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Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish (*Cherax quadricarinatus*)

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Abstract

Six practical diets containing increasing percentages of crude protein (CP) (30%, 35%, and 40%) with or without anchovy fish meal (FM) were fed to juvenile red claw crayfish (mean individual weight=1.12 g) during an 8-week feeding trial. Growth, survival, feed conversion ratio, and amino acid composition of tail-muscle meat of juvenile red claw were determined.

At the conclusion of the experiment, specific growth rate (SGR) and percent survival among treatments, which averaged 3.91%/day and 80.7%, overall, were not significantly different among treatments. The percent weight gain of red claw fed a diet containing 20% fish meal and 40% crude protein was significantly higher (1352%) than that of red claw fed a diet containing 0% fish meal and 30% crude protein (828%), but not different from red claw fed all other diets. Red claw fed Diet 3 had significantly higher FCR (5.73) compared to red claw fed Diet 6 (3.03) but not different from red claw fed the other four diets.

Results from this study indicate that juvenile red claw can be fed a practical diet containing 35% CP with 0% FM if a combination of less expensive plant protein ingredients (SBM, wheat, BGY, and milo) is added. CP levels can be reduced to 30% if 15% anchovy fish meal is included. Reducing CP levels and the reliance of fish meal in Australian red claw diets may help reduce operating costs and thereby increase producers profits.

Keywords: Red claw crayfish; *Cherax quadricarinatus*; Protein level; Fish meal; Diet

1. Introduction

Currently, the Australian red claw crayfish is being considered an aquaculture species in the United States and is commercially cultured in several countries, including Mexico and Australia. Research results have shown that red claw possess many biological and market prerequisites necessary to achieve successful culture. Red claw are fed inexpensive diets and grow rapidly (65–90 g) in a limited (117 days) growing season in temperate climate ponds (Thompson et al., 2004). They possess flexible eating habits, are highly

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marketable because they resemble marine lobsters, are generally nonaggressive in nature, and can command a high price ($13–17 per kg) in an established market. Further, there is no post-hatch larval phase, thereby precluding the requirement for larval culture as found for other crustacean species (Jones, 1995). Currently, the red claw commercial enterprise in the US is very small principally because hatchery-grown stock are not readily available. Growers must purchase stocking-size individuals from hatcheries in other countries (Australia or Mexico) and cost can range from $0.40–0.50 per juvenile. Recent efforts have been directed to determine specific nutrient requirements and evaluate inexpensive practical diets to help reduce production costs and possibly increase profits (Jacinto et al., 2003; Thompson et al., 2003a,b, 2004; Muzinic et al., 2004).

Protein is generally the most expensive component in a prepared diet for aquaculture. Lim (1997) stated that protein is the most critical ingredient in practical shrimp diets based upon cost and growth response. As protein levels influence diet costs, and diet costs can represent up to 70% of the total operating costs of an aquaculture enterprise, it is imperative that diets containing different percentages of protein be evaluated to determine the minimal level that meets essential amino acid requirements and contribute to maximum growth. Jacinto et al. (2003) reported that a practical diet containing 31% crude protein (CP) seems adequate for 1 g juvenile red claw, while Webster et al. (1994) reported that newly hatched (mean weight=0.022 g) red claw required 33% CP. However, both of these studies were conducted in aquaria, where the red claw were grown communally, and may have had access to supplemental nutrients when mortalities occurred in the culture system. To determine the protein requirement for red claw, individual culture units are needed to eliminate the possibility of the organism obtaining additional nutrients.

Fish meal (FM) is considered the most desirable animal protein ingredient in nutritionally complete diets for fish and crustaceans due to its high protein content and digestibility, serving as an excellent source of essential fatty acids and energy, and its high palatability. As FM is one of the most expensive macro-ingredients (used in high percentages) in an aquaculture diet, proportional reductions of FM in aquaculture diets is a current major research priority. Fish meal prices have risen and are likely to increase further with continued growth in demand. The response has been a steady rise in cost and an intense focus on finding substitutes. Naylor et al. (2000) stated that farm-raised fish fed FM also has produced negative publicity from the detection of industrial pollutants (PCBs and dioxin) that create the perception that aquaculture is not ecologically sound. Long-term availability of FM in the future is questionable. Thus, formulations of production diets will need to partially, or totally, reduce the percentages of FM without adversely affecting the growth and health of the cultured aquaculture species.

