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Effect of different dietary protein and lipid levels on growth and survival of juvenile Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens)

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Abstract

This study determined the effect of different dietary protein and lipid levels on growth and survival of juvenile redclaw *Cherax quadricarinatus*. Nine practical test diets were formulated to contain three crude protein (CP) levels [260, 310 and 360 g kg^{-1}, equivalent to 225, 260 and 296 g kg^{-1} digestible protein (DP) respectively] at three crude lipid (CL) levels (40, 80 and 120 g kg^{-1}, equivalent to 38, 67 and 103 digestible lipids respectively), with digestible protein : digestible energy (DP : DE) ranging from 14.6 to 22.6 mg protein kJ g^{-1}. Three replicate groups of 15 crayfish (initial weight mean ± SD, 0.71 ± 0.13 g) per diet treatment were stocked in 40 L tanks, at 28 °C for 60 days. The highest mean weight, specific growth rate and biomass, with values of 7.0 g, 3.67% day^{-1}, and 370.2 g m^{-2}, respectively, were achieved by feeding a diet with P : L = 310 : 80 ($P < 0.05$). The treatments resulted in a survival rate of 80–91%, feed conversion ratio of 1.08–1.61 and protein efficiency ratio of 2.24–3.08. Results indicated that a diet containing 270 g kg^{-1} DP (equivalent to 320 g kg^{-1} CP), 75 g kg^{-1} DL with a DP/DE of 18.4 mg protein kJ^{-1}, and 0.031 g protein per animal per day was optimum for juvenile *C. quadricarinatus* under the tested experimental conditions.

KEY WORDS: *Cherax quadricarinatus*, lipid, nutrition, protein, redclaw

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Introduction

Feed is one of the highest costs in the operation of an aquaculture enterprise (Cruz-Suárez *et al.* 2002), and in many cases reaches 50% of total expenses (Akiyama & Chwang 1990; Martínez-Córdova *et al.* 2003). The nutritional and energy requirements of aquatic animals can be provided by protein, carbohydrate and lipid sources; should one source be in short supply in the diet (Fotedar 2004). Protein is the most expensive component in balanced feeds and is probably the most important feed element for growth of cultured species (Cortés-Jacinto *et al.* 2003a; Johnston *et al.* 2003; Ward *et al.* 2003). To formulate a least-cost, balanced diet, it is necessary to establish the minimum protein level to provide essential amino acids to give acceptable growth (Guillaume 1997; Tacon & Akiyama 1997). Villarreal & Peláez (1999) and Lawrence & Jones (2002) have given general guidelines of protein levels recommended for commercial culture of redclaw.

In Mexico, redclaw crayfish *Cherax quadricarinatus* farms use commercially available pelletized marine shrimp diets with 350 g kg^{-1} crude protein (CP) (Cortés-Jacinto *et al.* 2003b). Keefe & Rouse (1999) showed that 330 g kg^{-1} protein in practical diet formulations seem adequate for juveniles up to 1 g. Survival, growth rate and feed efficiency of redclaw improved with a 310 g kg^{-1} CP diet (Cortés-Jacinto *et al.* 2003a). Dietary lipids play an important role as a source of energy and essential fatty acids (Hernández *et al.* 2002), thus there is an interest in determining the optimal amount of dietary lipid for maximum growth in redclaw crayfish. The optimal dietary lipid for freshwater prawn, *Macrobrachium rosenbergii*, ranges from 20 to 100 g kg^{-1} (D'Abramo & New 2000),

while a significant depression in weight gain was observed in the crayfish *Procambarus acutus acutus*, fed with 90 g kg^{-1} lipid (Davis & Robinson 1986). The crayfish *Astacus astacus*, fed 70 g kg^{-1} lipids had a high survival rate (Ackefors *et al.* 1992), and for *Procambarus clarkii*, the optimal lipid level was 60 g kg^{-1} (Jover *et al.* 1999).

