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The effects of soybean-based diets, with and without amino acid supplementation, on growth and biochemical composition of juvenile American lobster, *Homarus americanus*

Eric A.T. Floreto [a,*], Robert C. Bayer [b], Paul B. Brown [c]

[a] *136 Applefield Drive, Scarborough, Ontario, Canada M1P 3Y2*

[b] *Biosystems Science and Engineering, University of Maine, Orono, ME 04469-5735, USA*

[c] *Department of Forestry and Natural Resources, 1159 Forestry Building, Purdue University, West Lafayette, IN 47907-1159, USA*

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Abstract

The feasibility of feeding soy-based diets for the pound culture of the American lobster was investigated in a factorial study using diets (40% protein) containing various proportions of extruded-expelled, low-fat soybean meal (SBM) (0%, 25%, 50%, 75%, 87.5% and 100% of dietary protein) and fish meal (FM), with and without amino acid supplementation (arginine, leucine, methionine and tryptophan), in a 60-day feeding trial using late Stage 5 juveniles. The supplemental amino acids were added at levels to simulate the essential amino acid (EAA) profile of juvenile lobsters. The 0% SBM diet approximated salted fish and fish racks, the industry diet for pounded lobsters. A diet of fresh blue mussel, a component of the lobster's natural diet, was included for comparison. Supplementation and SBM levels of not more than 50% of dietary protein significantly resulted in higher body weight gains (BWGs) than diets without supplementation or with higher SBM levels. Survival was not significantly different for juveniles fed the supplemented diets and the non-supplemented diets containing FM. Juveniles fed the non-supplemented 100% SBM diet suffered early mortality. Supplementation significantly shortened molting cycles and was crucial for survival in juveniles fed the 100% SBM diet. Interactions between supplementation and level of dietary SBM on survival, BWG and duration of the molting cycle

* Corresponding author. Tel.: +1-416-438-3662.

E-mail addresses: eric_floreto@yahoo.com (E.A.T. Floreto), rbayer@maine.maine.edu (R.C. Bayer), pb@fnr.purdue.edu (P.B. Brown).

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were significant. Growth performance of juveniles fed blue mussel were comparable to those of juveniles fed the supplemented 50% SBM diet. The levels (% of protein) of arginine, phenylalanine and tryptophan were significantly higher in juveniles fed the supplemented diets, while tyrosine, aspartate, glutamate and serine were significantly higher in juveniles fed the non-supplemented diets. Arginine levels in juveniles fed the supplemented diets were nearly twice those of juveniles fed the non-supplemented diets. Interactions between SBM level and supplementation on lobster amino acid profile were not significant. Supplementation and decreasing levels of dietary SBM (lower content of 18:2 $n-6$, the major polyunsaturated fatty acid (PUFA) in SBM) were associated with better growth and resulted in increasing proportions (% of total) of 20:5 $n-3$, 22:6 $n-3$ and $n-3/n-6$ PUFA ratios in juvenile carcass. Based on weight gain, replacement of fish protein with SBM in practical diets at no more than 50% of dietary protein appears feasible, with multiple amino acid supplementation significantly enhancing growth performance.

Keywords: *Homarus americanus*; Soybean diets; Crustacean nutrition; Amino acids; Fatty acids; Amino acid supplementation

1. Introduction

In 1997, commercial harvest of market-size American lobsters, in the state of Maine, USA totaled nearly 20,700 metric tons, worth US$136 million (Maine Department of Marine Resources). At least 10% of this catch goes into a limited form of aquaculture called 'pounding' wherein soft-shelled lobsters caught in the summer and fall are held in tidal enclosures called 'lobster pounds' for a few months to harden their exoskeleton and gain some weight. The pounded lobsters are then sold at a higher price from winter to spring, when the supply from the wild decreases as lobsters move into deeper offshore waters.

Lobsters are held at high densities (10–11 m^{-2}) in the pounds and are typically fed salted herring/scraps or salted cod racks (a byproduct of the cod filleting industry, consisting of fish skeletons and some meat). With the occurrence of record lobster landings in 1998, pounding activity and the consequent demand for herring and cod racks is expected to increase. The local cod fishery is considered overexploited, and countermeasures, such as restrictions on the fishery, will curtail the supply of salted cod rack. Also, the use of herring, a fish fit for direct human consumption, as animal feed, raises ethical concerns. Thus, a formulated diet that replaces the current use of marine animal protein is highly desirable.

Considerable nutritional research has focused on the use of plant proteins to replace the limited and relatively expensive marine animal derived meals in formulated diets. Compared to other plant protein sources, soybean meal (SBM) is one of the most promising because of its availability, reasonable price, high digestibility and better amino acid profile. However, use of SBM is technically limited by its amino acid composition compared to fish meal (FM) (deficiency in the essential amino acids (EAAs) methionine, lysine and tryptophan), presence of antinutritional factors, and poor palatability (Lim and Dominy, 1990; Tacon and Akiyama, 1997). To date, maximum successful inclusion of dietary SBM in practical marine shrimp diets is not more than

50%, as higher levels resulted in lower growth (Akiyama, 1990; Lim and Dominy, 1990).

Approaches to improving the nutritional value of both plant and animal feed ingredients relative to amino acid composition include dietary supplementation of crystalline L-amino acids (Guillaume, 1997; Millamena et al., 1998), combining a variety of protein sources containing complementary EAA profiles such as legumes and grains (Audesirk and Audesirk, 1996), binding the supplemental amino acids in polymers (Chen et al., 1992) or plasteins (Teshima et al., 1992), development of transgenic seed plants (e.g. canola) with better protein digestibility or expressing growth factors in aquaculture species, and the search for better attractants to improve palatability (Lee and Meyers, 1997).

This study investigated the feasibility of replacing fish protein with SBM protein in practical diets for pounded American lobster. Diets containing different proportions of low-fat, extruded-expelled SBM (from 0% to 100% of dietary protein) and FM, were fed to juvenile lobsters to examine their growth and resulting biochemical composition. The study also investigated how amino acid supplementation of the diets would affect growth and subsequent biochemical profiles of the juveniles. Extruded-expelled SBM contains a higher content of lecithin and fat-soluble vitamins, and is environment-friendly because unlike solvent-extracted SBM, hexane is not used to remove the seed oil.

2. Materials and methods

2.1. Experimental diets

The experimental diets were formulated to contain 40% total crude protein by combining different proportions of low fat extruded-expelled SBM (Producers' Natural Processing, Brookston, IN, USA) and menhaden FM (Sigma, St. Louis, MO). Six levels (0%, 25%, 50%, 75%, 87.5% and 100%) of SBM as a percentage of the dietary protein, were tested, and corresponded to dietary levels of 0%, 20.8%, 41.7%, 62.5%, 72.9% and 83.3%, respectively. For the remainder of the description of the study, the diets will be referred to by their SBM inclusion level as a percent of dietary protein. Ingredient composition of the diets is presented in Table 1. Actual protein levels of the diets ranged from 38.3% to 43.8%. The additional amounts of protein may have originated from the cornstarch and amino acid supplements. The 0% SBM dietary treatment (100% of the protein is derived from FM) approximates the use of fish frames in lobster pounds, and is considered the control diet in this study. The fish frame diet was not included because of its higher content of protein (> 60%), unknown vitamin and mineral composition. A natural diet of slivers of mantle from fresh blue mussel, *Mytilus edulis*, a component of the lobster's diet in the wild, was also used for comparison. Levels of menhaden oil, soybean lecithin, cholesterol, mineral and vitamin mixes in the experimental diets were the same for all dietary treatments. Crude lipid was formulated to be 11% of the diet. However, actual gravimetric determination after lipid extraction (Bligh and Dyer, 1959) showed a range of 12.2–13.6%. The additional amounts of lipid possibly came from the cornstarch and fat-soluble vitamins. The EAA profiles of juvenile lobster (Boghen and

Table 1
Composition and proximate analyses of the test diets, with (+) and without (−) amino acid supplementation

Ingredients[a] (g/100 g dry diet)	Level of SBM (% of dietary protein)												Blue mussel
	0		25		50		75		87.5		100		
Diet type: amino acid supplement	−	+	−	+	−	+	−	+	−	+	−	+	
SBM (% of diet)	0	0	20.8	20.8	41.7	41.7	62.5	62.5	72.9	72.9	83.3	83.3	
Menhaden fish meal	65.3	65.3	48.9	48.9	32.6	32.6	16.3	16.3	8.2	8.2	–	–	
Cornstarch	16.8	16.8	12.7	12.7	7.0	7.0	2.0	2.0	2.2	2.0	0.2	–	
Menhaden oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Vegetable oil	1.4	1.4	1.0	1.0	0.6	0.6	–	0.2	–	0.2	–	–	
Soybean lecithin	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Mineral mix[b]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
Vitamin mix[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
CMC[d]	8.0	7.2	8.0	7.1	8.0	8.0	8.0	8.0	8.0	6.7	8.0	6.5	
Cellulose	0.1	–		–	1.6	0.5	2.5	1.2	0.2	–	–	–	
Amino acid supplementation													
Arginine	–	0.38	–	0.35	–	0.32	–	0.29	–	0.27	–	0.25	
Leucine	–	–	–	–	–	–	–	0.10	–	0.20	–	0.29	
Methionine	–	–	–	0.14	–	0.31	–	0.48	–	0.57	–	0.65	
Tryptophan	–	0.48	–	0.47	–	0.46	–	0.46	–	0.46	–	0.45	
Total	100	100	100	100	100	100	100	100	100	100	100	100	
Analyzed composition (as fed)													
Moisture	9.1	9.5	10.2	10.1	10.0	9.7	9.8	9.5	10.5	10.4	9.8	10.2	85.4
Crude protein[e]	39.4	41.4	38.7	43.8	38.3	42.1	40.9	42.8	38.8	41.5	39.6	42.4	55.0
Carbohydrate[e]	17.9	18.7	23.4	18.1	23.6	20.5	28.3	24.1	30.2	29.4	29.2	27.6	33.1
Crude lipid[e]	13.0	13.3	13.1	12.9	12.7	13.4	13.3	10.8	12.2	13.3	13.6	12.8	12.4
ME (calculated; kcal/100 g)[f]	391.9	400.3	383.7	393.6	371.6	383.1	359.8	374.4	362.6	378.1	361	374.8	–
P/E[g]	104.0	102.1	106.6	104.2	110.3	107.6	113.8	111.2	113.1	110.3	107	110.9	–

Castell, 1981) and the diets (calculated values) were compared and four EAAs (arginine, leucine, methionine and tryptophan) were identified for dietary supplementation to simulate the EAA profile of the lobster (Table 2). Diets containing no supplemental amino acids were also prepared, for a total of 12 experimental diets. Supplementation increased the dietary protein content by 0.9–1.6% (Table 1).

