Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus*

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Abstract

Australian red claw crayfish *Cherax quadricarinatus* is considered a popular crustacean species in several countries around the world because of its large size potential and resemblance to high-priced American lobsters. However, little is currently known of the nutrient requirements and practical diet formulations for red claw. Lecithin has been shown to be required in the diets of several crustacean species, but there are no reports of dietary lecithin requirements for red claw. A 10-week feeding trial was conducted in an effort to evaluate lecithin requirements for juvenile red claw. Juvenile red claw (mean individual weight of 1.6 ± 0.91 g) were individually stocked in a recirculating system at random into 80 plastic-mesh culture units, each containing its own individual water line. There were 20 red claw per treatment (diet). Water was recirculated through biological and mechanical filters. Four semi-purified diets were formulated to contain increasing percentages of commercial soya bean lecithin (0, 0.5, 1.0 and 2.0%). Diet ingredients included solvent-extracted menhaden fish meal (FM), casein, dextrin, wheat flour, pellet binder, vitamin and mineral mix, cod liver oil, and corn oil. Semi-purified diets were formulated to contain 40% protein using casein, menhaden FM, and wheat flour as protein sources. After 10 weeks, no significant differences ($P > 0.05$) were found in final weight, percentage weight gain and specific growth rate with average values of 13.0 g, 934%, and 3.14% day^{-1}, respectively. Percentage survival was high during the 10-week period (100, 95, 100 and 95%) as only two individuals died during the study; one of these, because of an escape from the culture unit. There was also no significant differences ($P > 0.05$) in percentage moisture, protein, fat and ash in whole-body red claw carcasses (wet-weight basis) among any treatment (diet) and averaged 77.1, 12.6, 1.3 and 6.2%, respectively. Based upon the present study, these results indicate that a diet containing 5% cod liver oil and 1% corn oil, and having no supplemental lecithin, may be sufficient for growth and survival of juvenile red claw crayfish.

KEY WORDS: *Cherax quadricarinatus*, diet, lecithin, nutrition, red claw crayfish

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Introduction

The Australian red claw crayfish *Cherax quadricarinatus* is considered a popular crustacean species in several countries around the world because of their potential large size and resemblance to high-priced American lobsters *Homarus americanus*. Despite the development of the red claw aquaculture industry in south-east Asia and Central/South America, production is still mainly concentrated in north-eastern Australia. Prices paid to producers for food sales of red claw average US \$5.00–6.00 kg^{-1}, with a premium price paid for larger (>120 g) individuals (C. Jones, Australia, pers. comm.). Currently, red claw production in USA is scattered and small-scale, with product in the 60–120 g range selling for US \$9–14 kg^{-1} (D. Rouse, Auburn University, pers. comm.). Research has shown that red claw may be a viable species for USA aquaculturists considering their many favourable culture traits including: rapid growth rates (100 g in 6 months); adaptability to crowding and nonaggressiveness; tolerance to a broad temperature range and water quality conditions relative to other exotic crustaceans grown in the USA; simplified life-cycle (no larval stage) which eliminates the need for expensive and sophisticated hatcheries for larval rearing; ease of pond-harvest by trapping via their response to water current; absence of major diseases;

consumption of a wide variety of foods including prepared diets (Masser & Rouse 1983; Webster *et al.* 1994).

Phospholipids comprise a group of polar lipids that are added to the diet of crustaceans as a source of energy, cell membrane components, to allow for the emulsification of lipids during digestion and absorption, and are the principal transport form of lipids in the crustacean haemolymph (Lee & Puppione 1978; Gong *et al.* 2001). Phospholipids (lecithin) have been reported to be necessary in the diets of several crustacean species for enhanced growth and survival including: American lobster (Conklin *et al.* 1980; D'Abramo *et al.* 1981); red swamp crayfish *Procambarus clarkii* (Lochmann *et al.* 1992); banana shrimp *Penaeus merguiensis* (Thongrod & Boonyaratpalin 1998); and western white shrimp *Litopenaeus vannamei* (Gong *et al.* 2001). However, these results are in contrast to other reports that stated that supplemental lecithin did not improve weight gain or survival of the freshwater prawn *Macrobrachium rosenbergii* (Hilton *et al.* 1984; Briggs *et al.* 1988; Kanazawa 1993).