As red claw are omnivorous, they may be able to utilize substitutes for FM such as plant-based protein sources (soybean meal) and thus reduce or eliminate dietary FM. Xue et al. (1999) reported that red claw have the enzyme polysaccharide hydrolase which enables them to digest plant material readily. Replacement of FM with less expensive ingredients, such as soybean meal (SBM), may contribute to a reduction in diet costs. In a recent study, Muzinic et al. (2004) reported that FM and shrimp meal could be completely replaced by soybean meal (SBM) and brewer's grains with yeast (BGY) in diets fed to juvenile red claw without adverse effects on growth and survival.

Currently, information about specific nutritional requirements and practical diet formulations for red claw has increased (Jacinto et al., 2003; Thompson et al., 2003a,b, 2004; Muzinic et al., 2004). Webster et al. (1994) stated that formulation of diets for intensive culture requires an understanding of nutritional requirements, and lack of such information may impede red claw aquaculture in the United States. In the absence of a commercially-available diet formulated specifically for red claw, producers may elect to use expensive high-quality, commercial shrimp diets, a decision that is probably a financial and nutritional waste. Another approach would be the use of low-quality diets that do not completely meet nutrient requirements of the species and thereby may reduce growth, health, and survival.

The objective of this study was to evaluate the effects of practical diets containing increasing percentages of crude protein (CP) (30%, 35%, and 40%), with or without anchovy fish meal (FM), on growth, survival, feed conversion ratio, and amino acid composition of tail muscle meat of juvenile red claw.

2. Materials and methods

2.1. Experimental diets

Six practical diets were formulated to contain increasing percentages of protein (30%, 35%, and 40%) (as fed basis) with or without anchovy fish meal (FM) based upon tabular values of protein content of ingredients (Lovell, 1998). Practical diets were formulated to contain the same ratio of protein/added lipid (5.7) and marine fish oil/corn oil ratio (2.5), respectively. The ingredient compositions of the diets are presented in Table 1. Diet 1 contained 30% CP with 0% FM and 42.5% SBM; Diet 2 contained 30% CP with 15% FM and 20% SBM; Diet 3 contained 35% CP with 0% FM and 56.5% SBM; Diet 4 contained 35% CP with 17.5% FM and 30.5% SBM; Diets 5 contained 40% CP with 0% FM and 71% SBM; and Diet 6 contained 40% CP with 20% FM and 40.5% SBM, and its ingredient composition was similar to a high-quality marine shrimp diet.

Table 1
Ingredient composition of the six practical diets containing three different protein levels (30%, 35%, and 40%) with or without fish meal fed to red claw crayfish

Ingredient	Diets					
	1 (30%)	2 (30%)	3 (35%)	4 (35%)	5 (40%)	6 (40%)
Anchovy fish meal (65%)	0.0	15.0	0.0	17.5	0.0	20.0
Soybean meal (48%)	42.5	20.0	56.5	30.5	71.0	40.5
Wheat flour (12.5%)	20.0	20.0	20.0	20.0	8.8	20.0
Milo (11.5%)	19.05	28.05	4.17	14.42	0.0	1.3
BGY (35%)[a]	5.0	5.0	5.0	5.0	5.0	5.0
Sardine oil	3.75	2.25	4.38	2.63	5.0	3.0
Corn oil	1.5	1.5	1.75	1.75	2.0	2.0
Vitamin mix[b]	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix[c]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin C (35% active)[d]	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5
Wheat gluten (80%)	4.0	4.0	4.0	4.0	4.0	4.0
Protein/added lipid ratio	5.71	5.71	5.71	5.71	5.71	5.71
Marine fish oil/corn oil	2.5	2.5	2.5	2.5	2.5	2.5

Values are percentages of the diet.

[a] BGY-35 (F.L. Emmert, Cincinnati, OH).

[b] 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; ethoxyquin, 99 mg.