Diet preparation and the coating of the crystalline L-amino acids with carboxymethylcellulose (CMC) as binder were patterned after that of Millamena et al. (1997). Cellulose was added as filler. The pH of the dough was adjusted to 7.0–7.5 by the dropwise addition of 3 N NaOH solution to neutralize the acidity brought about by the addition of the crystalline amino acids. The pH was determined by homogenizing 1 g of sample with 10 ml of distilled water in a test tube. The dough was extruded with a kitchen spaghetti maker, dried at room temperature (20°C) to a moisture content of around 10% (range of 9.1–10.5%) in a fume hood, and stored at −20°C until used.

2.2. Bioassay of *Homarus americanus*

Juvenile *H. americanus* (late Stage 5, average wet weight, 0.07 ± 0.02 g), produced from one breeder were air transported from the New England Aquarium in Boston, MA. Upon arrival, the juveniles were immediately transferred to individual plastic rearing containers (14 × 10 × 11.5 cm) filled with 1 l of 30‰ artificial seawater. The seawater was made by dissolving the appropriate amount of seasalts (Forty Fathoms Crystal Sea, Baltimore, MD) in 10 mg/l EDTA-treated distilled water. A piece of PVC pipe (5 cm length, 1.5 cm diameter) was placed on the bottom of each container for shelter. All materials used for the bioassay (rearing tank, airlines, airstones, shelters, etc.) were leached for a week in freshwater that was changed daily, prior to use. Culture water was changed daily, alternately at 50% and 100%. As part of the 50% water change procedure, uneaten food and feces were siphoned off, while, the juveniles were transferred to newly cleaned containers for the 100% water change. The water exchange rates ensured that water quality parameters (as measured in representative tanks by Hach test kits during 100% water change) exceeded optimum as described by Conklin and

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Notes to Table 1:

[a] SBM supplied by Producers' Natural Processing, Brookston, IN. All other ingredients were obtained from Sigma.

[b] Mineral mix (mg/100 g dry diet): calcium hydrogen phosphate dihydrate, 1000; dipotassium hydrogen phosphate, 600; sodium chloride, 800; magnesium sulfate, 60; zinc sulfate pentahydrate, 8; potassium iodide, 0.15; sodium selenite, 0.05; cobalt chloride hexahydrate, 0.1; cellulose, 35.

[c] Vitamin mix (mg/100 g dry diet): thiamine–HCl, 5; riboflavin, 8; Ca–pantothenate, 30; pyridoxine–HCl, 12; niacin, 50; D-biotin, 1; myo-inositol, 400; cyanocobalamin, 1; folic acid, 2; ascorbyl phosphate, 50; choline–Cl, 610; retinol palmitate, 50; cholecalciferol sulfate, 1.3; α-tocopherol, 30; menadione, 3; *p*-aminobenzoic acid, 40.

[d] Carboxymethylcellulose.

[e] Expressed as percent dry weight.

[f] Metabolizable energy, calculated using the physiological fuel values of Brett and Groves (1979) (kcal/g): protein, 5.091; carbohydrate, 4.111; lipid, 9.441; and digestibility by coefficients by Akiyama et al. (1989).

[g] Protein:energy ratio.

Table 2
The EAA composition of the experimental diets (g/100 g dry weight)

Amino acid	Juvenile[a] lobster (% of protein)	Calculated ratio in the 40% protein diet	Percent SBM in dietary protein						Blue mussel
			0	25	50	75	87.5	100	
Arginine	6.80	2.72	2.34[b]	2.37[b]	2.40[b]	2.43[b]	2.45[b]	2.47[b]	7.20
Histidine	2.10	0.84	0.94	0.93	0.92	0.91	0.91	0.90	1.12
Isoleucine	3.80	1.52	1.83	1.85	1.87	1.88	1.89	1.90	1.77
Leucine	6.40	2.56	3.03	2.84	2.65	2.46[b]	2.36[b]	2.27[b]	3.78
Lysine	4.00	1.60	3.07	2.83	2.58	2.34	2.22	2.10	3.66
Methionine	2.80	1.12	1.15	0.98[b]	0.81[b]	0.64[b]	0.55[b]	0.47[b]	1.53
Phenylalanine	3.90	1.56	1.57	1.63	1.70	1.77	1.80	1.83	1.83
Threonine	3.80	1.52	1.59	1.56	1.53	1.50	1.48	1.47	3.39
Tryptophan	2.30	0.92	0.44[b]	0.45[b]	0.46[b]	0.46[b]	0.46[b]	0.47[b]	0.21
Valine	4.60	1.84	2.13	2.04	1.95	1.86	1.81	1.77	1.74

[a]From Boghen and Castell, 1981.
[b]Supplemented with crystalline L-amino acid to approximate the calculated ratio in the 40% protein diet.

Chang (1993): NH_3–N, < 9.4 mg/l; NO_2–N, < 10 mg/l; NO_3–N, < 50 mg/l. Each container was provided with mild aeration from a single airstone, and except for a total of about 3 h daily, during visual inspections, water exchange and feeding, the cultures were kept in total darkness by covering with black plastic sheets. Ambient water temperature was 15–17°C. The juveniles were acclimated to the experimental routine for 10 days and during this period, were fed live adult *Artemia* that were reared on FM, as well as slivers of fresh blue mussel, once a day at 0800 hours. Heavy mortality (total of 25%) was observed during the first 3 days of the acclimation period and was attributed to transport stress, but was thereafter not observed. At the end of the 10-day acclimation period, the lobsters were weighed (Mettler H31, ± 0.1 mg) (0.10 ± 0.03 g) and randomly assigned to one of the 12 experimental diets and the blue mussel diet. Live adult *Artemia*, which was used in acclimation, was no longer available. The 100% SBM dietary treatments had four replicates (in anticipation of poor performance) while each of the other dietary treatments and the blue mussel diet had three replicates.

Juveniles were fed to excess once daily at 0800 hours after water change, and weighed every week or so (days of culture 1, 7, 11, 20, 31, 38, 45, 52 and 60) until the experiment was terminated at day 60. The frequent measurements allowed for close monitoring of growth rate. Juveniles at this stage molt about every 2 weeks. The juveniles were monitored in the dark several times a day for survival, molting and feeding. Because of the difficulty of weighing small amounts of diet, feed conversion ratios were not determined. Specific growth rate (SGR) was expressed as $\mu = \ln(w_1 - w_0)/(t_1 - t_0) \times 100$, where w_1 is final weight (g) at final time t_1, and w_0 is initial weight at initial time t_0. Exuviae were left in the tank for the newly molted juveniles to consume. Moribund lobsters were checked more frequently in a 'death watch' and quickly harvested when response to prodding with a glass rod no longer occurred. At harvest each lobster was placed in a tared vial and quickly frozen by a mixture of acetone and dry ice, freeze-dried at -50°C to constant weight (Mettler AE 240, ± 0.01

mg), pulverized with a glass rod, and stored at −20°C in nitrogen gas until further analyses.

2.3. Proximate analyses

Total protein was determined spectrophotometrically at 725 nm using a commercial Lowry-based, micro-protein determination kit (Sigma Diagnostics, St. Louis, MO). Total carbohydrate was assayed using the phenol–sulfuric acid reaction (absorbance at 470 nm, using glucose as the calibration standard) (Robyt and White, 1990). Spectrophotometric analyses were all performed in duplicate. Crude lipid was determined gravimetrically after extraction with $CHCl_3$–MeOH (Bligh and Dyer, 1959), and later used for fatty acid analysis.

2.4. Amino acid analysis

Tissue samples (2.0 ± 0.5 mg) were spiked with known amounts of norleucine as an internal standard and hydrolyzed in vacuo with 4 N methanesulfonic acid for 22 h at 100°C (Simpson et al., 1976). Dabsyl amino acids were prepared from the hydrolysates (Stocchi et al., 1989) and analyzed by HPLC (Hewlett Packard Series 1050 with automatic sampler) using a Supelcosil LC-DABS™ column (15 cm × 4.6 mm, 3 μm). Standard calibration mixtures were prepared from a commercial amino acid mixture (Standard H, Pierce Chemical, Rockford, IL) and from individual amino acids (Sigma).

2.5. Fatty acid analysis

Dry tissue samples were spiked with known amounts of tricosanoic acid (23:0) for quantitative estimates, and fatty acid methyl esters (FAMEs) were prepared from the lipids with BF_3–MeOH (Morrison and Smith, 1964). The FAMEs were taken up in hexane and assayed by GC–FID (Hewlett Packard 5890A) using a capillary column (Omegawax™ 320; 30 m × 0.32 mm; film thickness, 0.25 μm). Operating parameters were column temperature, 210°C, detector temperature, 250°C, carrier gas He, 30 ml/min, and FID temperature, 250°C. The FAMEs were identified by comparing their relative retention times and equivalent chain lengths with those of authentic standards (Sigma) and cod liver oil FAMEs.

