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Effect on Growth, Survival, and Fatty Acid Composition of Australian Red Claw Crayfish *Cherax quadricarinatus* Fed Practical Diets With and Without Supplemental Lecithin and/or Cholesterol

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Abstract

Red claw crayfish *Cherax quadricarinatus* is one of more than a hundred species of Australian freshwater crayfish. However, because of its rapid growth rate, communal and non-burrowing behavior, ease of spawning, wide temperature and dissolved oxygen tolerance, and no free-swimming larval stages, red claw may be the best candidate among the Australian crayfishes for semi-intensive or intensive aquaculture in the United States. The objective of the study was to examine the effects of growth, survival, and fatty acid composition of newly-hatched red claw when fed four practical diets with or without lecithin and cholesterol. However, little is known of its nutritional requirements or practical diet formulations. An 8-wk feeding trial was conducted in a recirculating system with newly-hatched juvenile (mean individual weight, 0.2 g) red claw, each stocked in separate plastic mesh culture units containing their own individual water line. Water was recirculated through biological and mechanical filters. Practical diets were formulated to be isonitrogenous (40% protein) and isocaloric (4 kcal available energy/g of diet) and contained menhaden fish meal (25%), soybean meal (35%, except in Diet 4), and shrimp meal (10%, except in Diet 4) as protein sources. Diet 1 had 0.5% lecithin added and 1.0% cholesterol added; Diet 2 contained 0% lecithin and 1.0% cholesterol; Diet 3 contained 0.5% lecithin and 0% cholesterol; and Diet 4 contained 0% lecithin and 0% cholesterol.

After 8 wk, juvenile red claw fed diets with 0% supplemental lecithin (Diets 2 and 4) had no significant difference ($P > 0.05$) in final weight and percentage weight gain (5.6 g and 2626%, respectively) compared to red claw fed the control diet (Diet 1) containing 0.5% lecithin. Red claw fed a diet without added cholesterol (Diet 3) had significantly ($P < 0.05$) lower final weight (3.6 g) and percentage weight gain (1,717%) compared to red claw fed the control diet (Diet 1). However, red claw fed Diet 4 (containing 0% added cholesterol and 0% added lecithin) showed no significant ($P > 0.05$) difference in final weight (5.1 g) and percentage weight gain (2,354%) compared to red claw fed all other diets. There was no significant difference ($P > 0.05$) among all diets for specific growth rate (SGR) which averaged 5.38%/d. Percentage survival was not significantly different among all treatments and was 76% for red claw fed Diet 1, 64% (Diet 2), 56% (Diet 3), and 80% (Diet 4). These results indicate that red claw fed Diet 4 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 and that the addition of dietary lecithin and cholesterol may not be necessary for good growth and survival of small (0.2 g) juvenile red claw. This may allow for less expensive diet formulations for use by producers of red claw crayfish.

Cherax quadricarinatus, red claw, is one of more than a hundred species of Australian freshwater crayfish. In terms of aquaculture, only three freshwater crayfish in the genus *Cherax* are commercially farmed in Australia: the yabby *Cherax destructor*; the marron *C. tenuimanus*; and the red claw. Research outside of Australia began during

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the late 1980s after people in the United States discovered that red claw possessed many advantageous characteristics in regard to their potential for U.S. aquaculture including: tolerance of a broad temperature range; tolerance of low dissolved oxygen concentrations (as low as 1 mg/L); achievement of substantial growth rates (100 g in a 6-mo growth season); non-burrowing and non-aggressive nature; acceptance of a wide variety of foods, including prepared diets; physically robust, which allows for ease of handling when out of water; ability to survive for long periods out of water, if kept moist during transit; ease of spawning and no free-swimming larval stages; ease of harvest via its response to water current using flow-traps; high percentage of dress-out meat (30% of the total body weight is edible tail meat), and good comparison in flavor and texture with other crustaceans, including the American lobster *Homorus americanus* (Masser and Rouse 1993; Webster et al. 1994).

The necessity to supplement cholesterol in crustacean diets has been evaluated extensively for both freshwater and marine crustacean species when fed purified, semi-purified, or practical diets. These include the freshwater crayfish *Pacifastacus leniusculus* (D'Abramo et al. 1985), juvenile American lobster (Castell et al. 1975; D'Abramo et al. 1984; Kean et al. 1985; Bordner et al. 1986), Karuma prawn *Penaeus japonicus* (Teshima et al. 1997), and mud crab *Scylla serrata* (Sheen 2000). However, these results are in contrast to reports that supplemental cholesterol is not essential in diets for freshwater prawn *Macrobrachium rosenbergii* (Briggs et al. 1988), red swamp crayfish *Procambarus clarkii* (Lochmann et al. 1992), and adult American lobster (Castell and Covey 1976).

