A Review of the Effects of Macroalgae in Shrimp Feeds and in Co-Culture

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Abstract

Most nutritional studies with seaweed meals or seaweed extracts have investigated low dietary inclusion rates (less than 10%) to establish their possible usefulness as functional (binder effect), nutritional, and nutraceutical (health protective effect) supplements in shrimp feeds. The optimum inclusion level varies depending on algae or consumer species. In many instances, the inclusion of algae in feed formulations has resulted in improved pellet quality (water stability, water holding capacity and texture), higher feed intake, improved feed efficiency, better growth performance and higher animal product quality (higher pigmentation, lower cholesterol content). Additionally, macroalgae contain some active compounds that can improve animal resistance against bacterial and virus diseases. Some seaweed species can be co-cultivated with shrimps, resulting in a sustainable alternative to reduce the need for artificial feed. In this paper the studies on the effects of seaweed inclusion in shrimp feeds or seaweed co-culture with shrimp will be reviewed.

Keywords: Shrimp, seaweed, macroalgae, feed, co-culture

Introduction

Several macro algae (*Ulva, Undaria, Ascophyllum, Porphyra, Sargassum, Polycavernosa, Gracilaria* and *Laminaria*) are widely used in fish diets and there are a number of studies into their effects that have been reviewed by Nakagawa and Montgomery (2007). In this paper we will focus on studies on shrimp; however, we provide occasional reference to some work on other aquatic species.

Some macroalgae that have been evaluated in shrimp feeds are *Macrocystis pyrifera, Ascophyllum nodosum, Kappaphycus alvarezii, Sargassum sp, Gracilaria heteroclada, Gracilaria cervicornis, Caulerpa sertularioides, Ulva clathrata, Enteromorpha* sp., *Hypnea cercivornis*, *Cryptonemia crenulata* and *Chnoospora minima.* The nutritional studies in shrimps that have investigated the effects of seaweed meal are summarized in Table1.

Table 1. Studies on the use of seaweed meal in shrimp feeds

Shrimp species	Title	References
P. monodon	Use of seaweed meals from ***Kappaphycus alvarezii*** and ***Gracilaria heteroclada*** as binders in diets of juvenile shrimp *Penaeus monodon*	Peñaflorida and Golez, 1996
P. monodon	The potential of ***Gracilaria spp***. meal for supplementation of diets for juvenile *Penaeus monodon* Fabricius	Briggs and Funge-Smith, 1996
P. californiensis	Efecto de la macrolaga ***Caulerpa sertularioides*** en el desarrollo del camarón café	Porchas Cornejo *et al.*. 1999
L. vannamei	Uso de harina de kelp ***Macrocystis pyrifera*** en alimentos para camarón	Cruz-Suárez *et al*., 2000
	Water stability and texture of shrimp pelleted feeds formulated with natural and synthetic binders	Cruz-Suárez *et al.*, 2002b
L. vannamei	Inclusión de harina de kelp (***Macrocystis pyrifera***) en alimentos balanceados para el camarón	Rivera, *et al*., 2002
L. vannamei	Uso de alga ***Sargassum sp***. en la elaboración de dietas para camarón y su impacto en los parámetros productivos	Cruz Suárez *et al.*, 2003
L. vannamei	***Sargassum spp***. como fuente potencial de alimento para camarón	Casas- Valdéz *et al.,* 2002
F. californiensis	Efecto del alga marina ***Sargassum spp***. sobre las variables productivas y la concentración de colesterol en el camarón café	Casas-Valdéz *et al*., 2006
L. vannamei	Efecto de la inclusión de alginato y harina de algas ***Sargassum sp y Macrocistys pyrifera*** sobre la estabilidad en agua, digestibilidad del alimento y sobre el crecimiento del camarón blanco	Suárez-García, 2006
L. vannamei	Inclusión de harina de Kelp (***Macrocystis pyrifera***) en alimentos balanceados para camarón	Marinho-Soriano *et al.,* 2007
L. vannamei	Comparison of ***Ulva clathrata*** and the kelps ***Macrocystis pyrifera*** and ***Ascophyllum nodosum*** as ingredients in shrimp feeds	Cruz- Suárez *et al.*, 2008b
L. vannamei	Seaweed meal as a protein source for the white shrimp *Litopenaeus vannamei*	Da Silva and Barbosa, 2008
	Harina de Kelp	Cruz-Suárez, *et al.*, 2007b
L. vannamei	Uso de harinas de sargaso (***Sargassum spp***.) y kelp (***Macrocystis Pyrifera***) en alimentos balanceados para el camarón ***Litopenaeus vannamei*** efectos sobre el crecimiento y la digestibilidad *in vivo*	Gutiérrez –Leyva, 2006

Chemical composition of seaweeds

The chemical composition of macroalgae varies with species, physiological status and environmental conditions; however, in general, the macroalgae are rich in nonstarch polysaccharides, vitamins and minerals (Mabeau and Fleurence1993; Wong and Cheung 2000). In most cases, the seaweeds are used in human or animal foods for their mineral contents or for the functional properties of their polysaccharides. Seaweeds are rarely promoted for the nutritional value of their proteins (Fleurence, 1999). The protein content of seaweed differs according to species and the seasonal period. Generally, the protein fraction of brown seaweeds is low (3 to 15% of the dry weight) compared with that of the green or red seaweeds (10 to 47% of the dry weight).