In USA, production of red claw may be intensive in nature, as intensive production is the most popular and widely used culture practice. Consequently, red claw grown intensively must rely more on a prepared diet, as little or no natural food items will be present in the pond to support their nutritional requirements. Little is known of the nutrient requirements and practical diet formulations for red claw. As diet costs represent between 30 and 70% of the total operating costs of an aquaculture enterprise, and because lecithin is a relatively expensive dietary ingredient (US $0.50–1.00 kg^{-1} of dietary ingredient; D. Brock, Rangen Inc., pers. comm.), it is important to determine the appropriate lecithin requirement for juvenile red claw in order to keep diet costs as low as possible. Currently, many producers of red claw utilize a modified marine shrimp diet to ensure adequate nutrition. The objective of the present study was to evaluate inclusion of supplemental soya bean lecithin on growth and survival of juvenile red claw crayfish fed casein/menhaden fish meal (FM) protein-based, semi-purified diets.

Materials and methods

Preparation of diets

Four semi-purified diets were formulated to contain increasing percentages of commercial soya bean lecithin (0, 0.5, 1.0 and 2.0%). In preparing diets, dry ingredients [casein, solvent-extracted menhaden FM, dextrin, wheat flour, dicalcium phosphate and carboxymethyl-cellulose (CMC)] were weighed for each diet and mixed together for 2 h (Table 1). Menhaden FM was extracted four times with a 2 : 1 (v/v) ethyl alcohol (95%) to FM for each extraction, followed by one extraction of 3 : 1 (v/v), and a final extraction with a 4 : 1 (v/v) ratio. During each extraction, FM was completely mixed with ethyl alcohol and allowed to settle for 10 min, followed by decanting of ethyl alcohol from the FM. After the final extraction, solvent-extracted FM was air-dried under a fume hood for 24 h and then stored in a freezer (−20 °C) until needed.

Table 1 The percentage composition of the four semi-purified diets fed to red claw crayfish

	Diets (percentage of lecithin)			
Ingredient (g kg^{-1})	1 (0)	2 (0.5)	3 (1.0)	4 (2.0)
Casein	200	200	200	200
Menhaden FM[1]	300	300	300	300
Dextrin	80	80	80	80
Wheat flour	228	223	218	208
Cod liver oil	50	50	50	50
Corn oil	10	10	10	10
Vitamin mix[2]	20	20	20	20
Mineral mix[3]	5	5	5	5
Vitamin C (Stay C-35%)	2	2	2	2
Dicalcium phosphate	20	20	20	20
CMC[4]	70	70	70	70
Cholesterol[5]	10	10	10	10
Lecithin[6]	0.0	5	10	20
Choline chloride	5	5	5	5

[1]Solvent extracted with ethyl alcohol.
[2]Vitamin mix was the Abernathy vitamin premix number 2 and supplied the following (mg or IU kg^{-1} of diet): biotin, 0.60 mg; B_{12}, 0.06 mg; E (as α-tocopheryl acetate), 50 IU; folic acid, 16.5 mg; myo-inositol, 132 mg; K (as menadione sodium bisulphate complex), 9.2 mg; niacin, 221 mg; pantothenic acid, 106 mg; B_6, 31 mg; riboflavin, 53 mg; thiamine, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; ethoxyquin, 99 mg.
[3]Mineral mix was Rangen trace mineral mix F1 for catfish with 0.3 mg selenium kg^{-1} diet added.
[4]CMC (United States Biochemical Corp., Cleveland, OH, USA).
[5]Cholesterol (Sigma Chemical Co., St Louis, MO, USA).
[6]Lecithin (Archer Daniels Midland Co., Decatur, IL, USA).