2.6. Statistics

Data for all measured parameters were analyzed using the software package, StatView SE + Graphics (Abacus Concepts, 1988, Berkeley, CA). Comparisons using two-way or one-way ANOVA were performed. Probabilities of $P < 0.05$ were considered significant. When differences were significant, treatment means were compared using Fisher's PLSD test. Treatment means were grouped and compared according to diet type and SBM level when interaction was not significant. When interaction was significant,

treatment means were compared independently of diet type or SBM level. Survival data and duration of molting cycles (expressed in days) were log transformed prior to ANOVA.

3. Results

Results on survival, SGR, maximum body weight gain (BWG), duration of the molt cycle and proximate composition of the juveniles are presented in Table 3.

3.1. Survival

Each juvenile lobster was a single observation; hence, survival was expressed as the number of days a replicate survived during the culture period. Amino acid supplementation or the presence of dietary FM resulted in significantly better survival than that for the 100% SBM diet, which did not contain any FM nor amino acid supplementation. Survival of juveniles fed the supplemented diets across all SBM levels were not significantly different from each other. Of the non-supplemented diets, juveniles fed 100% SBM had significantly lower period of survival than juveniles fed lower levels of dietary SBM. The earliest mortality in the 100% SBM dietary treatment occurred only after 25 days of culture. Results clearly demonstrated the benefit of either amino acid supplementation or the addition of FM in the diet. Except for the 100% SBM level, there were no significant differences in survival between supplementation and non-supplementation of amino acids, for the different dietary levels of SBM.

3.2. SGR and maximum BWG

The growth curves of the juveniles are presented in Fig. 1. A gradual decline in the slope of the growth curve occurred with increasing dietary inclusion of SBM. Slopes of the growth curve were significantly highest for dietary treatments containing not more than 25% SBM with amino acid supplementation, followed by the amino acid-supplemented 50% SBM diet, the non-supplemented 0% and 25% SBM diets (Table 3), and the supplemented 75% SBM diet. Supplementation significantly increased the slope of the growth curve at all SBM inclusion levels. SGRs likewise gradually decreased as levels of dietary SBM increased. Amino acid supplementation significantly improved SGR. Interaction was not significant. Juveniles fed the 100% SBM diet without supplementation exhibited the lowest SGR.

The maximum BWG was chosen for comparison because juveniles were observed to lose some weight prior to molting. Supplementing diets containing no more than 50% SBM with amino acids resulted in significantly higher Max BWG (Table 3). Interaction was significant. For non-supplemented diets, BWG was significantly higher for dietary treatments containing at most 25% SBM. However, with amino acid supplementation, this replacement level was increased to 50% SBM, without significant reduction in the BWG.

Table 3
Mean ± SE ($n = 3, 4$) survival, slope of the growth curve, SGR, maximum body weight gain (Max. BWG), molt cycle and proximate composition of juvenile lobsters fed various inclusion levels of low-fat soybean meal, with (+) and without (−) multiple amino acid supplementation. Data for the blue mussel diet and juveniles at the initial part of the study are shown for comparison

	Diet type: amino acid supplement	Level of SBM as percentage of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-way ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
Bioassay													
Survival (days)	−	60 ± 0^{a}	60 ± 0^{a}	60 ± 0^{a}	52 ± 8^{a}	57 ± 3^{a}	37 ± 5^{b}	53^{b}	58 ± 2	**	**	**	**
	+	60 ± 0^{a}	60 ± 0^{a}	59 ± 1^{a}	57 ± 3^{a}	60 ± 0^{a}	59 ± 1^{a}	59^{a}					
	Mean	60^{a}	60^{a}	60^{a}	55^{ab}	59^{a}	48^{b}						
Slope of growth curve (10^{-2})	−	0.41 ± 0.08^{c}	0.36 ± 0.05^{c}	0.28 ± 0.01^{d}	0.17 ± 0.04^{e}	0.16 ± 0.02^{e}	-0.003 ± 0.04^{f}	0.22^{b}	0.55 ± 0.08		**	**	**
	+	0.74 ± 0.01^{a}	0.68 ± 0.05^{ab}	0.66 ± 0.02^{b}	0.38 ± 0.03^{c}	0.18 ± 0.01^{e}	0.12 ± 0.02^{e}	0.44^{a}					
	Mean	0.57^{a}	0.52^{a}	0.47^{a}	0.27^{b}	0.17^{bc}	0.06^{c}						
SGR (%/day)	−	2.11 ± 0.17	1.83 ± 0.19	1.52 ± 0.01	1.40 ± 0.06	1.26 ± 0.04	0.40 ± 0.14	1.42^{b}	2.54 ± 0.3		**	**	ns
	+	3.09 ± 0.18	2.59 ± 0.23	2.64 ± 0.21	1.98 ± 0.16	1.44 ± 0.09	1.05 ± 0.16	2.08^{a}					
	Mean	2.60^{a}	2.21^{ab}	2.08^{ab}	1.69^{bc}	1.35^{cd}	0.72^{d}						
Max. BWG (%)	−	258.2 ± 38.4^{c}	208.4 ± 35.5^{cd}	148.5 ± 1.0^{e}	109.1 ± 17.4^{fg}	107.9 ± 11.6^{g}	15.4 ± 3.0^{h}	141.2^{b}	330.2 ± 56.9		**	**	*
	+	469.4 ± 12.3^{a}	382.3 ± 70.0^{ab}	408.0 ± 63.8^{ab}	178.2 ± 2.8^{d}	135.3 ± 10.6^{f}	84.7 ± 17.0^{g}	266.2^{a}					
	Mean	363.8^{a}	295.4^{a}	278.2^{a}	143.7^{b}	121.6^{b}	50.1^{b}						
Molt cycle (days)	−	17 ± 1^{abc}	18 ± 1^{bcd}	23 ± 1^{e}	18 ± 1^{bcd}	21 ± 2^{de}	did not molt^{g}	28^{a}	18 ± 1		**	**	**
	+	16 ± 1^{ab}	15 ± 1^{a}	20 ± 2^{cde}	19 ± 2^{bcde}	21 ± 1^{e}	26 ± 2^{f}	20^{b}					
	Mean	16^{a}	17^{a}	21^{a}	18^{a}	21^{a}	26^{b}						
Proximate composition (% of DW)													
Crude protein	−	63.1 ± 1.7	67.4 ± 1.1	61.4 ± 2.8	57.8 ± 0.6	53.2 ± 2.4	51.3 ± 2.2	59.1^{b}	58.3 ± 1.7	57.7 ± 1.1	**	**	ns
	+	70.0 ± 1.4	67.0 ± 2.6	66.1 ± 2.5	58.2 ± 2.0	57.8 ± 1.9	60.0 ± 3.2	63.0^{a}					
	Mean	66.5^{a}	67.2^{a}	63.8^{a}	58.0^{b}	55.5^{b}	56.3^{b}						
Carbohydrate	−	8.1 ± 0.5	7.4 ± 0.5	7.5 ± 0.3	7.0 ± 1.0	7.8 ± 0.2	6.7 ± 0.6	7.4^{a}	6.7 ± 0.5	8.2 ± 0.2	**	ns	ns
	+	6.5 ± 0.7	6.02 ± 0.7	5.7 ± 0.6	6.0 ± 0.4	5.0 ± 0.5	7.7 ± 0.7	6.2^{b}					
	Mean	7.3	6.7	6.6	6.5	6.4	7.3						
Crude lipid	−	4.4 ± 0.4^{e}	5.4 ± 0.5^{cd}	7.5 ± 0.3^{a}	6.2 ± 0.3^{bc}	8.0 ± 0.6^{a}	6.4 ± 0.4^{b}	6.3^{a}	4.6 ± 0.8	4.4 ± 0.1	**	**	**
	+	6.0 ± 0.2^{bc}	4.9 ± 0.4^{de}	5.1 ± 0.2^{de}	4.7 ± 0.7^{de}	5.9 ± 0.1^{bcd}	5.7 ± 0.4^{bcd}	5.4^{b}					
	Mean	5.2^{b}	5.1^{b}	6.3^{ab}	5.4^{b}	6.9^{a}	6.0^{ab}						

Treatment means with the same letter in the same row are not significantly different from each other ($P > 0.05$). ns, $P > 0.05$.
* $P < 0.05$.
** $P < 0.01$.