The content of crude protein, crude lipid, ash and fiber in *Macrocystis pyrifera* seaweed meals range from 5 to 14 , from .5 to 2 , from 31 to 45 and from 5 to 9 % respectively (Cruz-Suárez *et al.,* 2000; Rodríguez-Montesinos and Hernández-Carmona, 1991; Castro-Gonzalez *et al.*, 1994; Castro-Gonzalez *et al.,* 1991; Cruz-Suárez *et al*., 2008b), while, for *Ascophyllum* seaweed meals, these values varied from 5 to 10 , from 2 to 7 , from 15 to 21 and ≈8 % respectively (Sharp, 1987; Cruz-Suárez *et al*., 2008b) and for green seaweed meals from 7 to 29 , from 0.5 to 4, from 13 to 36 , and from 3 to 6 % respectively (Hashim and Mat-Saat, 1992; Wahbeh, 1997; Ventura and Castañón, 1998; Wong and Cheung, 2000, Wong and Cheung, 2001a; Aguilera-Morales *et al.,* 2005; Marsham *et al*., 2007; Cruz-Suárez *et al*., 2008b).

The amino acid composition of seaweeds has been frequently studied. For most seaweeds, aspartic and glutamic acids constitute together a large part of the amino acid fraction. In brown seaweeds, these two amino acids can represent between 22 and 44% of the total amino acids; in the green seaweeds up to 26 and 32% and in the red seaweed species 14 to 19% of the total amino acids (Fleurence, 1999). The fatty acid and pigment composition of seaweeds also differ between groups; brown andred seaweeds are a better potential sources of EPA and DHA than green ones (Ackman, 1981).

Brown seaweeds generally contain more vitamin C than their red and green counterparts. A summary of the typical whole composition of brown algae most commonly used in aquafeeds has been published by Cruz-Suárez *et al*. (2007b, 2008b).

The chemical composition of macroalgae reported in shrimp feed studies are presented in Table 2. Major nutrient (protein, carbohydrate and lipid) limitations may explain why the exclusive use of macroalgal feed (i.e. *G. cervicornis* meal) was unable to support growth and survival of white shrimp (Marinho-Soriano *et al*., 2007).

Table 2 Proximal composition of macroalgae reported in shrimp feed studies.

Proximate composition (%)							
	Moisture	Crude protein	Crude fat	Crude fiber	**NFE**	Ash	
Kappaphycus alvarezii	10.1	3.2	0.6	5.9	72.3	18.1	Peñaflorida *et al*.,1996
Gracilaria heteroclada	9.3	17.3	1.8	4.6	54.6	21.7	
Macrocystis pyrifera	7.4	6.1	0.7	10.5	44.2	31.1	Cruz-Suárez *et al*., 2000
Sargassum sp	9.7	6.3	0.4	5.6	46.0	32.0	Casas-Valdéz *et al*., 2002
Sargassum sp.	8.7	6.1	0.3	6.8	52.0	34.0	Casas-Valdéz *et al*., 2006
Gracilaria cervicornis		22.9	0.5		63.1		Marinho-Soriano *et al*., 2007
Ascophyllum nodosum	14.6	7.9	2.7	3.5	50.1	21.2	Cruz-Suárez *et al*., 2008b
Ulva clathrata	14.2	23.4	1.0	4.6	40.8	16.0	
Macrocystis pyrifera	11.2	7.7	2.0	9.3	38.9	31.0	
Cryptonemia crenulata	18.7	21.5	1.1		44.9	13.7	Da Silva and Barbosa, 2008
Hypnea cervicornis	24.3	19.6	1.0		41.5	13.7	
Caulerpa sertularioides fresh	91.1	2.4	0.5			2.2	Porchas *et al*., 1999
Ulva clathrata fresh	90.0	2.2	0.2	0.6	3.5	4.5	Cruz-Suárez *et al*., 2008a

Pellet quality

Several studies reported that seaweed meals can be binder for aquatic feeds (Table 3). The inclusion of algae in feed formulations has resulted in improved pellet quality (hydrostability, water holding capacity and texture), resulting in higher feed intake and improved feed efficiency. The optimum inclusion level varies depending on algae or consumer species.

Briggs and Funge-Smith (1996) investigated the effects of substituting wheat flour and soybean meal with various inclusion levels (0 to 30%) of the red seaweed *Gracilaria sp.* meal on the shrimp diet stability. Inclusion at up to 10% had no significant effects on diet water stability (after 12 h), compared with the control diet lacking seaweed. Diets containing 0-15% *Gracilaria* meal remained >88% water stable after 12 h. The 30% inclusion levels of *Gracilaria* meal resulted in a significant deterioration in diet water stability (86% after 12 h). Peñaflorida and Golez (1996) reported diet stabilities about 93-94% and 88% in shrimp diets supplemented with 5 to 10% *K. alvarezii* or *G. heteroclada* meals after one and four hour immersion in seawater. Cruz-Suárez *et al.* (2000) observed that the inclusion of 3 % of kelp meal resulted in better or similar shrimp pellet feed stability than for diets supplemented with a synthetic binder when feed were processed with a steam pelletizer. Marinho-Soriano *et al*. (2007) evaluated a feed made entirely of the rodophyta *Gracilaria cervicornis*, or a commercial shrimp feed; the stability in water (%) of the diets varied from approximately 82.6 % after 1h to 82.0% after 4 h of immersion in aerated seawater for *Gracilaria* pellets, and from approximately 91% after 1h to 89 % after 4h for commercial pellets. Recently Cruz Suárez *et al.* (2008b) showed that *Ulva* meal presented better binding properties than *Ascophyllum* and *Macrocystis* meals when included at 3.3% in shrimp diets.

The inclusion of macroalgae meal in feeds also affects pellet water absorption capacity (Table3).Kelp meal tends to increase the pellet water absorption, while artificial binders (Cruz-Suárez *et al.*, 2000; Cerecer-Cota *et al.*, 2005) significantly reduce this parameter. Seaweed's water absorption and gelling or binding capacity is modulated by the type and quantity of polysaccharides present (Percival, 1968; Sharp, 1987; Rodríguez-Montesinos and Hernández-Carmona, 1991; Ray and Lahaye, 1995a,b; Suzuki *et al*., 1996; Kuda *et al.*, 1997; Paradossi *et*

al., 1999; Jimenez-Escrig and Sanchez-Muñiz, 2000; Wong and Cheung, 2001b; Marais and Joseleau, 2001; Obluchinskaia *et al*., 2002; McHugh, 2003). When pure alginate is used as binder, the pellet holding water capacity is higher than when whole kelp (*Macrocystis or Sargassum*) meal is used (Cruz-Suárez *et al.*, 2000; Suárez-Garcia, 2006). Higher pellet water absorption was found for diets with *Ulva* than for diets with *Macrocystis* and *Ascophyllum* (132 vs 112%) (Cruz-Suárez *et al*., 2008b). The inclusion of 3.5% kelp meal in shrimp feeds produces a soft pellet texture, after immersion in water, increasing the feed intake (Cerecer-Cota, 2005).

