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Partial and total replacement of fish meal with soybean meal and brewer's grains with yeast in practical diets for Australian red claw crayfish *Cherax quadricarinatus*

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Abstract

Three feeding studies were conducted to evaluate the effects of total replacement of fish meal (FM) with a combination of soybean meal (SBM) and brewer's grains with yeast (BGY) in diets for two separate strains of juvenile Australian red claw crayfish. In Experiment 1, three practical diets were formulated to be isonitrogenous (40% protein) and isocaloric (4.0 kcal available energy/g diet) and contained either 25%, 10%, or 0% fish meal. Variable percentages of SBM (35%, 46.8%, and 79.8%, respectively) and BGY-35 (0%, 30%, and 5%, respectively) replaced the fish meal. In Experiments 2 and 3, four practical diets were formulated to be isonitrogenous (40% protein) and isocaloric (4 kcal available energy/g diet) containing 24% or 0% fish meal. Diet 1 contained 24% fish meal, 23% SBM, and 0% BGY-35. A variable percentage of SBM (56.75%, 47.75%, and 40.75%, respectively) and BGY-35 (10%, 20%, and 30%, respectively) replaced the fish meal in the remaining three diets.

In Experiment 1, after 8 weeks, juvenile red claw fed all three diets had no significant difference (P>0.05) in final weight, percentage weight gain, or survival, which averaged 7.90 g, 3848%, and 83%, respectively. In Experiment 2, after 8 weeks, juveniles fed all four diets had no significant difference in final weight, percentage weight gain, or specific growth rate which averaged 11.46 g, 977%, and 3.08%/day, respectively. Percentage survival was not significantly different among treatments and averaged 79%. In Experiment 3, after 8 weeks, juvenile red claw fed all four diets had

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no significant difference in final weight, percentage weight gain, or specific growth rate which averaged 16.22 g, 457%, and 2.34%/day, respectively. Percentage survival was not significantly different among treatments and averaged 98%.

These results indicate that fish meal and shrimp meal can be totally replaced with soybean meal and BGY in diets for juvenile red claw crayfish. This may allow for less expensive diets by red claw producers, which may increase profitability.

Keywords: Soybean meal; Brewer's grains with yeast; Australian red claw crayfish

1. Introduction

The Australian red claw crayfish is considered to be a popular crustacean species for aquaculture due to its potential large size and resemblance to American lobsters. Currently, red claw production in the United States is scattered and small scale, with animals in the 60–120 g range selling for US$9–14/kg (D. Rouse, Auburn University, personal communication). The red claw has many positive attributes that make it suitable for semi-intensive and intensive culture. They exhibit an unaggressive and nonburrowing behavior in captivity. Red claw tolerate relatively crowded conditions, with limited cannibalism occurring at adult densities greater than $60/m^2$; exhibit fast growth rates over a broad range of temperatures (23–31 °C); require relatively simple spawning techniques; and tolerate a wide range of water qualities, including low dissolved oxygen and elevated ammonia and nitrite levels. Furthermore, about 30% of the total body weight of the red claw is edible tail meat, compared to 15–20% for native crayfish (Masser and Rouse, 1997). Published research results devoted to red claw nutrition are limited.

Manomaitis (2001) examined the crude protein requirement of juvenile red claw (0.1 and 3.0 g). Experiment 1 used newly released juveniles (0.1 g) fed 25–40% protein diets. After 7 weeks, significant differences were observed in final weight, specific growth rate, and percentage weight gain of the juveniles with increasing protein levels in the diet. Manomaitis (2001) determined that the crude protein requirement of newly released juveniles to be at least 40%. Experiment 2 used 3.0 g juveniles (approximately 9 weeks post-release) fed 24–44% protein diets and after 10 weeks, no significant differences were observed in final weight, specific growth rate, and percentage weight gain of the juveniles fed any of the diets. Manomaitis (2001) concluded that a diet of about 30% protein should be utilized for 9- to 19-week red claw.

Thompson (2002) examined the growth of juvenile red claw (0.2 g fed practical diets, containing 40% protein), with or without supplemental lecithin and/or cholesterol. No significant differences were observed in final weight and percentage weight of red claw fed the control diet and a diet with no supplemental lecithin and cholesterol. They concluded that juvenile red claw fed a 40% protein diet containing 25% menhaden fish meal, 44.5% soybean meal, 0.5% choline chloride, 2% cod liver oil, and 1% corn oil may satisfy the lecithin and cholesterol requirements.

Diets are one of the major cost variables for most aquaculture species, representing up to 70% of operating costs. Fish meal (FM) is used as a major protein source within most finfish and crustacean diets (Lovell, 1989), due to the high nutrient quality and digestibility of fish meal protein. Long-term availability of fish meal is uncertain, and feeding fish and crustaceans, instead of people, a potential food-quality protein source is a subject of concern (Webster et al., 1992). Due to the high cost of fish meal and other considerations, there is interest in the partial or total replacement of this ingredient with less expensive plant protein meals without adversely affecting growth and health of culture species (Hardy, 1996).

Few studies have been conducted on the replacement of fish meal in crustacean diets. Piedad-Pascual et al. (1990) demonstrated that the weight gain and survival of juvenile tiger prawn (*Penaeus monodon*) were not different among those fed diets with lower amounts of defatted soybean meal (DSM) or those fed higher DSM diets, when completely replacing FM. Tidwell et al. (1993) concluded that growth, survival, and pond yield of freshwater prawn (*Macrobrachium rosenbergii*) were unaffected by either 50% or 100% replacement of fish meal with soybean meal and distillers dried grains with solubles (DDGS). Lim and Dominy (1990) suggested that the dietary level of soybean meal for culture of white shrimp (*Penaeus vannamei*) could be increased up to 56% if palatability and water stability of the diet pellet could be improved. However, when they replaced a marine animal-protein mix (comprised of anchovy fish meal, shrimp meal, and squid meal) completely with soybean meal, growth of the shrimp significantly decreased. Jones et al. (1996) found that higher SBM inclusion levels could be included in diets of higher protein content without significantly affecting growth of juvenile yabbies (*Cherax destructor*).