Although studies have used experimental diets for juvenile *Cherax* (Jones *et al.* 1996a,b; Hernández *et al.* 2002; Cortés-Jacinto *et al.* 2003a), little information is available on the nutrient requirement and practical diet formulation for redclaw (Cortés-Jacinto *et al.* 2003a; Thompson *et al.* 2003). Efficient diets contain sufficient non-protein energy sources (lipid and carbohydrate) that are metabolized to meet general energy requirements, allowing an organism to direct the maximum level of available dietary protein to growth (Johnston *et al.* 2003). A proper balance of protein and lipid is required to supply amino acids and calories for rapid growth and efficient protein utilization, and to maintain crayfish flesh composition that is high in protein and low in fat. It has been suggested that the optimal dietary P/E ratio appears to be in the range of 27 and 29 mg protein kJ^{-1} for juvenile crayfish (Ackefors *et al.* 1992). However, studies conducted on redclaw, show that optimum P/E ratio is 16.3 mg protein kJ^{-1} (Cortés-Jacinto *et al.* 2003a). This study was undertaken to determine the appropriate levels of dietary protein and lipid to support optimal growth and survival of the juvenile Australian redclaw crayfish.

Materials and Methods

Experimental diets and growth trial

Nine practical test diets (Table 1) were formulated using the Mixit-Win software (Agricultural Software Consultants, Inc., San Diego, CA, USA) to contain three levels of CP [260, 310 and 360 g kg^{-1}, equivalent to 225, 260 and 296 g kg^{-1} digestible protein (DP), respectively] at three crude lipid (CL) levels (40, 80 and 120 g kg^{-1}, equivalent to 38, 67, and 103 DL, respectively), with ratios of digestible protein : digestible energy (DP : DE) ranging from 14.6 to 22.6 mg protein kJ g^{-1} (Table 2). Table 3 presents the theoretical amino acid composition of diets. The amino acid profiles of diets were calculated from ingredient amino acid composition reported by National Research Council (1983), and Tacon (1990). DE of the diets was calculated based on conversion factors of 16.7 kJ g^{-1} protein and carbohydrate, and 37.6 kJ g^{-1} lipid (Cuzon & Guillaume 1997). The values of DP : DE ratios in each diet were calculated using digestible values of the ingredients (Table 4), based on the results of Campaña (2001).

All ingredients were sieved through a 500-μm mesh size. Each diet was prepared by mixing all the macro-ingredients in an electric mixer. The micro-ingredients (vitamin premix, mineral premix, choline chloride and calcium carbonate) that were mixed in a plastic container, were added to the macro-ingredients. Fish oil and soy lecithin homogenized into an emulsion was added to the mixture and then

Table 1 Content and composition (g kg^{-1}) of the experimental diets (wet weight basis)

	Diets (protein : lipid)								
Ingredients	260 : 40	260 : 80	260 : 120	310 : 40	310 : 80	310 : 120	360 : 40	360 : 80	360 : 120
Sardine meal[1]	133	138	143	220	225	231	308	312	318
Sorghum meal[1]	625	582	533	538	495	444	450	408	357
Fish oil[1]	0	19	41	0	19	42	0	20	42
Soy lecithin[1]	0	19	41	0	19	41	0	18	41

Common ingredients: soybean meal[1], 100; squid meal[2], 30; wheat meal[2], 30; binder (grenetine), 40; mineral premix[3], 25; vitamin premix[4], 4; ascorbic acid[5], 2; choline chloride[6], 1; calcium carbonate[7], 10.

[1]PIASA®, La Paz, B.C.S., México.

[2]Malta Texo de México, S.A. de C.V., Sinaloa, México.

[3]g kg^{-1} diet: KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $ZnSO_4 \cdot 7H_2O$, 0.09; $MnCl_2 \cdot 4H_2O$, 0.0234; $CuSO_4 \cdot 5H_2O$, 0.005; KI, 0.005; $CoCl_2 \cdot 2H_2O$, 0.0025; Na_2HPO_4, 2.37.