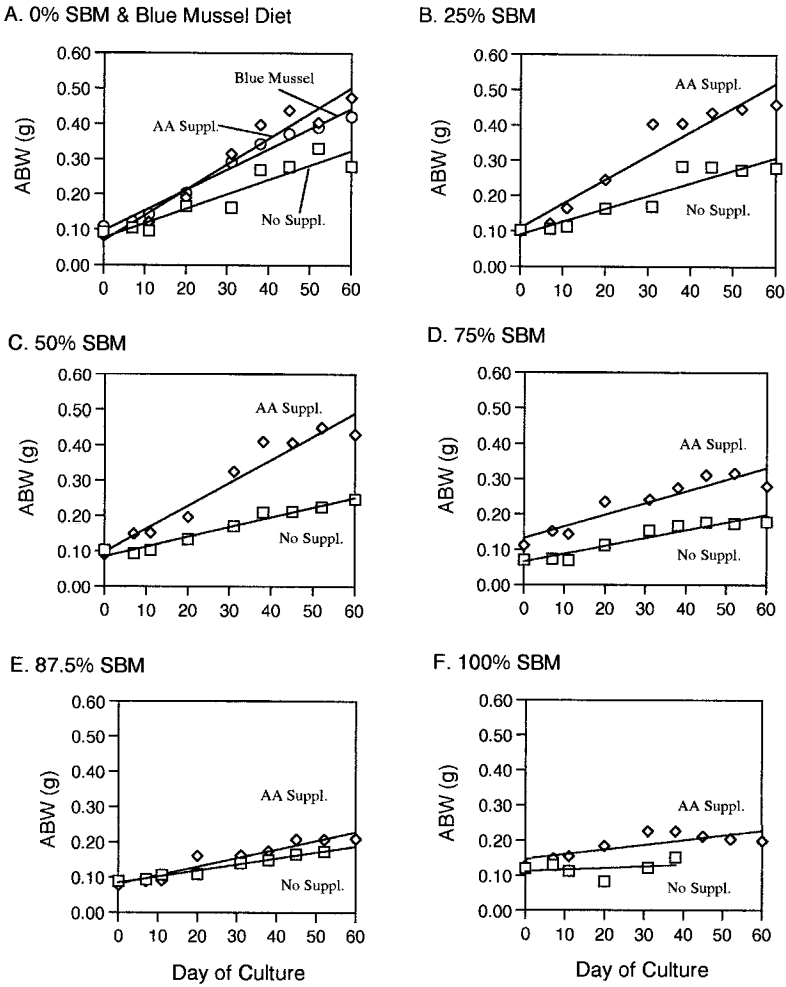

Fig. 1. Growth curves (mean, $n = 3$ or 4) of Stage 5 juvenile lobsters fed diets containing various proportions of low-fat soybean meal, with and without multiple amino acid supplementation.

3.3. Molting cycle

Juveniles, on the average, completed two to three molting cycles for the duration of the study. Molting cycles were significantly shorter in juveniles fed diets containing at least some amount of FM, compared to diets containing only SBM as the protein source (Table 3). Amino acid supplementation significantly shortened the molting cycle. Interaction was significant. Juveniles fed the 100% SBM diet with amino acid supplementation had the longest molting cycle, but juveniles on the non-supplemented counterpart diet did not molt at all and suffered early mortality. Therefore, the amino acid supplementation in the 100% SBM diet was crucial to effect molting, but

insufficient to reduce the length of the molting cycle to those of juveniles fed diets containing FM or the blue mussel diet.

3.4. Proximate composition

Amino acid supplementation significantly increased total protein content (Table 3). Juveniles fed diets containing not more than 50% SBM were composed of significantly higher total protein than those fed diets containing higher levels of SBM. Interaction was not significant. Total carbohydrate levels were significantly higher in juveniles fed the non-supplemented diets and were not significantly affected by the level of dietary SBM. Crude lipid was significantly higher in juveniles fed the non-supplemented diets. Although interaction was significant, there was no trend between increasing dietary SBM levels and carcass lipid levels of juveniles.

3.5. Amino acid profiles

The amino acid profiles (% of total protein) of the juveniles fed the different diets are presented in Tables 4 and 5. The major amino acids were the EAAs (Table 4) arginine and leucine, and the non-essential amino acids (NEAAs, Table 5) glycine, glutamate, aspartate, and proline. The dietary proportion of SBM, and the supplementation of diets with the four EAAs significantly affected the levels of several amino acids in lobster protein. Amino acid supplementation significantly increased the ratios of the EAAs arginine, phenylalanine and tryptophan, and significantly lowered the ratios of the EAA tyrosine, and the NEAAs aspartate, glutamate and serine. The levels of arginine were especially pronounced in juveniles fed the supplemented diets, nearly twice that in juveniles fed the non-supplemented counterpart diets (Table 4). The other two EAAs that were supplemented, leucine and methionine, did not produce significant differences in juvenile carcass relative to dietary SBM level or amino acid supplementation. None of the interaction effects was significant.

3.6. Fatty acid profiles

Dietary fatty acid profiles (% of total fatty acids) of the lobsters are presented in Tables 6 (saturated fatty acids), 7 (monounsaturated fatty acids) and 8 (polyunsaturated fatty acids, PUFA). Amino acid supplementation significantly reduced the proportion of the saturated fatty acids 17:0, 20:0 and 22:0 (Table 6); increased the proportion of the monounsaturated fatty acid $18{:}1n-7$ (Table 7); and increased the proportions of the PUFAs $20{:}4n-6$, $20{:}5n-3$ and $22{:}6n-3$ (Table 8). Total fatty acids (mg/g dry tissue, Table 6) of juvenile carcass from each of the dietary treatments were not significantly different from each other. The proportions of total PUFA, total $n-3$, and the $n-3/n-6$ ratio were significantly higher in juveniles fed the amino acid supplemented diets (Table 8). None of the interaction effects was significant.

The proportions of $16{:}1n-7$ and $20{:}1n-9$ were significantly higher in juveniles fed the 0% SBM diets than in juveniles fed diets containing higher levels of SBM (Table 7). Although results for $18{:}1n-7$ were significant, no correlation could be found

Table 4
The EAA profile (% of total protein) of juvenile lobster fed diets containing various inclusion levels of low-fat soybean meal

	Diety type: amino acid supplement	Level of SBM as percentage of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-way ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
Arginine	−	5.36 ± 0.49	6.28 ± 1.05	5.37 ± 0.74	5.70 ± 0.74	7.12 ± 0.72	6.93 ± 0.89	6.17[b]	9.91 ± 1.14	10.77 ± 0.04	**	ns	ns
	+	9.78 ± 0.53	11.80 ± 0.99	11.50 ± 1.13	10.46 ± 1.43	11.23 ± 1.63	10.78 ± 0.82	10.92[a]					
	Mean	7.57	9.04	8.44	8.08	9.18	8.86						
Histidine	−	5.90 ± 0.91	4.68 ± 0.72	6.03 ± 1.52	6.41 ± 0.61	5.63 ± 1.59	5.21 ± 0.69	5.62	4.35 ± 0.66	6.84 ± 0.40	ns	ns	ns
	+	5.71 ± 0.55	5.06 ± 1.39	4.56 ± 0.74	4.19 ± 0.53	4.74 ± 0.16	5.80 ± 0.53	5.05					
	Mean	5.81	4.87	5.29	5.30	5.18	5.50						
Isoleucine	−	2.69 ± 0.39	2.97 ± 0.39	3.71 ± 1.22	3.91 ± 0.36	2.09 ± 0.33	3.93 ± 1.13	3.25	3.36 ± 0.47	3.05 ± 0.14	ns	ns	ns
	+	2.24 ± 0.29	3.27 ± 0.44	2.80 ± 0.13	3.70 ± 0.78	2.49 ± 0.4	3.13 ± 0.88	2.95					
	Mean	2.46	3.12	3.25	3.81	2.29	3.53						
Leucine	−	7.27 ± 0.15	7.87 ± 0.26	7.44 ± 0.53	7.86 ± 0.16	7.32 ± 0.3	7.81 ± 0.31	7.61	8.36 ± 0.36	7.90 ± 0.07	ns	ns	ns
	+	7.24 ± 0.29	8.86 ± 0.64	8.71 ± 0.72	8.31 ± 0.55	8.39 ± 1.4	7.81 ± 0.45	8.20					
	Mean	7.26	8.37	8.07	8.09	7.85	7.81						
Lysine	−	5.08 ± 0.47	4.27 ± 0.61	4.40 ± 1.33	5.35 ± 0.57	4.16 ± 0.2	4.65 ± 0.17	4.65	3.28 ± 0.34	6.03 ± 0.03	ns	ns	ns
	+	5.23 ± 0.51	3.60 ± 0.46	2.84 ± 0.99	4.01 ± 0.34	4.03 ± 0.5	4.47 ± 0.66	4.05					
	Mean	5.15	3.94	3.62	4.68	4.10	4.56						
Methionine	−	2.97 ± 0.18	2.92 ± 0.07	2.89 ± 0.14	3.18 ± 0.30	2.71 ± 0.5	2.91 ± 0.35	2.93	3.46 ± 0.28	2.77 ± 0.08	ns	ns	ns
	+	3.46 ± 0.44	2.88 ± 0.16	3.04 ± 0.26	2.52 ± 0.17	2.73 ± 0.2	2.89 ± 0.33						
	Mean	3.22	2.90	2.96	2.85	2.72	2.90						