Brewer's grains with yeast (BGY) increases nutrient value and palatability in many animal diets (dairy cattle, beef cattle, swine, and equines) and pet foods. Brewery grains and yeast are the two main by-products of the brewing industry. Diet formulations that incorporate SBM and BGY may be less expensive than diets containing FM. The results of investigations designed to evaluate the effects of partial and total replacement of fish meal with a combination of SBM and BGY-35 in diets for juvenile red claw crayfish are reported.

2. Materials and methods

2.1. Experimental diets

2.1.1. Experiment 1

Three experimental diets were formulated to contain decreasing percentages of fish meal (FM), and increasing percentages of soybean meal. The ingredient compositions of the diets are presented in Table 1. Diet 1 was similar to a high-quality marine shrimp diet containing 25% menhaden FM, 35% SBM, and 10% shrimp meal. Diet 2 contained 10% FM, 46.8% SBM, and 0% shrimp meal, while diet 3 contained 0% FM, 79.8% SBM, and 0% shrimp meal. It has been reported that a combination of protein sources may be used to replace fish meal completely in aquaculture diets (Webster et al., 1992), so brewer's grains with yeast (BGY) was added to diets 2 and 3.

Table 1
Percent composition of the ingredients of three practical diets (Experiment 1) containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

Ingredient	Diets		
	1	2	3
Menhaden fish meal (67%)	25.0	10.0	–
Soybean meal (47%)	35.0	46.8	79.8
Shrimp meal (44%)	10.0	–	–
BGY-35 (35%)	–	30.0	5.0
Wheat flour (11%)	16.3	–	–
Cod liver	2.0	3.0	5.0
Lecithin[a]	0.5	–	–
Cholesterol[b]	1.0	–	–
Vitamin mix[c]	2.0	2.0	2.0
Mineral mix[d]	0.5	0.5	0.5
Other[e]	7.7	7.7	7.7

Values in parentheses are percent of protein in diet ingredient.

[a] Lecithin (Archer Daniels Midland).

[b] Cholesterol was analytical grade, purchased from Sigma (St. Louis, MO).

[c] Vitamin mix was the Abernathy vitamin premix no. 2 and supplied the following (mg or IU/kg of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopheryl acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 132 mg; K (as menadione sodium bisulfate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; and ethoxyquin, 99 mg.

[d] Mineral mix was Rangen trace mineral mix F1 for catfish with 0.3 mg selenium/kg diet added.

[e] Other ingredients included were at levels common to all diets: corn oil at 1.0%, dicalcium phosphate at 1.0%, Stay C at 0.2%, choline chloride at 0.5%, and carboxymethylcellulose (CMC; United States Biochemical, Cleveland, OH) at 5.0% as binder.

Dry ingredients (menhaden fish meal, soybean meal, shrimp meal, wheat, corn meal, carboxymethylcellulose binder, and BGY-35) for each diet were weighed (Mettler PM 4600, Mettler Instrument, Highstown, NJ) and mixed for 2 h using a Hobart mixer (A-200 T; Hobart, Troy, OH). The remaining dietary ingredients (cholesterol, dicalcium phosphate, vitamin mix, mineral mix, Stay C, and choline chloride) were weighed and then added and mixed with the previous dry ingredients for an additional 30 min. All dry ingredients were then thoroughly mixed with water to obtain a 25% moisture level. Diets were passed through a commercial food chopper (General Slicing MC-100; General Slicing, Murfreesboro, TN) with a 1-cm die and dried (26 °C) for 48 h in an industrial convection oven (Grieve PL-326; Grieve, Round Lake, IL).

Once the diets were dry (at a constant weight), they were broken to the desired pellet size and sieved (2-mm opening mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburgh, PA). After the diet had been sieved, cod liver oil, corn oil, ethoxyquin (0.2% of lipids), and liquid commercial soybean lecithin (if added; Archer Daniels Midland, Decatur, IL) were added to the diets and mixed by hand until all pellets were coated uniformly. Addition of lipids after pellets had been dried was done to ensure that n-3 highly unsaturated fatty acids (HUFAs) would not be subjected to possible high temperatures of the pelleting process, and thus, HUFAs would be conserved (Thompson, 2002; Thompson et al., 2003). Diets were stored in plastic containers and kept frozen (− 20 °C) until fed.

Diets were analyzed to determine percent moisture, protein, lipid, fiber, and ash. Moisture was determined by drying (100 °C) until a constant weight was achieved; protein was determined by macro-Kjeldahl method; lipid was determined by the acid hydrolysis method; fiber was determined by using the fitted glass crucible method; and ash was determined by placing diets in a muffle furnace (600 °C) for 24 h and weighing residue (AOAC, 1990). The nitrogen-free extract (NFE), i.e. carbohydrate, was determined by difference [NFE = 100 − (%protein+%lipid+%fiber+%ash)]. Available energy (AE) was calculated from physiological fuel values of 4.0, 4.0, and 9.0 kcal/g for protein, carbohydrate (NFE), and lipid, respectively (Garling and Wilson, 1977; Webster et al., 1999). Diets used in Experiment 1 were analyzed for cholesterol, amino acid composition, and fatty acid composition by a commercial analytical laboratory (Woodson-Tenent Lab, Dayton, OH). Proximate composition (which includes percent moisture, protein, fat, fiber, ash), percent of NFE, available energy, and cholesterol content of the three practical diets are presented in Table 2, amino acid compositions of practical diets are presented in Table 3, and fatty acid compositions (percent of total) of the three practical diets are presented in Table 4.

2.1.2. Experiments 2 and 3

Four experimental diets were formulated to contain increasing percentages of BGY, to correspond with the elimination of FM from three of the diets. Diet 1 was formulated to be similar to a high-quality marine shrimp diet, and contained 24% anchovy FM, 23% SBM, and 10% shrimp meal. Diets 2–4 contained no FM, 10% shrimp meal, and different combinations of BGY-35 and SBM, respectively (Table 5). Diets were prepared and analyzed as discussed previously. Proximate composition,

Table 2
Proximate composition and cholesterol content of Experiment 1 practical diets containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

	Diets		
	1	2	3
Moisture (%)	11.98	11.00	11.22
Protein (%)[a]	43.91	43.71	44.37
Lipid (%)[a]	9.27	10.36	9.72
Fiber (%)[a]	3.07	3.03	3.49
Ash (%)[a]	12.20	8.20	7.77
NFE[b]	31.55	34.70	34.65
Available energy[c]	3.85	4.07	4.04
Cholesterol (mg/100 g)	815.0	49.1	28.4

[a] Dry matter basis.

