Aquaculture Nutrition **2006 12**; 227–238

Effects of feeding practical diets containing different protein levels, with or without fish meal, on growth, survival, body composition and processing traits of male and female Australian red claw crayfish (*Cherax quadricarinatus*) grown in ponds

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Abstract

Four practical diets containing 2% of crude protein (CP) (180 and 280 g kg^{-1}), with or without menhaden fish meal (FM), were fed to Australian red claw *Cherax quadricarinatus* during a 97-day feeding trial. Growth, survival, body composition and processing traits of pond-cultured red claw were determined. Juvenile red claw (mean individual weight of 5.75 ± 3.3 g) were randomly stocked into twelve 0.04-ha ponds at a rate of 1000 per pond (25 000 ha^{-1}), and each diet was fed to three ponds. At harvest, the final mean weight of red claw fed Diet 4 (0 g kg^{-1} FM and 280 g kg^{-1} CP) and Diet 3 (113 g kg^{-1} FM and 280 g kg^{-1} CP) was significantly ($P < 0.05$) higher (62.4 and 58.5 g, respectively) than red claw fed Diet 1 (73 g kg^{-1} FM and 180 g kg^{-1} CP; 51.7 g) and Diet 2 (0 g kg^{-1} FM and 180 g kg^{-1} CP; 53.0 g). Red claw fed diets containing 280 g kg^{-1} CP, with or without FM, had significantly higher percent weight gain (894 and 959%, respectively) compared to red claw fed 180 g kg^{-1} CP, with or without FM (778 and 799%, respectively). Feed conversion ratio, percent survival, and total yield among treatments, which averaged 3.55, 65.2%, and 724 kg ha^{-1} overall, were not significantly different. Results from this study indicate that pond-cultured red claw stocked at 25 000 ha^{-1} can be fed a practical diet containing 280 g kg^{-1} CP with 0 g kg^{-1} FM if a combination of plant-protein ingredients (soybean meal, distillers' dried grains with solubles, and milo) is added; however, if the percentage of dietary protein level is 180 g kg^{-1}, growth is reduced even if FM is added at 73 g kg^{-1} of the diet. Use of diets containing plant-protein sources may help reduce diet costs to producers and thereby, increase profits.

KEY WORDS: body composition, *Cherax quadricarinatus*, diet, essential amino acid index, fish meal, protein level, processing traits, red claw crayfish

Received 19 August 2005, accepted 19 January 2006

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Introduction

Globally, interest in the culture of the Australian red claw crayfish *Cherax quadricarinatus* has increased during the past several years and is commercially cultured in several countries, including China, Mexico, and Australia. It has been shown that red claw possess many favourable culture traits essential for commercial culture (Rouse *et al.* 1991). Red claw can be fed prepared diets, and they grow rapidly (65–90 g) in a limited (117 days) growing season in temperate-climate ponds (Thompson *et al.* 2004a). Preliminary marketing studies conducted at our facility show that red claw are highly desired by consumers because of their excellent tail-meat flavour, lobster-like appearance, are larger than shrimp, and have excellent storage quality. Further, there is no larval phase as found for other crustacean species (Jones 1995) and this negates the need for expensive and sophisticated hatcheries.

However, there are some difficulties in farming red claw in the United States. Commercial culture of red claw is small scale and scattered principally because hatchery-grown, stocking-size juveniles are not readily available in the United States. Producers must purchase these individuals from hatcheries in other countries (Australia or Mexico) and costs can range from \$0.40 to \$0.50 (US) per juvenile (with transport costs). Likewise, diet costs can represent up to 70% of the total operating expenses of an aquaculture enterprise; thus, recent efforts to determine specific nutrient requirements and evaluate inexpensive practical diets have been devoted to reduce diet costs and possibly increase profits (Jacinto *et al.* 2003; Thompson *et al.* 2003a,b; Muzinic *et al.* 2004; Thompson *et al.* 2004a; Jacinto *et al.* 2005; Thompson *et al.* 2005).

Development of a cost-effective and nutritionally balanced formulated diet is essential for red claw producers. Protein is generally the most expensive component in a prepared diet for a crustacean species (Lim 1997). Information on the optimal dietary protein requirements for red claw grown in indoor experimental systems has been examined more extensively than ponds. Jacinto *et al.* (2003) reported that a practical diet containing 310 g kg^{-1} crude protein (CP) seems adequate for 1 g juvenile red claw, while in one of the first studies to evaluate prepared diets for red claw, Webster *et al.* (1994) reported that newly hatched (mean weight of 0.022 g) red claw required 330 g kg^{-1} CP. Manomaitis (2001) reported that the recommended CP level in the diets for 0.02 g juvenile red claw was 400 g kg^{-1}, while larger (3.03 g) juveniles could be fed a diet with 300 g kg^{-1} protein. In ponds, Thompson *et al.* (2004a)) reported that red claw (mean weight of 4.6 g stocked at a density of 25 000 ha^{-1}) could be fed a diet containing 220 g kg^{-1} CP.

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. However, FM is one of the most expensive macro-ingredients in an aquaculture diet. Naylor *et al.* (2000) reported that FM prices have risen and are likely to increase further with continued growth in demand. In addition, long-term availability of FM in the future may be questionable. Replacement of FM with less expensive protein sources may contribute to a reduction in diet costs. Muzinic *et al.* (2004) reported that juvenile red claw can be fed a practical diet in which all of the FM is replaced by 800 g kg^{-1} soybean meal (SBM) and 50 g kg^{-1} brewer's grains with yeast (BGY) when grown in an indoor recirculating system. Thompson *et al.* (2005) reported that juvenile red claw could be fed a practical diet with a combination of plant-protein ingredients, and without FM, if the protein level is 350 g kg^{-1}. However, if the percentage of dietary protein is 300 g kg^{-1}, it appears that FM may be needed in the diet when red claw are grown in an indoor recirculating system.

