

A Professorial Chair Lecture on:

THE USE OF ALGAE IN THE FEED FORMULATION FOR AQUACULTURE SPECIES

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SEARCA Professorial Chair Awardee (April 2015-March 2016)

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Augusto E. Serrano, Jr.

The use of seaweeds in the feed formulation for aquaculture species

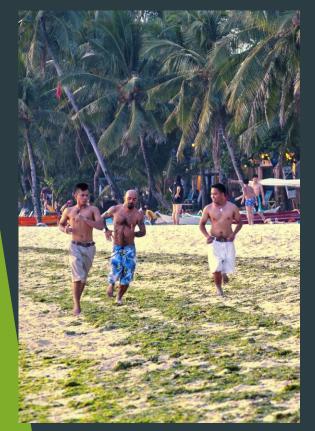
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Unutilized, nuisance



Hundred Islands, Alaminos, Pangasinan

Unutilized, nuisance







Boracay Island, Aklan

Unutilized, nuisance



Underground River, Puerto Princesa, Palawan

Nuisance



Could be used as ingredient in aquafeeds for shrimps





Rhizoclonium spp. grows in any idle fishpond



Rhizoclonium riparium at Dagupan, Pangasinan



DOST-PCAARD and UP Visayas (3 years)











Aquafeeds Program

ISP Interventions, Deliverable Potential Impacts



Seaweeds

Industry Problems	S & T Gaps	S & T Interventions	Deliverables	Potential Impact
 High cost of imported ingredients. Volatile prices of raw materials 	 Cheap, locally available feed ingredients 	• Utilize macroalgae iUlva lactuca, Enteromorpha (Ulva) intestinalis, Sargassum and Rhizoclonium as feed ingredient	 Information on nutritive value of PC and raw meal of seaweeds (proximate analysis, amino acid profile, digestibility) Biological value of raw meal and PC of seaweeds to shrimp and tilapia Feed formula for shrimp and tilapia 	Reduce cost of the feeds for tilapia and shrimp Reduce the cost of marketable sized tilapia and shrimp

Selected seaweeds



Ulva lactuca

Rhizoclonium riparium var implexum



Enteromorpha intestinalis



Characterization as ingredient

Biochemical

- Proximate analyses
 - Meal or Protein concentrate (PC)
- Amino acid profile
 - Meal or PC

Biological (lab)

- *In vivo* digestibility
- Growth performance
- Feed efficiency
- Body composition
- Immunological responses

Penaeus monodon or Litopenaeus vannamei

Objective







Feeding trial facilities



Source and preparation

Sources

- **Enteromorpha intestinalis Arevalo and Dumangas**
- Ulva lactuca Zamboanga
- Preparation
 - ► Meal
 - Cleaning (debris, other animals)
 - ▶ Shade-dried, oven-dried
 - pulverized
 - Protein concentrate (PC)
 - Juiced
 - Acidified
 - Precipitate oven dried, pulverized

Meal







Protein concentrate















Formulae

Weight gain, WG (g) = FABW - IABW

Where FABW = final average body weight (g); and IABW = initial average body weight (g)

- SGR (Specific Growth Rate, % body weight day⁻¹) = 100*(In FABW In IABW) / D
- Where D = days of culture

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► FCE (Feed Conversion Efficiency, %) = 100*(FI /WG)

Where FI= total feed intake of individual fish

FCR (feed conversion ratio) = WG/FI

- PER = WG / (FI*feed protein (in decimal))
- PR (Protein Retention, %) = 100*(% final carcass CP in decimal x FABW (g)) (% initial carcass CP in decimal x IABW (g)) / (FI*diet CP in decimal (g))

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- LR (Lipid Retention, %) = 100*(% final carcass body CL in decimal x FABW (g)) -(% initial carcass CL in decimal x IABW (g)) / (FI*diet CL in decimal (g))
- PG (Protein gained, g) = FABW*initial body CP in decimal IABW*final body CP
- Survival (%) = 100*Final number of fish replicate container⁻¹/Initial number of fish replicate container⁻¹