Several crustaceans have been reported to require supplemental lecithin to enhance growth and survival when fed purified or practical diets (Conklin et al. 1980; D'Abramo et al. 1981; Lochmann et al. 1992). However, these results are in contrast to reports that found that supplemental lecithin did not improve weight gain or survival of the juvenile freshwater prawn (Hilton et al. 1984; Briggs et al. 1988; Kanazawa 1993). Since many crustaceans require lecithin and cholesterol to be added to their diet, these two nutrients are usually added to high-quality commercial shrimp diets. However, lecithin and cholesterol are expensive. Since diet costs can be as much as 70% of the total operating expenses for an aquaculture enterprise, it is imperative that the least expensive diet be formulated that meets the nutrient requirements of the species.

There is very little information on the nutritional requirements and practical diet formulations for red claw. Investigation of the nutritional requirements of juvenile red claw crayfish is important since production methods in the U.S. will be based on semi-intensive or intensive culture. Likewise, a complete diet is needed for any intensive indoor nursery operations established for red claw. Currently, there are no data in the literature on the qualitative and quantitative requirements for lecithin and cholesterol for red claw using practical diets under laboratory conditions. The goal of the study was to examine the effects on growth, survival, and fatty acid composition of newly-hatched red claw when fed four practical diets with or without lecithin and cholesterol.

Materials and Methods

Preparation of Diets

In preparing diets, dry ingredients (menhaden fish meal, soybean meal, shrimp meal (if added), wheat flour, corn meal, and carboxymethylcellulose binder) were weighed (Mettler PM 4600, Mettler Instrument Corp., Hightstown, New Jersey, USA) and mixed together for 2 h to ensure they were thoroughly mixed (Table 1). Dietary ingredients (cholesterol (if added), dicalcium phosphate, vitamin mix, mineral mix, vitamin C, and choline chloride) were

TABLE 1. *Ingredient composition of four practical diets with and without added lecithin and/or cholesterol fed to red claw crayfish. Values are percentages of the diet.*

Ingredient	Diets			
	1	2	3	4
Menhaden fish meal (67%)	25.0	25.0	25.0	25.0
Soybean meal (47%)	35.0	35.0	35.0	44.5
Shrimp meal (44%)	10.0	10.0	10.0	0.0
Wheat flour (11%)	16.3	16.3	16.3	16.3
Corn meal	0.0	0.5	1.0	2.0
Cod liver oil	2.0	2.0	2.0	2.0
Corn oil	1.0	1.0	1.0	1.0
Lecithin[a]	0.5	0.0	0.5	0.0
Cholesterol[b]	1.0	1.0	0.0	0.0
Dicalcium phosphate	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
Vitamin C[e]	0.2	0.2	0.2	0.2
CMC[f]	5.0	5.0	5.0	5.0
Choline chloride	0.5	0.5	0.5	0.5

[a] Commercial soybean lecithin (Archer Daniels Midland Co., Decatur, Illinois, USA).

[b] Cholesterol (Sigma Chemical Co., St. Louis, Missouri, USA).

[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, 160 mg; B_6, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; ethoxyquin, 99 mg.

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

[e] Vitamin C (Roche's Stay C at 35% active).

[f] CMC (Carboxymethylcellulose; United States Biochemical Corp., Cleveland, Ohio, USA).

weighed, mixed with the previous dry ingredients for 30 min, and then mixed with water to obtain a 25% moisture level. Diets were then passed through an extruder with a 1-cm die to form "spaghetti-like" strands and air-dried using a convection oven (Grieve Corporation, Round Lake, Illinois, USA). After drying, all diets were broken into pellets of appropriate size and then sieved (2-mm opening mesh) using a U.S.A. standard testing sieve (Fisher Scientific, Pittsburgh, Pennsylvania, USA). After sieving, cod liver oil, corn oil, ethoxyquin (0.2% of lipid), and liquid commercial soy lecithin (L. Colbert, Archer Daniels Midland Co., Decatur, Illinois, USA) (according to treatment) were slowly added to the diet and mixed until all pellets were uniformly coated based on appearance of pellets. Pellets were mixed until all had a sheen from the added lipids. The oils were mixed after pelletizing to prevent destruction of essential fatty acids and other nutrients, such as choline, due to heat generation during processing. Diets were stored in plastic containers in a freezer (−20 C) until fed.