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

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

2.2. Preparation of diets

Dry ingredients were weighed (Mettler PM 4600, Mettler Instrument, Hightstown, NJ), combined, and mixed together thoroughly for 1 h using a Hobart mixer (A-200 T; Hobart, Troy, OH). Warm tap water was then added to obtain a 25% moisture level. Diets were single extruded through a 1-cm die to form "spaghetti-like" strands and air-dried in a convection oven (Grieve, Round Lake, IL). After drying, all diets were broken into pellets of appropriate size using a Disk Mill (S.500; GlenMills, Clifton, NJ) and then sieved (2-mm opening mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburgh, PA). After sieving, a combination of sardine oil and corn oil that had previously been mixed together were slowly added to the diet and mixed until all pellets were uniformly coated. The oils were added after pelletizing to avoid destruction of essential fatty acids (HUFAs) during processing (Thompson et al., 2003a,b). Diets were stored in plastic containers at −20 °C until fed.

2.3. Diet analysis

The composition of each of the experimental diets was analyzed to determine percent moisture, protein, lipid, fiber, and ash. Moisture was determined by placement of a 2-g sample into a convection oven (135 °C) for 2 h until constant weight (Association of Official Analytical Chemists [AOAC], 1990, procedure 930.15); protein was determined by combustion (AOAC, 1990, procedure 990.03); lipid was deter-

Table 2
Results of proximate analysis of six practical diets containing three different protein levels (30%, 35%, and 40%) with or without fish meal fed to red claw crayfish

	Diets					
	1 (30%)	2 (30%)	3 (35%)	4 (35%)	5 (40%)	6 (40%)
Moisture (%)	9.9	10.8	11.8	9.7	12.1	10.1
Crude protein (%)[a]	35.32	35.56	40.50	40.99	45.24	46.07
Crude lipid (%)[a]	9.53	8.98	10.81	10.00	11.83	10.62
Fiber (%)[a]	2.22	2.02	2.38	2.10	2.84	2.00
Ash (%)[a]	5.89	7.17	6.70	8.15	7.61	9.09
NFE[b]	47.04	46.27	39.61	38.76	32.48	32.22

Values are means of two replications per diet.
[a] Dry-matter basis.
[b] NFE=nitrogen-free extract (by difference).

mined by the acid hydrolysis method (AOAC, 1990, procedure 954.02); fiber was determined by using the fritted-glass crucible method (AOAC, 1990, procedure 962.09); and ash was determined by placing a 2-g sample in a muffle furnace (600 °C) for 2 h (AOAC, 1990, procedure 942.05). The nitrogen-free extract (NFE) 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). Proximate composition of each of the six practical diets are presented in Table 2, and amino acid compositions are presented in Table 3. Proximate and amino acid composition was determined by a commercial analytical laboratory (Eurofins Scientific, Des Moines, IA).

2.4. Experimental system and maintenance

A feeding trial was conducted using 150 plastic-mesh culture units (12.7 cm^3; Plastic Window Breeder-Fine, Lustar Products, Springfield, NJ) located within five rectangular fiberglass tanks (325.5-l; 236×102×15 cm) at the Aquaculture Research Center, Kentucky State University. Dechlorinated city (tap) water was recirculated through a 2000-l biological and mechanical filtration system containing vertical polyester screens and polyethylene bio-balls (Red Ewald, Karnes City, TX) and then passed through a propeller washed bead filter (Aquaculture Systems Technologies, New Orleans, LA) to remove solid and nitrogenous wastes and provide substrate for nitrifying bacteria. Each culture unit was equipped with an individual water tube connected to a

Table 3
Amino acid composition of six practical diets containing three different protein levels (30%, 35%, and 40%) with or without fish meal fed to red claw crayfish