The following generalized quadratic equation was used:

$$R = a + bI + cI^2$$

Where: R = measured response (i.e. WG or SGR), I = dietary nutrient concentration, and a, b, and c are constants that are calculated to provide the best fit of the data. The value of I that produces the maximum response (i.e. I_{max}) is calculated as follows:

$$I_{max} = -0.5 (b / c)$$

Biochemical evaluation

Proximate analysis

Seaweed	СР	CL	Fiber	Ash	NFE
Ulva intestinalis (meal)	9.9	0.1	5.9	33.1	51.0
(PC)	31.6	27.6	1.37	31.4	8.0
<i>Ulva lactuca</i> (meal)	14.5	0.5	4.4	31.7	48.9
(PC)	43.9	22.3	3.2	16.8	13.8
Sargassum spp. (meal)	11.0	0.4	11.0	34.7	42.9
Rhizoclonium riparium var implexum (meal)	18.8	0.2	21.35	38.6	21.05
(PC)	25.4	0.22	2.4	48.8	23.18

Amino acid profile- *Rhizoclonium* spp.

Essential amino acid index (EAAI), chemical score (CS) and A/E ratios of Rhizoclonium meal collected from Arevalo, Iloilo and EAA requirement levels for the Nile tilapia and the tiger prawn

Table 4

Specification -	Rhizoclonium *1	Penaeid req. *3	A/E	**	(A/E Rhizoclonium)/(A/E req.) (%)	
	AA (% (CP)	Rhizoclonium meal	Penaeid shrimp	Penaeid shrimp	
Arginine	5.54	5.8	11.47	14.08	81.5	
Histidine	1.89	2.1	3.91	5.10	76.7	
Isoleucine	5.30	3.5	10.97	8.50	99.7	
Leucine	8.53	5.4	17.65	13.11	134.6	
Lysine	5.68	5.3	11.76	12.86	91.5	
Phenylalanine	6.20	7.1	12.83	17.23	74.5	
Methionine	1.77	3.6	3.66	8.74	41.9	
Threonine	5.05	3.6	10.45	8.74	119.6	
Tryptophan	0.94	0.8	1.95	1.94	100.5	
Valine	7.42	4.0	15.36	9.71	158.2	
Total	48.32	41.2	-		-	
EAAI	-	-	-	0.92	-	
CS	-		-	41.9		

A of Knizocionium sp. collected from the two sites

*2 Santiago & Lovell (1988)

*3 Akiyama (1992)

*4 A/E is the ratio of a given essential amino acid to the total essential amino acids

AA - amino acid

CP - crude proteine

Amino acid profile - *Rhizoclonium* spp.

		(A/E Ingredient)/(A/E req.)(%)						
	RM	RM (Leganes)	PCRM	PCRM	RM (Arevalo)	RM	PCRM	PCRM (Leganes)
		Nile tila	pia			Penaeid	shrimp	
Arginine	108.1	135.3	352.1	134.43	81.4	101.9	265.1	101.4
Histidine	93.1	109.1	222.26	314.52	89.8	89.8	183.3	259.0
Isoleucine	99.8	99.8	102.6	73.09	129.2	129.2	71.2	94.6
Leucine	117.4	114.6	114.6	87.99	134.6	133.9	69.8	103.4
Lysine	96.2	96.2	51.6	67.54	91.3	78.4	48.9	64.1
Phenylalanine	128.5	114.3	128.5	98.50	74.6	66.3	36.7	57.2
Methionine	62.2	74.7	139.3	173.22	42.0	50.5	94.1	116.9
Threonine	117.3	72.9	78.3	104.61	119.5	74.3	79.7	106.5
Tryptophan	74.6	63.1	68.1	93.08	100.0	84.5	91.2	124.7
Valine	129.2	133.1	71.9	91.76	158.3	163.1	88.1	112.5
Total	1385.6	1013.1	1329.3	1238.75	1020.7	971.9	1028.1	1140.3
EAAI* ⁵	100.0	98.4	112.5	111	68.8	91.8	87.4	105.0
CS *6	62	63	52	68	42	51	49	64
Limiting AA	Met	Tryp	Lys	Lys	Met	Met	Lys	Lys

Biological evaluation

A. Apparent Digestibility Coefficients

Dry matter Digestibility

Seaweed	P. monodon/P. vannamei
Ulva lactuca (meal)	71.5 (P.m.)
Ulva lactuca (PC)	88.8 (P.m.)
Sargassum spp. meal	84.8 (P.v.)
Rhizoclonium (meal)	60.0 (p.v.)