Dietary ingredients (mineral mix, vitamin mix, vitamin C, cholesterol and choline chloride) were weighed, mixed with the previous dry ingredients for 30 min, and then mixed with water to obtain a 40% moisture level. Diets were then passed through an extruder with a 1.2-cm die to form 'spaghetti-like' strands and air-dried using a convection oven (Grieve Corporation, Round Lake, IL, USA). After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills, Inc., Clifton, NJ, USA). Diets were sieved (2 mm opening mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburgh, PA, USA), and liquid commercial soya bean lecithin (L. Colbert, Archer Daniels Midland Co., Decatur, IL, USA), cod liver oil, corn oil and ethoxyquin

Table 2 Mean (±SE) of the fatty acid composition (percentage of relative) of semi-purified diets fed to red claw crayfish. Values are means of two replications per diet

	Diet (percentage of lecithin)			
Fatty acid	1 (0)	2 (0.5)	3 (1.0)	4 (2.0)
C14:0	2.24 ± 0.01	2.23 ± 0.01	2.17 ± 0.0	2.00 ± 0.00
C16:0	15.25 ± 0.01	15.51 ± 0.01	15.48 ± 0.02	15.27 ± 0.02
C16:1*n*–7	2.86 ± 0.01	2.80 ± 0.00	2.73 ± 0.01	2.53 ± 0.01
C18:0	4.56 ± 0.01	4.67 ± 0.00	4.69 ± 0.01	4.63 ± 0.01
C18:1*n*–9	19.41 ± 0.01	19.30 ± 0.00	19.04 ± 0.06	18.87 ± 0.00
C18:2*n*–6	32.00 ± 0.18	32.29 ± 0.02	32.68 ± 0.11	34.73 ± 0.06
C18:3*n*–3	4.29 ± 0.01	4.33 ± 0.00	4.43 ± 0.01	4.73 ± 0.00
C20:0	0.34 ± 0.01	0.32 ± 0.0	0.32 ± 0.00	0.33 ± 0.03
C20:1*n*–9	1.07 ± 0.01	1.06 ± 0.00	1.04 ± 0.00	0.97 ± 0.00
C20:4*n*–6	1.05 ± 0.01	1.01 ± 0.00	1.01 ± 0.01	0.92 ± 0.00
C20:5*n*–3	3.64 ± 0.04	3.50 ± 0.01	3.47 ± 0.03	3.23 ± 0.02
C22:0	0.30 ± 0.00	0.32 ± 0.00	0.33 ± 0.01	0.34 ± 0.01
C22:1*n*–9	0.67 ± 0.01	0.71 ± 0.01	0.69 ± 0.00	0.63 ± 0.01
C22:6*n*–3	6.49 ± 0.07	6.24 ± 0.07	6.26 ± 0.04	5.72 ± 0.02
Other	5.83	5.71	5.66	5.10

(0.2% of lipid) were added to the diet and mixed until all pellets were uniformly coated. Diets were stored in plastic container's in a freezer (−20 °C) until fed. Diets were analysed for fatty acid composition by a commercial analytical laboratory (Woodson-Tenent Labs, Dayton, OH, USA) and are shown in Table 2.

Experimental system and maintenance

A 10-week feeding trial was conducted in 80 individual plastic-mesh culture units (12.7 × 12.7 × 12.7 cm; Plastic Window Breeder-Fine; Lustar Products Company, Springfield, NJ, USA) and located within four rectangular, fiberglass tanks (236.22 × 101.6 × 15.24 cm) at the Aquaculture Research Center, Kentucky State University, KY, USA. Water was recirculated through a 2000-L biological and mechanical filtration system containing vertical polyester screens and polyethylene bio-balls to remove unwanted solids and provide substrate for living *Nitrosomonas* and *Nitrobacter* bacteria. Water was supplied to each culture unit at a rate of 0.8 L min^{-1} via individual water lines connected to plastic aquarium pipe valves. Water temperature in the recirculating system was maintained at 27–29 °C through the use of water heaters. Continuous aeration in the culture system was provided by a blower and diffuser tubing inside the fiberglass tanks. Approximately 5% of the total water volume was replaced daily with dechlorinated city water. Lighting was provided by overhead fluorescent ceiling lights on a 14 : 10 h light : dark cycle. Sodium bicarbonate and crushed coral were added to the recirculating system to maintain alkalinity levels.