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able 3. Effect of macroalgae meal inclusion on shrimp pellet quality.

Algae meal	Inclusion level %	feed technology	dietary protein %	ellet water stability %1h	pellet water absorption % 1h	Ref.
	Control starch	mincer		94		1
Kappaphycus alvarezii	3,5,7,10		38-40	94		
Gracilaria heteroclada	3,5,7,10			93		
	Control wheat	mincer		94		
Kappaphycus alvarezii	5,10			94		
Gracilaria heteroclada	5, 10			93		
Gracilaria spp.	5 to 30			(0 to 15%) > 88 (30%) 86		2
Macrocystis pyrifera *Chile*	Control wheat gluten	mincer	35	82.2		3
	4			82		
	8			77.7		
Macrocystis pyrifera *Mexico*	Control alginate	mincer	30	91.8	180	
	2			88.8	130	
	4			87.4	150	
Macrocystis pyrifera *Mexico*	Control synthetic binder	steam pelleted	40	91.5	70	
	3.2		25	94.3	104	
	3.2		25	95.6	-	
Macrocystis pyrifera *Mexico*	Control Synt. binder	steam pelleted	30,30,35,40	97,98,98,96	71,65,65,66	
	3		30,30,35,40	97,98,97,96	117,102,121,130	
	5		30,30,35,40	95,97,96,95	130,135,145,137	
	3+synt		40	96	93	
	alginate	mincer	30	91.7a	191 d	4
Sargassum sp.	2			88.1b	129 ab	
	4			80.9c	139 b	
Macrocystis pyrifera	4			87.1b	153 c	
	control			87.7b	117 a	
Gracilaria cervicornis	0	pelleted mincer	35	91		5
	100		23	82.6		
Ulva clathrata	3.3	mincer	34	96.3	132	6
Ascophyllum nodosum				95.6	112	
Macrocystis pyrifera				94	112	

1) Peñaflorida *et al*., 1996; 2) Briggs and Funge, 1996; 3) Cruz- Suárez *et al*., 2000; 4) Suárez-García, 2006; 5) Marinho-Soriano *et al*., 2007; 6) Cruz- Suárez *et al*., 2008b

Shrimp performance

In Table 4 are presented the studies where the effects of macroalgae meal dietary inclusion have been considered in terms of shrimp performance. In the majority of studies the inclusion of macroalgae meal improves growth, feed intake and protein efficiency at low inclusion levels (<10%).

Peñaflorida and Golez (1996) observed the best weight gain in small shrimp *P. monodon* (200 mg) fed diet including 5% *Kappaphycus alvarezii* meal and the lowest growth with the supplementation of 3% *Gracilaria heteroclada* meal; however, in a second feeding trial (500 mg initial weight), differences were not significant. Briggs and Funge-Smith (1996) found that shrimp-SGRs were very high with diets containing 0 to 15% *Gracilaria*, but lower with a 30% inclusion level; this negative effect was attributed to the high ash content, low protein content, or high level of soluble fiber present in the experimental diet with a high seaweed inclusion level. Rivera *et al.* (2002) also reported positive effects on growth of small shrimp *L. vannamei* when 10% of *Macrocystis* meal dissolved in water was used to coat pelletized diet, and poorer growth with higher (15 and 20%) seaweed levels.

On the other hand, although a significant proportion (50%) of the daily ration offered to *L. vannamei* was replaced by *Gracilaria* seaweed in the Marinho-Soriano *et al*. study (2007), the growth performance achieved (SGR =4.7%) was not significantly different with the values reported for the shrimp fed a commercial diet (5.1%).

Cruz-Suárez *et al.* (2000) found a significant increase in growth rate (53-68%) in white shrimp *L. vannamei* juveniles (450 mg) fed diets containing 2 or 4% of Mexican kelp meal (*Macrocystis pyrifera*) compared to a control diet. Nevertheless, when Chilean kelp meal (*Macrocystis pyrifera*) was tested (4 and 8% inclusion levels) in *L. vannamei* (643 mg), a slight increment in weight gain, but not significant, was observed. Suárez-Garcia (2006) concluded that the inclusion of *Sargassum sp* meal (2-4% inclusion levels) or kelp meal *M. pyrifera* (4% inclusion level) produced growth similar to the control diet or a diet containing 3% of pure alginate as a binder. The same result was obtained by Casas-Valdez *et al*. (2006) with 4% *Sargassum* meal diets fed to shrimp *Farfantantepenaeus californiensis*. In contrast, Gutiérrez-Leyva (2006) found an

improvement of SGF of shrimp *L. vannamei* fed diets with increasing levels (1, 4, 7, 10%) of *Macrocystis* or *Sargassum* meals, with 10% inclusion level giving the best growth rate. Feeding shrimp a diet supplemented with 3.3% *Ulva* meal resulted in better growth than feeding them diets supplemented with *Ascophyllum* and *Macrocystis* meals, however differences were not significant (Cruz-Suárez *et al*., 2008b). The rhodophytes *Hypnea cervicornis* and *Cryptonemia crenulata* used as a source of protein (seaweed powder) in shrimp diets at different percentages: 39%; 26%, 13%, and 0% (control diet) exhibited no significant differences between treatments ($P>0.05$) in terms of final biomass, biomass gain and specific growth rate (Da Silva and Barbosa, 2008).