Biological evaluation

Feeding/growth trials

Table 1. Composition and proximate analysis of diets used in Experiment 1 which contained graded amounts of *Enteromorpha intestinalis* meal (EIM) fed to the Nile tilapia *Oreochromis niloticus* juvenile replacing soybean meal (g kg⁻¹) (Aquino et al 2014)

% EIM inclusion	0	3.9	7.8			
% SBM replacement	0	15	30			
Fish meal (sardine)	310.0	310 0	310 0			
Soybean meal	260.0	221.0	182.0			
EIM	0.0	39.0	78.0			
Copra meal	74.8	74.8	74.8			
Rice bran	120.9	120.9	120.9			
Leucaena meal (leaf)	101.0	101.0	101.0			
Cod liver oil	30.0	30.0	30.0			
Vegetable oil	20.0	20.0	20.0			
Vitamin mix	21.7	21.7	21.7			
Mineral mix	21.6	21.6	21.6			
Cornstarch	30.0	30.0	30.0			
Carboxymethylcellulos	e 10.0	10.0	10.0			
TOTAL	1000.0	1000.0	1000.0			
	Proximate compo	Proximate composition (dry basis)				
DM	949.2	945.3	944.1			
СР	400.2	388.1	374.9			
CL	99.4	94.3	94.1			
Crude fiber	64.7	61.8	63.7			
Ash	140.5	149.1	156.7			
NFE	295.2	306.8	310.8			

Experiment 1 Results

Table 2 Oreochromis niloticus

Enteromorpha intestinalis meal (EIM)

% EIM incl.	0	3.9	7.8
% SBM repl.	0	15	30
IABW	0.03	0.03	0.03
FABW	6.97 ^a	8.25 ^a	4.54 ^b
WG	7.00 ^a	8.28 ^a	4. 57 ^b
SGR	6.79 ^a	6.99 ^a	6.29 b
FCR	1.00	1.06	1.10
Survival	95.6	93.3	93.3
PR	29.6	29.7	26.7
LR	9.59 ^a	7.45 ^b	6.87 ^b

Increasing the inclusion level of EIM beyond 3.9% (15% SBM replacement) resulted in significantly poorer WG, SGR and FI but resulted in statistically similar FCR and survival rate

(Hasan and Chakrabarti 2009)	-progressive decrease in performance when 15%-20% algal meal was added to the diet					
(Azzaza et al 2008)	- <i>Ulva rigida</i> as SBM replacement up to 20 % in the diet of Nile tilapia did not affect growth performance and feed efficiency					
Guroy et al (2007)	- 10-15% <i>Ulva rigida</i> meal to the diet of the Nile tilapia was optimum for growth performance					
Ergun et al (2008)	- 5% Ulva rigida meal was optimum for the Nile tilapia					
Diler et al (2007)	15% Ulva rigida meal optimally replaced wheat meal in Cyprinus carpio					
Mustafa & Nakagawa (1995)	5% - 15% Ulva rigida in the diets of black sea bream (Acanthopagrus schlegeli), red sea bream (Pagrus major), rainbow trout (Oncorhynchus mykiss) improves the growth performance					

- Why only 3.9%
 - higher inclusion of EIM could have resulted in a considerable increase in phenolic compounds causing poor digestibility of dietary protein.
 - could be due to high carbohydrate and ash contents in EIM.

Conclusion of Experiment 1.

EIM could replace 15 % of the SBM in the diet of Nile tilapia without exhibiting adverse effect on WG, SGR, FCR, PR and survival rate.