Amino acid	Diets					
	1 (30%)	2 (30%)	3 (35%)	4 (35%)	5 (40%)	6 (40%)
Alanine	1.43 (4.05)	1.62 (4.56)	1.52 (3.75)	1.89 (4.61)	1.76 (3.89)	2.04 (4.43)
Arginine	1.98 (5.61)	1.91 (5.37)	2.36 (5.83)	2.11 (5.15)	2.47 (5.46)	2.44 (5.30)
Aspartic acid	3.33 (9.43)	3.12 (8.77)	3.91 (9.65)	3.75 (9.15)	4.46 (9.86)	4.32 (9.38)
Cystine	0.53 (1.50)	0.45 (1.27)	0.66 (1.63)	0.63 (1.54)	0.72 (1.59)	0.68 (1.48)
Glutamic acid	7.45 (21.09)	7.06 (19.85)	7.92 (19.56)	8.16 (19.91)	8.45 (18.68)	8.63 (18.73)
Glycine	1.44 (4.08)	1.63 (4.58)	1.64 (4.05)	1.97 (4.81)	1.86 (4.11)	2.19 (4.75)
Histidine	0.78 (2.21)	0.78 (2.19)	0.90 (2.22)	0.89 (2.17)	0.98 (2.17)	1.01 (2.19)
Isoleucine	1.31 (3.71)	1.31 (3.68)	1.48 (3.65)	1.50 (3.66)	1.64 (3.63)	1.69 (3.67)
Leucine	2.47 (6.99)	2.49 (7.00)	2.69 (6.64)	2.84 (6.93)	3.03 (6.70)	3.08 (6.69)
Lysine	1.54 (4.36)	1.66 (4.67)	1.89 (4.67)	1.98 (4.83)	2.14 (4.73)	2.31 (5.01)
Methionine	0.47 (1.33)	0.60 (1.69)	0.55 (1.36)	0.77 (1.88)	0.59 (1.30)	0.83 (1.80)
Phenylalanine	1.58 (4.47)	1.50 (4.22)	1.77 (4.37)	1.75 (4.27)	1.99 (4.40)	1.93 (4.19)
Proline	2.21 (6.26)	2.15 (6.05)	2.28 (5.63)	2.54 (6.20)	2.47 (5.46)	2.62 (5.69)
Serine	1.65 (4.67)	1.56 (4.39)	1.88 (4.64)	1.89 (4.61)	2.15 (4.75)	2.08 (4.51)
Threonine	1.30 (3.68)	1.35 (3.80)	1.49 (3.68)	1.64 (4.00)	1.73 (3.82)	1.83 (3.97)
Tyrosine	1.02 (2.89)	1.01 (2.84)	1.16 (2.86)	1.19 (2.90)	1.32 (2.92)	1.35 (2.93)
Valine	1.46 (4.13)	1.50 (4.22)	1.64 (4.05)	1.73 (4.22)	1.84 (4.07)	1.95 (4.23)

Values are percentage of the diet. Values in parentheses are expressed as percentage of dietary protein.

plastic aquarium pipe valve that supplied water at a rate of 0.8 l/min. Each culture unit contained a 5-cm section of 1-in. diameter PVC pipe for shelter. Water temperature was maintained at 27–29 °C by the use of an immersion heater located in the biological and mechanical filtration system, and continuous aeration was provided through air diffuser tubing inside each fiberglass tank.

Approximately 5% of the total water volume was replaced daily with dechlorinated city (tap) water. Lighting was provided by overhead fluorescent ceiling lights on a 12:12-*L*/*D* cycle. Sodium bicarbonate and crushed coral were added in the recirculating system to maintain alkalinity levels near 100 mg/l. Each culture unit was cleaned every other day to remove uneaten diet and feces; molts were not removed.

Water quality parameters were checked three times weekly. Dissolved oxygen was measured using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, OH); water temperature was measured using a thermometer; total ammonia and nitrite were determined using a DREL/2000 spectrophotometer (HACH, Loveland, CO); pH was determined with an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, OH); and total alkalinity and chlorides were determined using a digital titrator (HACH).

2.5. Stocking and feeding

Juvenile red claw (*Cherax quadricarinatus*) averaging 1.12±0.77 g (S.D.), produced from a single female, were obtained from Auburn University, Auburn, AL, and individually and randomly stocked into 150 plastic-mesh culture units within the five rectangular tanks at 25 culture units per treatment. A tank was randomly selected, and an individual red claw was then stocked into a randomly selected culture unit within that tank. This process was continued until all red claw were stocked. After stocking, one of the six experimental diets was randomly chosen for each culture unit. During the first week after stocking, any mortalities were replaced with an individual of similar weight. Thereafter, no mortalities were replaced.

Red claw were fed one of six practical diets formulated to contain increasing percentages of protein (30%, 35%, and 40%), with or without FM, and fed three times daily (800, 1200, and 1500 h) to "controlled excess" based on the observation of the amount of uneaten diet for 8 weeks.

2.6. Data collection

At the conclusion of the feeding trial, each red claw was individually weighed on an electronic scale (AB54-S; Mettler Toledo, Columbus, OH). Growth parameters and feed efficiency 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 (%)=$100[(W_t - W_i)/W_i]$; feed conversion ratio=total diet fed (kg)/total wet weight gain (kg).