[b] NFE = nitrogen-free extract.

[c] Available energy was calculated as 4.0, 4.0, and 9.0 kcal/g of protein, carbohydrate (NFE), and lipid, respectively.

Table 3
Amino acid composition of Experiment 1 practical diets containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

Amino acid	Diets		
	1	2	3
Alanine	1.91 (4.35)	1.81 (4.14)	1.69 (3.81)
Arginine[a]	2.15 (4.90)	2.20 (5.03)	2.47 (5.57)
Aspartic acid	3.50 (7.97)	3.42 (7.82)	4.01 (9.04)
Cystine	0.45 (1.03)	0.56 (1.28)	0.58 (1.31)
Glutamic acid	5.63 (12.82)	6.01 (13.75)	6.47 (14.58)
Glycine	1.93 (4.40)	1.64 (3.75)	1.63 (3.67)
Histidine[a]	1.25 (2.85)	1.13 (2.59)	0.96 (2.16)
Isoleucine[a]	1.52 (3.46)	1.51 (3.46)	1.62 (3.65)
Leucine[a]	2.58 (5.88)	2.75 (6.29)	2.80 (6.31)
Lysine[a]	2.28 (5.19)	1.95 (4.46)	2.16 (4.87)
Methionine[a]	0.71 (1.62)	0.71 (1.62)	0.57 (1.29)
Phenylalanine[a]	1.54 (5.15)	1.66 (3.80)	1.74 (3.92)
Proline	2.26 (3.51)	2.14 (4.90)	2.02 (4.55)
Serine	1.59 (3.62)	1.67 (3.82)	1.85 (4.17)
Threonine[a]	1.45 (3.30)	1.40 (3.20)	1.46 (3.29)
Tyrosine[a]	0.84 (1.91)	0.91 (2.29)	0.96 (2.16)
Valine[a]	1.79 (4.08)	1.77 (4.05)	1.79 (4.03)

Values are percentage of the diet (values in parentheses are expressed as percentage of dietary protein).
[a] Essential amino acid.

NFE, and available energy of each of the four practical diets are presented in Table 6, while amino acid composition of each of the four practical diets is presented in Table 7.

Table 4
Fatty acid composition (percent of total) of Experiment 1 practical diets containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

Fatty acid	Diets		
	1	2	3
14:0	3.05	1.65	1.68
16:0	22.97	18.10	15.21
16:1n-7	3.25	1.90	2.06
18:0	6.50	3.80	3.87
18:1n-9	24.19	18.99	20.49
18:2n-6	24.80	40.51	42.91
18:3n-3	2.24	4.30	4.90
20:1n-9	1.42	0.76	0.52
20:4n-6	0.61	0.63	0.52
20:5n-3	1.83	2.15	2.32
22:6n-3	4.47	3.80	2.58
Others	4.67	3.41	2.94

Table 5
Percent composition of ingredients of four practical diets (Experiments 2 and 3) containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

Ingredient	Diets			
	1	2	3	4
Anchovy fish meal (71%)	24.0	–	–	–
Soybean meal (47%)	23.0	56.75	47.75	40.75
Shrimp meal (44%)	10.0	10.0	10.0	10.0
BGY-35 (35%)	–	10.0	20.0	30.0
Wheat flour (11%)	20.0	4.0	3.0	–
Corn meal	6.25	–	–	–
Cod liver oil	2.5	6.0	6.0	6.0
Lecithin[a]	1.0	1.0	1.0	1.0
Cholesterol[b]	1.0	1.0	1.0	1.0
Vitamin mix[c]	2.0	2.0	2.0	2.0
Mineral mix[d]	0.5	0.5	0.5	0.5
Other[e]	8.75	8.75	8.75	8.75

Values in parentheses are percent of protein in diet ingredient.
[a] Lecithin (Archer Daniels Midland).
[b] Cholesterol was analytical grade, purchased from Sigma.
[c] Vitamin mix was the Abernathy vitamin premix no. 2 and supplied the following (mg or IU/kg of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as alpha-tocopheryl acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 132 mg; K (as menadione sodium bisulfate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; and ethoxyquin, 99 mg.
[d] Mineral mix was Rangen trace mineral mix F1 for catfish with 0.3 mg selenium/kg diet added.
[e] Other ingredients included were at levels common to all diets: corn oil at 2.0%, dicalcium phosphate at 1.0%, Stay C at 0.25%, choline chloride at 0.5%, and wheat gluten at 5.0%.

2.2. Experimental system and feeding

2.2.1. Experiment 1

The feeding trial was conducted at the Aquaculture Research Center, Kentucky State University, in plastic-mesh culture units (12.7 cm^3; Plastic Window Breeder-Fine, Lustar Products, Springfield, NJ) which were positioned within four rectangular fiberglass tanks (325.5 l; 236.22 × 101.6 × 15.24 cm). Each unit was secured to a fiberglass tank with clear 100% silicone rubber sealant. Water was recirculated through a system consisting of a 2000-l vertical screen filter utilizing high-density polyester screens and polyethylene "bio-balls" to remove particulates and provide substrate for nitrifying bacteria (Red Ewald, Karnes City, TX). Water temperature was maintained between 27 and 29 °C. Continuous aeration was provided by a blower and air-stones. All culture units were cleaned by siphoning every other day to remove uneaten diet. Each unit was supplied with an individual water line supplying water at a rate of 0.8 l/min.