The active compound of macroalgae responsible for growth improvement has not been clearly defined and the benefit has been attributed to their vitamin and mineral content, lipid mobilization and improved absorption and assimilation efficiency ratios. The seasonal and species variations of this active compound and difference in composition of control diets may explain the diverse results obtained among studies. Improvement in growth due to seaweed inclusion was also noted in fish by Hashim and Mat Saat (1992), Nakagawa *et al.* (1984), and Nakagawa *et al.* (1987).

Feed intake

A higher feed intake has been observed in shrimp fed diets supplemented with different inclusion levels of *M. pyrifera* or *Sargassum* (Cruz-Suárez *et al*., 2000; Suárez-Garcia, 2006; Rivera *et al*., 2002; Gutiérrez-Leyva, 2006). Feeding shrimp a diet supplemented with 3.3% *Ulva* meal resulted in similar feeding intake as for diets supplemented with *Ascophyllum* and *Macrocystis* meals (Cruz-Suárez *et al*., 2008; Table 4). It has been reported that some compounds from seaweed extracts, such as amino acids, digalactosyl-diacylglycerol, 6-sulfoquinovosyldiacylglycerol, phosphatidylethanolamine and phosphatidylcholine can act as attractants in pelleted diets for abalone (Sakata and Ina, 1985; Sakata *et al.*, 1991) and dimethyl-beta-propiontheine (DMTP) for fish (Segovia *et al*., 2007). Green algae, particularly the Ulvales, are a good source of dimethyl sulfonyl propionate (DMSP) (Van Alstyne *et al*., 2001). DMSP has been shown to act as an

attractant, and to give improved growth performance in shrimp (Meng-Qing *et al*., 2001); DMSP increased feed efficiency as well as feed intake.

Feed conversion ratio (FCR)

Changes in FCR due to inclusion of seaweed have been reported. FCR in *Penaeus monodon* was 14% lower in a diet with 10% *Gracilaria heteroclada* (Peñaflorida and Golez, 1996). In contrast, the inclusion or *Macrocystis* and *Sargassum* meal sometimes tended to increase and other times to diminish the FCR (Cruz-Suárez *et al*., 2000; Rivera *et al*., 2002; Suárez-Garcia, 2006; Gutierrez-Leyva, 2006). The FCR obtained with *Ulva* meal was significantly lower than that obtained with *Macrocystis* and *Ascophyllum* (Cruz-Suárez *et. al*., 2008b). The inclusion of *Hypnea cervicornis* and *Cryptonemia crenulata* meals, 39% and 26%, exhibited better feed conversion (1.79:1 and 1.82:1) than diets with lower content (2.04:1 and 2.08:1) ($P<0.05$) (Da Silva and Barbosa, 2008).

Protein efficiency ratio (PER)

Positive results on protein efficiency ratio due to the inclusion of seaweeds have been found in white shrimp *L. vannamei* (Cruz-Suárez *et al.,* 2000a, see Table 4). The improvement in protein utilization may vary with seaweed species. Shrimp fed *Ulva* diet presented higher PER compared with shrimp fed diets containing *Macrocystis* and *Ascophyllum* meals (Cruz-Suárez *et al.*, 2008b). In snakehead fry (Hashim and Mat-Saat, 1992), abalone (Viera *et al*., 2005) and rohu (Bindu and Sobha, 2004), improved protein efficiency ratios have been observed with the inclusion of pure carragenan, *Ulva* meal, *H. spinella, G. cornea, Ulva fasciata*, *Spyridia insignis* and *Sargassum wightii.* In contrast, Nakagawa *et al.* (1997) and Valente *et al.* (2006) concluded that the supplementation of *Gracilaria bursa-pastoris*, *Ulva rigida, Gracilaria cornea* and *Ascophyllum* in feeds for sea bass did not modify PER. It has been postulated that algae can increase absorption and assimilation of dietary protein (Yone *et al*., 1986a,b), or modulate lipid metabolism (Nakagawa *et al*., 1984, 1987, 1997.)

Table 4. Effect of macroalgae meal inclusion on shrimp performance.