Table 3

Composition and proximate analysis of diets used in Experiment 2 containing graded levels the protein concentrate of *Enteromorpha intestinalis* (EIPC) fed to the Nile tilapia *Oreochromis niloticus* juvenile replacing soybean meal (g kg⁻¹) (Serrano Jr. and Aquino 2014)

% EIPC inclusion	0	3.9	7.8	11.7
% SBM replacement	0	15	30	45
Fish meal (sardine)	310.0	310.0	310.0	310.0
Soybean meal	260.0	221.0	182.0	143.0
EIPC	0.0	39.0	78.0	117.0
Copra Meal	74.8	74.8	74.8	74.8
Rice Bran	120.9	120.9	120.9	120.9
<i>Leucaena</i> leaf meal	101.0	101.0	101.0	101.0
Cod liver oil	30.0	30.0	30.0	30.0
Vegetable oil	20.0	20.0	20.0	20.0
Vitamins	21.7	21.7	21.7	21.7
Minerals	21.6	21.6	21.6	21.6
Cornstarch	30.0	30.0	30.0	30.0
Carboxymentylcellulose	10.0	10.0	10.0	10.0
TOTAL	1000.0	1000.0	1000.0	1000.0
	Prox	imate composition (dry	basis)	
DM 949	.2	950.7	949.8	947.4
CP 400	.2	375.1	375.3	382.2
CL 99.4		98.5	96.3	95.5
Crude Fiber 64.7	1	62.4	61.5	62.7
Ash 140	.5	150.2	164.0	183.2
NFE 295	.2	313.9	302.8	276.3

Experiment 2
Table 4
Oreochromis niloticus X
Enteromorpha
intestinalis protein
concentrate (EIPC)

% EIPC incl.	0	3.9	7.8	11.7
% SBM rep.	0	15	30	45
WG	7.00 ^a	5.37 ^{ab}	4.67 ^b	4.20 ^b
SGR	6.79 ^a	6.48 ^{ab}	6.30 ^b	6.18 ^b
FCR	1.00	1.05	1.01	1.02
Survival	95.6	88.9	91.1	91.1
PR	29.6	29.6	29.0	28.2
LR	9.59 ^a	7.36 ^b	7.58 ^b	7.52 ^b

- Tilapia fed 3.9% (15% SBM replacement) exhibited statistically similar WG and SGR with those fed the control diet
- 'unpalatable' components might have also been concentrated.
 - Azaza et al (2008) 30 % Ulva rigida meal contains on a dry weight basis 2.65% saponins, 0.22% tannins and 0.61% phytic acid; lower digestible energy
 - Serrano (2013) may not necessarily result in the negative performance as we have shown in the common carp previously
- There is no available data with which to compare the results of Experiment 2.
- Feed efficiency (i.e. FCR), survival and PR were not affected by the dietary treatments.

Conclusion of Experiment 2.

EIPC produced from the combined procedures of acidification and heat treatment could be included at 3.9% (i.e. 15% SBM replacement) of the diet without adversely affecting SGR, feed and nutrient efficiencies and survival but with possible slight reduction in body CL.

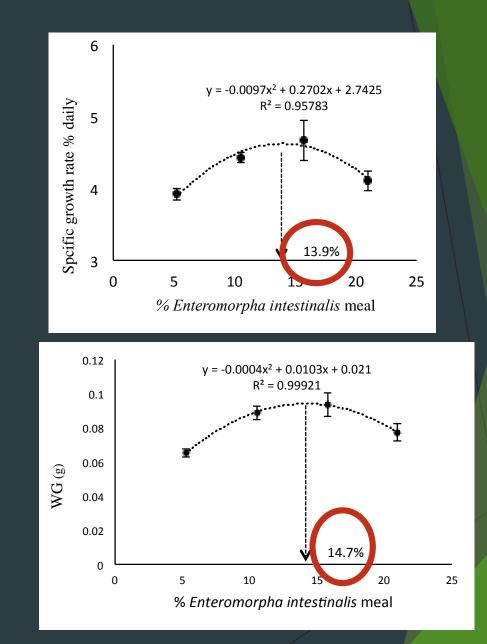
Table 5

Composition and proximate analysis of diets used in Experiment 3 containing graded levels of *Enteromorpha* intestinalis meal (EIM) fed to the black tiger shrimp *Penaeus monodon* postlarvae replacing soybean meal (g kg⁻¹) (Serrano Jr. and Tumbokon 2015)