Water quality parameters were measured three times per week. Water temperature was measured using a thermometer and dissolved oxygen was measured using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, OH). Total ammonia and nitrite were determined using a DR/2000 spectrophotometer (HACH, Loveland, CO); pH was determined with an electric pH meter (pH pen, Fisher Scientific, Cincinnati,

Table 6
Mean proximate composition (± S.E.) of Experiments 2 and 3 practical diets containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish; $n=2$

	Diets			
	1	2	3	4
Moisture (%)	11.26 ± 0.16	10.92 ± 0.20	10.95 ± 0.17	7.03 ± 0.12
Protein (%)[a]	41.90 ± 0.20	44.55 ± 0.45	43.25 ± 0.15	43.70 ± 0.30
Lipid (%)[a]	11.05 ± 0.35	13.85 ± 0.25	13.90 ± 0.50	14.00 ± 0.20
Fiber (%)[a]	3.10 ± 0.20	4.45 ± 0.15	4.80 ± 0.20	5.80 ± 0.20
Ash (%)[a]	12.15 ± 0.05	10.25 ± 0.05	10.10 ± 0.10	9.80 ± 0.00
NFE[b]	31.80 ± 0.00	26.90 ± 0.00	27.95 ± 0.65	26.70 ± 0.10
Available energy[c]	3.94 ± 0.02	4.11 ± 0.01	4.10 ± 0.01	4.08 ± 0.00

[a] Dry matter basis.
[b] NFE = nitrogen-free extract.
[c] Available energy was calculated as 4.0, 4.0, and 9.0 kcal/g of protein, carbohydrate (NFE), and lipid, respectively.

OH); and total alkalinity and chlorides were determined with a digital titrator (HACH).

Juvenile red claw (*Cherax quadricarinatus*) (0.2 ± 0.05 g), from the same female, were obtained from the research hatchery at Auburn University, Auburn, AL and randomly stocked into individual plastic-mesh rearing units, with 25 replicates per treatment. Individual weights of 12 juveniles were determined using an electronic scale (Mettler

Table 7
Amino acid composition of Experiments 2 and 3 practical diets containing different levels of soybean meal and brewer's grains with yeast fed to red claw crayfish

Amino acid	Diets			
	1	2	3	4
Alanine	1.74 (4.15)	1.50 (3.37)	1.54 (3.56)	1.64 (3.75)
Arginine[a]	1.93 (4.61)	2.22 (4.98)	2.28 (5.27)	2.37 (5.42)
Aspartic acid	3.17 (7.57)	3.66 (8.22)	3.77 (8.72)	3.77 (8.63)
Cystine	0.41 (0.98)	0.54 (1.21)	0.54 (1.25)	0.57 (1.30)
Glutamic acid	6.31 (15.06)	7.07 (15.87)	7.22 (16.69)	7.56 (17.30)
Glycine	1.99 (4.75)	1.53 (3.43)	1.57 (3.63)	1.67 (3.82)
Histidine[a]	0.80 (1.91)	0.84 (1.89)	0.85 (1.97)	0.86 (1.97)
Isoleucine[a]	1.38 (3.29)	1.51 (3.39)	1.52 (3.51)	1.56 (3.57)
Leucine[a]	2.36 (5.63)	2.52 (5.66)	2.54 (5.87)	2.64 (6.04)
Lysine[a]	1.86 (4.44)	1.82 (4.09)	1.78 (4.12)	1.77 (4.05)
Methionine[a]	0.64 (1.53)	0.54 (1.21)	0.56 (1.29)	0.60 (1.37)
Phenylalanine[a]	1.54 (3.68)	1.71 (3.84)	1.72 (3.98)	1.80 (4.12)
Proline	2.14 (5.11)	2.05 (4.60)	2.10 (4.86)	2.18 (4.99)
Serine	1.50 (3.58)	1.71 (3.84)	1.71 (3.95)	1.81 (4.14)
Threonine[a]	1.30 (3.10)	1.32 (2.96)	1.34 (3.10)	1.41 (3.23)
Tyrosine[a]	0.90 (2.15)	1.04 (2.33)	1.01 (2.34)	1.05 (2.40)
Valine[a]	1.65 (3.94)	1.75 (3.93)	1.77 (4.09)	1.85 (4.23)

Values are percentage of the diet (values in parentheses are expressed as percentage of dietary protein).
[a] Essential amino acid.

AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) prior to stocking to obtain an average initial weight. Within the first week after stocking, any mortalities were replaced to eliminate any mortality arising from stocking. Red claw were fed one of three practical diets, formulated to contain 40% protein, to excess, based on the observation of the amount of uneaten diet, three times daily (0800, 1200, and 1500 h) for 8 weeks. Each rearing unit was supplied with a 5-cm section of 1-in.-diameter PVC pipe for shelter.

At the conclusion of the feeding trial, all red claw were individually weighed (wet weight) on an electronic scale (Mettler AT261 Delta Range). For each treatment, eight red claw were randomly sampled for each treatment and flash-frozen with liquid nitrogen (?196 °C), for subsequent analysis of whole-body fatty acid composition. Of those, two sets of four red claw were combined, representing two replicates per treatment. After freezing, red claw legs, claws, and uropods were removed from the body. The remaining whole-body samples were shattered into pieces and stored in screw-topped glass vials under nitrogen. The glass vials were immediately cap-sealed with Teflon tape and covered with aluminum foil. Samples were stored in a freezer (− 30 °C) until lipid extraction.

2.2.2. Experiment 2

Juvenile red claw (1.2 ± 0.6 g), from the same female, were obtained from an Ecuadorian strain of brood stock and spawned at the Aquaculture Research Center at Kentucky State University. Individual weight of each juvenile was determined on an electronic scale (Mettler AT261 Delta Range, Mettler Instruments) prior to stocking. All other experimental procedures and methods were the same as those described in Experiment 1.

At the conclusion of the feeding trial (8 weeks), red claw were individually weighed (wet weight) on an electronic scale (Mettler AT261 Delta Range). For each treatment, 12 red claw juveniles were randomly sampled for proximate analysis. They were sacrificed by submersion in ice water for approximately 1.5 h. Three sets of four red claw were combined per treatment, representing three replicates. At the completion of the ice submersion, red claw legs, claws, and uropods were removed from the body. The remaining whole-body samples were chopped into pieces and stored in plastic Ziploc sample bags. Samples were stored in a freezer (− 30 °C) until analysis was conducted by a commercial analytical lab (Woodson-Tenent Lab).