Experimental System and Maintenance

An 8-wk feeding trial was conducted in 100 plastic-mesh culture units (12.7 × 12.7 × 12.7 cm; Plastic Window Breeder-Fine, Lustar Products Company, Springfield, New Jersey, USA) and located within four rectangular fiberglass tanks (236 × 102 × 15 cm) at the Aquaculture Research Center, Kentucky State University. Water was recirculated through a 2,000-L biological and mechanical filtration system containing vertical polyester screens and polyethylene bio-balls to remove solid and nitrogenous wastes. Each culture unit had an individual water tube connected to a plastic aquarium pipe valve that supplied water at a rate of

0.8 L/min. Water temperature was maintained at 27 to 29 C by the use of an immersion heater, and continuous aeration was provided by a blower and air diffuser tubing inside each fiberglass tank. Approximately 5% of the total water volume was replaced daily with dechlorinated tap water. Lighting was provided by overhead fluorescent ceiling lights on a 14:10 h light : dark cycle. Sodium bicarbonate and crushed coral were added in the recirculating system to maintain alkalinity levels between 140 to 160 mg/L. Sodium bicarbonate (baking soda) was added (as needed) when total alkalinity levels dropped below the desired target level. Crushed coral was covered in a nylon sock and remained submersed in the biofiltration unit during the entire 8-wk study.

Each culture unit was cleaned every other day to remove uneaten feed and feces; however, red claw molts were left for the red claw to consume. Water quality parameters were checked three times weekly on water samples collected from the biofilter. Dissolved oxygen was measured using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, Ohio, USA); water temperature was monitored using a thermometer; total ammonia-N, nitrite-N, total alkalinity, and chloride were measured using a DREL/2000 spectrophotometer (Hach Co., Loveland, Colorado, USA); pH was monitored using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA).

Feeding Trial and Experimental Diets

Newly-hatched juvenile red claw 0.2 ± 0.05 g (SD), produced from a single female, were obtained from Auburn University, Auburn, Alabama, and individually stocked into 100 plastic-mesh culture units; 25 culture units per treatment. Juvenile red claw were randomly stocked into all four tanks. A tank was randomly selected and an individual red claw was stocked into a randomly selected culture unit in that tank. This process was continued until all red claw were stocked. One of four practical diets was randomly chosen for each culture unit. Twelve juvenile red claw were individually weighed using an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) prior to stocking to obtain an overall average weight.

Juveniles were fed to excess three times daily (0730, 1230, and 1600 h) one of four practical diets formulated to contain 40% protein based upon tabular values of diet ingredients (Lovell 1998). Practical diets 1, 2, and 3 were formulated to contain 25% menhaden fish meal, 35% soybean meal, 10% shrimp meal, 16.3% wheat flour, 2% cod liver oil, and 1% corn oil (Table 1). Diet 1 served as a control and had 0.5% lecithin and 1.0% cholesterol added; Diet 2 contained 0% lecithin and 1.0% cholesterol; and Diet 3 contained 0.5% lecithin and 0% cholesterol. Since shrimp meal may contain cholesterol and essential fatty acids/phospholipids, Diet 4 was formulated to contain 25% menhaden fish meal, 44.5% soybean meal, 16.3% wheat flour, 0% shrimp meal, 0% lecithin, and 0% cholesterol.

All diets were formulated to be isonitrogenous (40% protein as-fed basis) and isocaloric (4.0 kcal available energy/g of diet). Due to differences in proximate composition of the diet ingredients from tabular values (NRC 1993), diets varied somewhat in actual chemical analysis from calculated values. There were no mortalities within the first week after stocking and no mortalities were subsequently replaced during the study.

Diets were analyzed for percentage moisture, protein, fat, fiber, and ash. Moisture was determined by drying (100 C) until constant weight; protein by macro-Kjeldahl method; lipid by the acid hydrolysis method; fiber by using the fritted glass crucible method; and ash by placing diets in a muffle furnace (600 C) for 24 h (AOAC 1990). Carbohydrate (NFE) was determined by difference [NFE = 100 − (% protein + % lipid + % fiber + % ash)]. Available energy (AE) was calculated from physiologi-

cal 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 were also analyzed for cholesterol, amino acid composition, and fatty acid composition by a commercial analytical laboratory (Woodson-Tenent Lab, Dayton, Ohio, USA).