% EIM incl.	0	5.25	10.5	15.75	21
% SBM repl.	0	15	30	45	60
Fish meal (Danish)	300.0	300.0	300.0	300.0	300.0
Squid Meal	29.0	29.0	29.0	29.0	29
Carboxymet hylcellulose	80.5	80.5	80.5	80.5	80.5
Lignobond	15.0	15.0	15.0	15.0	15
Vitamins	10.0	10.0	10.0	10.0	10
Minerals	10.0	10.0	10.0	10.0	10
DicalPhos	20.0	20.0	20.0	20.0	20
BHT	0.5	0.5	0.5	0.5	0.5
Bread flour	11.7	11.7	11.7	11.7	11.7
Cod liver oil	63.0	63.0	63.0	63.0	63
Lecithin	5.0	5.0	5.0	5.0	5
Soybean meal	350.0	297.5	245.0	192.5	142.5
EIM	0.0	52.5	105.0	157.5	210
IUIAL	1000.0	1000.0	1000.0	1000.0	1000
	Proximate	analysis (%, dry basis	5)		
DM	89.6	91.6	91.9	92.1	92.4
СР	45.8	43.3	40.59	38.1	35.7
CL	11.6	10.9	10.1	10.1	10.7
Crude fiber	2.8	2.6	2.6	2.6	2.6
NFE	27.9	29.4	33.0	33.0	33.6
Ash	11.0	12.6	13.9	14.9	16.0

Experiment 3	% EIM incl.	0	5.25	10.5	15.8	21.0
	% SBM rep.	0	15	30	45	60
	IABW	0.01	0.01	0.01	0.01	0.01
Table 6	FABW	0.14 ^{ab}	0.11 ^a	0.14 ^{ab}	0.16 ^b	0.12 ^{ab}
Penaeus monodon	WG	0.08	0.07	0.09	0.09	0.08
X	SGR	4.78 ^{ab}	4.48 ^b	5.09 ^{ab}	5.19 ^a	4.80 ^{ab}
Enteromorpha	Survival	84.4	75.6	84.5	77.8	91.1
intestinalis meal (EIM)	FCR	1.46	1.47	1.54	1.24	1.35
	PER	1.56	1.59	1.60	2.12	2.08

Figure 1. Responses of the shrimp to varying levels of dietary EIM fitted into a quadratic model.



- CP did not affect growth (see PER) probably due to the compensatory increased in FI.
- Probably there was a balanced amino acid similar to another chlorophyte, *Rhizoclonium riparium var implexum* which exhibited essential amino acid index (EAAI) for the shrimp *Penaeus monodon* of 0.97 (Bunda et al. 2015)

Conclusion of Experiment 3.

The optimum inclusion level of EIM in the diet of the black tiger shrimp ranged from 13.9 to 14.7% or its equivalent range of 39.7% to 42% SBM replacement. However, the highest inclusion of 21.0% or 60% replacement of SBM in the diet could be used without deleterious effects on the growth performance and feed efficiency of the shrimp.

Table 7 **Composition and** proximate analysis of diets used in Experiment 4 containing graded levels of Enteromorpha intestinalis meal (EIM) fed to the black tiger shrimp Penaeus monodon postlarvae replacing soybean meal (g kg⁻¹) (Serrano Jr. et al 2015)