While information about specific nutritional requirements and practical diet formulations for red claw is increasing (Jacinto *et al.* 2003; Thompson *et al.* 2003a,b; Hernandez *et al.* 2004; Muzinic *et al.* 2004; Thompson *et al.* 2004a; Jacinto *et al.* 2005; Thompson *et al.* 2005), there is no information on the partial or total replacement of FM with varying dietary protein levels for red claw grown in ponds. The purpose of the present study was to determine growth, survival, body proximate composition, and processing characteristics of red claw fed diets with two different protein levels, which have either no FM present or FM present in the diet.

Materials and methods

Description and stocking of ponds

Juvenile red claw (5.75 ± 3.3 g) were obtained from a commercial supplier (Central Queensland Crayfish, Queensland, Australia). Red claw were placed into aerated water and actively moving individuals were stocked into twelve 0.04-ha ponds, average water depth approximately 1.1 m, on 12 June 2004 at a rate of 1000 red claw per pond (25 000 ha^{-1}). Groups of 100 live red claw were hand counted at random until all ponds had been stocked. Three replicate ponds were assigned to each of the four diets containing two protein levels (180 and 280 g kg^{-1}) with or without menhaden FM.

Practical diets and feeding rates

Four practical diets were formulated to contain two levels of protein (180 and 280 g kg^{-1}), with or without menhaden FM, based upon values of protein content of ingredients supplied by the feed mill (Table 1). Dietary ingredients were processed into 5-mm sinking pellets by a commercial feed mill (Farmers Feed, Lexington, KY, USA). Two feedings, each consisting of one-half of the total daily ration, were distributed over the entire surface area of each pond between 08:00 and 08:30 h, and between 15:30 and 16:00 h for 97 days. All red claw were fed the same amount of diet based upon a percentage of estimated body weight and the feeding schedule was devised by C.D. Webster (unpublished data) with survival assumed to be 65%. Amount of diet fed was adjusted every 2 weeks.

Diet analysis

Experimental diets were analysed to determine moisture, protein, lipid, fibre, and ash. Moisture was determined by the placement of a 2-g sample into a convection oven (135°C) for

Table 1 Ingredient and chemical composition (g kg^{-1}) of four practical diets containing two levels of protein with or without FM fed to red claw

Ingredient	Diet 1	2	3	4
Menhaden FM (62%)	73	0.0	113	0.0
Soybean meal (48%)	0.0	95	120	353
Distillers' grains with solubles (28%)	183	183	300	300
Milo (10%)	631	604	340	210
Wheat gluten (72%)	26	26	40	40
Menhaden fish oil	10	15	15	25
Corn oil	10	10	5.0	5.0
Vitamin and mineral mix[1]	3.0	3.0	3.0	3.0
Stay C (35% active)[2]	1.0	1.0	1.0	1.0
Choline chloride	3.0	3.0	3.0	3.0
Dicalcium phosphate	10	10	10	10
Lignin sulphonate (binder)	50	50	50	50
Chemical analysis				
Moisture	137.0	133.0	133.0	139.0
Crude protein[3]	178.2	182.6	278.4	327.1
Crude lipid[3]	90.5	91.7	101.7	102.1
Fibre[3]	53.0	52.0	53.0	37.0
Ash[3]	69.0	52.0	74.0	65.7
NFE[4]	609.3	621.7	492.9	468.1
Available energy[5] (kJ)	16.7	16.7	16.7	17.2

FM = fish meal.

Values in parentheses are percentage protein of ingredient. Proximate analysis values are means of two replications per diet.

[1] 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 alpha-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; thiamin, 43 mg; D_3, 440 IU; A (as vitamin A palmitate), 4399 IU; ethoxyquin, 99 mg. Mineral mix was Rangen trace mineral mix for catfish with 0.3 mg selenium kg^{-1} diet added.

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

[3] Dry-matter basis.

[4] NFE = nitrogen-free extract (by difference).

[5] Available energy was calculated as 16.7, 16.7, and 37.7 kJ g^{-1} of protein, carbohydrate, and lipid, respectively.

2 h until constant weight (AOAC procedure 930.15, 1995); protein was determined by combustion (AOAC procedure 990.03, 1995); lipid was determined by the acid hydrolysis method (AOAC procedure 954.02, 1995); fibre was determined by using the fritted-glass crucible method (AOAC procedure 962.09, 1995); and ash was determined by placing a 2-g sample in a muffle furnace (600°C) for 2 h (AOAC 942.05, 1995). The nitrogen-free extract (NFE) was determined by difference [NFE = 100 − (% protein + % lipid + % fibre + % ash)]. Available energy was calculated from estimated physiological fuel values of 16.7, 16.7, and 37.7 kJ g^{-1} for protein, carbohydrate (NFE), and lipid, respectively (Garling & Wilson 1977; Webster *et al.* 1999). Proximate and essential amino acid composition of each of the four practical diets are presented in Tables 1 and 6 and were determined by a commercial analytical laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA). Diet 4 had a higher percentage of protein than anticipated, probably due to differences in the protein content of the SBM actually used to make the diets from the value supplied at the time of diet formulation.

Water quality management

Water temperature and dissolved oxygen were measured in all ponds twice daily (09:00 and 15:30 h) using a YSI Model 58 oxygen meter (YSI, Yellow Springs, OH, USA). A 1/2-HP electric aerator (Air-O-Lator, Kansas City, MO, USA) was positioned in the centre of each pond and run continuously throughout the duration of the study. The pH was measured daily (15:30 h) using an electric YSI Model 60 pH meter (YSI). All other water quality parameters were measured thrice weekly (13:00 h), which included total ammonia and nitrite using a DREL/2000 spectrophotometer (HACH, Loveland, CO, USA). Alkalinity was determined by titration using a digital titrator (HACH).