Cystine	−	1.01 ± 0.13	1.09 ± 0.27	1.40 ± 0.40	1.01 ± 0.14	0.76 ± 0	0.97 ± 0.08	1.04	1.14 ± 0.22	1.22 ± 0.01	ns	ns	ns
	+	1.37 ± 0.26	0.75 ± 0.08	1.20 ± 0.32	0.71 ± 0.05	1.05 ± 0.1	1.21 ± 0.15	1.05					
	Mean	1.19	0.92	1.30	0.86	0.90	1.09						
Phenylalanine	−	4.57 ± 0.15	5.25 ± 0.48	4.95 ± 0.38	5.28 ± 0.20	4.91 ± 0.37	5.42 ± 0.56	5.08[b]	6.06 ± 0.31	4.85 ± 0.09	*	ns	ns
	+	5.33 ± 0.22	5.60 ± 0.15	6.00 ± 0.45	5.46 ± 0.22	5.88 ± 0.45	5.13 ± 0.48	5.55[a]					
	Mean	4.95	5.43	5.47	5.37	5.40	5.28						
Tyrosine	−	3.75 ± 0.19	2.86 ± 0.32	4.33 ± 0.63	3.70 ± 0.32	2.33 ± 0.40	3.38 ± 0.21	3.39[a]	1.61 ± 0.18	4.17 ± 0.09	**	ns	ns
	+	2.50 ± 0.83	1.36 ± 0.23	2.02 ± 0.60	2.30 ± 0.65	1.80 ± 0.44	2.63 ± 0.58	2.13[b]					
	Mean	3.12	2.11	3.17	3.00	2.06	3.00						
Tryptophan	−	0.32 ± 0.02	0.57 ± 0.15	0.43 ± 0.10	0.41 ± 0.03	0.40 ± 0.02	0.44 ± 0.04	0.43[b]	0.74 ± 0.06	0.41 ± 0.02	**	ns	ns
	+	0.53 ± 0.08	0.64 ± 0.09	0.60 ± 0.09	0.54 ± 0.03	0.50 ± 0.06	0.51 ± 0.07	0.55[a]					
	Mean	0.43	0.60	0.52	0.46	0.44	0.47						
Threonine	−	5.41 ± 0.26	5.63 ± 0.99	4.44 ± 1.30	6.25 ± 0.64	5.46 ± 0.50	5.52 ± 0.34	5.45	4.91 ± 0.21	4.46 ± 0.15	ns	ns	ns
	+	4.96 ± 0.30	4.99 ± 0.23	5.17 ± 0.36	5.03 ± 0.32	4.97 ± 0.49	4.34 ± 0.07	4.88					
	Mean	5.18	5.31	4.81	5.64	5.21	4.93						
Valine	−	3.60 ± 0.51	3.52 ± 0.44	3.48 ± 0.35	4.22 ± 0.59	2.67 ± 0.14	4.01 ± 0.38	3.61	3.73 ± 0.47	3.56 ± 0.03	ns	ns	ns
	+	3.17 ± 0.48	4.00 ± 0.31	2.95 ± 0.33	3.88 ± 0.51	3.23 ± 0.37	3.33 ± 0.34	3.42					
	Mean	3.39	3.76	3.21	4.05	2.95	3.67						
Total EAA	−	46.70 ± 2.21	47.91 ± 2.44	47.18 ± 3.34	53.29 ± 1.80	45.55 ± 1.85	51.19 ± 2.37	48.77	50.92 ± 2.09	56.03 ± 0.33	ns	ns	ns
	+	51.52 ± 2.24	52.59 ± 1.95	46.85 ± 5.17	46.88 ± 4.73	50.88 ± 1.70	52.01 ± 2.01	50.22					
	Mean	49.11	50.25	47.02	50.09	48.21	51.60						

Explanations are the same as in Table 3.

* $P < 0.05$.

** $P < 0.01$.

Table 5
The NEAA profile (% of total protein) of juvenile lobsters fed diets containing various inclusion levels of low-fat soybean meal

	Diet type: amino acid supplement	Level of SBM as percentage of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-way ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
Aspartate[#]	−	8.30 ± 0.30	7.74 ± 1.08	7.85 ± 0.84	7.73 ± 0.41	8.13 ± 0.79	7.97 ± 0.56	7.95[a]	4.04 ± 0.45	6.34 ± 0.10	**	ns	ns
	+	6.62 ± 0.66	5.01 ± 0.58	6.14 ± 0.56	6.37 ± 0.25	5.40 ± 0.71	6.79 ± 0.58	6.10[b]					
	Mean	7.46	6.38	6.99	7.05	6.77	7.38						
Glutamate[£]	−	10.14 ± 0.37	9.47 ± 1.32	11.86 ± 1.85	9.45 ± 0.50	9.94 ± 0.96	9.74 ± 0.69	10.08[a]	6.05 ± 0.47	8.41 ± 0.17	**	ns	ns
	+	8.10 ± 0.81	6.13 ± 0.72	7.50 ± 0.68	7.80 ± 0.30	6.60 ± 0.87	8.30 ± 0.70	7.45[b]					
	Mean	9.12	7.80	9.68	8.62	8.27	9.02						
Serine	−	5.32 ± 0.53	5.65 ± 1.01	4.37 ± 1.35	5.59 ± 0.35	6.18 ± 0.53	5.67 ± 0.52	5.47[a]	4.03 ± 0.20	3.64 ± 0.04	**	ns	ns
	+	4.96 ± 0.05	4.08 ± 0.34	4.62 ± 0.45	3.76 ± 0.42	4.36 ± 0.18	4.27 ± 0.55	4.34[b]					
	Mean	5.14	4.87	4.50	4.68	5.27	4.97						
Glycine	−	15.06 ± 0.57	14.20 ± 1.65	12.45 ± 1.20	11.77 ± 1.39	16.79 ± 2.64	12.40 ± 0.20	13.78	13.43 ± 1.46	9.08 ± 0.05	ns	ns	ns
	+	29.57 ± 1.42	15.56 ± 1.15	15.30 ± 2.24	16.89 ± 1.08	16.65 ± 3.37	14.56 ± 1.06	17.90					
	Mean	22.31	14.88	13.87	14.73	16.72	13.63						
Alanine	−	6.83 ± 0.69	5.96 ± 1.14	5.78 ± 1.30	6.63 ± 1.18	7.62 ± 0.13	6.31 ± 0.51	6.51	6.39 ± 0.18	5.40 ± 0.12	ns	ns	ns
	+	6.26 ± 0.62	5.98 ± 0.35	5.32 ± 1.03	4.92 ± 0.62	6.32 ± 0.19	5.60 ± 0.29	5.73					
	Mean	6.55	5.97	5.55	5.78	6.97	5.96						
Proline	−	4.97 ± 0.25	9.08 ± 3.56	11.12 ± 4.97	5.54 ± 0.58	5.77 ± 1.27	9.49 ± 2.53	7.76	15.13 ± 2.51	11.10 ± 0.07	ns	ns	ns
	+	8.44 ± 1.74	10.64 ± 2.05	9.71 ± 1.81	9.35 ± 1.59	9.79 ± 0.14	8.07 ± 1.31	9.27					
	Mean	6.71	9.86	10.42	7.45	7.78	8.78						
Total NEAA	−	53.30 ± 2.21	52.09 ± 2.44	52.82 ± 3.34	46.71 ± 1.80	54.45 ± 1.85	48.81 ± 2.37	51.23	49.07 ± 2.09	43.99 ± 0.33	ns	ns	ns
	+	48.48 ± 2.24	47.41 ± 1.95	53.15 ± 5.17	53.12 ± 4.73	49.12 ± 1.70	47.99 ± 2.01	49.78					
	Mean	50.89	49.75	52.98	49.91	51.79	48.40						

Explanations are the same as in Table 3.
[#]Aspartic acid and asparagine.
** $P < 0.01$.
[£]Glutamic acid and glutamine.

Table 6
The total fatty acids (mg/g DW) and saturated fatty acid profile (% of total fatty acids) of juvenile lobsters fed diets containing various inclusion levels of low-fat soybean meal

	Diet type: amino acid supplement	Level of SBM as percentage of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-ay ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
Σ Fatty acids (mg/g DW)	−	19.84 ± 1.91	20.93 ± 1.45	27.06 ± 3.00	25.36 ± 1.99	22.92 ± 2.93	19.48 ± 1.85	22.43	24.77 ± 2.11	31.30 ± 0.85	ns	ns	ns
	+	25.69 ± 2.84	20.61 ± 1.48	21.18 ± 1.52	19.82 ± 1.22	22.05 ± 1.69	20.20 ± 2.19	21.52					
	Mean	22.76	20.77	24.12	22.59	22.49	19.84						
Fatty acid													
14:0	−	0.60 ± 0.04	0.53 ± 0.03	0.55 ± 0.12	0.61 ± 0.03	0.58 ± 0.04	0.66 ± 0.15	0.59	0.95 ± 0.17	0.91 ± 0.03	ns	ns	ns
	+	0.96 ± 0.08	0.65 ± 0.17	0.52 ± 0.21	0.40 ± 0.04	0.42 ± 0.02	0.41 ± 0.03	0.55					
	Mean	0.78	0.59	0.54	0.50	0.50	0.53						
15:0	−	1.27 ± 0.90	0.29 ± 0.07	0.38 ± 0.03	0.28 ± 0.04	0.31 ± 0.02	0.49 ± 0.15	0.50	0.54 ± 0.08	0.68 ± 0.01	ns	ns	ns
	+	0.36 ± 0.01	0.34 ± 0.07	0.21 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	0.27					
	Mean	0.81	0.32	0.29	0.25	0.27	0.36						
16:0	−	15.19 ± 1.43	15.05 ± 1.45	14.94 ± 1.35	15.21 ± 0.16	15.14 ± 1.08	15.82 ± 0.76	15.26	13.56 ± 0.33	14.01 ± 0.20	ns	ns	ns
	+	17.99 ± 0.40	16.85 ± 0.75	15.20 ± 0.49	15.86 ± 0.32	15.99 ± 0.16	15.08 ± 0.33	16.10					
	Mean	16.59	15.95	15.07	15.53	15.56	15.45						
17:0	−	0.76 ± 0.17	1.29 ± 0.62	0.99 ± 0.36	0.61 ± 0.01	0.91 ± 0.24	0.69 ± 0.08	0.87[a]	0.85 ± 0.09	0.72 ± 0.01	*	ns	ns
	+	0.64 ± 0.01	0.71 ± 0.06	0.54 ± 0.10	0.62 ± 0.02	0.61 ± 0.01	0.66 ± 0.05	0.63[b]					
	Mean	0.70	1.00	0.76	0.62	0.76	0.68						
18:0	−	5.95 ± 0.69	6.21 ± 0.86	6.51 ± 0.61	6.82 ± 0.79	6.66 ± 0.57	6.23 ± 0.96	6.39	6.26 ± 0.35	5.08 ± 0.05	ns	ns	ns
	+	5.86 ± 0.84	6.66 ± 0.13	5.95 ± 0.77	6.95 ± 0.14	6.94 ± 0.10	7.10 ± 0.36	6.61					
	Mean	5.91	6.44	6.23	6.89	6.80	6.67						
20:0	−	0.47 ± 0.06	0.46 ± 0.09	0.50 ± 0.02	0.50 ± 0.05	0.69 ± 0.08	0.53 ± 0.15	0.52[a]	0.28 ± 0.02	0.35 ± 0.01	**	ns	ns
	+	0.37 ± 0.06	0.45 ± 0.16	0.32 ± 0.01	0.29 ± 0.02	0.41 ± 0.03	0.37 ± 0.04	0.37[b]					
	Mean	0.42	0.46	0.41	0.42	0.52	0.45						
22:0	−	1.10 ± 0.11	0.98 ± 0.17	0.87 ± 0.38	1.01 ± 0.09	1.70 ± 0.08	1.25 ± 0.43	1.16[a]	0.42 ± 0.02	0.45 ± 0.01	**	ns	ns
	+	0.59 ± 0.17	0.83 ± 0.32	0.52 ± 0.08	0.64 ± 0.13	0.85 ± 0.03	0.90 ± 0.14	0.73[b]					
	Mean	0.84	0.90	0.69	0.84	1.27	1.08						
Σ Saturates	−	25.69 ± 1.07	25.43 ± 1.27	25.11 ± 0.98	25.37 ± 0.97	26.15 ± 1.13	25.93 ± 2.24	25.63	23.36 ± 0.82	22.60 ± 0.31	ns	ns	ns
	+	27.49 ± 0.64	26.54 ± 1.55	23.38 ± 1.10	24.90 ± 0.40	25.61 ± 0.32	24.84 ± 0.77	25.43					
	Mean	26.32	25.99	24.24	25.14	25.88	25.38						