All culture units were siphoned every other day to remove uneaten diet and wastes while leaving red claw molts. Water quality parameters were checked three times weekly. Dissolved oxygen was measured using a YSI Model 58 oxygenmeter (YSI Industries, Yellow Springs, OH, USA); water temperature was measured using a thermometer; total ammonia nitrogen, nitrite, total alkalinity and chloride were measured using a DREL/2000 spectrophotometer (Hach, Loveland, CO, USA); pH was monitored using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH, USA).

Feeding trial

Juvenile (0.2 g) red claw were obtained from Auburn University, Auburn, Alabama, and grown until they averaged 1.6 ± 0.91 g (SD). Individuals were stocked at random into 80 plastic-mesh culture units. There were 20 red claw per treatment (diet) and each culture unit was considered a replication, respectively. Individual weights of each juvenile red claw were measured using an electronic scale (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) prior to stocking. Juveniles were fed to excess three times daily (07:30, 12:30, and 16:00 hours) for 10 weeks. Semi-purified diets were formulated to contain 40% protein using casein, menhaden FM, and wheat flour as protein sources. Mortalities was monitored daily and replaced during the first week of the study, with no replacements thereafter.

At the end of 10 weeks, each red claw was individually weighed and sexed. For each treatment, six females and six males (48 total) were randomly sampled, chill-killed using an ice-water bath, and analysed for body composition. After

chill-killing, legs and claws were removed from the body, and the whole-body diced with a cleaver. There were two replicates per sex per treatment. Proximate analysis was conducted at a commercial analytical laboratory (Woodson-Tenent Labs, Dayton, OH, USA).

Growth performance of red claw were measured in terms of final individual weight (g), percentage weight gain, specific growth rate (SGR % day^{-1}), and percentage survival. Whole-body composition of red claw was measured for percentage moisture, protein, fat and ash. Moisture was determined by placing a 2-g sample in an oven (125 °C) for 4 h and dried until constant weight. Protein was determined by the nitrogen analyser (AOAC method 990.03); lipid was determined by ether extraction; and ash was determined by placing a sample in a muffle furnace at 600 °C for 4 h until constant weight (AOAC 1995).

Growth parameters were calculated as follows: SGR (% day^{-1}) = $[(\ln W_t - \ln W_i)/T] \times 100$, 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 (%) = $100\ [(W_t - W_i)/W_i]$.

Statistical analysis

Data was analysed by analysis of variance (ANOVA) using the SAS General Linear Models procedure, using SAS software version 8.0 (SAS 1999). Duncan's multiple range test was used to compare differences among individual means at the $P = 0.05$ level of significance. Broken-line regression was used to determine the break-points in the growth curve which estimated the lecithin requirement of red claw (Robbins *et al.* 1979). This model assumes that when the lecithin requirement is met, growth rate reaches a plateau. Percentage and ratio data were arcsine-transformed prior to statistical analysis (Zar 1984). Values are presented untransformed to facilitate interpretation.

Results

Water quality

Over the duration of the study, water quality parameter averaged (±SD): water temperature, 27.7 ± 0.49 °C; dissolved oxygen, 7.47 ± 0.52 mg L^{-1}; total ammonia nitrogen, 0.24 ± 0.12 mg L^{-1}; nitrite, 0.03 ± 0.02 mg L^{-1}; alkalinity, 169 ± 75.5 mg L^{-1}; chlorides, 74.5 ± 18.1 mg L^{-1}; pH, 8.35 ± 0.28. These water quality values were within acceptable limits for indoor production of red claw (Masser & Rouse 1997).

Growth

There were no significant difference ($P > 0.05$) in initial weight, and after 10 weeks final mean weight, percentage weight gain and mean SGR were not significantly different ($P > 0.05$) among treatments with parameters averaging 13.0 g, 934%, and 3.14% day^{-1}, respectively (Table 3). Percentage survival was high during the 10-week period (100, 95, 100 and 95%) (Table 3) as only two individuals died during the study; one of these due to an escape from the culture unit. There were no significant differences ($P > 0.05$) in percentage moisture, protein, fat, or ash in whole-body red claw carcass (wet-weight basis) among any treatment (diet) with values averaging 771, 126, 13 and 62 g kg^{-1}, respectively (Table 4).