Shrimp species	Algae	Inclusion level %	SGR (% day)	weight gain %	Consum g	FCR	PER	Survival %	Ref.
P. monodon (0.02, 0.05g) 56 days		control	4.98			3.8		87	1
	K. alvarezii	3,5,7,10	(5%) 5.21			(5%)3.3		(3%) 93	
	G. heteroclada	3,5,7,10	(10%) 5.12			(10%) 3.4		83	
		control	4.17			3.3		83	
	K. alvarezii	5,10	3.7			(10%) 2.9		(10%) 83	
	G. heteroclada	5, 10	3.7			(10%) 2.9		(5%) 87	
P. monodon 60 days	***Gracilaria spp***.	5 to 30	(0-15%)7.9-8			(0 to		(0 to 15%)	2
		control	(30%) 7.3			15%)3.1-3.5		48-56	
L. vannamei (0.6 g) 28days	***M. pyrifera*** Chile	control	2.94	128	2.12a	2.63	1.11	100	3
		4	3.12	139	2.49b	2.8	1.04	100	
		8	3.27	151	2.99c	3.12	0.96	100	
(.45g) 28 days	***M. pyrifera*** Mexico	control	4.74a	279.2a	1.68a	1.36a	2.70b	97	
		2	5.93b	427.3b	4.07b	2.03b	1.79a	100	
		4	6.18b	470.0b	4.32b	2.04b	1.74a	95	
(.53 g) 28 days	***M. pyrifera*** Mexico	control	3.4	157.6a	1.40a	1.68	1.37	98	
		3.2	3.3	150.6a	1.47a	1.85	1.83	100	
		3.2	3.9	195.0b	2.00b	1.94	1.98	98	
L. vannamei (400mg)	***M. pyrifera***	control		76a	5	1.9b		100	
		10		100b	6.6	1.7a		100	
		15		75a	6.9	2.3b		86	
		20		62a	6.6	2.8b		77	
L. vannamei (0.16g) 28 days	***Sargassum sp***.	0		309a	.83a	1.8a		62bc	4
		2		468bc	1.26 b	1.9a		50ab	
		4		561c	1.26 b	1.8a		45a	
	M. pyrifera	4		404ab	1.29 b	2.2a		52ab	
		control		402ab	1.12 b	2.0a		72c	
F. californiensis (0.5 g) 45 days	***Sargassum spp***.	0 control	3.64		1.03	1.78		70	5
		4	3.49		1.15	1.7		77	
L. vannamei (0.34g) 30days	***G. cervicornis*** (Rhodophyta)	0	5.11	363.81		1.56		100	6
		100	0.44	16.06		30		40	
		50	4.71	313.93		1.84		100	
L. vannamei (1.6 g) 28 days	***U. clathrata***	3.3		203	5.5	1.71b	1.99b	95	7
	A. nodosum			187	5.7	1.92ab	1.70a	100	
	M. pyrifera			169	5.6	2.1a	1.54a	90	
L. vannamei (1.1 g) 45days		control	3.5		1.8ab	2.1a	1.5ab	93.3a	8
	M. pyrifera	1	3.8		2.05ab	1.9ab	1.6ab	90a	
		4	3.6		1.81ab	1.9ab	1.6ab	100a	
		7	3.6		1.58 b	1.6b	1.9a	96.7a	
		10	3.9		2.17 ab	1.8ab	1.7ab	86.7a	
	Sargassum sp	1	3.5		1.79 ab	1.9ab	1.6ab	96.7a	
		4	3.9		1.93 ab	1.7ab	1.8ab	93.3a	
		7	3.7		1.85 ab	1.8ab	1.7ab	86.7a	
		10	3.9		2.57 a	2.1a	1.4b	96.7a	
L. vannamei 10-day-old post-larvae 45 days aged	***Hypnea cervicornis* and *Cryptonemia crenulata***	39	5.65a			1.8b		96.2a	9
		26	5.68a			1.8b		97a	
		13	5.17a			2.0a		97a	
		0	4.68a			2.1a		95.2a	

1) Peñaflorida *et al*., 1996; 2) Briggs and Funge, 1996; 3) Cruz-Suárez *et al*., 2000; 4) Suárez-García, 2006; 5) Casas-Valdez *et al*., 2006; 6) Marinho-Soriano *et al*., 2007; 7) Cruz- Suárez *et al*., 2008b; 8) Gutiérrez-Leyva, 2006; 9) Da Silva and Barbosa, 2008.

Digestibility

The protein and dry matter digestibility of diets supplemented with low kelp meal concentration, was enhanced in one study and in others it was diminished (Table 5).

Table 5. Apparent digestibility of diets supplemented with seaweed meals

Shrimp species	Algae	Inclusion level %	APD %	ADMD %	Reference
L. vannamei *3.8 g*	***M. pyrifera*** Mexico	control	80.3a	67.9a	Cruz-Suárez *et al.*, 2000
		2	82.4b	74.1b	
		4	82.9b	77.5c	
L. vannamei *9-14 g*	***Sargassum sp***.	0	88a	78.2a	Suárez-Garcia, 2006
		2	84.9a	71.4a	
		4	86.7a	77.8a	
	M. pyrifera	4	84.5a	75.1a	
		control	88.1a	81.3a	
L. vannamei *5-7g*	***Sargassum sp***.	0	81.4a	69.6ab	Gutiérrez -Leyva, R.2006
		4	79.7ab	68.9ab	
		10	80.5a	69.1ab	
	M. pyrifera	4	81.1a	70.1a	
		10	73.3b	60.0b	

Survival and disease resistance

Most of the studies with macroalgae meal supplementation reported excellent survival in shrimp at inclusion levels lower than 10% of the diet.

Peñaflorida and Golez (1996) observed better survival in *P. monodon* fed low inclusion levels of *K. alvarezii* and *G. heteroclada* and increased mortality with increasing levels of these algae. Cruz-Suárez *et al*. (2000) and Suárez-Garcia (2006) also reported excellent survival in shrimp *L. vannamei* fed diets containing (2-4%) kelp meal *M. pyrifera* and *Sargassum spp*. Da Silva and

Barbosa (2008) also reported high survival with diets containing high levels (39, 26 and 13 %) of *Hypnea cervicornis* and *Cryptonemia crenulata* meals. Hashim and Mat-Saat (1992) found a significant improvement in survival of snakehead fry fed a diet supplemented with pure carragenan, followed by diets supplemented with *Polycavernosa spp* meal, *Gracilaria* meal and *Ulva* meal, while the inclusion of *Sargassum* meal resulted in a lower survival.

Several studies reported that dietary supplementation with seaweed meal or their extracts, due to the presence of some compounds (such as fucoidan, alginates, laminarins, carrageenans, etc.) can enhance the immune resistance and improve survival when shrimp are challenged with some bacteria or virus (Table 6).

Table 6. Studies on the immunostimulatory, antibacterial or antiviral effect of seaweed meal or their extracts on penaeid shrimp.