Explanations are the same as in Table 3.
* $P < 0.05$.
** $P < 0.01$.

Table 7
The monounsaturated fatty acid profile (% of total fatty acids) of juvenile lobsters fed diets containing various inclusion levels of low-fat soybean meal

	Diet type: amino acid supplement	Level of SBM as percentage of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-way ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
16:1$n-7$	−	3.19 ± 0.48	2.23 ± 0.29	2.56 ± 0.36	2.84 ± 0.15	1.90 ± 0.19	2.70 ± 0.82	2.58	6.23 ± 0.51	6.68 ± 0.16	ns	*	ns
	+	4.07 ± 0.14	2.82 ± 0.51	2.20 ± 0.58	2.56 ± 0.31	2.20 ± 0.14	1.96 ± 0.17	2.60					
	Mean	3.63[a]	2.53[b]	2.34[b]	2.70[b]	2.05[b]	2.33[b]						
17:1	−	1.02 ± 0.17	1.44 ± 0.55	1.26 ± 0.76	0.98 ± 0.05	1.15 ± 0.39	0.59 ± 0.07	1.05	1.44 ± 0.13	1.02 ± 0.20	ns	ns	ns
	+	0.86 ± 0.12	1.20 ± 0.11	0.67 ± 0.22	0.90 ± 0.20	0.94 ± 0.25	0.72 ± 0.19	0.87					
	Mean	0.94	1.32	0.97	0.94	1.05	0.66						
18:1$n-9$	−	6.97 ± 0.60	5.98 ± 0.86	6.02 ± 0.79	7.14 ± 1.27	5.95 ± 0.47	9.81 ± 2.49	7.13	5.16 ± 0.19	12.25 ± 0.08	ns	ns	ns
	+	7.79 ± 0.41	7.81 ± 0.90	8.83 ± 2.32	7.02 ± 0.41	7.29 ± 0.55	6.44 ± 0.27	7.47					
	Mean	7.38	6.89	7.43	7.08	6.62	8.13						
18:1$n-7$	−	5.25 ± 0.48	3.75 ± 0.37	3.96 ± 0.94	4.72 ± 0.44	3.72 ± 0.26	6.01 ± 0.84	4.65[b]	6.23 ± 0.39	8.21 ± 0.05	*	*	ns
	+	6.24 ± 0.59	5.27 ± 0.89	4.58 ± 0.24	5.45 ± 0.52	4.39 ± 0.47	5.58 ± 0.27	5.27[a]					
	Mean	5.75[a]	4.51[ab]	4.27[b]	5.09[a]	4.06[b]	5.80[a]						
20:1$n-9$	−	1.16 ± 0.04	1.01 ± 0.01	0.76 ± 0.11	0.70 ± 0.12	0.56 ± 0.01	0.83 ± 0.13	0.83	2.07 ± 0.06	2.20 ± 0.02	ns	**	ns
	+	1.51 ± 0.09	0.86 ± 0.10	1.09 ± 0.30	0.78 ± 0.11	0.82 ± 0.02	0.43 ± −	0.99					
	Mean	1.34[a]	0.92[b]	0.92[b]	0.73[b]	0.69[b]	0.75[b]						
Σ Monoenes	−	19.34 ± 0.17	14.85 ± 1.06	15.14 ± 1.57	15.40 ± 1.09	16.11 ± 1.91	20.36 ± 2.16	17.22	21.13 ± 1.55	31.19 ± 1.13	ns	ns	ns
	+	20.84 ± 0.17	18.20 ± 1.71	14.14 ± 0.03	16.79 ± 0.52	15.77 ± 0.90	15.50 ± 0.60	16.95					
	Mean	20.24	16.86	14.74	16.23	15.91	17.93						

Explanations are the same as in Table 3.
* $P < 0.05$.
** $P < 0.01$.

between the level of 18:1$n-7$ in juvenile carcass and level of dietary SBM. Increasing levels of dietary SBM gradually increased the proportion of linoleic acid, 18:2$n-6$, in lobster carcass ($r^2 = 0.91$, $P < 0.01$, Table 8). This fatty acid is the major PUFA in SBM. Juveniles fed 100% SBM diets contained levels of 18:2$n-6$ that were up to 2.6 times higher than those of juveniles fed the 0% SBM diets. Lobsters fed the 87.5% and 100% SBM diets had levels of 20:5$n-3$ that were significantly lower than that of lobsters fed diets containing no SBM. The relative proportions of 22:6$n-3$ in lobsters fed the 0% and 25% SBM diets were significantly higher than in juveniles fed the 87.5% and 100% SBM diets. Juveniles fed the 100% SBM diets had the lowest levels of 22:6$n-3$. The dietary level of SBM inversely correlated with the levels of 20:5$n-3$ ($r^2 = 0.91$, $P < 0.01$) and 22:6$n-3$ ($r^2 = 0.88$, $P < 0.05$) in the lobster tissue. These fatty acids are also the major PUFAs in FM. Total $n-3$ levels were significantly higher in juveniles fed the 0% and 25% SBM diets than those of juveniles fed the other dietary SBM levels. Total $n-6$ levels were significantly higher in juveniles fed the diets containing at least 50% SBM as dietary protein, followed by the 25% and the 0% SBM diets. Juveniles fed the 0% SBM diet had a significantly higher total $n-3/n-6$ ratio, followed by those on the 25% SBM diet and those on diets containing higher proportions of SBM. The tissues of juveniles fed the blue mussel diet had total $n-3$ levels that were comparable to those of juveniles fed the 0% SBM diet. However, due to a significantly lower total $n-6$ level, juveniles fed this natural diet had a significantly higher total $n-3/n-6$ ratio than that of juveniles fed the experimental diets.

4. Discussion

Crustacean nutrition studies typically use at least 15 animals per treatment. However, statistically significant results were obtained in this study in spite of only three or four replicates being used per dietary treatment. This was attributed to the high level of control in the experiment made possible with: the use of hatchery-reared, sibling juveniles; the rearing and chemical analyses of each juvenile as a single observation (instead of pooling several animals together); and the use of well-acclimated, healthy animals for the experiment. The factorial experimental design also allowed statistical evaluation of 19 animals per dietary type (with or without amino acid supplementation) and six or eight animals per dietary SBM level, when interaction was not significant.

Results showed that juvenile lobsters could utilize low-fat SBM without significant reduction in weight gain, as long as dietary levels did not exceed 50% SBM as dietary protein (42% of the diet), and there was amino acid supplementation. This level is higher than that found for *Penaeus vannamei* (28% of the diet, Lim and Dominy, 1990) and for *Penaeus japonicus* (14% soybean protein or 35% of the diet, Kanazawa, 1992), but closely agrees with results of other workers for *Penaeus monodon* (50% of the diet, Akiyama, 1990; 45% of the diet, Che, 1992). Higher dietary levels were associated with lower survival, lower BWG and a longer duration of the molting cycle.