[4]Except where units are given, values are in mg kg^{-1} diet: Vit. A retinol, 5000 UI; Vit. D_3, 4000 UI; Vit. E α-tocopheryl acetate, 100; Vit. K menadione, 5; thiamin, 60; riboflavin, 25; pyridoxin, 50; pantothenic acid, 75; niacin, 40; biotin, 1; inositol, 400; cyanocobalamin, 0.2; folic acid, 10.

[5]Stay-C (35% active agent). (Roche®).

[6]Choline chloride (65% active agent).

[7]ACS reagent (Sigma®).

Table 2 Proximate composition of the experimental diets (g kg^{-1} dry matter)

	Diets (protein : lipid)								
	260 : 40	260 : 80	260 : 120	310 : 40	310 : 80	310 : 120	360 : 40	360 : 80	360 : 120
Protein[1]	267.0 ± 0.1	266.7 ± 0.7	266.6 ± 1.2	316.8 ± 0.6	314.8 ± 0.3	315.3 ± 1.2	363.9 ± 0.99	362.9 ± 1.66	365.8 ± 0.9
Ether extract[1]	43.2 ± 0.6	87.8 ± 0.3	122.5 ± 0.2	48.5 ± 0.9	83.0 ± 0.7	122.0 ± 0.88	49.2 ± 0.3	87.0 ± 0.9	121.0 ± 0.8
Ash[1]	69.8 ± 0.2	68.6 ± 0.3	81.7 ± 0.3	82.6 ± 0.6	83.0 ± 0.5	69.0 ± 0.2	95.3 ± 0.4	97.6 ± 0.3	96.2 ± 0.8
Fibre[1]	3.9 ± 0.4	6.9 ± 0.7	2.5 ± 0.1	2.7 ± 0.2	3.9 ± 0.1	6.9 ± 0.1	15.3 ± 1.8	10.4 ± 2.3	12.3 ± 0.3
NFE[1]	547.7	499.5	450.5	486.9	444.2	415.0	410.6	353.2	335.5
Gross energy (kJ g^{-1})	17.5	18.0	19.2	17.5	18.2	19.1	17.9	18.7	19.4
Digestible protein[2]	229	227	226	264	263	261	300	299	297
Digestible energy[3]	140	146	154	136	143	150	133	139	147
DP : DE (mg protein kJ^{-1})	16.3	15.5	14.6	19.4	18.4	17.4	22.6	21.5	20.2

[1]Mean ± SD, $n = 3$; NFE, nitrogen free extract, calculated by difference.
[2]Calculated using ingredient protein digestibility values (Campaña 2001).
[3]Computed as 16.7 kJ g^{-1} protein and carbohydrates and 37.6 kJ g^{-1} lipids (Cuzon & Guillaume 1997).

Table 3 Amino acid composition (g kg^{-1} of diet) of diets containing different protein and lipid levels fed to juvenile crayfish *C. quadricarinatus*

	Diets (protein : lipid)								
Amino acid	260 : 40	260 : 80	260 : 120	310 : 40	310 : 80	310 : 120	360 : 40	360 : 80	360 : 120
Arginine	14.0	14.2	14.4	18.1	18.3	18.5	22.2	22.4	22.6
Histidine	6.1	6.2	6.3	8.5	8.5	8.7	10.8	10.9	11.0
Isoleucine	9.1	9.2	9.3	11.8	11.9	12.0	14.6	14.6	14.8
Leucine	17.6	17.6	17.6	21.9	21.9	22.0	26.3	26.3	26.3
Lysine	13.7	13.9	14.3	18.9	19.1	19.5	24.1	24.4	24.7
Methionine	4.6	4.7	4.8	6.4	6.4	6.5	8.1	8.2	8.3
Phenylalanine	9.3	9.3	9.4	11.7	11.8	11.9	14.2	14.2	14.3
Tyrosine	6.0	6.2	6.3	8.4	8.6	8.7	10.8	11.0	11.1
Serine	10.2	10.4	10.7	14.4	14.6	14.9	18.6	18.8	19.1
Threonine	8.6	8.8	8.9	11.6	11.7	11.9	14.6	14.7	14.8
Valine	9.7	9.8	9.9	12.6	12.7	12.8	15.6	15.7	15.8

Values are calculated using ingredient amino acid values obtained by Tacon (1990) and National Research Council (1983).