2.2.3. Experiment 3

Juvenile red claw (3.1 ± 0.9 g) were obtained from Central Queensland Crayfish (Biloela, Queensland, Australia). It was uncertain whether or not the juveniles came from one or more females. They were randomly stocked into individual plastic-mesh rearing units, with 30 replicates per treatment. Individual weight of each juvenile was determined on an electronic scale (Mettler AT261 Delta Range, Mettler Instruments) prior to stocking. All other experimental procedures and methods were the same as those described in Experiment 1.

At the conclusion of the feeding trial (8 weeks), red claw were individually weighed (wet weight) on the Mettler electronic scale. For each treatment, 8 red claw were randomly sampled and sacrificed for amino acid analysis by submersion in ice water for approximately 1.5 h. Two sets of four red claw were combined per treatment, representing two replicates. After chilling, tail muscle was removed from the exoskeleton of each red claw, and then diced. The replicate samples were stored in plastic Ziploc sample bags. Samples were stored in a freezer (−30 °C) until analyzed by a commercial analytical lab (Woodson-Tenent Lab).

2.3. Statistical analysis

Growth performance of red claw crayfish was measured in terms of final individual weight (g), percentage weight gain, specific growth rate (SGR, %/day), and percentage survival. Growth parameters were calculated as follows: SGR (%/day)=$[(\ln W_t - \ln W_i)/T] \times 100$, where W_t and W_i are the final and initial individual weights of the red claw, respectively, and T is the length of the culture period in days; weight gain (%) = $100[(W_t - W_i)/W_i]$. Fatty acid composition (Experiment 1), proximate composition (Experiment 2), and amino acid composition (Experiment 3) were compared. Amino acid compositions of diets were compared to amino acid composition of red claw tail muscle (Experiment 3) using the essential amino acid index (EAAI).

Data were analyzed by analysis of variance (ANOVA) using the SAS General Linear Models (GLM) procedure, using SAS software version 8.0 (SAS, 1999; Cary, NC) to determine whether growth was significantly different among treatment means. If ANOVA indicated significant treatment differences, Duncan's multiple range test was used to compare differences between individual means at the $P = 0.05$ level of significance. A chi-square test comparing proportions was used to determine if survival was diet dependent. Percentage and ratio data were arcsine-transformed prior to statistical analysis (Zar, 1984). Data are presented as untransformed values.

3. Results

3.1. Experiment 1

The mean ± S.D. of each of the measured water quality variables was as follows: water temperature, 28.0 ± 0.6 °C; dissolved oxygen, 7.3 ± 0.2 mg/l; total ammonia nitrogen, 0.2 ± 0.2 mg/l; nitrite, 0.03 ± 0.01 mg/l; alkalinity, 157.0 ± 36.1 mg/l; chlorides, 61.0 ± 23.6 mg/l; and pH, 8.4 ± 0.1. These mean water quality conditions were within acceptable limits for indoor production of red claw crayfish (Masser and Rouse, 1997).

The mean final weight and percentage weight gain of red claw treatments were not significantly different among all treatments and collectively averaged 7.9 g and 3848% (Table 8). However, the specific growth rate (SGR) for red claw fed diet 3 (6.7%/day) was significantly higher for than that of red claw fed diet 1 (5.7%/day; Table 8). Survival was not significantly different among treatments and averaged 83%, overall.

The fatty acid composition of the whole-body red claw (expressed as percent of total fatty acids) are presented in Table 9. Among red claw fed the three diets, there was no significant difference in the levels of palmitoleic acid (16:1n-7) and oleic acid (18:1n-9). Red claw fed diet 2 (10% FM) had significantly higher percentages of palmitic acid (16:0), linoleic acid (18:2n-6), and linolenic acid (18:3n-3) compared to those of red claw fed diet

Table 8
Means (± S.E.) of final individual weight, percentage weight gain, specific growth rate (SGR), and percent survival of red claw fed three practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 1)

	Diets		
	1	2	3
Final weight (g)	6.97 ± 1.0	7.61 ± 1.07	8.97 ± 5.4
Weight gain (%)	3384 ± 499	3702 ± 532	4382 ± 275
SGR (%/day)	5.74 ± 0.40^{b}	5.87 ± 0.40^{ab}	6.72 ± 0.11^{a}
Survival (%)	76.0	84.0	88.0

Means within a row having different superscript letters are significantly different ($P<0.05$).

1 (Table 9). Red claw fed diet 1 (25% FM) contained significantly higher percentages of stearic acid (18:0), arachidonic acid (20:4n-6), and docosahexaenoic acid (22:6n-3) compared to those of red claw fed diet 2, while red claw fed diet 1 had significantly higher percentages of eicosapentaenoic acid (20:5n-3) compared to that of red claw fed diet 3 (0% FM).

Fatty acid composition of the whole-body juvenile red claw indicates that they can store highly unsaturated fatty acids (HUFA). Between 5.5% and 12.0% of the fatty acids in the whole body were comprised of EPA and DHA, although only 5.0–6.0% of both fatty acids were comprised in the diets. Also, 18:1n-9 and 18:2n-6 comprised between 44.0% and 58.5% of the fatty acids in the whole body. The two most predominant saturated fatty acids were 16:0 and 18:0 (Table 9).