Producto	shrimp species	administration	Seaweed species	Results
Fucoidan	*P. japonicus*[1]	Oral	60 and 100 mg fucoidan from *C. okamuranos* semipure/ kg/day	Control WSSV, 77% de survival
Seaweed meal	*P. vannamei*[2,13]	Oral	*M. pyrifera* meal 1-4% in diets	Partial control WSSV
Fucoidan	*P. monodon*[4]	Oral	*Sargassum polycystum*	Reduce the impact of WSSV
Alginates	*L. vannamei*[5]	injected intramuscularly	Sodium Alginate (10, 20 o 50 μg/g)	Increased immune ability as well as resistance to *V. alginolyticus* infection. Phenoloxidase activity fagocitic activity, bacterial clearance increased significantly when shrimp were injected.
Ulva secondary metabolites	*P. monodon*[6]	Oral	*Ulva fasciata diet*	Increase defense factors such haemogram, agglutination index, phagocytic rate, bacterial clearance and serum bactericidal activity
Hot water extract	*L. vannamei*[8]	injected intramuscularly	*Gracilaria tenuistipitata* (4 o 6 μg/g)	Increased immune ability as well as resistance to *V. alginolyticus* infection. Total hemocyte count, phenoloxidase activity and respiratory burst increased significantly when shrimp were immersed in seawater containing the extracts or when shrimp were injected.
Seaweed extracts	*P. indicus*[9]	injected intramuscularly	*C. racemosa, D. dichotoma, E. compressa, G. crassa, G. edulis, H. clathraus, H. musciformis, P. boergeseni, S. wieghti y T. conoides*	Some extracts inactivated partialy WSSV
Hot water extract	*P. vannamei*[10]	Immersion injected intramuscularly	*Sargassum duplicatum* (100, 300 y 500 mg/L) immersed or inyected with hot water extract (2, 6, 10 y 20 μg/g).	Increased immune ability as well as resistance to *V. alginolyticus* infection. Total hemocyte count, phenoloxidase activity and respiratory burst increased significantly when shrimp were immersed in seawater containing the extracts or when shrimp were injected.
Fucoidan extracted three times with 0.05 N HCl at 95 °C for 12 h.	*P. monodon*[11]	injected intramuscularly	*Sargassum polycystum*	Produced a significantly increased expression ($p<0.05$) of the ribosomal protein L26 (RPL26) gene, a macrophage activator gene in *P. monodon*
Fucoidan	*P.*	oral	*Cladosiphon*	Partial control against WSSV

Producto	shrimp species	administration	Seaweed species	Results
	vannamei[12]		*okamurnus*	infection, variable response

[1]Takahashi *et al*., 1998; [2]Cruz-Suárez *et al*., 2002a; [3]Hennequart *et al*., 2004; [4]Chotigeat *et al*., 2004; [5]Cheng *et al*., 2004; [6]Selvin *et al*., 2004; [7]Immanuel *et al*., 2004; [8]Hou and Chen, 2005; [9]Balasubramanian *et al*., 2006; [10]Yeh *et al*., 2006; [11]Deachamag *et al*., 2006; [12]Cruz-Suárez *et al*., 2007a.

Pigmentation

Crustacean pigmentation is affected by dietary pigment source, dosage level, duration of feeding, dietary composition, degree of carotenoid esterification, etc. (Meyers and Latscha, 1997). Menasveta *et al*. (1993) evaluated the pigmentation efficiency of feeds supplemented with 50 ppm astaxanthin vs 5% of extracted brown algae (*Chnoospora minima*) on *P. monodon*, and they found that carotenoides present in the brown algae feed increased carotenoid content in prawn, but the deposition levels were 2 to 3 times lower than those produced by the axtaxanthin supplemented feed. Cruz- Suárez *et al.* (2008b) showed that shrimp fed diets supplemented with 3.3% *Ulva clathrata* meal were highly pigmented in comparison with shrimps fed *Macrocystis* and *Ascophyllum* meals. The pigments present in *Ulva clathrata* (80% lutein) seem to be better metabolized and deposited in shrimp carcass than oxidized forms such as fucoxanthin present in kelp meals, which is in line with the observations reported by Meyers and Latcha (1997) on crustacean carotenoid metabolism. Ulva pigmentation efficiency is further better when shrimp are fed with the live co-cultured algae (Cruz-Suárez *et al*., 2008a).

Brown algae are rich in carotenoids especially in fucoxanthin, and some chlorophyll α and χ, β-carotene and xanthophylls violaxanthin (Barret and Anderson, 1980; Strand *et al*., 1998; Burtin, 2003; Dhargalkar and Kavlekar, 2004), while green algae, such as *Ulva sp., Ulva clathrata* and *Chaetoniorpha torta,* contain chlorophyll α and β, β-carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin and neoxanthin (Dhargalkar and Kavlekar, 2004; Burtin, 2003). Red algae contain red phycoerythrin and blue phycocyanin, β-carotene and α-carotene and their dihydroxylated derivatives: zeaxanthin and lutein (Burtin 2003).

Carcass composition

Cholesterol and lipid carcass composition of shrimp can be reduced with the consumption of *Sargassum* meal (Casas-Valdéz *et al*., 2006) and *Ulva clathrata* (Cruz-Suárez, 2008a). *Ulva* is known to change fat deposition and mobilization patterns in sea bream (Nakagawa *et al*., 1987), apparently resulting in a more efficient use of fat deposits so weight loss during winter stress is reduced, as well as other compositional changes. The effect may be due at least in part to cysteinolic acid, a non-protein amino acid similar to taurine. Cysteinolic acid, like taurine, can form conjugates with cholesterol in the formation of bile salts, at least in sea bream (Une *et al*., 1991), and this may be the basis for its effects on fat and cholesterol metabolism. Conceivably, animals naturally adapted to diets that include cysteinolic acid require it for efficient fat metabolism. Cysteinolic acid could play other roles in metabolism as well. For example, it could be converted to cysteine.

Single cell seaweed detritus as hatchery diets for shrimp

The use of seaweeds detritus as larvae feed has been reported by Japanese researchers. The formation of algal detrital particles in a completely cell-detached form, namely, single cell detritus (SCD) was reported for the first time by Uchida *et al.* (1999) during the microbial degradation process of macroalgal thalli. SCD displays 3 characteristics suitable for feed for aquatic hatchery animals: 1) The size of SCD is in the 2-14 μm range, which is similar to that of dietary phytoplankton; 2) The cell wall components of SCD are partially degraded which facilitates digestion; 3) Bacterial cells are attached to SCD, which modifies the algal detritus to protein-rich particles. The use of SCD as a potential hatchery diet instead of phytoplankton culture, which is labor-intensive, was successfully demonstrated based on feeding experiments with *Artemia*. Further modification of SCD could include the attachment of bacteria to SCD which would exert beneficial effects on the hatchery animals. Use of SCD diets in fish feeding regimes is an attempt to introduce the concept of detrital food web to aquaculture systems, which could contribute to the development of sustainable fish nursery systems (Uchida *et al*., 1997; Uchida and Murata, 2002).