After weighing, all experimental red claw within each treatment were chill-killed using an ice-water bath, the tail muscle was then removed from each body and stored frozen in Ziplock® bags until analyzed. There were two replicate samples of pooled tail-muscle samples per treatment. Amino acid analysis was conducted (wet-weight basis) by a commercial analytical laboratory (Eurofins Scientific, Memphis, TN). The essential amino acid index (EAAI) was then determined. This method compares the essential amino acid composition of each of the experimental diets to those in the tail muscle. EAAI provides a method to determine if the organism is obtaining adequate levels of essential amino acids.

2.7. Statistical analysis

Data were calculated for percent weight gain, specific growth rate (SGR), percent survival, feed conversion ratio (FCR), and essential amino acid index (EAAI).

Data were analyzed according to a 3×2 factorial design using the SAS General Linear Models (GLM) procedure (SAS software version 8.2; SAS, 1999) for significant differences among treatment means when analyzed by protein level, fish meal level, and their interaction. Duncan's multiple range test was used to compare differences among individual means at the P=0.05 level of significance. A Chi-squared test was used to determine if survival was independent of diet type. All percentage and ratio data were transformed to arc sine values prior to statistical analysis (Zar, 1984).

3. Results

During the study, water quality parameters averaged (±S.D.): water temperature, 28.1±7.1 °C; dissolved oxygen, 6.9±0.3 mg/l; total ammonia nitrogen, 0.34±0.08 mg/l; nitrite, 0.002±0.004 mg/l; total alkalinity, 108±38.7 mg/l; chloride, 77.1±64.7 mg/l; pH, 7.58±0.29. These water quality averages were within acceptable limits for indoor production of red claw (Masser and Rouse, 1997).

Proximate composition (which includes percent moisture, protein, lipid, fiber, and ash) and NFE of the six practical diets are presented in Table 2, and amino acid composition of the diets are presented in Table 3.

Initial weight did not differ significantly among treatments (P>0.05). After 8 weeks, specific growth rate (SGR) and percent survival did not differ significantly among treatments, averaging 3.91%/day and 80.7%, respectively (Table 4). The percent weight gain of red claw fed Diet 6 was significantly higher (1352%) compared to that of red claw fed Diet 1 containing 0% FM and 30% protein (828%) but not different from those of red claw fed all other diets. Thus, red claw fed diets without fish meal with protein levels lower than 35% had a reduced percent weight gain. Red claw fed Diet 3 (0% FM and 35% protein) had a significantly higher FCR (5.73) compared to that of red claw fed Diet 6 (3.03), but not different from those of red claw fed the other four diets.

When analyzing data among the three protein levels (30%, 35%, and 40%), which includes with and without FM, there were no significant differences for percent weight gain, SGR, and percent survival among treatments. The response values averaged 1016%, 3.91, and 80.7%, respectively.

Percent weight gain, SGR, and percent survival of red claw fed diets containing FM were not significantly different compared to those of red claw fed diets containing no FM. Mean values of these responses were 1012%, 3.91, and 80.7%, respectively.

Mean (±S.E.) essential amino acid composition of the tail-muscle meat of red claw and EAAI results for each of the dietary treatments are presented in Table 5. Red claw fed Diet 2 had significantly higher percentage of methionine (0.42%) compared to that of red claw fed Diet 5 (0.32%), but not different (P>0.05) from red claw fed all other treatments. All other analyzed amino acids were not significantly different (P>0.05) among red claw fed the six diets. EAAI for diets in this study ranged from 176 to 185 for red claw fed 30% protein, 208 to 212 for red claw fed 35% protein, and 237 to 254 for red claw fed 40% protein when compared to red claw tail muscle (Table 5). Elimination of fish meal with increased soybean meal resulted in only modest change in the EAAI for each dietary protein level (30%, 35%, 40%).