Growth performance and body analysis of red claw were measured in terms of final individual weight (g), percentage weight gain, specific growth rate (SGR, %/d), percentage survival, and body analysis in terms of fatty acid composition. Growth parameters were calculated as follows: SGR (%/d) = $[(\ln W_t - \ln W_i)/T] \times 100$, 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[(Wt − Wi)/Wi].

At the conclusion of the study, each red claw was individually weighed on an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland). Fatty acid analysis was measured on whole-body samples of red claw. For each treatment, four red claw were randomly sampled and flash-frozen with liquid nitrogen (−196 C). After freezing, red claw legs, claws, and uropods were removed from the body. The remaining whole-body was shattered into pieces and stored in screw-topped glass vials under nitrogen. The glass viles were immediately cap-sealed with teflon tape and then covered in aluminum foil. Samples were stored in a freezer (−30 C) until lipid extraction. There were two replicates per treatment. Fatty acid analysis was conducted at a commercial analytical laboratory (Woodson-Tenent Labs, Dayton, Ohio, USA).

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) using the SAS General Linear Models (GLM) procedure, using SAS software version 8.0 (SAS 1999) for significant differences among treatment means. Duncan's multiple range test was used to compare differences among individual means at the $\alpha = 0.05$ level of significance. A chi-square test was used to determine if survival rate is independent of diet type. All percentage and ratio data were transformed to arc sin values prior to statistical analysis (Zar 1984). Values are presented untransformed to facilitate interpretation.

Results

Over the duration of the study, water quality parameter averaged (± SD): water temperature, 28.0 ± 0.6 C; dissolved oxygen, 7.3 ± 0.20 mg/L; total ammonia-N, 0.21 ± 0.15 mg/L; nitrite-N, 0.03 ± 0.01 mg/L; total alkalinity, 157 ± 36 mg/L; chloride, 61.0 ± 23.6 mg/L; pH, 8.39 ± 0.08. These water quality averages were within acceptable limits for indoor production of red claw (Masser and Rouse 1997).

Proximate composition (which includes percentage moisture, protein, fat, fiber, ash), NFE, available energy, and cholesterol content of the four practical diets are shown in Table 2, amino acid composition of the diets is presented in Table 3, and fatty acid composition (% relative) of the diets is shown in Table 4.

After 8 wk, juvenile red claw fed diets with 0% supplemental lecithin (Diets 2 and 4) were not significantly different in final weight and percentage weight gain (5.6 g and 2,626%, respectively) from red claw fed the control diet (Diet 1) containing 0.5% supplemental lecithin, 1.0% cholesterol, and 10% shrimp meal (Table 5). Red claw fed a diet without added cholesterol (Diet 3) had significantly lower final weight (3.6 g) and percentage weight gain (1,717%) compared to red claw fed the control diet (Diet 1). However, red claw fed Diet 4 (containing 0% added cholesterol and 0% added lecithin) showed no significant difference in final weight (5.1 g) and percentage weight gain (2,354%) compared to red claw fed all other diets. There was no significant difference among all diets for specific growth rate (SGR) which averaged

TABLE 2. *Proximate composition and cholesterol content of practical diets with and without added lecithin and/or cholesterol fed to red claw crayfish.*

	Diets			
	1	2	3	4
Moisture (%)	12.0	11.1	8.3	8.3
Protein (%)[a]	43.91	44.29	44.36	45.90
Lipid (%)[a]	9.27	8.33	7.91	7.03
Fiber (%)[a]	3.07	2.81	2.73	1.50
Ash (%)[a]	12.20	11.14	11.80	8.94
NFE[b]	31.55	33.43	33.20	36.63
Available energy[c]	3.85	3.86	3.81	3.94
Cholesterol (mg/100 g)	815.0	911.0	90.60	68.40

[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, and lipid, respectively.

5.38%/d. Percentage survival was also not significantly different among all treatments and was 76% for red claw fed Diet 1, 64% (Diet 2), 56% (Diet 3), and 80% (Diet 4). During the period between 35 d to 56 d of the study, survival of red claw fed Diet 3 decreased from 80% to 56%; however, the percentage survival of red claw fed the other three diets did not show any dramatic decreases during this period. Red claw fed Diet 1 decreased from 84% to 76%; those fed Diet 2 decreased from 76% to 64%; and those fed Diet 4 had no decrease and remained at 80%.