A technology to produce shrimp hatchery diets from brown and green macroalgae was developed and patented by Uchida and Numaguchi (1996, USA patent 5801050). "Algal detritus particles are prepared by contacting algae with marine bacteria capable of attaching to and decomposing the algae under conditions sufficient to induce decomposition of the structural components of the algal tissue, thereby forming detritus suitable for use as a primary feed for marine organisms. Marine bacteria which belong to the genus *Alteromonas* can be used. Algae belonging to the class Phaeophyceae or Chlorophyceae preferably are used as the starting material in the invention. *Laminaria, Eisenia, Ecklonica, Undaria* and *Sargassum* are examples of types of Phaeophyceae which are useful. These algae can be decomposed by the above described marine bacteria to form particles suitable for use as a primary feed. *Ulva pertusa, Monostroma, Enteromorpha* and *Acetabuleria* are examples of types of Chlorophyceae. The cell walls of these plants can be decomposed by the action of the above marine bacteria, resulting in formation of protoplasmic detritus suitable for use as primary feed comprising particles having substantially the same diameter. Bacteria which are most preferred for accomplishing the present invention include *Pseudoalteromonas espejiana*. The proliferation of bacteria is faster and requires less use of resources such as light or heat compared with that of phytoplankton or zooplankton, which are used for feed at present. Thus, using the present method, detritus feed can be produced at low cost and with less labor. The invention provides an avenue for utilizing undeveloped or underdeveloped marine resources, such as *Ulva pertusa*, into value-added products in the marine environment. The concept of making use of and/or recycling marine resources has the advantage of preserving the environment" (Uchida and Numaguchi (1996, USA patent 5801050).)

The Fisheries College and Research Institute (FCRI) in India, has also developed a single cell diet that can be used as a cost-effective substitute for micro algae cultures for shrimp larvae reared at marine hatcheries. Named 'Marine Single Cell Detritus,' the product was derived from seaweeds. The feed was developed through enzymatic and fermentative treatment of seaweeds in two phases. In the first stage, the seaweeds were treated with an enzyme which led to the formation of single cell units. The 'enzymatic digest' was then treated with bacteria and yeast in the 'fermentative phase' to form the product. A series of tests conducted by the institute proved that MSCD had distinct advantages over traditional feeds like micro algae cultures and imported feeds like 'artemia cysts,' used widely in marine hatcheries across the country now. The MSCD

is 20% less expensive than artemia cysts and its manufacturing process is simpler than that of micro algae cultures. Besides, the new diet possesses bioremediation properties which control water quality. The 'probiotic' characteristics of the feed helps the fish develop infection resistance. The MSCD has 35% crude protein, making it nutritious. It can be stored in room temperature for a year (http://www.hinduonnet.com/thehindu/thscrip/p...007073157820100.htm&date=2007/07/31/&prd=th& (1 of 2)).

Fresh algae and shrimp/macroalgae co-culture

There are few reports about the nutritional benefits that shrimp can get as a result of fresh algae consumption or about shrimp/macroalgae integrated cultures (Table 7).

The use of *Enteromorpha sp* as part of fresh food to induce maturation of prawn *Penaeus indicus* and *L. stylirostris* was reported by Emerson (1980), Emerson *et al*. (1983), and Bray and Lawrence, (1988, 1990) respectively. Moss (1994) studied the nutritional contribution of different macro- and microalgae to juvenile shrimp growth. Shrimp juvenile white shrimp, *Penaeus vannamei* fed a diatom culture composed primarily of *Chaetoceros sp*. were significantly heavier ($p < 0.05$) than shrimp fed a monoculture of the green alga *Nannochloropsis oculuta,* fronds from the leafy macroalga *Ulva sp*., or fronds from the filamentous macroalga *Enteromorpha* sp. after 5 days. Porchas-Cornejo *et al.* (1999) reported that shrimp *Farfantepenaeus californiensis* increases its growth rate 3 fold in the presence of the algae *Caulerpa sertularioides*. Lombardi *et al*. (2006) tested the feasibility of co-culturing the Pacific white shrimp *Litopenaeus vannamei* and the Philippines seaweed *Kappaphycus alvarezii* in floating cages; juveniles (2.39±1.62 g) were transferred to 6 experimental grow-out cages at a density of 100 shrimp/m^2. Commercial pellets consisting of 35% crude protein were supplied once a day and shrimp were harvested after 103 days of growth. Thalli of the seaweed *K. alvarezii* were fixed on ropes and attached to floating tubes and set up inside 3 shrimp grow-out cages. Shrimp yield reached production rates as high as 3.23 kg/m^2/yr with a mean of 2.36±0.76 kg/m^2/yr. Seaweed production reached rates of 23.70 kg/m^2/yr with a mean of 17.47±5.71 kg/m^2/yr. There were no negative interferences in co-culturing shrimp and seaweed inside the

same cage. Student's t-test showed no statistical differences between the two treatments (monoculture and polyculture) for shrimp weight gain, survival rate, and feed conversion ratio.

Van Tri and Thi Thanh Ha (2004) evaluated 3 diet formulas: **live seaweed** + mixed feed; dry seaweed + mixed feed; and dry seaweed on N5 Nauplius *Litopenaeus vannamei* of the same size-collected from a hatchery at a density of 90 individuals/liter. The authors showed that white leg shrimp larvae feeding on live seaweed and mixed feed have shorter metamorphosis period than those feeding on dry seaweed and mixed feed or dry seaweed only. Larvae fed on live seaweed together with mixed feed had higher growth rate (in length and weight) and higher survival rate (48-53%) than those fed on dry seaweed and mixed feed or dry seaweed only.