No significant differences in amino acid levels were found among red claw fed the three protein

Table 4
Mean (±S.E.) initial weight, percent weight gain, specific growth rate (SGR), feed conversion ratio (FCR), and percent survival of red claw crayfish fed three different protein levels (30%, 35%, and 40%) with or without fish meal

	Diets					
	1 (30%)	2 (30%)	3 (35%)	4 (35%)	5 (40%)	6 (40%)
Initial wt. (g)	1.0±0.2	1.2±0.2	1.0±0.1	1.1±0.2	1.2±0.2	1.2±0.2
Weight gain (%)	828±126^{b}	989±140^{ab}	919±118^{ab}	927±162^{ab}	1056±122^{ab}	1352±191^{a}
SGR (%/day)	3.61±0.3	3.87±0.3	3.88±0.2	3.60±0.3	4.12±0.3	4.39±0.3
FCR	4.69±0.2^{ab}	4.10±0.7^{ab}	5.73±1.2^{a}	4.81±0.6^{ab}	4.43±0.7^{ab}	3.03±0.4^{b}
Survival (%)*	76.0	84.0	80.0	84.0	76.0	84.0

Means having different superscripts within a row are significantly different (P<0.05).

* A Chi-squared test showed no significant difference (P>0.05) in survival with respect to diet type (χ^2 test statistic (TS)=1.2404; Critical value (CV) with 5 df=11.070).

Table 5
Essential amino acid composition (percentage of diet) and the essential amino acid index (EAAI) of the diets and essential amino acid composition of juvenile red claw tail muscle

Amino acid	Diets						Muscle[a]
	1 (30%)	2 (30%)	3 (35%)	4 (35%)	5 (40%)	6 (40%)	
Arginine	1.98	1.91	2.36	2.11	2.47	2.44	1.45±0.09
Histidine	0.78	0.78	0.90	0.89	0.98	1.01	0.35±0.02
Isoleucine	1.31	1.31	1.48	1.50	1.64	1.69	0.68±0.04
Leucine	2.47	2.49	2.69	2.84	3.03	3.08	1.18±0.07
Lysine	1.54	1.66	1.89	1.98	2.14	2.31	1.23±0.07
Methionine	0.47	0.60	0.55	0.77	0.59	0.83	0.36±0.02
Phenylalanine	1.58	1.50	1.77	1.75	1.99	1.93	0.63±0.04
Threonine	1.30	1.35	1.49	1.64	1.73	1.83	0.68±0.04
Tyrosine	1.02	1.01	1.16	1.19	1.32	1.35	0.54±0.04
Valine	1.46	1.50	1.64	1.73	1.84	1.95	0.70±0.04
EAA index[b]							
Muscle	185.65	176.89	208.86	212.46	237.95	254.47	

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

[b] $EAAI = \sqrt[n]{[(aa_1/AA_1)(aa_2/AA_2)\ldots(aa_n/_{Aan})]}$, 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 total number of amino acids evaluated.

levels (30%, 35%, and 40%) with and without FM. No significant differences in amino acid levels were found among red claw fed diets containing FM relative to those of red claw fed diets containing no FM.

4. Discussion

The results of the present study indicate that juvenile red claw can be fed a practical diet with a combination of plant-protein ingredients and without fish meal (FM) if the protein level is 35%; however, if the percentage of dietary protein is 30%, it appears that FM may be needed in the diet. These data are the first to indicate that the quantity and quality of protein are important for growth of (1 g) juvenile red claw grown indoors. Muzinic et al. (2004) reported that juvenile (0.2–3.1 g) red claw can be fed a practical diet in which all of the FM is replaced by 80% soybean meal (SBM) and 5% brewer's grains with yeast (BGY); however, that diet contained 40% protein.

Webster et al. (1992) suggested that feeding of diets containing a combination of protein sources may allow the use of a high percentage of SBM without growth reduction. Inclusion of several high-quality plant protein sources in the diets of our study may have sufficiently satisfied the requirements of the essential amino acids. The levels of amino acids in the diets fed in the present study compare favorably to those in practical diets that have been successfully used in other studies with juvenile red claw (Thompson et al., 2003b; Muzinic et al., 2004). Results of the present study also suggest that levels of dietary crude protein (CP) can be reduced to 30% if a level of 15% anchovy fish meal is included. Jacinto et al. (2003) reported that 1 g juvenile red claw, grown communally, required 31% dietary protein for optimal growth using a diet which contained 20% sardine meal. Webster et al. (1994) indicated that small (mean weight=0.022 g) juvenile red claw required 33% dietary protein (28% menhaden fish meal included) to achieve highest growth when grown communally in aquaria that were part of an indoor recirculating system. Manomaitis (2001) evaluated protein requirement of two age classes of juvenile red claw crayfish and reported that small (0.02 g) red claw required 40% dietary protein, while a diet containing 30% protein was sufficient for larger (3.0 g) red claw. All diets in the study contained high (>29%) levels of menhaden fish meal. In a recent study, Thompson et al. (2004) reported that red claw (4.6 g) could be fed a diet containing 22% protein when grown in ponds, although the diet did contain some (7%) fish meal. It was suggested that natural food items in the pond may have satisfied part of the protein requirements of red claw, which are omnivorous detritivores. However, the possibility of red claw consuming additional nutrients in the present study appears minute, since they were grown in individual culture units in an indoor recirculating system.