3.2. Experiment 2

The mean ± S.D. of each of the measured water quality variables was as follows: water temperature, 28.2 ± 0.9 °C; dissolved oxygen, 6.6 ± 0.3 mg/l; total ammonia nitrogen

Table 9
Means (± S.E.) of fatty acid composition (percent of total) of whole-body red claw fed three practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 1); $n=2$

Fatty acid	Diets		
	1	2	3
16:0	14.18 ± 0.13^{b}	15.60 ± 0.48^{a}	14.75 ± 0.03^{ab}
16:1n-7	1.33 ± 0.41	1.59 ± 0.04	1.55 ± 0.08
18:0	8.25 ± 0.54^{a}	4.81 ± 0.41^{b}	5.34 ± 0.28^{b}
18:1n-9	24.00 ± 0.87	24.22 ± 0.92	26.28 ± 0.55
18:2n-6	20.05 ± 0.20^{b}	32.21 ± 0.67^{a}	32.06 ± 0.18^{a}
18:3n-3	1.37 ± 0.01^{b}	2.40 ± 0.14^{a}	2.50 ± 0.10^{a}
20:4n-6	3.74 ± 0.49^{a}	1.46 ± 0.14^{b}	1.08 ± 0.15^{b}
20:5n-3	7.94 ± 1.38^{a}	4.39 ± 0.11^{ab}	4.07 ± 0.18^{b}
22:6n-3	4.17 ± 0.13^{a}	2.10 ± 0.08^{b}	1.53 ± 0.11^{c}
Others	14.97	11.26	10.87

Means within a row having different superscript letters are significantly different ($P<0.05$).

Table 10
Means (± S.E.) of final individual weight, percentage weight gain, specific growth rate (SGR), and percent survival of Ecuadorian strain red claw fed four practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 2)

	Diets			
	1	2	3	4
Final weight (g)	12.25 ± 1.26	11.04 ± 1.42	11.54 ± 1.18	11.06 ± 1.16
Weight gain (%)	910 ± 115	827 ± 183	1014 ± 138	1154 ± 162
SGR (%/day)	3.13 ± 0.18	2.66 ± 0.28	3.21 ± 0.20	3.31 ± 0.23
Survival (%)	76.0	80.0	80.0	80.0

Means were not significantly different at $P<0.05$.

(TAN), 0.3 ± 0.1 mg/l; nitrite 0.01 ± 0.01 mg/l; alkalinity, 91.4 ± 10.5 mg/l; chlorides, 60.0 ± 8.2 mg/l; and pH, 8.2 ± 0.2. These mean water quality conditions were within acceptable limits for indoor production of red claw crayfish (Masser and Rouse, 1997).

The mean final weight, percentage weight gain, and specific growth rate (SGR) of red claw were not significantly different among all treatments and collectively averaged 11.5 g, 977%, and 3.1%/day, respectively (Table 10). Survival was not significantly different among all treatments and averaged 79%, overall.

Results of the proximate composition analysis of the whole-body red claw are presented in Table 11. Percentage moisture, and protein, and lipid, when expressed on a dry matter basis, were not significantly different among all treatments and averaged 75.5%, 54.1% (13.2%), and 2.8% (0.7%), respectively. When expressed on a dry matter basis, the level of ash (31.8%) contained in red claw fed diet 2 was significantly higher than that of red claw fed diet 1, diet 3, or diet 4, collectively averaging 28.6%.

3.3. Experiment 3

The mean ± S.D. of each of the measured water quality variables was as follows: water temperature, 28.6 ± 1.0 °C; dissolved oxygen, 6.7 ± 0.3 mg/l; TAN, 0.3 ± 0.1 mg/l; nitrite, 0.03 ± 0.03 mg/l; alkalinity, 89.7 ± 13.3 mg/l; chlorides, 48.3 ± 11.4 mg/l; and pH,

Table 11
Mean proximate composition (± S.E.) of whole-body Ecuadorian strain red claw fed four practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 2); $n=3$

	Diets			
	1	2	3	4
Moisture (%)	73.66 ± 0.58	76.65 ± 1.60	74.81 ± 0.77	76.75 ± 1.88
Protein (%)	53.33 ± 0.88	53.49 ± 0.62	53.27 ± 1.30	56.13 ± 0.80
Lipid (%)	3.35 ± 0.53	1.83 ± 0.91	3.13 ± 0.28	2.80 ± 0.01
Ash (%)	28.67 ± 0.83^{b}	31.82 ± 1.20^{a}	28.80 ± 1.11^{b}	28.33 ± 0.25^{b}

Values are expressed on a dry matter. Means within a row having different superscript letter are significantly different ($P<0.05$).

Table 12
Means (± S.E.) of final individual weight, percentage weight gain, specific growth rate (SGR), and percent survival of Australian strain red claw fed four practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 3)

	Diets			
	1	2	3	4
Final weight (g)	16.83 ± 1.39	14.78 ± 0.87	16.78 ± 1.07	16.53 ± 0.83
Weight gain (%)	475 ± 44	438 ± 33	443 ± 51	475 ± 39
SGR (%/day)	2.37 ± 0.12	2.31 ± 0.10	2.27 ± 0.11	2.41 ± 0.09
Survival (%)	93.0	100.0	100.0	100.0

Means were not significantly different at $P<0.05$.

8.2 ± 0.2. These mean water quality conditions were within the acceptable limits for indoor production of red claw (Masser and Rouse, 1997).

The mean final weight, percentage weight gain, and SGR of red claw were not significantly different among all treatments and collectively averaged 16.2 g, 458%, and 2.3%/day (Table 12). Survival was not significantly different among all treatments and averaged 98%, overall.

The results of the amino acid analysis of the tail muscle of the red claw are presented in Table 13. Levels of most amino acids were not significantly different among red claw fed the four diets. The percentages of cystine and methionine in red claw fed diets 1–3 were

Table 13
Mean amino acid composition (± S.E.) of the tail-muscle portion of Australian strain red claw fed four practical diets containing different levels of soybean meal and brewer's grains with yeast (Experiment 3); $n=2$