Harvest

Ponds were harvested between 13 and 20 September 2004. Three days prior to harvest, the water level in each pond was lowered to approximately 0.5 m at the drain end. On the day of the harvest, the standpipe in each pond was lowered so that all water was completely drained and red claw were then manually removed. Red claw were hand collected and individually weighed on electronic scales (AB54-S; Mettler Toledo, Columbus, OH, USA) and identified (by sex) to be either male, female, or having both morphological traits via intersex.

Growth parameters and feed efficiency were calculated as follows:

$$\text{Weight gain (\%)} = 100[(W_t - W_i)/W_i];$$

$$\text{Feed conversion ratio} = \text{total diet fed (kg)}/\text{total wet weight gain (kg)}.$$

After weighing, twenty males and twenty females from each pond (sixty males and sixty females per treatment) were randomly sampled, chill-killed using an ice-water bath, and the tail muscle was removed from each body and stored in ziplock bags and frozen (−15°C) until analysed. Amino acid analysis was conducted (wet-weight basis) by a commercial analytical laboratory (Eurofins Scientific, Memphis, TN, USA) and essential amino acid index (EAAI) was calculated as follows:

$$\text{EAAI} = \sqrt[n]{[(\text{aa}_1/\text{AA}_1)(\text{aa}_2/\text{AA}_2)\ldots(\text{aa}_n/\text{AA}_n)]},$$

where EAAI is the nth root of the essential amino acids in the test diet (aa) to the content of each of those amino acids in the reference tissue (AA) and n is the total number of amino acids evaluated (Tidwell *et al.* 1993).

Moisture, protein, lipid and ash of the tail muscle was analysed and analysis procedures were as described for the diets except for moisture (AOAC procedure 950.46; 1995) and lipid was determined by ether extraction (AOAC procedure 960.39; 1995).

Processing traits were measured before the tail muscle meat was removed from the body. Whole-body traits were measured for claw weight, tail weight, tail muscle weight, and cephalothorax weight, respectively.

Statistical analysis

Overall growth data were analysed by ANOVA using the SAS General Linear Models (SAS software version 8.2; SAS 1999). Means of the four treatments (diets) were compared using least-significant difference (LSD). Data were analysed by a two-factor ANOVA with protein level and diets, with or without FM, as the factors, and feed conversion ratio (FCR), percent survival, and total yield (kg ha^{-1}) as the three dependent variables. Data were also analysed by a three-factor ANOVA with protein level, diets (with or without FM), and gender as the factors, and final weight and percent weight gain as the two dependent variables. Essential amino acid composition and proximate composition of juvenile male and female red claw tail muscle, and processing data were analysed by ANOVA and means were compared by LSD (SAS 1999). All percentage and ratio data were transformed to arc sin values prior to statistical analysis (Zar 1984). All statistical computations were performed at the $P < 0.05$ probability level.

Results

Water quality

All ponds were continuously aerated throughout the duration of the study and O_2 concentration averaged 7.0 mg L^{-1} for the morning and 9.42 mg L^{-1} for the afternoon (Table 2). Although there were significant differences ($P < 0.05$) in pH and temperature, the minimum and maximum values were within acceptable limits for red claw (Masser & Rouse 1993). The average pH and morning/afternoon water temperatures were similar among the four dietary treatments, which averaged 8.65, 24.7 (am), 26.3 (pm), respectively (Table 2). Average morning and afternoon water temperature was also analyzed (by month). Results indicate that the months of June and July were significantly ($P < 0.05$) higher (25.7 and 25.5°C) compared to August (23.9°C) and September (23.3°C) for morning water temperature. The afternoon water temperature showed that June (27.5°C) was significantly ($P < 0.05$) higher compared to the other three months of the study, which were 27.1°C (July), 25.5°C (August), and 24.8°C (September), respectively. This is a common occurrence for pond water temperatures in Kentucky (Tidwell *et al.* 1995; Webster *et al.* 2001). There were no significant differences ($P > 0.05$) in total ammonia nitrogen (TAN), nitrite, and alkalinity, which averaged 0.31, 0.004, and 92.0 mg L^{-1}, respectively (Table 2).

Growth and production

After 97 days, the final mean weight of red claw fed Diet 3 and Diet 4 was significantly ($P < 0.05$) higher (58.5 and

Table 2 Means (± SE) of water quality parameters measured in ponds of red claw fed four practical diets containing two levels of protein with or without fish meal

	Diet			
Parameter	1	2	3	4
DO (mg L^{-1}; a.m.)	7.30 ± 0.03^{a}	7.08 ± 0.04^{b}	7.02 ± 0.04^{b}	6.54 ± 0.04^{c}
DO (mg L^{-1}; p.m.)	8.90 ± 0.05^{c}	9.68 ± 0.09^{a}	9.45 ± 0.07^{b}	9.65 ± 0.08^{ab}
pH	8.57 ± 0.03^{b}	8.66 ± 0.04^{ab}	8.66 ± 0.03^{ab}	8.70 ± 0.05^{a}
Temperature (°C; a.m.)	24.7 ± 0.11^{b}	24.3 ± 0.12^{c}	24.7 ± 0.11^{b}	25.2 ± 0.11^{a}
Temperature (°C; p.m.)	26.3 ± 0.11^{b}	25.9 ± 0.11^{c}	26.3 ± 0.11^{b}	26.8 ± 0.11^{a}
TAN (mg L^{-1})	0.29 ± 0.01^{a}	0.33 ± 0.02^{a}	0.29 ± 0.01^{a}	0.31 ± 0.02^{a}
Nitrite (mg L^{-1})	0.001 ± 0.0^{a}	0.005 ± 0.0^{a}	0.001 ± 0.0^{a}	0.007 ± 0.0^{a}
Alkalinity (mg L^{-1})	94 ± 2.5^{a}	89 ± 2.7^{a}	94 ± 2.5^{a}	90 ± 2.5^{a}

Means within a row having different superscripts are significantly different ($P < 0.05$).
DO = dissolved oxygen; TAN = total ammonia nitrogen.