Overall mean of final individual weight, percentage weight gain and SGR among 36 males and 42 females harvested were not significantly different ($P > 0.05$) among all treatments with averages of 12.58 and 13.45 g (for males and females, respectively), 901 and 964% (for males and females, respectively), and 3.05 and 3.22% day^{-1} (for males and females, respectively). However, when analysed by treatment (diet), female red claw fed diet 3 had significantly higher ($P < 0.05$) percentage weight (1143%) and SGR

Table 3 Mean (±SE) of initial individual weight, final individual weight, percentage weight gain, SGR, and percentage survival of red claw crayfish fed four semi-purified diets with various percentages (0, 0.5, 1.0 and 2.0) of soya bean lecithin

	Diet (percentage of lecithin)			
Parameter	1 (0)	2 (0.5)	3 (1.0)	4 (2.0)
Initial weight (g)	1.35 ± 0.20	1.73 ± 0.22	1.74 ± 0.22	1.46 ± 0.19
Final weight (g)	12.19 ± 1.14	12.89 ± 1.16	14.79 ± 1.57	12.27 ± 1.35
Weight gain (%)[1]	1079 ± 152	904 ± 136	866 ± 103	888 ± 113
SGR (% day^{-1})	3.32 ± 0.18	3.04 ± 0.21	3.10 ± 0.15	3.10 ± 0.17
Survival (%)	100	95.0	100	95.0

[1]Due to variability of individual red claw final weights, determination of percentage weight gain cannot accurately be calculated using the treatment means for final weight and initial weight. Percentage weight gain was calculated for each individual red claw from their respective dietary treatments based upon each individual's initial and final weights.

No significant differences ($P > 0.05$) were found for any listed parameter.

Table 4 Mean (±SE) of whole-body dl moisture, protein, fat and ash (wet-weight basis) of red claw crayfish fed four semi-purified diets with various percentages (0, 0.5, 1.0 and 2.0) of soya bean lecithin. Values are means of four replications per diet (g kg^{-1})

	Diet (percentage of lecithin)			
	1 (0)	2 (0.5)	3 (1.0)	4 (2.0)
Moisture (g kg^{-1})	764 ± 9	777 ± 15	788 ± 6	753 ± 12
Protein (g kg^{-1})	132 ± 5	123 ± 5	119 ± 3	129 ± 5
Fat (g kg^{-1})	11 ± 1	11 ± 2	12 ± 3	16 ± 3
Ash (g kg^{-1})	64 ± 3	59 ± 7	55 ± 1	68 ± 3

No significant differences ($P > 0.05$) were found among all treatments.

Table 5 Mean (±SE) of initial individual weight, final individual weight, percentage weight gain and SGR of male (M) and female (F) red claw crayfish fed diets containing various (0, 0.5, 1.0 and 2.0) percentages of lecithin

		Diet (percentage of lecithin)			
Variable	Sex	1 (0)	2 (0.5)	3 (1.0)	4 (2.0)
Initial weight (g)	M	1.29 ± 0.29a	1.93 ± 0.31a	1.82 ± 0.34a	1.38 ± 0.25a
	F	1.39 ± 0.28a	1.55 ± 0.32a	1.67 ± 0.28a	1.54 ± 0.28a
Final weight (g)	M	12.27 ± 0.98a	14.52 ± 2.14a	11.78 ± 1.78a	11.82 ± 1.51a
	F	12.12 ± 1.83a	11.41 ± 0.96a	17.80 ± 2.28a	12.67 ± 2.24a
Weight gain (%)[1]	M	1257 ± 310a	857 ± 197a	589 ± 70b	976 ± 198a
	F	960 ± 150a	947 ± 197a	1143 ± 151a	808 ± 125a
SGR (% day^{-1})	M	3.48 ± 0.32a	2.94 ± 0.35a	2.69 ± 0.15b	3.17 ± 0.29a
	F	3.21 ± 0.21a	3.14 ± 0.26a	3.51 ± 0.17a	3.03 ± 0.20a
Number of males		8	9	10	9
Number of females		12	10	10	10

[1]Due to variability of individual red claw final weights, determination of percentage weight gain cannot accurately be calculated using the treatment means for final weight and initial weight. Percentage weight gain was calculated for each individual red claw from their respective dietary treatments based upon each individual's initial and final weights.