Table 4 Proximate analysis of the ingredients from experimental diets

Ingredients	Protein[1]	Ether extract[1]	Ash[1]	Fibre[1]	NFE	APD (%)	ALD (%)
Sardine meal	689 ± 1.9	84 ± 0.2	157 ± 0.5	1 ± 0.1	69	72.4	83.5
Squid meal	820 ± 0.5	40 ± 0.4	102 ± 1.2	17 ± 1.4	21	70.8	84.6
Sorghum meal	99 ± 0.1	34 ± 1.5	17 ± 0.2	20 ± 0.8	830	89.6	85.6
Soybean meal	477 ± 0.8	5 ± 0.5	70 ± 0.2	21 ± 1.6	427	91.8	93.6
Wheat meal	139 ± 0.7	6 ± 0.3	10 ± 0.3	0 ± 0.0	845	90.5	95.0

[1]Dry matter (g kg^{-1}), mean ± SD, $n = 3$
NFE, nitrogen free extract, calculated by difference.
APD, apparent protein digestibility, values obtained by Campaña (2001) for the same set of ingredients.
ALD, apparent lipid digestibility, values obtained by Campaña (2001) for the same set of ingredients.

sufficient distilled water was added to provide 30% of the weight of ingredients. The dough was then passed through a meat grinder (Tor-Rey™, Monterrey, N.L., México) to form 2-mm pellets, which were dried in an air flux oven (Hafo Series 1600; Sheldon Manufacturing, Inc., Cornelius, OR, USA) at 40 °C for 8 h. Proximate analyses of ingredients and diets were carried out according to the methods of the AOAC (1995). Gross energy of the diet was

measured in an adiabatic bomb calorimeter (model 1261; 3 Parr Moline, IL, USA).

Diet efficacy was measured as a function of the weight increase, and by the calculation of the following parameters, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR).

Intermolt crayfish were weighed using an electronic digital balance (Ohaus Navigator^{TM}; Ohaus Scale Corp., Florham Park, NJ, USA) every 2 weeks.

Culture conditions

The trial was conducted at the Nutrition Laboratory of CIBNOR (La Paz, B.C.S., Mexico) for 60 days. A 36-unit static water experimental system was used, and each tank had a 40-L capacity. Nine dietary treatments were tested in triplicate, using juvenile redclaw crayfish (0.71 ± 0.13 g) selected from a reproduction pond at CIBNOR. Crayfish were stocked in fibreglass units at a density of 15 specimens per tank, and the diets were randomly assigned to each tank. Crayfish that died during the first 3 days of experiment were replaced with animals held under identical conditions.

All tanks were cleaned daily in the morning before feeding. Faeces and dead animals were removed daily from each tank. Dry feed intake was determined by feeding to apparent satiation. Juveniles redclaw were initially fed at a rate of 5% of biomass day^{-1}. Each day, 30% was fed at 08:00 hours, 30% at 14:00 hours and 40% at 20:00 hours (according to Cortés-Jacinto *et al*. (2003b). The following morning leftover feed, which could be readily identified by its swollen pellet shape, was removed and quantified by concentrating on a Whatman no. 1 filter paper with a vacuum pump (GastTM; MFG Corp., Benton Harbor, MI, USA), before drying at 50 °C for 18 h in an air flux oven (Hafo Series 1600, VWR 4 1680).The ration adjusted accordingly to minimize the amount of uneaten feed. The correction was repeated weekly.