62.4 g, respectively) compared to that of red claw fed Diet 1 (51.7 g) and Diet 2 (53.0 g) (Table 3). Likewise, red claw fed Diets 3 and 4 had significantly higher percent weight gain (894 and 959%, respectively) compared to that of red claw fed Diet 1 (778%) and Diet 2 (799%). However, FCR, percent survival, and total yield did not differ significantly ($P > 0.05$) among treatments, averaging 3.55, 65.2%, and 724 kg ha^{-1}, respectively (Table 3).

When analysing data by a two-factor ANOVA with protein level (which includes diets with or without FM) or FM level (which includes both protein levels) as the factors, no significant difference ($P > 0.05$) was found among the three dependent variables (FCR, percentage survival, and total yield).

Table 4 reports the results of a three-factor ANOVA in which protein level and gender exerted significant ($P < 0.05$) effect on both final mean weight and percent weight gain. However, inclusion of FM in diets had no significant ($P > 0.05$) impact on the two dependent variables. Table 4 shows that the higher protein level (280 g kg^{-1}) significantly ($P < 0.05$) increased final individual weight and percent weight gain of red claw fed FM (59.0 g and 902%, respectively) and no FM (62.3 g and 957%, respectively) compared to those fed diets with the lower protein level (180 g kg^{-1}) with FM (51.3 g and 782%, respectively) and no FM (53.2 g and 803%, respectively). Results indicate that male red claw had significantly ($P < 0.05$) higher final individual weight and percent weight gain fed diets with FM (60.0 g and 929%, respectively) and without FM (63.5 g and 977%, respectively) compared to female red claw fed diets with FM (50.4 g and 755%, respectively) and no FM (52.0 g and 783%, respectively).

Table 4 Results of a three-factor ANOVA, with protein level, diets with or without fish meal (FM), and gender being the factors, and final weight and percent weight gain being the dependent variables

Factors	Dependent variables	
	Final wt. (g)	Wt. gain (%)
FM		
Protein (g kg^{-1})		
280	59.0 ± 2.74^a	902 ± 46.5^a
180	51.30 ± 3.35^b	782 ± 54.0^b
Gender		
Male	59.96 ± 3.28^a	929 ± 45.6^a
Female	50.35 ± 2.15^b	755 ± 36.6^b
No FM		
Protein (g kg^{-1})		
280	62.28 ± 3.92^a	957 ± 66.6^a
180	53.21 ± 2.89^b	803 ± 49.0^b
Gender		
Male	63.45 ± 3.47^a	977 ± 58.9^a
Female	52.0 ± 2.62^b	783 ± 44.5^b

Reported means were taken over data referring to the corresponding treatments of the three factors (presence of FM, protein level, and gender), which are shown in the row and column headings. Inclusion or exclusion of FM had no significant ($P > 0.05$) effect on final weight or percent weight gain so means are grouped by inclusion or exclusion of FM. Means within a column with different superscripts are significantly different ($P < 0.05$).

Percentages of males and females at harvest and final weight

There were no significant ($P > 0.05$) differences in the percentages of males and females harvested from ponds among treatments. Overall, the percentage of males averaged 45.6% and the percentage of females averaged 52.7%. However, the percentage of intersex of red claw fed Diet 4 was significantly ($P < 0.05$) higher (2.5%) compared to that of red claw fed Diet 3 (1.0%), but not different from that of red claw fed all other diets.

Final individual weight of males fed Diet 4 was significantly higher (68.9 g) compared to that of males fed Diet 1 (56.5 g), but not different from males fed the other two diets. There were no significant differences in the final mean weight of females fed any treatment (diet), which averaged 51.2 g.

Body composition

Proximate compositions of male and female red claw tail muscle are presented in Table 5. There were no significant differences ($P > 0.05$) in moisture, protein, lipid and ash (wet-weight basis) in the tail muscle of males fed any diet

Table 3 Overall means (± SE) of final weight, percent weight gain, specific growth rate, FCR, percentage survival, and total yield of red claw fed four practical diets containing two levels of protein with or without fish meal

	Diet			
	1	2	3	4
Final weight (g)	51.71 ± 3.73^b	52.97 ± 3.16^b	58.54 ± 1.67^a	62.35 ± 3.87^a
Weight gain (%)	778 ± 63^b	799 ± 54^b	894 ± 28^a	959 ± 66^a
FCR	4.13 ± 0.67^a	3.48 ± 0.37^a	3.25 ± 0.27^a	3.33 ± 0.28^a
Survival (%)	63.0 ± 8.6^a	70.1 ± 2.4^a	66.6 ± 4.6^a	60.9 ± 2.1^a
Yield (kg ha^{-1})	645 ± 116^a	733 ± 65^a	768 ± 61^a	748 ± 59^a

Means within a row having different superscripts are significantly different ($P < 0.05$).
FCR = feed conversion ratio

Table 5 Means (± SE) moisture, protein, fat, and ash (wet-weight basis) of tail muscle (g kg^{-1}) of male and female red claw fed four practical diets containing two levels of protein with or without fish meal

	Diet			
	1	2	3	4
Males				
Moisture	802.0 ± 8.0[a]	795.0 ± 9.0[a]	810.0 ± 5.0[a]	800.0 ± 6.0[a]
Protein	171.0 ± 5.0[a]	172.0 ± 10.0[a]	145.0 ± 12.0[a]	171.0 ± 5.0[a]
Fat	2.0 ± 0.0[a]	2.0 ± 0.0[a]	2.0 ± 0.0[a]	2.0 ± 0.0[a]
Ash	13.0 ± 0.0[a]	13.0 ± 0.0[a]	13.0 ± 0.0[a]	13.0 ± 0.0[a]
Females				
Moisture	791.0 ± 1.0[ab]	795.0 ± 4.0[ab]	800.0 ± 4.0[a]	786.0 ± 6.0[b]
Protein	178.0 ± 4.0[ab]	167.0 ± 9.0[bc]	152.0 ± 6.0[c]	190.0 ± 8.0[a]
Fat	2.0 ± 0.0[a]	2.0 ± 0.0[a]	2.0 ± 0.0[a]	2.0 ± 0.0[a]
Ash	14.0 ± 0.0[a]	13.0 ± 0.0[a]	13.0 ± 0.0[a]	14.0 ± 0.0[a]