Mean between males and females in the same column for each variable with different letters are significantly different ($P < 0.05$).

(3.51% day^{-1}) compared with male red claw (589 and 2.69% day^{-1}), respectively (Table 5). Broken-line regression analysis showed that the four diets had no distinct impact on final weight.

Discussion

Although dietary lecithin is an essential nutrient for some crustaceans, our study indicates that red claw juveniles fed a semi-purified diet containing up to 2% of a commercial soya bean lecithin did not improve growth or survival compared with the basal diet lacking supplemental lecithin. These results suggest that a diet containing 5% cod liver oil and 1% corn oil, and having no supplemental lecithin, may be sufficient for growth and survival of juvenile red claw crayfish. Red claw fed the basal diet had 100% survival and slightly higher percentage weight gain and SGR compared with red claw fed the other diets, although differences were not statistically different.

In the present study, growth rates and survival of red claw fed all diets were similar to, or higher than, values previously reported in literature. It has been reported that red claw (average initial weight 3.22 g), grown in individual containers for 10 weeks and fed four protein levels (24, 31, 37 and 44%), had mean SGRs of 1.73, 2.00, 1.92 and 1.87% day^{-1} and survival percentages were 80, 87, 100 and 100% for the four diets, respectively (L. Manomaitis, Auburn University, pers. comm.).

Phospholipids from soya bean lecithins are mixtures of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid which may vary in purity and composition and that PC has been assumed to be the major active component in lecithin for enhancing growth. However, the active components of soya bean lecithin is unclear. Gong *et al.* (2001) reported that growth-enhancing effects of juvenile shrimp *L. vannamei* fed three different types of commercial soya bean lecithin with various percentages of PC levels (21.7, 12.5 and 7.5) were not different, and suggested that PL other than PC may also affect shrimp growth. In our study, the lecithin composition of our commercial lecithin contained 16% (PC), 12% (PE), 9% (PI) and 3% phosphatidic acid, with the remaining components consisting of soya bean oil, ash, glycolipids, carbohydrates and water.

It has been established that crustaceans can synthesize phospholipids, such as PC. However, some crustacean species require a dietary source of phospholipids for good growth and survival. The need for supplementary phospholipids has been demonstrated in various crustacean species. Conklin *et al.* (1980) found that the inclusion of at least 7.5% (dry-weight basis) of soya lecithin in a casein-based purified diet was critical for the survival of juvenile American lobsters. The absence of supplemental soya bean lecithin dramatically reduced survival and caused 'molt death syndrome' which is characterized by the animal's inability to successfully complete the molting process. Gong *et al.* (2001) evaluated the dietary phospholipid requirements of the juvenile western white shrimp *L. vannamei* fed semi-purified diets. They concluded that juvenile shrimp had increase growth when supplemental soya bean lecithin was added at 3–5% of the diet. Lochmann *et al.* (1992) reported that red swamp crayfish fed a practical diet without supplemental lecithin had reduced weight gain compared with crayfish fed the complete diet containing 6% lecithin.

Although the beneficial effect of soya bean lecithin on marine shrimp growth has been reported by some investigators, results from the present study are in agreement with those of the freshwater prawn, *M. rosenbergii*. Hilton *et al.* (1984) indicated that a semi-purified diet containing up to 10% lecithin with 6% capelin oil was not required in postlarval prawns when grown communally. Briggs *et al.* (1988) demonstrated that a diet containing no supplemental lecithin and 6% cod liver oil (0.048% total phospholipids), had growth and survival comparable with those fed diets containing 5% supplementary soya lecithin. Briggs *et al.* (1988) stated that supplementary lecithin was not essential in semi-purified diets, and phospholipid levels should be readily achievable in practical dietary formulations which could result in cost savings in future diet formulated for juvenile freshwater prawn. Kanazawa (1993) reported that adding 1 and 2% soya bean lecithin to diets containing either casein-based or crab protein-based diets did not improve growth or survival of the freshwater prawn. Kean *et al.* (1985) suggested that the American lobster may have a limited capacity for PC synthesis, but that supplemental lecithin was not necessary if the diet had crab protein, instead of casein, as the protein source.