Fish meal 380.0 380.0 380.0 380.0 380.0 (Danish) 29.0 29.0 29.0 29.0 29.0 Soybean Meal 350.0 298.0 245.0 193.0 Bread Hour 80.0 80.0 80.0 80.0 Cod Liver Oil 63.0 63.0 63.0 63.0 Lecithin 5.0 5.0 5.0 5.0 Carboxymethylce 37.5 37.5 37.5 37.5 Lignobond 15.0 15.0 15.0 15.0 Vitamins 10.0 10.0 10.0 10.0 Minerals 10.0 20.0 20.0 20.0 20.0 BHT 0.5 0.5 0.5 0.5 0.5 UPC 0.0 52.0 105.0 158.0 TOTAL 1000.0 1000.0 1000.0 10000.0 DM 95.8 96.0 95.2 95.1 CP 41.3 41.4 <t< th=""><th></th><th></th><th></th><th></th><th></th></t<>					
Fish meal 380.0 380.0 380.0 380.0 380.0 (Danish) 29.0 29.0 29.0 29.0 29.0 Soybean Meal 350.0 298.0 245.0 193.0 Bread Hour 80.0 80.0 80.0 80.0 Cod Liver Oil 63.0 63.0 63.0 63.0 Lecithin 5.0 5.0 5.0 5.0 Carboxymethylce 37.5 37.5 37.5 37.5 Lignobond 15.0 15.0 15.0 15.0 Vitamins 10.0 10.0 10.0 10.0 Minerals 10.0 20.0 20.0 20.0 20.0 BHT 0.5 0.5 0.5 0.5 0.5 UPC 0.0 52.0 105.0 158.0 TOTAL 1000.0 1000.0 1000.0 10000.0 DM 95.8 96.0 95.2 95.1 CP 41.3 41.4 <t< td=""><td>% ULPC incl.</td><td>0</td><td>5.2</td><td>10.5</td><td>15.8</td></t<>	% ULPC incl.	0	5.2	10.5	15.8
(Danish) 380.0 29.0 20.0 20.0 20.0 20.0 20.0 20.0 20.0 20.0 20.0 <td>% SBM repl.</td> <td>0</td> <td>15</td> <td>30</td> <td>45</td>	% SBM repl.	0	15	30	45
Soybean Meal350.0298.0245.0193.0Bread Hour80.080.080.080.0Cod Liver Oil63.063.063.063.0Lecithin5.05.05.05.0Carboxymethylce37.537.537.537.5Hulose37.537.537.537.5Lignobond15.015.015.015.0Vitamins10.010.010.010.0Minerals10.020.020.020.0phosphate20.020.020.020.0BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.110.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	Fish meal (Danish)	380.0	380.0	380.0	380.0
Bread Hour 80.0 63.0	Squid Meal	29.0	29.0	29.0	29.0
Cod Liver Oil63.063.063.063.0Lecithin5.05.05.0Carboxymethylce37.537.537.5Ilulose37.537.537.5Lignobond15.015.015.0Vitamins10.010.010.0Minerals10.010.010.0Dicalcium phosphate20.020.020.0BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	Soybean Meal	350.0	298.0	245.0	193.0
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Minerals10.010.010.010.0Dicalcium phosphate20.020.020.020.0BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4		15.0	15.0	15.0	15.0
Dicalcium phosphate20.020.020.020.0BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	Vitamins	10.0	10.0	10.0	10.0
phosphate20.020.020.020.0BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4		10.0	10.0	10.0	10.0
BHT0.50.50.50.5ULPC0.052.0105.0158.0TOTAL1000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	Dicalcium phosphate	20.0	20.0	20.0	20.0
TOTAL1000.01000.01000.0Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	BHT	0.5	0.5	0.5	0.5
Proximate Analysis (%, dry basis)DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	ULPC	0.0	52.0	105.0	158.0
DM95.896.095.295.1CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	TOTAL	1000.0	1000.0	1000.0	1000.0
CP41.341.440.740.1CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4		Proxi	mate Analysis (%, o	dry basis)	
CL10.810.710.410.2Crude Fiber2.52.02.92.9Ash15.315.115.915.4	DM	95.8	96.0	95.2	95.1
Crude Fiber2.52.02.9Ash15.315.115.915.4	СР	41.3	41.4	40.7	40.1
Ash 15.3 15.1 15.9 15.4	CL	10.8	10.7	10.4	10.2
	Crude Fiber	2.5	2.0	2.9	2.9
NFE 26.0 26.9 25.3 26.4	Ash	15.3	15.1	15.9	15.4
	NFE	26.0	26.9	25.3	26.4

Experiment 4
Table 8
Penaeus monodon X
<i>Ulva lactuca</i> meal (ULM)

% ULM inclusion	0	5.2	10.5
% SBM replacement	0	15	30
IABW	0.11	0.11	0.11
FABW	1.45	1.22	1.03
SGR	3.02 ^a	2.82 ^{ab}	2.61 ^b
FCE	0.67	0.65	0.66
PG	0.20 ^a	0.17 ^b	0.16 ^b
PER	13.1	14.8	14.3
Survival	87.0	93.0	87.0