Amino acid	Diets			
	1	2	3	4
Alanine	0.97 ± 0.02	0.98 ± 0.06	1.02 ± 0.02	0.94 ± 0.01
Arginine[1]	1.75 ± 0.04	1.76 ± 0.17	1.89 ± 0.02	1.25 ± 0.44
Aspartic acid	1.36 ± 0.08	1.42 ± 0.04	1.42 ± 0.03	1.18 ± 0.09
Cystine	0.19 ± 0.01[a]	0.18 ± 0.01[a]	0.19 ± 0.00[a]	0.16 ± 0.01[b]
Glutamic acid	2.75 ± 0.09	2.70 ± 0.24	2.82 ± 0.06	2.45 ± 0.12
Glycine	0.87 ± 0.05	0.85 ± 0.08	0.91 ± 0.05	0.81 ± 0.05
Histidine[1]	0.37 ± 0.02	0.37 ± 0.03	0.39 ± 0.01	0.34 ± 0.02
Isoleucine[1]	0.73 ± 0.04	0.74 ± 0.07	0.78 ± 0.01	0.66 ± 0.03
Leucine[1]	1.28 ± 0.06	1.29 ± 0.11	1.37 ± 0.02	1.17 ± 0.07
Lysine[1]	1.35 ± 0.06	1.34 ± 0.12	1.42 ± 0.03	1.21 ± 0.06
Methionine[1]	0.44 ± 0.02[a]	0.42 ± 0.01[ab]	0.45 ± 0.01[a]	0.39 ± 0.00[b]
Phenylalanine[1]	0.64 ± 0.03	0.65 ± 0.06	0.70 ± 0.02	0.60 ± 0.02
Proline	0.55 ± 0.03	0.59 ± 0.06	0.64 ± 0.01	0.47 ± 0.07
Serine	0.57 ± 0.04	0.58 ± 0.02	0.59 ± 0.02	0.48 ± 0.06
Threonine[1]	0.56 ± 0.04	0.58 ± 0.03	0.59 ± 0.02	0.48 ± 0.06
Tyrosine[1]	0.55 ± 0.03	0.56 ± 0.05	0.59 ± 0.02	0.54 ± 0.01
Valine[1]	0.76 ± 0.03	0.77 ± 0.07	0.82 ± 0.02	0.70 ± 0.03

Means within a row having different superscript letters are significantly different ($P<0.05$).

[1] Essential amino acid.

Table 14
Essential amino acid composition of the diets (percentage of diet) and juvenile red claw tail muscle and essential amino acid index (EAAI) of the diets

Amino acid	Diets				Muscle[a]
	1	2	3	4	
Arginine	1.93	2.22	2.28	2.37	1.66 ± 0.13
Histidine	0.80	0.84	0.85	0.86	0.37 ± 0.01
Isoleucine	1.38	1.51	1.52	1.56	0.73 ± 0.02
Leucine	2.36	2.52	2.54	2.64	1.28 ± 0.04
Lysine	1.86	1.82	1.78	1.77	1.33 ± 0.04
Methionine	0.64	0.54	0.56	0.60	0.42 ± 0.01
Phenylalanine	1.54	1.71	1.72	1.80	0.65 ± 0.02
Threonine	1.30	1.32	1.34	1.41	0.55 ± 0.02
Tyrosine	0.90	1.04	1.01	1.05	0.56 ± 0.01
Valine	1.65	1.75	1.77	1.85	0.76 ± 0.02
EAA index[b]					
Muscle	179.96	188.73	190.05	197.00	

[a] Means of two replications (± S.E.) for muscle tissue. Muscle values are for comparative purposes and are not included in statistical analysis.

[b] $EAAI = \sqrt[n]{[(aa_1/AA_1)(aa_2/AA_2)\ldots(aa_n/AA_n)]}$, where EAAI is the nth root of the essential amino acids in the test diet (aa) to the content of each of those amino acids in the reference tissue (AA) and n is the number of amino acids evaluated.

significantly higher than those of red claw fed diet 4. EAAI for diets in this study ranged from 179 to 197 when compared to red claw muscle (Table 14).

4. Discussion

Results of these studies indicate that a practical diet containing 40% protein and formulated to contain soybean meal, BGY, 5% cod liver oil, 1% corn oil, and 0% FM, without added lecithin and cholesterol, may be adequate for good growth and survival of juvenile (0.2–3.1 g) red claw crayfish. Growth and survival of red claw juveniles in the present studies are comparable to those of previous nutrition studies (Manomaitis, 2001; Thompson, 2002; Thompson et al., 2003). In these studies, average percent weight gain ranged from 297% to 2634% and SGR ranged from 2.0% to 5.9%/day.

The search for economically viable diets with formulations that have ingredients not derived from the marine environment must remain a high priority to ensure the development of sustainable and profitable aquacultural industries (Jones et al., 1996). Although FM has high levels of available energy, excellent amino acid profiles, and is very digestible, it is one of the most expensive ingredients in prepared fish diets. Future prices of fish meal will probably rise due to the fact that the present global supply of FM will likely remain static or decline because world capture fisheries have reached a plateau (New, 1991) despite the increased usage of FM, not only for crustacean culture, but also for the husbandry of finfish, pets, and livestock. Protein sources that can be utilized to replace marine protein sources include terrestrial plant and animal sources.

Of all plant protein ingredients, SBM has been the most extensively evaluated and most commonly used in commercial aquaculture diets (Lovell, 1988; Akiyama, 1991). In marine shrimp, SBM has a higher protein digestibility value than FM, squid meal, and shrimp meal (Akiyama, 1991). Akiyama and FSGP Aquaculture Research (1990) fed tiger prawn (*P. monodon*) diets where plant proteins comprised 72.2% and 50.3% of the total protein (27.8% and 49.7% animal protein, respectively) and stated that no differences in growth or survival were observed after 42 days. Piedad-Pascual et al. (1990) found no significant differences in weight gain of tiger prawn fed different levels of SBM (up to 55% SBM) which completely replaced FM. Tidwell et al. (1993) stated that variable percentages of SBM (25%, 15%, and 26.5%) and 40% of DDGS partially or completely replaced FM in the diets of the freshwater shrimp (*M. rosenbergii*) grown in ponds, so that average yield, survival, and individual weight did not differ among all treatments.

In the present studies, it appears that FM and shrimp meal can be completely replaced by soybean meal (with BGY) without adverse effects on growth and survival. Growth of some fish species has been decreased when fed diets in which all the FM has been replaced with SBM (Webster et al., 1992; McGoogan and Gatlin, 1997). However, Webster et al. (1992) suggested that feeding diets containing a combination of protein sources may allow the use of a high percentage of SBM without growth reduction.