The results of fatty acid composition of whole-body red claw (% relative) after the 56-d feeding trial are presented in Table 6. There was no significant difference in the percentage of eicosapentaenoic (20:5n–3; EPA) and oleic (18:1n–9) acids in whole-body red claw fed any of the diets. However, red claw fed Diet 1 had significantly

TABLE 3. *Amino acid composition of practical diets with and without added lecithin and/or cholesterol fed to red claw crayfish. Values are percentage of the diet. Values in parentheses are expressed as percentage of dietary protein.*

	Diets			
Amino acid	1	2	3	4
Alanine	1.91 (4.35)	1.93 (4.36)	1.96 (4.41)	2.00 (4.35)
Arginine	2.15 (4.90)	2.19 (4.94)	2.11 (4.75)	2.38 (5.17)
Aspartic acid	3.50 (7.97)	3.46 (7.81)	3.39 (7.64)	3.81 (8.28)
Cystine	0.45 (1.03)	0.45 (1.02)	0.47 (1.06)	0.51 (1.11)
Glutamic acid	5.63 (12.82)	5.63 (12.10)	5.41 (12.18)	6.20 (13.48)
Glycine	1.93 (4.40)	1.94 (4.38)	1.89 (4.26)	2.00 (4.35)
Histidine	1.25 (2.85)	1.21 (2.73)	1.25 (2.82)	1.30 (2.83)
Isoleucine	1.52 (3.46)	1.54 (3.48)	1.48 (3.33)	1.67 (3.63)
Leucine	2.58 (5.88)	2.61 (5.89)	2.52 (5.68)	2.83 (6.15)
Lysine	2.28 (5.19)	2.32 (5.24)	2.22 (5.00)	2.48 (5.39)
Methionine	0.71 (1.62)	0.72 (1.63)	0.75 (1.69)	0.77 (1.67)
Phenylalanine	1.54 (3.51)	1.56 (3.52)	1.51 (3.40)	1.70 (3.70)
Proline	2.26 (5.15)	1.86 (4.20)	1.91 (4.30)	2.05 (4.46)
Serine	1.59 (3.62)	1.60 (3.61)	1.53 (3.45)	1.74 (3.78)
Threonine	1.45 (3.30)	1.45 (3.27)	1.40 (3.15)	1.56 (3.39)
Tyrosine	0.84 (1.91)	0.86 (1.94)	0.84 (1.89)	1.22 (2.65)
Valine	1.79 (4.08)	1.77 (4.00)	1.73 (3.90)	1.89 (4.11)

TABLE 4. *Fatty acid composition (% relative) of practical diets with and without lecithin and/or cholesterol fed to red claw crawfish.*

Fatty acid	Diets 1	2	3	4
14:0	3.05	3.48	3.06	2.34
16:0	23.00	24.60	22.37	17.60
16:1n-7	3.25	3.71	3.25	2.65
18:0	6.50	7.19	6.50	4.98
18:1n-9	24.19	24.36	22.94	19.47
18:2n-6	24.80	20.65	26.20	31.31
18:3n-3	2.24	1.86	2.49	3.27
20:0	0.41	0.46	0.38	0.31
20:1n-9	1.42	1.62	1.34	1.09
20:4n-6	0.61	0.46	0.57	1.09
20:5n-3	1.83	1.86	1.91	3.58
22:0	0.41	0.46	0.38	0.31
22:1n-9	0.81	0.93	0.96	0.62
22:6n-3	4.47	4.87	4.40	7.94
Other fatty acids	3.01	3.49	3.25	3.44

lower percentages of palmitic (16:0) and palmitoleic (16:1n–7) acids compared to all other diets. Red claw fed Diets 1 and 2 had significantly lower percentages of linoleic (18:2n–6) and linolenic (18:3n–3) acids compared to all other diets. Red claw fed Diet 4 had significantly lower percentages of stearic (18:0) and arachidonic (20:4n–6) acids compared to all other diets. Red claw fed Diets 1 and 2 had significantly higher percentages of decosahexaenoic (22:6n–3; DHA) acids compared to those fed all other diets.

