability while the higher WA capacity.

Shrimp growth performance

Results of macroalgae inclusion in shrimp feeds may vary according to the consumer species, the type of algae and inclusion level tested. Peñaflorida & Golez (1996) observed a better weight gain in small shrimp *Penaeus monodon* (200 mg) fed diet including 50 g kg^{-1} *Kappaphycus alvarezii* meal and a lower growth with the supplementation of 30 g kg^{-1} *Gracilaria heteroclada* meal; however, in a second feeding trial with bigger shrimp (500 mg), differences were not significant. Briggs & Funge-Smith (1996) (cited by Peñaflorida & Golez 1996) also observed a significant reduction in growth of *P. monodon* fed diets containing 300 g kg^{-1} of the red seaweed *Gracilaria* spp.; negative effects could be attributed to the high ash content, low dietary protein content or the high levels of soluble fibre present in the experimental diets owing to high seaweed inclusion levels.

Cruz-Suárez *et al*. (2000) found a significant increase in growth (530–680 g kg^{-1}) in white shrimp *L. vannamei* juveniles (450 mg) fed diets containing 20 or 40 g kg^{-1} of Mexican kelp meal (*M. pyrifera*), although when Chilean kelp meal (*M. pyrifera*) was tested (40 and 80 g kg^{-1} inclusion levels) in *L. vannamei* (643 mg), a slight increment in weight gain, but not significant, was observed. The inclusion of *Sargassum* sp. meal (20–40 g kg^{-1} inclusion levels) or kelp meal *M. pyrifera* (40 g kg^{-1} inclusion level) produced growth similar to the control diet or a diet containing 30 g kg^{-1} of pure alginate as a binder (Suárez-García 2006). This latter result is in agreement to that reported by Valente *et al*. (2006) who observed a significant reduction in growth of sea bass fed diets containing more than 100 g kg^{-1} of *Gracilaria bursa-pastoris* meal.

In the present study, feeding the feed supplemented with ULVA meal to shrimp resulted in better growth than feeding them diets supplemented with ASC and MAC meals; high protein and low ash content in ULVA meal may have contributed to this difference, although inclusion rate was low, and ULVA does not show any noticeable advantage for its essential amino acids content.

Feed intake, FCR and PER

Feed intake was slightly lower with the ULVA treatment, but the differences were far from significance; therefore, better FCR with ULVA (significantly lower than with MAC by 20%) must be imputed principally to higher growth.

Changes in FCR owing to inclusion of seaweed have already been reported: FCR in *P. monodon* was 14% lower in a diet with 100 g kg^{-1} *Gracilaria heteroclada* (Peñaflorida &

Golez 1996), although results in this study were variable. Green algae, particularly the Ulvales, are a good source of dimethyl sulphonyl propionate (DMSP; Van Alstyne *et al*. 2001); DMSP has been shown to act as an attractant, and gives improved growth performance in shrimp (Men-Qing *et al*. 2001). DMSP increased feed efficiency as well as feed intake.

Some positive effect on PER has been reported in white shrimp *L. vannamei* fed diets supplemented with MAC meals (Cruz-Suárez *et al*. 2000). In the present study, shrimp fed ULVA diet presented higher PER than shrimp fed diets containing MAC and ASC meals, and the reason for this improvement in protein efficiency seems still to be found, as ULVA amino acid profile was generally low, especially for lysine and methionine.

Pigmentation

Colour development depends on the carotenoid content of the feed (Moretti *et al*. 2006) although it has been reported that dietary carotenoids were responsible for less than 20% of the flesh pigmentation in aquatic organisms (Torrisen *et al*. 1989; Storebakken & No 1992). Carotenoid pigmentation is affected by dietary pigment source, dosage level, duration of feeding, dietary composition, degree of carotenoid esterification, etc. (Meyers & Latscha 1997; Bjerkeng 2000; Buttle *et al*. 2001; Gomes *et al*. 2002; White *et al*. 2002).

The present study showed that shrimp utilized the lutein present in *Ulva* better than oxidized forms such as fucoxanthin present in kelp meals, which is in line with the observations reported by Meyers & Latscha (1997) on the crustacean carotenoid metabolism; animals often demonstrate a marked degree of selectivity in absorbing specific carotenoids or metabolically transforming them, and also the type of the pigments absorbed and the specific rate of absorption vary considerably between families and species. For example, *P. monodon* failed to absorb fucoxanthin isolated from barnacles (Meyers & Latscha 1997). These findings are in agreement with those reported by Cruz-Suárez *et al*. (2000) in *L. vannamei* fed diets containing different inclusion levels of *M. pyrifera*: dietary supplementation with *M. pyrifera* did not provide pigmentation to *L. vannamei*. In contrast, *L. vannamei* is able to use the pigments lutein and zeaxanthin of the Aztec marigold 'cempasúchil' (*Tagetes erecta*) flower (Vernon-Carter *et al*. 1996; Arredondo-Figueroa *et al*. 1999; Göçer *et al*. 2006). As well, Menasveta *et al*. (1993) fed a diet supplemented with 5% *Chnoora minima* brown algae to duplicated groups of *P. monodon* juveniles affected by the blue discolouration syndrome, without success, while an astaxanthin supplement allowed a significant colouration recovery after 2 weeks of experiment.

Conclusions

ULVA meal improved pellet DM hydrostability and pellet WA in comparison with MAC and ASC meals. Growth performance was slightly improved with respect to that provided by brown seaweeds, while improvement was significant for FCR and PER, without or with correction for the preprandial nutrient losses in seawater; better feed efficiency obtained with *U. clathrata* at low dietary inclusion level could not be imputed to any change in the dietary essential amino acid supply.

Body pigmentation improved in shrimp fed ULVA in comparison with those fed MAC, and this is clearly related to the capacity of *L. vannamei* to easily convert lutein (but fucoxanthin) to astaxanthin.

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