Amino acid balance, achieved through the addition of dietary FM (minimum of 12.5% as dietary protein, in this study) or multiple supplementation (arginine, leucine, methionine and tryptophan), was necessary for molting. However, only an inclusion of

Table 8
The PUFA profile (% of total fatty acids) of juvenile lobsters fed diets containing various inclusion levels of low-fat soybean meal

	Diet type: amino acid supplement	Level of SBM as % of dietary protein						Diet type mean	Blue mussel diet	Initial	Two-way ANOVA		
		0	25	50	75	87.5	100				Diet type	SBM level	Intxn.
18:2$n-6$	−	7.82 ± 1.68	11.07 ± 3.33	14.09 ± 2.85	18.26 ± 1.51	15.98 ± 0.85	17.75 ± 3.43	14.35	1.96 ± 0.22	4.66 ± 0.04	ns	**	ns
	+	7.68 ± 0.90	12.95 ± 1.57	18.21 ± 0.19	16.18 ± 1.57	17.44 ± 0.97	19.84 ± 1.64	15.62					
	Mean	7.75^{c}	12.01^{b}	16.15^{ab}	17.22^{a}	16.71^{a}	18.80^{a}						
18:3$n-3$	−	2.72 ± 1.27	2.43 ± 0.37	2.32 ± 0.58	2.63 ± 0.26	2.46 ± 0.17	4.20 ± 0.83	2.87	0.97 ± 0.09	4.41 ± 0.04	ns	ns	ns
	+	1.52 ± 0.16	2.03 ± 0.14	2.68 ± 0.26	2.31 ± 0.09	2.50 ± 0.35	2.92 ± 0.26	2.36					
	Mean	2.12	2.23	2.50	2.47	2.48	3.56						
18:4$n-3$	−	0.43 ± 0.05	0.33 ± 0.10	0.35 ± 0.05	0.34 ± 0.03	0.62 ± 0.18	0.54 ± 0.05	0.42	0.50 ± 0.16	0.42 ± 0.02	ns	ns	ns
	+	0.38 ± 0.02	0.48 ± 0.24	0.17 ± 0.01	0.19 ± −	0.24 ± −	1.35 ± 1.12	0.58					
	Mean	0.40	0.39	0.28	0.30	0.49	1.03						
20:2$n-6$	−	1.88 ± 0.07	4.13 ± 0.52	3.90 ± 1.38	3.65 ± 0.42	2.52 ± 1.43	2.75 ± 0.47	3.17	1.05 ± 0.07	0.91 ± 0.03	ns	*	ns
	+	1.86 ± 0.03	2.71 ± 0.42	3.57 ± 0.25	4.04 ± 0.31	4.52 ± 0.33	3.62 ± 0.24	3.40					
	Mean	1.87^{b}	3.28^{a}	3.73^{a}	3.85^{a}	3.72^{a}	3.19^{a}						
20:4$n-6$	−	3.50 ± 0.39	2.93 ± 0.38	2.64 ± 0.44	2.54 ± 0.27	2.31 ± 0.26	2.48 ± 0.33	2.72^{b}	4.80 ± 0.08	3.49 ± 0.03	**	ns	ns
	+	3.65 ± 0.16	3.29 ± 0.39	2.60 ± 0.63	3.46 ± 0.28	3.25 ± 0.12	3.26 ± 0.10	3.26^{a}					
	Mean	3.58	3.11	2.65	3.00	2.78	2.87						
20:3$n-3$	−	0.45 ± 0.07	0.68 ± 0.23	0.47 ± 0.08	0.42 ± 0.10	0.44 ± 0.06	0.57 ± 0.05	0.51	0.25 ± 0.01	0.57 ± 0.01	ns	ns	ns
	+	0.39 ± 0.03	0.42 ± 0.02	0.59 ± 0.04	0.50 ± 0.01	0.61 ± 0.02	0.50 ± 0.03	0.50					
	Mean	0.42	0.55	0.53	0.45	0.52	0.53						

20:5$n-3$	−	16.40 ± 1.47	13.64 ± 2.68	11.69 ± 1.99	11.75 ± 1.50	10.11 ± 1.48	10.01 ± 0.46	12.27^{b}	19.66 ± 0.66	10.76 ± 0.10	**	*	ns
	+	16.61 ± 0.13	15.03 ± 2.16	16.32 ± 0.11	15.39 ± 1.09	14.76 ± 1.00	12.81 ± 0.75	14.96^{a}					
	Mean	16.51^{a}	14.33^{ab}	13.54^{ab}	13.57^{ab}	12.43^{b}	11.61^{b}						
22:5$n-3$	−	0.79 ± 0.19	1.38 ± 0.67	1.72 ± −	0.54 ± 0.03	0.91 ± 0.03	0.47 ± 0.09	0.89	0.90 ± 0.03	0.90 ± 0.02	ns	ns	ns
	+	0.87 ± 0.09	0.80 ± −	0.67 ± −	0.59 ± −	0.51 ± −	0.42 ± 0.08	0.67					
	Mean	0.83	1.19	1.20	0.56	0.78	0.44						
22:6$n-3$	−	11.38 ± 0.92	12.58 ± 0.18	8.71 ± 1.62	8.45 ± 1.02	7.49 ± 0.72	6.18 ± 0.22	8.97^{b}	11.71 ± 0.57	8.57 ± 0.71	**	**	ns
	+	13.01 ± 0.80	10.94 ± 1.97	10.54 ± 2.16	11.22 ± 0.19	10.72 ± 1.09	8.44 ± 0.50	10.69^{a}					
	Mean	12.19^{a}	11.76^{ab}	9.63^{bcd}	9.83^{abc}	9.10^{cd}	7.31^{d}						
Σ PUFA	−	46.90 ± 0.12	47.16 ± 4.17	48.75 ± 5.18	50.89 ± 1.79	45.04 ± 1.38	45.54 ± 3.97	47.46^{b}	45.88 ± 2.94	37.33 ± 0.44	**	ns	ns
	+	47.16 ± 1.32	50.03 ± 5.19	55.93 ± 2.01	54.38 ± 0.51	55.29 ± 0.85	53.75 ± 1.01	52.81^{a}					
	Mean	47.06	48.88	52.34	52.64	51.19	49.65						
Σ $n-3$	−	32.00 ± 1.09	28.51 ± 4.03	24.98 ± 3.12	24.68 ± 2.20	22.39 ± 1.79	21.02 ± 0.61	25.36^{b}	34.76 ± 1.21	26.21 ± 0.60	**	**	ns
	+	33.60 ± 0.71	29.87 ± 3.61	27.86 ± 5.17	30.27 ± 1.19	29.34 ± 1.63	25.97 ± 1.00	29.30^{a}					
	Mean	32.80^{a}	29.19^{ab}	26.42^{bc}	27.48^{bc}	25.87^{bc}	23.50^{c}						
Σ $n-6$	−	12.90 ± 0.14	22.08 ± 1.42	22.28 ± 3.31	24.27 ± 1.98	22.03 ± 0.90	23.88 ± 3.55	21.66	9.51 ± 0.53	10.89 ± 0.30	ns	**	ns
	+	13.34 ± 0.78	19.22 ± 2.22	24.86 ± 0.32	23.88 ± 1.51	25.45 ± 0.80	27.12 ± 1.41	22.44					
	Mean	13.17^{c}	20.36^{b}	23.31^{a}	24.04^{a}	24.08^{ab}	25.50^{a}						
Σ $n-3/n-6$	−	2.56 ± 0.02	1.48 ± 0.08	1.13 ± 0.08	1.11 ± 0.05	0.95 ± 0.04	0.94 ± 0.13	1.29^{b}	4.58 ± 0.30	30.3 ± 0.16	*	**	ns
	+	2.53 ± 0.11	1.55 ± 0.01	1.33 ± 0.04	1.28 ± 0.12	1.16 ± 0.10	0.97 ± 0.08	1.45^{a}					
	Mean	2.54^{a}	1.52^{b}	1.21^{c}	1.21^{c}	1.07^{c}	0.95^{c}						

Explanations are the same as in Table 3.

* $P < 0.05$.

** $P < 0.01$.

FM, regardless of supplementation, resulted in molting cycles comparable to those of juveniles fed lower SBM levels ($\leq 75\%$) or fresh blue mussel. This observation implies that other nutrients in FM may be necessary for normal molting, possibly the essential fatty acids or EFAs of the $n-3$ series. Sheen and Wu (1999) reported protracted molting cycles in juvenile mud crab *Scylla serrata* fed diets deficient in $n-3$ and $n-6$ highly unsaturated fatty acids or HUFAs. Kanazawa et al. (1985) reported that 0.5–1.0% of dietary $n-3$ HUFAs produced the best survival and growth in larval shrimp, *P. japonicus*, while D'Abramo (1997) noted that EFA requirement of crustaceans generally does not exceed 1% of the diet. Our results establish the importance of a high dietary $n-3/n-6$ ratio. Though the amounts of total $n-3$ HUFAs in the 100% SBM diet (which produced poor growth and longer molting cycles), and the blue mussel diet (which produced good growth and shorter molting cycles), were similar (1.5% of dry diet, Table 9), the blue mussel diet had a very high $n-3/n-6$ ratio which probably explains the superior results of the blue mussel diet compared to those obtained with the feeding of the 100% SBM diet. Dietary $n-3/n-6$ ratios that reflected the ratios in reproductive tissues resulted in better reproductive performance in the mud crab *S. serrata* (Millamena and Quinitio, 2000). Dietary lipid influences tissue lipid composition in crustaceans (D'Abramo and Sheen, 1993). In fish, the enzymes that esterify fatty acids into phospholipids do not have absolute specificities for particular fatty acids, and this results in tissue fatty acid profiles that reflect that of the diet (Sargent et al., 1999). The $n-3$ HUFA profiles of the juveniles were inversely proportional to the dietary inclusion level of SBM. Possibly, below a threshold dietary $n-3/n-6$ ratio, the lobster cannot preferentially sequester $n-3$ over $n-6$ HUFAs (Table 5), even if dietary $n-3$ HUFAs were supplied (though addition of FM) at amounts that supposedly satisfy EFA requirements. This problem appeared to have been alleviated with amino acid supplementation of the 100% SBM diet (successful molting of juveniles fed this diet), because a good dietary amino acid balance apparently improves utilization of dietary EFAs (Teshima, 1997). A good dietary amino acid balance also prevented molt death syndrome in juvenile American lobsters fed crab protein instead of casein (Kean et al., 1985).