Conflicting reports for a need of supplemental lecithin in some crustacean diets, both fresh and saltwater, may have several possible explanations. One possible reason for not observing a lecithin requirement for the levels used in the present study may be the inclusion of cholesterol in the diet. As the cholesterol requirement of red claw is not known, and lack of dietary cholesterol may result in reduced growth and/or survival in crustaceans (Kean *et al.* 1985), it was decided to add cholesterol to the diets. Gong *et al.* (2001) stated that juvenile western white shrimp, *L. vannamei* required 3.0% lecithin in a diet and that this level was higher compared with other species because of their use of cholesterol-free diets. Teshima *et al.* (1986b) assumed that dietary phospholipids may provide specific lipid classes in the formation of lipoproteins and that dietary phospholipids improved the mobilization of cholesterol from the gut to the hepatopancreas, haemolymph and muscle in *Penaeus japonicus*. Further, dietary phospholipids, especially PC, was hypothesized to act as an acyl donor for lecithin–cholesterol acyltransferase which converts free cholesterol into a sterol ester. Thus, lecithin–cholesterol–lipoprotein would interact in a beneficial manner for improved shrimp growth (Teshima *et al.* 1986c).

While this interaction may exist, it is yet unproven. Gong *et al.* (2001) did not evaluate diets with supplemental cholesterol to determine the validity of a lecithin–cholesterol interaction. Indeed, Gong *et al.* (2000) reported no interaction between soya bean lecithin and cholesterol in western white shrimp and stated that phospholipids other than PC, such as PE and PI, were active components of soya bean lecithin. Other reports have concluded that no interaction between lecithin and cholesterol occurs. Teshima *et al.* (1982) suggested that the improved growth and survival observed in *P. japonicus* fed diets containing supplemental cholesterol were not affected by dietary levels of soya bean phospholipid. Kean *et al.* (1985) found no interaction between added lecithin and cholesterol in juvenile American lobsters *H. americanus* when fed diets containing 0, 3, or 6% soya lecithin and 0, 0.25, 0.5 and 1.0% cholesterol. These authors stated that a protein (amino acid) and lecithin interaction might be involved. Briggs *et al.* (1988) likewise reported no interaction between supplemental cholesterol and lecithin in diets for freshwater prawn *M. rosenbergii*. Addition of three levels of cholesterol (0, 0.5 and 1.0%) and two levels of lecithin (0 and 5%) did not improve final weight, percentage weight gain, or mean moult rate of juvenile freshwater prawn. Chen & Jenn (1991) fed the marine shrimp, *P. penicillatus*, diets containing 0, 0.5, or 1.0% cholesterol and 0, 1.25, 2.5 and 5% soya PC and found no interaction.

With the interaction between lecithin and cholesterol vaguely defined and largely unproven, and the cholesterol requirement unknown in red claw, it is felt that addition of cholesterol to diets used in the present study was warranted. Based upon previous reports (Kean *et al.* 1985; Briggs *et al.* 1988; Chen & Jenn 1991; Gong *et al.* 2000), it appears that addition of cholesterol to diets should have had little effect on lecithin requirement for juvenile red claw.

A second possible reason for our results could be that choline may be an important vitamin for some crustaceans to achieve good growth and survival. Diets that are not very water stable could be susceptible to choline leaching. Lecithin, which has PC as one of its constituents, could serve as a source for choline and thus supply the vitamin. Liu *et al.* (1993) reported that the fleshy prawn *Penaeus chinensis* required 4000 mg kg^{-1} diet of choline for good growth and survival. In our study, 0.5% choline chloride was added in the diets which may have been sufficient in meeting the choline requirement of juvenile red claw. Further, lipid was top-dressed onto the diet pellets after processing which may have offered additional water stability so that leaching of nutrients was minimized.