Means within a row having different superscripts (a, b) are significantly different ($P < 0.05$) among dietary treatments. Moisture, protein, fat, and ash were not significantly different ($P > 0.05$) for males and females within each column (diet). Values are means of three replicates per diet for both sexes.

averaging 802, 165, 2.0, and 13.0 g kg^{-1}, respectively. There were no significant differences ($P > 0.05$) in lipid and ash (wet-weight basis) in the tail muscle of females among treatments, and averaged 2.0 and 14.0 g kg^{-1}, respectively. However, female red claw fed Diet 4 had significantly higher protein (190 g kg^{-1}) in the tail muscle compared to females fed Diet 3 (152 g kg^{-1}) and Diet 2 (167 g kg^{-1}), but not different from that of females fed Diet 1 (178 g kg^{-1}). Although moisture in the tail muscle of females fed Diet 4 was significantly ($P < 0.05$) lower (786 g kg^{-1}) compared to females fed Diet 3 (800 g kg^{-1}), it was not significantly different ($P > 0.05$) compared to females fed Diet 1 and Diet 2 (791 and 795 g kg^{-1}, respectively).

When analysed within the same treatment (diet), there were no significant differences ($P > 0.05$) in proximate compositions of tail muscle between male and female.

Essential amino acid of male and female red claw tail-muscle and EAAI

There were no significant differences in the essential amino acid composition of the tail-muscle meat of male and female red claw among the four treatments (diets). When analysed within the same treatment (diet), there were also no significant differences in amino acids among male and female red claw. Likewise, no significant differences in essential amino acid levels of tail muscle were found among male and female red claw fed the two protein levels (180 and 280 g kg^{-1}) in diets with and without FM. No significant differences in essential amino acid levels were found among male and female red claw fed diets containing FM relative to those of red claw fed diets containing no FM. Means among the four treatments (diets) of each essential amino acid of male and female red claw tail muscle are presented in Table 6.

EAAI for diets in this study ranged from 0.75 to 0.78 for male red claw and 0.72 to 0.80 for female red claw fed 180 g kg^{-1} protein when compared to the tail muscle (Table 6). For those red claw fed the diet with 280 g kg^{-1} protein, EAAI ranged from 1.15 to 1.50 for male red claw and 1.20 to 1.43 for female red claw when compared to the tail muscle.

Processing traits

Processing traits of male and female red claw are presented in Table 7. Male red claw fed Diet 3 had significantly

Table 6 Essential amino acid composition (g kg^{-1}) and the EAAI of the diets and essential amino acid composition (g kg^{-1}) of juvenile red claw (male and female) tail muscle

	Diet				
Amino acid	1	2	3	4	Muscle[1]
Males					
Arginine	8.8	9.5	14.5	18.3	17.8 ± 0.7
Histidine	4.0	4.5	6.3	8.0	4.5 ± 0.2
Isoleucine	5.4	6.0	9.2	12.4	7.9 ± 0.2
Leucine	14.6	15.5	22.5	26.8	14.3 ± 0.3
Lysine	6.2	6.4	11.1	13.5	14.8 ± 0.3
Methionine	3.1	2.7	4.3	4.3	4.3 ± 0.1
Phenylalanine	7.0	7.8	11.3	14.7	7.8 ± 0.2
Threonine	6.8	6.9	11.2	13.0	7.3 ± 0.2
Tyrosine	4.3	5.3	7.8	10.2	6.6 ± 0.1
Valine	7.4	7.7	11.5	14.2	8.3 ± 0.2
EAA index[2]	0.75	0.78	1.15	1.50	
Females					
Arginine	8.8	9.5	14.5	18.3	17.7 ± 0.3
Histidine	4.0	4.5	6.3	8.0	4.3 ± 0.1
Isoleucine	5.4	6.0	9.2	12.4	7.9 ± 0.2
Leucine	14.6	15.5	22.5	26.8	14.6 ± 0.3
Lysine	6.2	6.4	11.1	13.5	14.8 ± 0.3
Methionine	3.1	2.7	4.3	4.3	4.4 ± 0.2
Phenylalanine	7.0	7.8	11.3	14.7	7.9 ± 0.2
Threonine	6.8	6.9	11.2	13.0	7.5 ± 0.2
Tyrosine	4.3	5.3	7.8	10.2	6.6 ± 0.2
Valine	7.4	7.7	11.5	14.2	0.84 ± 0.2
EAAI[2]	0.72	0.80	1.20	1.43	

EAAI = essential amino acid index.

[1] Means of three replications (±SE) for muscle tissue. Muscle values are presented for comparison and are not included in the statistical analysis.

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

($P < 0.05$) higher claw weight (17.9 g) compared with males fed Diet 4 (13.7 g), but not different from males fed Diet 1 (16.3 g) and Diet 2 (15.6 g). Male red claw fed Diet 3 also had significantly higher weight of tail (with shell) and weight of tail muscle meat (no shell) (23.6 and 16.8 g, respectively) compared with male red claw fed Diet 1 (19.6 and 14.1 g) and Diet 2 (20.6 and 14.6 g, respectively), but not different from male red claw fed Diet 4 (21.9 and 15.5 g, respectively). Male red claw fed Diet 3 also had a significantly higher cephalothorax weight (32.2 g) compared with males fed Diet 1 (27.8 g), but not different from males fed the other two diets.