- Shrimp fed with the control diet and with that containing 15% ULM resulted in significantly higher SGR than those fed with 30% ULM but FABW of those fed diet with 30% ULM were all similar
- In terms of FCE, PG, and PER, no significant differences were observed in all treatments.
- Other studies
 - Incorporating 10% seaweed to the diets of *Penaeus monodon* and *Litopenaeus vannamei* resulted in higher WG, FCR was lowered by 0.1 point, improved color of shrimp, 25% lower mortality rates, improved taste and texture of the shrimp (Ocean Harvest Technology 2010).

Conclusion of Experiment 4.

- All the parameters (survival rate, FCE, PR and LR) were not affected by the diet;
- Both inclusion levels of 5.2% and 10.5% (15% and 30% SBM replacement) resulted in similar growth performance (FABW and SGR)

Table 9

Composition and proximate analysis of diets used in Experiment 5 containing graded levels of Ulva lactuca protein concentrate (ULPC) fed to the black tiger shrimp Penaeus monodon postlarvae replacing soybean meal (g kg⁻¹) (Serrano Jr. and Santizo 2014)

% ULM inclusion	0	5.2	10.5
% SBM replacement	0	15	30
Fish meal (Danish)	380.0	380.0	380.0
Squid Meal	29.0	29.0	29.0
Soybean Meal	350.0	298.0	245.0
Bread flour	80.0	80.0	80.0
Cod Liver Oil	63.0	63.0	63.0
Lecithin	5.0	5.0	5.0
Carboxymethylcellulose	37.5	37.5	37.5
Lignobond	15.0	15.0	15.0
Vitamin	10.0	10.0	10.0
Mineral	10.0	10.0	10.0
Dicalcium phosphate	20.0	20.0	20.0
внт	0.5	0.5	0.5
ULM	0.0	52.0	105.0
TOTAL	1000.0	1000.0	1000.0
	Proximate Analy	sis (%, dry basis)	
DM	95.8	95.1	95.1
СР	41.3	38.5	37.3
CL	10.8	10.4	10.2
Crude Fiber	2.5	2.2	2.7
NFE	26.0	29.9	28.9
Ash	15.3	14.0	16.1

Experiment 5	% ULPC	0	5.2	10.5	15.8
Table 10	% SBM rep.	0	15	30	45
	IABW	0.11	0.11	0.11	0.11
Penaeus monodon	FABW	1.45 ^a	1.23 ^{ab}	1.16 ^{ab}	0.98 ^b
X	SGR	3.02 ^a	2.84 ^{ab}	2.76 ^{ab}	2.57 ^b
	FCR	1.49	1.52	1.54	1.59
Ulva lactuca protein	PER	13.12	14.33	14.60	13.45
concentrate (ULPC)	PG	0.20 ^a	0.19 ^{ab}	0.17 ^{ab}	0.15 ^b
	Survival	87	96	98	91

- only shrimps the diet containing 15.8% ULPC or 45% SBM replacement exhibited inferior FABW and SGR
- Probably due to the reduced feed intake (FI) of the shrimp, suggesting reduced palatability

Rainbow trout	100% substitution of fish meal resulted in either reduced growth (Medale et al 1998) or no negative effects (Kaushik et al 1995)
Other fish	Reduced growth due probably to reduced FI as a result of reduced palatability (Davis et al 1995)
	Due to saponins, tannins and phytic acid (Azaza et al 2008)

Summary & Recommendation

	% inclusion	% SBM repl.
Enteromorpha intestinalis		
	Meal form	
Nile tilapia	3.9%	15%
Black tiger shrimp	21%	60%
	PC form	
Nile tilapia	3.9%	15%
Ulva lactuca		
	Meal form	
Black tiger shrimp	10.5%	30%
	PC form	
Black tiger shrimp	10.5%	30%

Conclusion of Experiment 5.

ULPC could be included in the diet of *Penaeus monodon* fry up to 10.5% (i.e. 30% SBM replacement) without reducing growth and PR of the black tiger shrimps;

at 15.8% inclusion level (i.e. 45% SBM replacement), growth was reduced.

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