At present, the amino acid requirements of red claw have not been determined. Muzinic et al. (2004) have examined the amino acid composition of practical diets containing different levels of SBM and BGY, as replacements for FM, and results suggest that the amino acid levels in a 40% crude protein diet were adequate for good growth and survival of juvenile red claw crayfish. All diets fed to red claw in the present study had amino acid values similar to those in the Muzinic et al. (2004) study. These levels may have met or exceeded the animal's requirements as indicated by the EAAI values. A complementary blend of soybean meal and other plant protein sources used to replace FM in a 35% protein diet appear to provide sufficient levels of essential amino acids to meet requirements. This observation is in agreement with the results obtained by Webster et al. (1992).

Determination of the exact amino acid requirements in crustaceans is difficult (Shiau, 1998). Although a variety of methods have been used in previous feeding trials (Arai, 1981; Sick and Millikin, 1983), the essential amino acid index (EAAI), as proposed by Tacon and Cowey (1985), was used to compare the essential amino acid patterns within the diets to those in the tail muscle. For each dietary protein level (30%, 35%, and 40%), elimination of fish meal and a corresponding substitute with increased contents of soybean meal resulted in only modest change in the EAAI. For red claw fed diets containing 35% and 40% protein, the EAAI slightly decreased when fish meal was not included and soybean meal levels were increased. Based upon the EAAI index, red claw fed diets with no added FM may be obtaining adequate levels of essential amino acids. Results of the present study are similar to those of Muzinic et al. (2004) who found that a decrease in the dietary content of fish meal with a corresponding substitution with soybean meal and BGY caused very little change in EAAI when diets were compared to red claw tail muscle.

FCR values in the present study (3.03–5.73) were higher than previously reported values. Jacinto et al. (2003) reported that FCR values ranged from 1.04 to 1.56 when red claw were grown communally. They stated that red claw were fed to apparent satiation twice daily at levels closely matching the maximum food ingested with little or no remaining food pellets. Manomaitis (2001) also reported FCR values that ranged from 1.14 to 1.61 for red claw grown individually in an indoor recirculating system. In that study, red claw were fed in the morning and evening, and feeding rates were based on the feeding response of each experimental crawfish. In the present study, red claw were fed pellets according to the observed level of feeding activity three times daily. However, while the amount of diet offered was not too extreme, uneaten diet was present in the culture units and may explain why FCR values were higher in the present study. It is important to refrain from withholding food from organisms during a feeding trial, and overfeeding is more desirable than underfeeding (Tacon and Cowey, 1985). This is because underfeeding could result in suboptimal growth of the organism and lead to erroneous conclusions of diet suitability based upon the faulty growth data.

Percent survival reflect results of other studies with red claw. Survival ranged from 79–98% in Muzinic et al. (2004); Jacinto et al. (2003) reported survival of 65–89%; Thompson et al. (2003a) reported survival of 95–100%; Thompson et al. (2003b) reported survival of 56–80% for juvenile red claw fed experimental diets; and Webster et al. (1994) reported survival of 50–71%.

The results of the present study indicate that juvenile red claw can be fed a diet with 30% CP when 15% fish meal is added; however, the removal of 15% fish meal requires a diet containing 35% CP when the ingredients in the present study are used. Reducing protein and fish meal levels and feeding a plant protein-based diet, which takes advantage of their omnivorous feeding habits, may help reduce operating costs and thereby increase producer's profits. Additional research devoted to the use of plant protein ingredients to replace fish meal in diets for crustacean species should be conducted.

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