The decrease in the dietary $n-3/n-6$ ratio in diets containing increasingly higher SBM levels was inherent in the current diet formulation using low-fat SBM, which therefore contained 18:2$n-6$, as well as the gradual elimination of $n-3$ HUFA-containing FM. The greater EFA activity of 18:2$n-6$ for freshwater species probably explains the success of higher SBM incorporation in diets for the freshwater prawn, *Macrobrachium rosenbergii* (Koshio et al., 1992). In production ponds (which may also derive much nutrition from natural productivity), FM was totally replaced by SBM (Tidwell et al., 1993). For marine aquaculture species, which require $n-3$ HUFAs, however, the addition of high levels of fish oil to offset the high content of 18:2$n-6$ inherent in low-fat SBM could be limited by the maximum amount of total lipid that can be incorporated into crustacean diets without causing a reduction in growth. Recommended levels of dietary lipid range from 5% to 14% (D'Abramo, 1997; Shivaram and Raj, 1997; Sheen and Wu, 1999). Hence, when using plant protein sources which generally contain C_{18} PUFAs as the dominant PUFA, attention must be paid to not only the amino acid balance, but also the $n-3/n-6$ PUFA ratio.

Table 9
The fatty acid composition (mg/g dry weight) of the experimental diets

Fatty acid	Diet type: amino acid supplement	Level of SBM (% of dietary protein)												Blue mussel
		0		25		50		75		87.5		100		
		+	−	+	−	+	−	+	−	+	−	+	−	
Saturates														
14:0		5.9	6.3	5.0	4.8	3.2	3.1	2.0	2.1	1.5	1.5	1.1	1.1	1.5
15:0		0.6	0.7	0.6	0.5	0.4	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.2
16:0		19.8	21.0	19.3	18.6	16.5	15.9	14.2	15.1	14.0	13.1	13.4	13.8	5.1
18:0		3.8	4.1	4.2	3.9	3.9	3.8	3.7	4.0	3.8	3.7	3.9	4.1	0.9
20:0		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	–
Σ Saturates		31.5	33.6	30.5	29.1	25.1	24.3	21.1	22.4	20.4	19.3	19.4	20.2	8.0
Monounsaturates														
16:1 $n-7$		8.9	9.5	7.5	7.2	4.9	4.7	3.1	3.2	2.4	2.3	1.7	1.7	3.5
18:1 $n-9$		6.8	7.2	10.2	9.4	11.9	1.7	13.6	14.4	14.8	14.8	16.3	18.5	0.4
18:1 $n-7$		2.7	2.9	2.5	2.4	2.0	1.9	1.7	1.7	1.6	1.1	1.6	–	1.2
20:1 $n-9$		0.8	1.1	0.2	0.9	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.1	1.1
Σ Monounsaturates		21.6	23.1	23.3	21.7	20.9	20.1	19.6	20.7	19.8	19.3	20.4	21.2	7.6
Polyunsaturates														
18:2 $n-6$		8.3	9.0	21.9	20.1	32.8	31.8	40.7	43.2	46.1	40.7	51.3	49.2	0.3
18:3 $n-3$		2.3	2.5	4.5	4.2	6.1	5.9	7.3	7.8	8.2	7.9	9.0	9.4	0.2
18:4 $n-3$		2.2	2.4	2.0	1.8	1.3	1.2	0.9	0.9	0.7	0.7	0.6	0.6	0.5
20:4 $n-6$		1.3	1.3	1.0	0.9	0.6	0.6	0.3	0.4	0.2	0.2	0.1	0.1	1.6
20:4 $n-3$		1.1	1.2	1.0	0.9	0.6	0.6	0.4	–	0.4	0.4	0.3	–	0.1
20:5 $n-3$		9.3	9.7	7.9	7.3	5.0	4.8	3.1	3.2	2.4	2.4	1.8	1.81	0.2
22:5 $n-3$		2.1	2.2	1.8	1.6	1.1	1.0	0.7	0.6	0.5	0.6	0.4	0.4	0.5
22:6 $n-3$		10.6	10.7	8.9	8.2	5.6	5.2	3.4	3.7	3.0	3.0	2.3	2.5	2.9
Σ PUFA		37.9	39.7	49.9	45.9	53.8	51.6	57.1	59.9	61.7	56.1	66.0	64.2	17.4
Σ Fatty acids		98.4	103.1	108.9	102.1	102.7	98.8	99.5	105.1	103.2	97.2	107.1	107.7	35.9
Σ $n-3$		25.8	28.9	26.3	24.1	19.9	18.9	15.8	16.2	15.2	15.0	14.5	14.8	15.0
Σ $n-6$		10.0	10.7	23.6	21.4	33.8	32.6	41.2	43.7	46.4	47.8	51.5	49.4	2.1
Σ $n-3/n-6$ ratio		2.6	2.7	1.1	1.1	0.6	0.6	0.4	0.4	0.3	0.3	0.3	0.3	7.1
Percent dietary $n-3$ HUFA		2.6	2.9	2.6	2.4	2.0	1.9	1.6	1.6	1.5	1.5	1.5	1.5	1.5

Juveniles fed 100% SBM contained significantly higher levels of 16:1$n-7$ and 20:1$n-9$ than juveniles fed the other diets. Sheen and Wu (1999) also noted an increase in the level of 16:1 for juvenile mud crab *S. serrata* fed lipid-deficient diets and stated that it might be due to biosynthesis to meet body requirements.

Arginine and its derivatives are major energy reserves of ATP in muscles of crustaceans, which rely on phosphoarginine for ATP metabolism (Hird, 1986). Hence, this EAA must be provided at dietary levels that are higher than what would be needed for weight gain. Recent investigations with the shrimp, *P. monodon*, led to an estimate of 5.3% of dietary protein as the arginine requirement (Millamena et al., 1998). The arginine content of the blue mussel diet was also very high relative to the arginine content of the experimental diets (Table 2). The technique of coating the crystalline amino acids in CMC (Millamena et al., 1997) as performed in this study, may also have prevented/reduced leaching and allowed for the controlled release of these nutrients in the gut (Chen et al., 1992). Except for the high levels of arginine in juveniles fed supplemented diets, there were no pronounced differences in the amino acid profiles of the experimental lobsters. The slightly elevated levels of aspartate and glutamate in juveniles fed the non-supplemented diets, coupled with the lower growth performance of juveniles on these diets, may indicate reduced utilization of these amino acids which are used to form nucleic acids. Higher body levels of aspartate in the prawn, *P. japonicus*, were associated with reduced catabolism of this amino acid caused by a pyridoxine deficiency (Giri et al., 1997). The higher levels of tyrosine and serine in the carcass of juveniles fed non-supplemented diets may indicate reduced utilization or increased biosynthesis of these amino acids to meet body requirements. The lack of any significant results in the amino acid profile of the juveniles relative to SBM level indicates that SBM is a suitable protein source as far as amino acid profile is concerned.

Better growth performance in crustaceans has been obtained when practical protein sources are supplemented with crystalline amino acids (Guillaume, 1997). Fernandez and Sukumaran (1995) reported better growth rate, food conversion, and protein efficiency ratio in the shrimp, *Penaeus indicus*, when a squid meal-based diet was supplemented with arginine, lysine, methionine and tryptophan. Despite the addition of the four crystalline amino acids (arginine, methionine, leucine and tryptophan), such that all the supplemented diets theoretically had amino acid profiles simulating that of juvenile lobster, BWG was inversely proportional to the dietary inclusion of SBM. Several reasons have been offered to explain the poor performance of fish and crustacean diets containing very high SBM levels: the carbohydrate fraction lowers the digestibility of SBM (Akiyama, 1991); the higher fiber content may increase gastric emptying time (Shiau, 1997) and not allow for more efficient nutrient absorption in the gut; poor palatability leads to poor consumption; the cell wall may make the protein unavailable; or higher exposure to still unknown antinutritional factors (Guillaume, 1997).

Low palatability of diets containing $\geq 50\%$ SBM as dietary protein was observed in this study. Juveniles fed these diets ignored, fled or even transported the pellet out of their shelters each feeding time. This behavior was not observed with diets containing the EAA supplements, which are (except for tryptophan) known to be crustacean attractants (Lee and Meyers, 1997). Given no other choice for food, juveniles eventually

had to consume at least part of these apparently unpalatable diets for sustenance. Juveniles were observed to consume the pellets after they had stayed in the tank for several minutes (probably until the appropriate attractants had leached out). In contrast, diets containing no or low levels of SBM were readily received. Juveniles even grabbed at the pellet offered from the tip of the forceps during feeding time, or actively sought it out if the pellet was dropped a distance from their shelters. Though these four EAAs were likewise present in the non-supplemented diets, albeit in lower levels, they existed in the protein-bound form and would therefore be unavailable as water-soluble attractants. The only attractant for the 0% SBM non-supplemented diet would be the fish oil, which apparently was not enough. Hence, this diet required sufficient attractant to elicit a feeding response.

The fleeing behavior exhibited by the juveniles also suggests that SBM itself may contain deterrents to feeding. The volatile substances responsible for its odor are suspect as these may be the most readily soluble in water. If so, plant meals destined for use in aquaculture diets will need additional processing to remove these volatiles. Concerns about antinutritional factors from soybean have been largely addressed in the terrestrial feed industry through better meal processing techniques (Witte, 1995). However, substances with antinutritional activity specifically for aquatic animals have not been investigated and may persist under current processing methods.

Presently, the major drawback in the development of practical or formulated diets for lobsters is the relatively low price of herring and cod racks (US$0.28/kg). The practical diet must also yield growth rates that are at least as good as what is currently achieved with cod racks fed to pounded lobsters, a goal that has not yet been demonstrated. The future prospect of scarcity in the supply of cod rack should be anticipated with continued research into formulated diets for lobsters. The greater incorporation of plant proteins into formulated diets for carnivorous aquatic species will help conserve the limited sources of marine protein, and, in the long run, contribute to a reduction in the cost of farmed seafood.

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