Discussion

Results of the present study indicate that supplemental lecithin may not be needed in a practical diet for small (0.2 g) juvenile red claw when fed a diet containing 40% protein and formulated to contain 25% menhaden fish meal, 44.5% soybean meal, 0.5% choline chloride, 2% cod liver oil, and 1% corn oil (Diet 4). This diet may meet any lecithin requirements of newly-hatched juvenile red claw since supplementation of 0.5% lecithin did not increase growth performance significantly. In the present study, the lecithin composition contained 16% phosphatidylcholine (PC), 12% phoshatidylethanolamine (PE), 9% phosphatidylinositol (PI), and 3% phosphatidic acid, with the remaining components consisting of soybean oil, ash, glycolipids, carbohydrates, and water. These results are consistent with a recent study at Kentucky State University (unpublished data) which concluded that lecithin supplementation up to 2.0% did not improve weight gain or survival of young juvenile red claw (1.6 g initial mean weight) when grown individually and fed a semi-purified diet. The present study suggests that supplemental soybean lecithin may not be needed when a complementary blend of fish and corn oils, fish meal (animal protein) and soybean meal (plant protein) mixtures, and the addition of 0.5% choline chloride are provided in practical diet for red claw.

In the present study, a diet without cholesterol (Diet 4) did not adversely affect growth or survival of red claw. When analyzed, the diet had 0.07% cholesterol so it may be that the dietary cholesterol require-

TABLE 5. *Means (± SE) of final individual weight, percentage weight gain, specific growth rate (SGR), and percentage survival of red claw crayfish fed four practical diets with and without added lecithin and/or cholesterol. Means within a row having different superscripts are significantly different (P < 0.05).*

	Diets 1	2	3	4
Final wt. (g)	7.0 ± 1.0^{a}	6.0 ± 0.84^{ab}	3.6 ± 0.8^{b}	5.1 ± 0.8^{ab}
Weight gain (%)	3,384 ± 499^{a}	2,897 ± 422^{ab}	1,717 ± 394^{b}	2,354 ± 396^{ab}
SGR (%/day)	5.74 ± 0.40^{a}	5.66 ± 0.36^{a}	4.68 ± 0.37^{a}	5.42 ± 0.27^{a}
Survival (%)[1]	76.0^{a}	64.0^{a}	56.0^{a}	80.0^{a}

[1] A chi-square test showed no significant ($P > 0.05$) in survival rate with respect to diet type (χ^2 with 3 df = 4.2543; *CV* value = 7.8150).

TABLE 6. *Means (± SE) of fatty acid composition of whole-body red claw (% relative) fed four practical diets with and without added lecithin and/or cholesterol. Values are means for two replications per treatment. Means within a row having different superscripts were significantly different (P < 0.05).*

	Diets			
Fatty acid	1	2	3	4
16:0	14.2 ± 0.13^{b}	15.2 ± 0.08^{ab}	15.9 ± 0.42^{a}	16.3 ± 0.74^{a}
16:1n-7	1.3 ± 0.41^{b}	2.0 ± 0.06^{ab}	1.9 ± 0.12^{ab}	2.3 ± 0.17^{a}
18:0	8.2 ± 0.54^{a}	7.1 ± 0.64^{ab}	7.2 ± 0.62^{ab}	5.8 ± 0.34^{b}
18:1n-9	24.0 ± 0.87^{a}	24.8 ± 0.49^{a}	24.3 ± 0.81^{a}	24.5 ± 0.01^{a}
18:2n-6	20.0 ± 0.20^{b}	19.7 ± 1.10^{b}	23.2 ± 1.30^{ab}	25.8 ± 0.60^{a}
18:3n-3	1.4 ± 0.01^{b}	1.7 ± 0.17^{b}	1.8 ± 0.21^{ab}	2.4 ± 0.13^{a}
20:4n-6	3.7 ± 0.49^{a}	2.80 ± 0.33^{ab}	2.5 ± 0.58^{ab}	1.8 ± 0.06^{b}
20:5n-3	7.9 ± 1.38^{a}	7.1 ± 0.63^{a}	5.9 ± 0.92^{a}	4.4 ± 0.19^{a}
22:6n-3	4.2 ± 0.13^{a}	4.6 ± 0.02^{a}	3.4 ± 0.27^{b}	2.7 ± 0.12^{c}
Other	15.0	14.9	13.8	14.1