Female red claw fed Diet 3 had significantly higher claw weight (9.7 g) compared with females fed all other diets (Table 7). In weight of tail (with shell), females fed Diet 1 (17.5 g) were significantly lower compared to that of red claw fed Diet 3 and Diet 4 (19.9 and 19.5 g, respectively), but not different to female red claw fed Diet 2 (18.4). Female red claw fed Diet 3 and 4 had significantly ($P < 0.05$) higher weight of tail muscle meat (no shell) compared to that of red claw fed Diet 1 and Diet 2. Female red claw fed Diet 3 had significantly cephalothorax weight (26.4 g) compared with females fed Diets 1 and 2, but not different from females fed Diet 4. Males had significantly ($P < 0.05$) higher percentages of all processing traits in all treatments compared to females except tail muscle weight (in grams) of red claw fed Diet 2 (Table 7).

Harvest percentage of male and female red claw within different weight categories

Figures 1 and 2 show the different weight categories for both male and female red claw. For each treatment, a higher number of males of weights above 60 g (considered to be highly marketable and commanding a high price from consumers) were harvested compared to that of female red claw in the same weight class, which comprised 32.1% of the males compared to only 13.7% of the females fed Diet 1; 37.9% of the males compared to 17.6% of the females fed Diet 2; 50.6% for males compared to 28.5% for females fed Diet 3; and 57% of the males compared to 33.6% of the females fed Diet 4. Conversely, results indicate that a higher number of females weighing between 21 and 60 g (commanding a lower price from consumers) were harvested compared to that of male red claw in the same weight class, which comprised 83.2% of the females compared to 65.2% of the males fed Diet 1; 81.1% of the females compared to 60.5% of the males fed Diet 2; 70.7% of the females compared to 48.4% of the males fed Diet 3; and 65.9% of the females compared to 42.4% of the males fed Diet 4.

Discussion

The results of the present study indicate that red claw grown in ponds can be fed a practical diet with a combination of plant-protein ingredients, and without FM, if the protein level is 280 g kg^{-1}. However, when red claw were fed diets containing 180 g kg^{-1} CP, even with 73 g kg^{-1} FM included, growth was reduced. These data are the first to indicate that the quantity and quality of protein is important for the growth of red claw grown in ponds. In the present study, Diet 4 had a higher percentage of protein than formulated, probably due to differences in the actual percentage of protein in SBM from the value stated by the feed mill prior to diet formulation. However, the additional protein should not have influenced growth or survival of red claw. Thompson *et al.* (2004a) reported that the growth of pond-cultured red claw was not different when fed diets containing 220, 320, or 420 g kg^{-1} protein. However, results from the present study do indicate that diets containing 180 g kg^{-1} protein, as

Table 7 Means (±SE) of claws, tail, tail muscle, and cephalothorax (wet-weight basis) of male and female red claw fed four practical diets containing two levels of protein, with or without fish meal

	Diet			
	1	2	3	4
Males				
Claws (g)	16.3 ± 1.2^{ab,x}	15.6 ± 1.2^{ab,x}	17.9 ± 1.1^{a,x}	13.7 ± 1.0^{b,x}
Tail (g)	19.6 ± 0.8^{b,x}	20.6 ± 1.0^{b,x}	23.6 ± 0.9^{a,x}	21.9 ± 0.8^{ab,x}
Tail muscle (g)	14.1 ± 0.6^{b,x}	14.6 ± 0.7^{b,x}	16.8 ± 0.7^{a,x}	15.5 ± 0.7^{ab,x}
Cephalothorax (g)	27.8 ± 1.1^{b,x}	29.4 ± 1.5^{ab,x}	32.2 ± 1.2^{a,x}	29.9 ± 1.1^{ab,x}
Females				
Claws (g)	7.8 ± 0.3^{b,y}	8.3 ± 0.4^{b,y}	9.7 ± 0.4^{a,y}	7.8 ± 0.3^{b,y}
Tail (g)	17.5 ± 0.6^{b,y}	18.4 ± 0.6^{ab,x}	19.9 ± 0.5^{a,y}	19.5 ± 0.6^{a,y}
Tail muscle (g)	11.7 ± 0.4^{b,y}	11.8 ± 0.4^{b,y}	13.2 ± 0.4^{a,y}	13.1 ± 0.4^{a,y}
Cephalothorax (g)	24.1 ± 0.8^{bc,y}	23.5 ± 0.9^{c,y}	26.4 ± 0.6^{a,y}	26.2 ± 0.7^{ab,y}

Means within a row having different superscripts (a, b) are significantly different ($P < 0.05$) among dietary treatments. Means between males and females in the same column for each respective variable with different superscripts (x, y) are significantly different ($P < 0.05$).

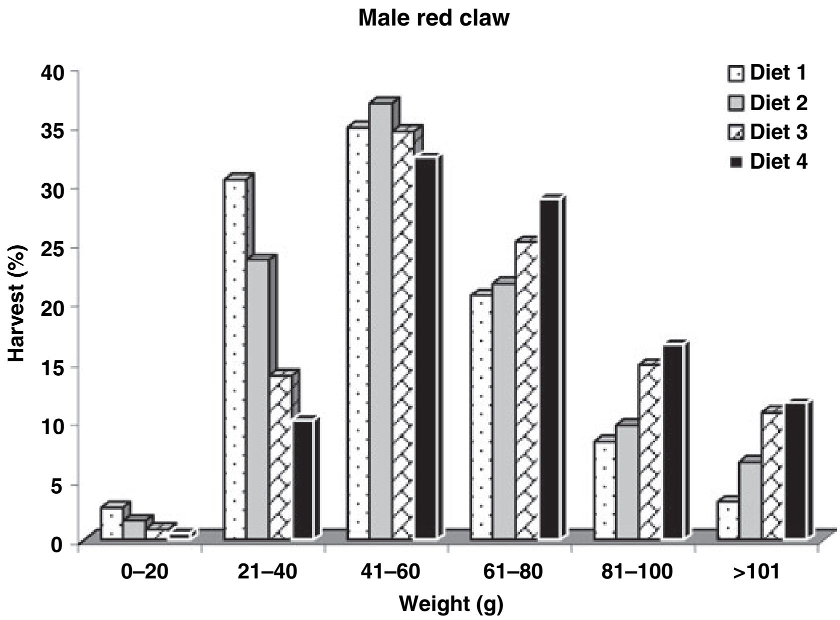

Figure 1 Harvest percentage of male red claw, within different weight categories, from ponds fed four practical diets containing two levels of protein, with or without fish meal. Percentages represent means of three replicates (ponds) per treatment (diet).