The use of *Ulva* as food for shrimp in co-culture experiment has been proved (Cruz-Suárez *et al*., 2008a) to be beneficial as an alternative to improve the utilization of commercial feed and increase the economical value of the shrimp produced due to a larger size and a higher pigmentation. Shrimps in co-culture groups fed *Ulva* and pelleted feed showed significantly better performance than the shrimp fed *Ulva* or feed alone. The consumption of co-cultivated *Ulva clathrata* by shrimp *L. vannamei* improved the pelleted feed utilization and the growth rate: with 10 to 45% less commercial feed, growth rate was improved by 60%. Additionally, shrimp carcass quality improved with the level of *Ulva* consumed: lipids diminished, while total carotenoides, total and esterified astaxanthin increased following quadratic models. The co-cultivated *Ulva clathrata* also modified water quality: decreasing water turbidity and total phytoplankton cell number (Cruz-Suárez *et al.*, 2008a).

In the shrimp/macroalga integrated system, nitrogenous enriched waste water of cultured shrimp may be transformed into a valuable algal biomass, seaweeds production being an added income as feed for shrimp (Evans and Langdon, 2000; Schuenhoff *et al*., 2003; Neori *et al*., 2004). Several studies have shown that culture of *Ulva spp*. in nutrient-rich waters increases its protein content from 11% to over 32% in dry weight (Shpigel *et al*., 1999; Boarder and Shpigel, 2001). This biofilter produced *Ulva* has been shown to provide good growth rates for abalone *H. tuberculata* (Neori *et al*., 1998; Shpigel *et al*., 1999), *H. discus hannai* (Corazani and Illanes, 1998; Shpigel *et al*., 1999) and *H. roei* (Boarder and Shpigel, 2001). The evaluation of the

suitability of macroalgae cultivated in an integrated biofilter unit as a potential feed for the shrimp is a sustainable alternative to diminish shrimp production cost and environmental impact.

Polyculture of seaweeds in shrimp ponds or in the effluent ponds is one of the Best Management Practices suggested by Food and Agriculture (FAO) experts.

Table 7. Studies on the use of fresh algae as feed and shrimp/macroalgae co-culture

Shrimp species	Title	References
P. indicus	Induced maturation of prawn *Penaeus indicus*. (***Enteromorpha sp***. Fresh)	Emerson, W. D. 1980.
P. indicus	Growth and Maturation of *Penaeus indicus* under blue and green light. (***Enteromorpha sp.*** Fresh)	Emerson, W. D., Hayes, D.P., and Ngonyame, M., 1983.
Litopenaeus vannamei	Growth rates, nucleic acid concentrations, and RNA/DNA ratios of juvenile white shrimp, fed different algal diets. ***Chaetoceros sp.*** green algae ***Nunnochloropsis oculutu,*** fronds from the leafy macroalga, ***Ulva sp***., or fronds from the filamentous macroalga, ***Enteromorpha sp***. after 5 days.	Moss, 1994
P. stylirostris	Reproduction of eyestalk-ablated *Penaeus stylirostris* fed various levels of total dietary (***Enteromorpha sp.*** Fresh)	Bray *et al*., 1990a
P. monodon	Influence of dietary fatty acids on reproduction of *P. stylirostris* (***Enteromorpha sp***. Fresh)	Bray and Lawrence, 1988.
Penaeus californiensis	Efecto de la macrolaga C***aulerpa sertularioides*** en el desarrollo del camaron café	Porchas Cornejo *et al*., 1999
Litopenaues vannamei	Impacts of feed on the growth of white leg shrimp larvae *Litopenaeus vannamei* (Boone,1931).	Van and Thanh , 2004.
Litopenaeus vannamei	Cage polyculture of the Pacific white shrimp and the Philippines seaweed ***Kappaphycus alvarezii***	Lombardi *et al*., 2006.
Litopenaues vannamei	Shrimp and green algae ***Ulva clathrata*** co-culture to optimize commercial feed utilization.	Cruz- Suárez *et al*., 2008a.
Litopenaues vannamei	Cultivation of seaweed ***Ulva clathrata*** within shrimp ponds	Tomena and Copertino XIX ISS 2007 (214)
Litopenaues vannamei	Biofiltering efficiency, uptake and assimilation rates of ***Ulva clathrata*** cultiivated in shrimp aquaculture waste water	Copertino and Tomena XIX ISS 2007 (125)
P. monodon	Possible implications of the co-cultivation of blacktiger shrimp and cladophoraceae species on southwest asin shrimp farm	Tsutsui *et al*.,. XIX ISS 2007 (66)
	Development of abandoned shrimp ponds for restoration of wild Gracilaria along the coast of Pattan Bay, south of Thailand	Rapeepom *et al*., XIX ISS 2007 (37)
P. monodon	Profitable environmental remediation with seaweeds in an intensive marin shrimp culture	Hamano *et al*., . XIX ISS 2007 (232)

Conclusions

Algal meal inclusion in aquafeeds generally improves pellet stability and pellet water absorption. Growth performance, FCR, PER and disease resistance generally are improved at low inclusion levels. Additionally, shrimp product quality may also be improved by a better body pigmentation and lower lipid and cholesterol content. The use of fresh seaweeds for brood stock and fermented seaweeds for larvae feeds seems to be an interesting and promising alternative for these shrimp life stages. The shrimp/algae co-culture using live algae as a suitable feed for shrimp represent a sustainable alternative to diminish the need for artificial feed. The benefit of co-culture is amplified by the fact that co-cultured algae are enriched in highly available nutrients and variations in composition may be less than in the wild.

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