ment for red claw is 0.07% or less which is similar to values reported for the freshwater prawn (0.12% of the diet; Briggs et al. 1988) and red swamp crayfish (0.18% of the diet; Lochmann et al. 1992). Cholesterol is thought to be an essential nutrient for development, growth, and survival of all crustacean species. Crustaceans lack the ability to synthesize sterols (Teshima 1972). Sterols are considered essential nutrients for crustaceans because of the important role of cholesterol as a cell constituent and as a metabolic precursor of steroid hormones and molting hormones (Teshima 1972). Studies of several crustacean species have shown that the absence of cholesterol in a diet has increased mortality and reduced growth. For example, D'Abramo et al. (1985) reported that a dietary sterol content between 0.5 and 1.0% (dry weight) is necessary for optimal growth of the juvenile freshwater crayfish *Pacifastacus leniusculus*. They stated that mortality was characterized by the inability of juveniles, particularly larger individuals, to extricate themselves successfully from their old exoskeleton via "molt death syndrome." This condition has been associated with other crustaceans such as the juvenile American lobster (Conklin et al. 1980; Bowser and Rosemark 1981). Juvenile American lobsters have been shown to require a cholesterol range between 0.12 and 0.5% of the diet (Castell et al. 1975; D'Abramo et al. 1984; Kean et al. 1985; Bordner et al. 1986). However, Castell and Covey (1976) found contrasting results as supplement cholesterol did not improve growth in the adult American lobster, 300–600 g in body weight. Teshima et al. (1997) indicated that the optimal dietary cholesterol requirement for juvenile Karuma prawn ranged from 0.26% to 0.6%. Sheen (2000) found that the optimal dietary cholesterol requirement for the juvenile mud crab was 0.51% for maximum growth.

However, other studies have shown that some crustacean species do not require cholesterol in a diet. Briggs et al. (1988) found that juvenile freshwater prawn did not require supplementary cholesterol nor lecithin in a semi-purified diet. There was no advantage by supplementing 0.5 and 1% cholesterol, in combination with either 0% or 5% supplementary lecithin, and these levels proved sufficient to promote growth, survival, and molting rates. They indicated that diets without supplementary cholesterol contained 0.12% cholesterol that was derived from the endogenous cod liver lipid component of the basal diet. Similar results were found in the freshwater red swamp crayfish. Lochmann et al. (1992) indicated that omission of supplementary cholesterol in the diet did not reduce crayfish weight gain and reported that diets without supple-

mental cholesterol (analyzed to be 0.18% of diet) was sufficient to meet or exceed the sterol requirement of juvenile red swamp crayfish because supplementation of 1% cholesterol did not promote additional weight gain.

A possible reason why growth in red claw fed a diet with 0% added cholesterol and 0.5% added lecithin (Diet 3) was significantly different, compared to red claw fed a diet containing 0% added cholesterol and 0% added lecithin (Diet 4), may be due to the fact that some red claw fed Diet 3 appeared to have a slow feeding response or reduced attractiveness of the diet. Daily observations at the start and conclusion of the feeding trial revealed that some red claw fed Diet 3 did not appear to aggressively consume the diet or grow from their initial stocking size. These conflicting results may indicate that some red claw fed Diet 3 never learned how to consume the pellets efficiently. Previous feeding trials (unpublished data) at our laboratory using commercial shrimp diets found this behavior in particularly small (<1.0 g) juvenile red claw. It may be that a high number of slow-feeding newly-hatched juvenile red claw were stocked into culture units representing Diet 3 in the present study. After 5 wk, mortalities increased possibly due to starvation.

Red claw fed Diet 4 (0% added lecithin and 0% added cholesterol) contained a combination of polyunsaturated fatty acids (PUFA), such as linoleic (18:2n–6), linolenic (18:3n–3), and oleic (18:1n–9) acids, and highly unsaturated fatty acids (HUFA) such as eicosapentaenoic (20:5n–3, EPA) and decosahexaenoic (22:6n–3, DHA) acids in the diet (Table 4), which may have satisfied the essential fatty acid requirements of small juvenile red claw.

The results of the present study indicate that small red claw (0.2 g) may not require supplementary lecithin or cholesterol added to a practical diet formulated to contain 25% menhaden fish meal, 44.5% soybean meal, 0.5% choline chloride, 2% cod liver oil, and 1% corn oil. Future studies should continue to evaluate nutritional requirements of red claw using practical diet formulations, such as fish meal replacement, cholesterol requirement, and evaluating the diet formulations from the present study on larger (>1.0 g) red claw.

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