Brewer's grains with yeast does not have any known anti-nutritional factors, such as gossypol; however, published data on the use of brewer's grains with yeast in aquaculture diets are limited. Results from the present studies indicate that brewer's grains with yeast can be included in practical diets for red claw, at levels up to 30%, without adverse effects on growth and survival. This is in agreement with Grant (1985) who evaluated the potential brewer's grain meal (BGM) as a diet ingredient for rainbow trout. Ingredient levels were varied in isonitrogenous and isocaloric open-formula trout diets containing 0, 9.3%, 18.7%, 28.0%, and 37.3% BGM, respectively (up to 100% replacement of plant protein). After the 66-day feeding trial, rainbow trout had no differences in weight gains, feed conversion ratios, and percentage survival among treatments. Grant (1985) concluded that BGM was a good diet ingredient for rainbow trout that could replace soybean and cottonseed meals at relatively high levels (37%).

Diets used in all three experiments of the present studies contained both n-6 and n-3 highly unsaturated fatty acids such as linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids (Table 4), which may have satisfied the essential fatty acid requirements of small juvenile red claw. Weight gain of Japanese prawn, *Penaeus japonicus*, improved when a 1% level of either 18:2n-6 and 18:3n-3 was added to diets containing either oleic acid, pollack (*Theragra chaleogramma*) residual liver oil, soybean oil, or short-necked clam (*Tapes phillipinarium*) lipids (Kanazawa et al., 1977, 1979a,b). Shewbert and Mies (1973) found that a level between 1% and 2% of linolenic acid achieved the best response. Read (1981) found that a 1% addition of either 18:2n-6 or 18:3n-3 to diets fed to *Penaeus indicus* improved growth and survival. Xu et al. (1993, 1994) observed that the relative nutritive value of dietary essential fatty acids for the Chinese prawn (*Penaeus chinensis*) increased from 18:2n-6 to 18:3n-3 to 20:4n-6 to 22:6n-3.

D'Abramo (1997) stated that the HUFA requirement of freshwater crustaceans appears to be approximately 1/10 of the level suggested for marine crustaceans. This lower requirement is probably reflective of the lower HUFA levels that characterize the whole-body tissue and diet of freshwater crustaceans. Weight gain of freshwater prawns (*M. rosenbergii*) was not significantly increased by additions of linoleic or linolenic acid in pure triacylglycerol form either alone or in various combinations (Sheen, 1989). D'Abramo and Sheen (1993) observed the addition of either a mixture of HUFA, or of either 20:4n-6 or 22:6n-3 alone, in diets fed to juvenile freshwater prawns significantly increased weight gain relative to equivalent levels of 18:3n-3 or 18:2n-6. Dietary levels as low as 0.075% were effective in eliciting a significant increase in weight gain relative to a control diet containing a mixture of saturated and mono-unsaturated fatty acids. In the present study, the diets contained higher HUFA levels than 0.075%, therefore the diets were satisfactory for good growth of freshwater crustaceans Sandifer and Joseph (1976) demonstrated superior growth in juvenile freshwater prawns fed diets supplemented with 3% shrimp head oil (rich in 20:5n-3 and 22:6n-3).

The amino acid composition of the diets used in the present studies (Tables 3 and 7) appears to have been adequate for good growth and survival of juvenile red claw crayfish. Decreasing the fish meal content of the diet and substituting soybean meal and BGY caused very little change in EAAI. Tidwell et al. (1993) found decreasing the fish meal content and substituting soybean meal and DDGS caused almost no change in EAAI when diets were compared to freshwater prawn muscle. Piedad-Pascual et al. (1990) compared EAAI ratios in diets containing various levels of defatted soybean meal with the EAAI in tissue of the juvenile tiger shrimp (*P. monodon*). EAAI ranged from 75 to 70, decreasing as the level of fish meal decreased, and SBM increased.

There are no data available on amino acid requirements of red claw and there is very limited information about the essential amino acid dietary requirements of crustaceans. Millamena et al. (1997) determined the threonine requirement for juvenile tiger shrimp. The shrimp were fed amino acid test diets (40% protein) with graded levels of threonine (1.8–5.3% of dietary protein). Results showed that the quantitative threonine requirement for growth is 3.5% of dietary protein or 1.4% of the diet. On the other hand, Teshima et al. (2002) determined that the threonine requirement for the kuruma prawn [*Penaeus (Marsupenaeus) japonicus*] is 2.3% of dietary protein. The amount of threonine in the present diets ranged from 2.96% to 3.30% of dietary protein (1.30–1.46% of the diet). In Experiment 3, the amino acid threonine composition in the tail-muscle portion of red claw was 0.55%.

In Experiment 3, the amino acid composition of the tail-muscle portion of red claw was: 0.37% for histidine, 0.73% for isoleucine, 1.28% for leucine, 0.65% for phenylalanine, 0.43% for methionine, 0.76% for valine, 1.33% for lysine, and 1.66% for arginine (Table 13). Millamena et al. (1999) determined the quantitative requirements of post-larvae tiger shrimp for several other amino acids. They concluded that the optimum dietary requirements for essential amino acids, in percent of diet (and percent of dietary protein), are as follows: 0.8% (2.2%) for histidine, 1.01% (2.7%) for isoleucine, 1.7% (4.3%) for leucine, and 1.4% (3.7%) for phenylalanine. Teshima et al. (2002) reported a lower dietary requirement of the same essential amino acids for the kuruma prawn: 1.1% for histidine, 2.3% for isoleucine, 3.4% for leucine, 2.6% for phenylalanine, 1.3% for methionine, 2.4% for valine, 3.2% for lysine, and 2.9% for arginine, in percent dietary protein, when fed a diet containing 50% protein. All diets fed to red claw in the present study had amino acid values within the levels suggested for either tiger shrimp or kuruma prawn.

In conclusion, it appears that juvenile (0.2–3.1 g) red claw can be fed a diet in which all of the FM has been replaced by 80% SBM and 5% BGY. This may allow for a decrease in diet costs for producers and potentially increase profits, as well as reducing the reliance on FM as an ingredient in diets for red claw crayfish.

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