A third possible reason for our results could be that a blend of 5% cod liver oil and 1% corn oil added to the diets met the essential fatty acid requirements of juvenile red claw. As phospholipids are essential components of cell membranes, and essential fatty acids may be required by crustaceans for proper growth and survival, addition of the two lipid sources to diets fed in our study may have negated any need for fatty acids from lecithin. These lipids provided a good mixture of polyunsaturated fatty acids such as linoleic (18:2*n*−6), linolenic (18:3*n*−3), and oleic (18:1*n*−9) acids, and highly unsaturated fatty acids such as eicosapentaenoic (20:5*n*−3) and docosahexaenoic (22:6*n*−3) acids (Table 2).

A fourth possible explanation for our results could be that some crustaceans require sufficient amounts of lecithin (phospholipids) in their diet to increase digestion and absorption via emulsification of lipids. Other studies have suggested that the addition of dietary lecithin increases the transport rates of lipids out of the midgut (hepatopancreas) and into the haemolymph (D'Abramo *et al.* 1985; Teshima *et al.* 1986a). While some crustaceans may require lecithin to mobilize lipids from the midgut to haemolymph, red claw may not.

Finally, it could be that the dietary protein sources used in the present study may have been sufficient to result in good growth and survival of red claw. Kean *et al.* (1985) reported that juvenile American lobster did not have a requirement for dietary lecithin when rock crab *Cancer irroratus* muscle was used as the principal protein source, instead of casein. These results are contrary to previous reports (Conklin *et al.* 1980; D'Abramo *et al.* 1981) which used casein as the primary protein source. Kean *et al.* (1985) suggested that the casein-based purified diets may have insufficient levels of certain dietary amino acids for optimal American lobster growth compared with the rock crab protein. However, Mu *et al.* (2000) stated that protein digestibility and amino acid availability of casein was similar to FM, shrimp meal, soya bean meal, Spirulina meal and gelatin in juvenile Chinese hairy crab *Eriocheir sinensis* and suggested that amino acid availability values are more useful than protein digestibility values for comparison of protein quality. In our study, a blend of casein and solvent-extracted menhaden FM was added in the diet, which may have resulted in a balanced amino acid composition for red claw.

In the present study, female red claw fed diet 3 had significantly higher percentage weight gain and SGR percentage compared with male red claw fed the same diet. These results are consistent with other studies (L. Manomaitis, Auburn University, pers. comm.) which found that female red claw had higher weight gain compared to male red claw when fed all treatments, and had significant difference in weight gain ($P < 0.05$) at the 44% protein level than lower protein diets. However, it is not clear why females fed diet 3 (inclusion of 1.0% lecithin) had higher percentage weight gain and SGR values. There was no significant difference in red claw initial weights between males and females, ratios of males and females were equal, and diet 3 had 100% survival after 10 weeks. In addition, red claw fed diets 1 (0% lecithin), 2 (0.5% lecithin) and 4 (inclusion of 2.0% lecithin) had no significant difference ($P > 0.05$) in final weight, percentage weight gain, and SGR percentage when compared by sex (Table 5).

In conclusion, these results indicate that a diet containing a blend of 5% cod liver oil, 1% corn oil, 0.5% choline chloride added to the diet, and having no supplemental lecithin, may be sufficient for red claw crayfish. There were no differences in growth or body composition of red claw fed diets containing 0% lecithin and with 0.5, 1.0 and 2.0% lecithin added. The discrepancies between the present study and some other reports may arise from the difference in species, developmental state, or experimental conditions. Eliminating lecithin in the diet may allow for less-expensive diet formulations for red claw and may reduce diet costs for producers, who may feed a marine shrimp diet, thereby increasing returns. As diet cost represents the largest variable cost in a commercial aquaculture operation, increased knowledge of specific dietary requirements is essential for formulating a cost-effective commercial red claw diet. Future research areas should evaluate diets with higher percentages of lecithin and/or reduced levels of lipids, continue to assess specific nutrient requirements, such as cholesterol, and examine practical diet formulations of red claw.

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