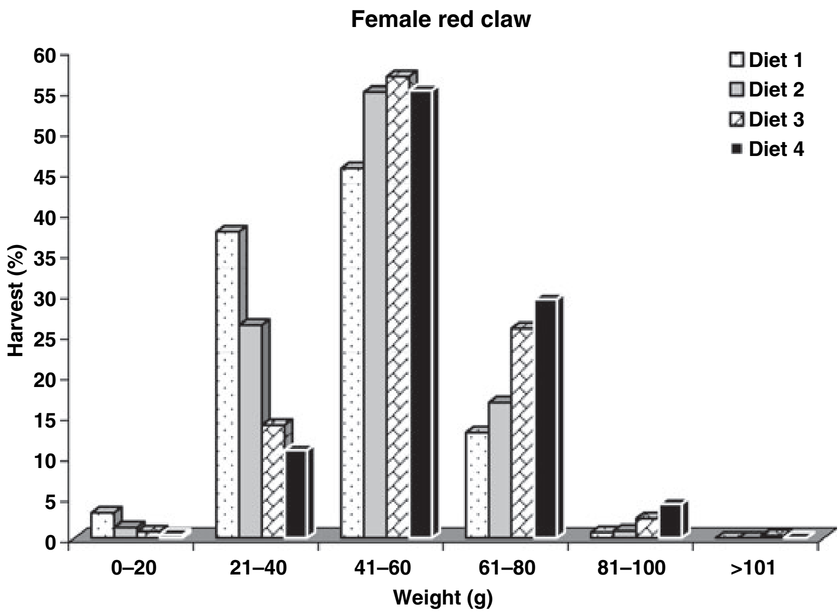

Figure 2 Harvest percentage of female red claw, within different weight categories, from ponds fed four practical diets containing two levels of protein, with or without fish meal. Percentages represent means of three replicates (ponds) per treatment (diet).

formulated in the present study, may not be suitable for red claw when grown in ponds at the stocking density used in the present study. Data from the present pond study were different than found in the laboratory. Thompson *et al.* (2005) found that juvenile red claw (mean weight of 1.12 g) can be fed a diet with 300 g kg^{-1} CP when 150 g kg^{-1} FM is added; however, when FM was not added, a diet with 350 g kg^{-1} CP was required to be fed, when red claw were grown indoors using a recirculating system.

Other reports have examined CP requirements and FM replacement in diets for red claw using indoor systems. Webster *et al.* (1994) indicated that small (mean weight of 0.022 g) juvenile red claw required 330 g kg^{-1} dietary protein (280 g kg^{-1} menhaden FM 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 and reported that small (0.02 g) red claw required 400 g kg^{-1} dietary protein, while a diet containing 300 g kg^{-1} protein was sufficient for larger (3.0 g) red claw. All diets in that study contained high (>290 g kg^{-1}) levels of menhaden FM. Jacinto *et al.* (2003) reported that 1 g juvenile red claw, grown communally, required 310 g kg^{-1} dietary protein for optimal growth using a diet which contained 200 g kg^{-1} sardine meal. Muzinic *et al.* (2004) found that juvenile (0.2–3.1 g) red claw, grown individually in an indoor recirculating system, can be fed a practical diet in which all of the FM is replaced by 800 g kg^{-1} SBM and 50 g kg^{-1} BGY; however, that diet contained 400 g kg^{-1} protein.

While the possibility of red claw consuming additional nutrients is small in the indoor studies when individual culture units are used, this is not the case in pond studies. In the present pond study, natural food items in the pond may have satisfied a part of the protein requirement of red claw, which are omnivorous detritivores. Consequently, this may explain why pond-cultured red claw can be fed a diet containing lower dietary protein levels without added FM, compared to red claw grown indoors. However, no measurement of pond organic matter was made during the present study (although at harvest, pond bottoms were devoid of any organic matter). Thus, it is difficult to state the impact that natural foods contributed to the diet of red claw, especially as the red claw grow larger.

Average final weights in the present study were similar to, or higher than, those stated in some reports (Brummett & Alon 1994; Karplus *et al.* 1995; Salame & Rouse 2000; Webster *et al.* 2004). However, the cooler-than-normal summer in the present study allowed for below normal water temperatures, and may have resulted in slower growth compared to other studies (Rouse & Kahn 1998; Thompson *et al.* 2004a).

Feed conversion ratio values in the present study (3.25–4.13) were lower, or similar to, previously reported values (Rouse & Kahn 1998; Jones & Ruscoe 2000; Thompson *et al.* 2004a; Webster *et al.* 2004), while mean survival rates (65.2%) and total yield (724 kg ha^{-1}) of red claw fed the four treatments in the present study were similar to, or higher than, other reports (Brummet & Alon 1994; Rouse & Kahn 1998; Salame & Rouse 2000; Thompson *et al.* 2004a,b; Webster *et al.* 2004).

At harvest, the percentage of male and female red claw from all treatments was 45.7% male, 52.6% female, and only 1.7% intersex animals. Intersex red claw are those animals that exhibit both male and female secondary sexual characteristics (Medley & Rouse 1993). Thompson *et al.* (2004a)) reported similar percentages of males and females at harvest (48.5% male and 51.5% female). In that study, there were very few (<1%) intersex animals harvested and were counted as males. Webster *et al.* (2004) reported that 48.2% of the harvested red claw were male, 51.8% were female, and <1% were intersex. Percentages of males and females in the present study are consistent with other reports (Karplus *et al.* 1995; Pinto & Rouse 1996; Rouse & Kahn 1998).

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. Thompson *et al.* (2005) stated that in the absence of a commercially available diet formulated specifically for red claw, producers may feed expensive high-quality, commercial shrimp diets, a decision that may be financially and nutritionally wasteful. Another approach would be the use of low-quality diets that do not completely meet nutrient requirements of the species and may reduce growth, health and survival. At present, the amino acid requirements of red claw have not been determined. However, determination of amino acid requirements in crustaceans is difficult (Shiau 1998).

Although a variety of methods have been used in previous feeding trials (Arai 1981; Sick & Millikin 1983), the *n*th 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), as proposed by Tacon & Cowey (1985), was used in the present study to compare the essential amino acid patterns within the diets to those in the tail muscle of red claw (Tidwell *et al.* 1993; Muzinic *et al.* 2004; Thompson *et al.* 2005). While most reports have used the amino acid composition of whole-body tissue of the animal to determine protein requirement (Philipps & Brockway 1956; Deshimaru & Shigeno 1972; Penaflorida 1989), or have shown that there is a significant correlation between the dietary amino acid requirement of the organism and the essential amino acid composition of whole-body tissue (Cowey & Tacon 1983; Wilson & Poe 1985), we used the tail muscle of the red claw for essential amino acid composition.

We feel that use of tail muscle from crustaceans is appropriate based on the fact that tail muscle comprises a vast majority of the whole-body dry-matter protein. Further, it has been reported that the essential amino acid composition of whole-body and tail muscle are very similar (Reed & D'Abramo 1989; Mente *et al.* 2002). Reed & D'Abramo (1989) stated that the dietary amino acid composition of two reference diets and the corresponding amino acid composition of either the whole-body, or tail-muscle tissue, were highly correlated for juvenile Malaysian prawn (*Macrobrachium rosenbergii*). Likewise, Mente *et al.* (2002) stated that total essential amino acid and non-essential amino acid concentrations in the tail muscle and whole-animal tissues of *Litopenaeus vannamei* were not significantly different from each other when shrimp were unfed, or fed with one of three diets. It was found that, while there were some variation in the free pool concentration of individual amino acids in the tail muscle, the total level of essential and non-essential amino acids was stable. Mente *et al.* (2002) suggested that intracellular amino acid pools were not altered by passive movements of amino acids, but were regulated by active transmembrane transport. Thus, the free amino acid patterns of whole-body and tail muscle in *Litopenaeus vannamei* appear stable.

In the present study, calculation of EAAI of the four diets indicated that Diet 1 and Diet 2 (180 g kg^{-1} protein) were deficient in one or more essential amino acids. Oser (1959) developed criteria, later used by Penaflorida (1989), for protein quality of feedstuffs. Good-quality protein sources had EAAI greater than or equal to 0.90, useful protein sources had EAAI of 0.80, and inadequate when sources below 0.70. Although the amino acid requirements of red claw have not been determined, results in the present study indicate that diets containing 280 g kg^{-1} CP, with or without FM, may have sufficiently satisfied the requirements of the essential amino acids of male and female red claw as indicated by the EAAI values which ranged from 1.15 to 1.49. Both were higher than the 0.90 value, indicating that the diets were of high-quality. Conversely, EAAI values of male and female red claw fed 180 g kg^{-1} CP, with or without FM, were as low as 0.72 indicating that these diets may be deficient in one or more essential amino acids. While greater than 0.70, they were below the numerical value considered to be a useful protein source (0.80).

Processing yield data was evaluated to compare the overall meat yields between male and female red claw. Data in the present study indicate that males grow larger, have a higher percentage of their body weight comprised of claw (chelae), higher tail (outer shell) weight, and slightly higher tail muscle (shell off) weight compared to that of females. These results are in agreement with previous red claw studies (Gu *et al.* 1994; Curtis & Jones 1995; Thompson *et al.* 2004a,b). Results also show that a higher percentage of males of weights above 60 g were harvested compared to that of female red claw for each dietary treatment, overall. Red claw in this weight class (>60 g) are considered to be quite marketable and may result in higher prices paid to producers. Overall, our data suggest that there is an advantage of growing all-male monosex populations of red claw for commercial pond production. However, this practice may be labour intensive, since currently no technology exists on an efficient and inexpensive means to hand-sex stocking-size juveniles.

In conclusion, the results of the present study indicate that red claw grown in ponds can be fed a diet with 280 g kg^{-1} CP with 0 g kg^{-1} FM if a combination of plant-protein ingredients (SBM and DDGS) is added; however, it appears that 180 g kg^{-1} CP with 0 or 73 g kg^{-1} FM is not adequate for optimal growth. Reducing protein and FM levels in red claw diets may help reduce operating costs, and thereby increase producer's profits.

Acknowledgements

The authors would like to thank B.R. Lee, M.S. Tee, Sam Wise, and D.R. Wynn for technical assistance; Matthew Smith for feeding and water quality assistance; Michelle Coyle, Rafael Cuevas, Jason Donaher, Pauline Jackson, Robert Janes, Parag Kulkarni, Steven Mims, Noel Novelo, Richard Onders, Daniel Yancey, and Dave Yasharian for help in harvesting and weighing red claw; and Michelle Coyle for typing this manuscript. This research was partially funded by a USDA Capacity Building Grant, a grant from the Kentucky Soybean Promotion Board and Kentucky Soybean Association, and a USDA grant under agreement KYX-80-00-10A to Kentucky State University.

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