Basic physicochemical factors of water quality were measured. Temperature and dissolved oxygen were measured twice daily in all tanks using a dissolved oxygen meter (model 55-DO®; YSI Incorporated, Yellow Springs, OH, USA); pH was measured once a day with a pH meter (American Marine®, Ridgefield, CT, USA); N-nitrate, N-nitrite, and total ammonium nitrogen were measured weekly with a spectrophotometer (model DREL-2010; The Hach Company, Loveland, CO, USA). Temperature was maintained at 28.01 ± 0.33 °C during the 60 days of culture with 100-W heaters (Aquarium Pharmaceuticals, Inc. Paris, France). A 5-hp blower (Sweetwater®, Apopka, FL, USA) and air stones maintained dissolved oxygen levels above 6.11 ± 0.09 mg L^{-1}. Approximately 30% of the water volume in each tank was replaced daily with fresh tap water. The natural photoperiod was 14L : 10D. Crayfish habitat was provided in the form of bundles of an open weave synthetic mesh (similar to that used for onion bags) held in place with a little rock weight.

Biochemical analysis of muscle

At the end of the trial, experimental organisms were pooled from the three replicates per treatment, and five crayfish were randomly selected from each treatment and killed for analysis. A portion of muscle of each sampled crayfish was homogenized in 1.2% NaCl saline solution, according to the methods described by García-Guerrero *et al*. (2003). To quantify proteins, the homogenate was first digested for 30 min at ambient temperature with 0.5 N NaOH; protein concentration was determined by the Bradford (1976) method, using albumin as standard. For carbohydrates, proteins were precipitated with 20% trichloroacetic acid and centrifuged at 2608 × ***g*** for 10 min at 4 °C; carbohydrates were then quantified from the supernatant by the Anthrone method (Van Handel 1965), using glucose as standard. To determine total lipids, an adaptation of the method of Barnes & Blackstock (1973) was used: 0.1 mL homogenate was mixed with 1 mL 76% H_2SO_4, and incubated at 80 °C for 10 min. The acid solution obtained was mixed with phosphosulphovanillin reagent. Concentration was calculated using a mixture of triacylglycerols (12 mg mL^{-1}) and cholesterol (8 mg mL^{-1}) as standard.

Statistical analysis

Final weight, biomass (g m^{-2}), SGR, PER, feed intake, protein intake, FCR, water quality data and muscle composition data were subjected to a one-way ANOVA. Differences were considered significant at $P < 0.05$. Individual differences between dietary treatments were determined by Duncan's new multiple range test (Zar 1999) in the statistical computer package (StatSoft® Inc., Tulsa, OK, USA). The interaction between protein and lipid was evaluated by a completely randomized block design with two factors. The lipids factor had three levels, 40, 80 and 120 g kg^{-1}, while protein factor had three levels, 260, 310 and 360 g kg^{-1}. Survival percentages were transformed by the square root of the arcsine before statistical analyses (Zar 1999). Growth response (in terms of average weight gain in grams) to DP concentrations was fitted to a quadratic model (second-order polynomial) (Zeitoun *et al*. 1976; Gurure *et al*. 1995; Shearer

2000). This model fits dose–response relations on the basis of mathematical and/or biological principles (Gurure *et al.* 1995).

Results

Over the duration of the study, water quality parameters averaged (±SD) were: temperature, 28.01 ± 0.33 °C; dissolved oxygen, 6.11 ± 0.09 mg L^{-1}; total ammonia nitrogen, 0.11 ± 0.06 mg L^{-1}; nitrite, 0.08 ± 0.04 mg L^{-1}; nitrate, 5.67 ± 0.06 mg L^{-1}; pH, 7.90 ± 0.13. These values were within acceptable limits for indoor production of redclaw crayfish (Masser & Rouse 1997). Survival of juvenile crayfish was in the range of 80–91%. The amino acid composition for the nine practical diets is shown in Table 3.

Dietary protein concentration significantly affected growth of juvenile crayfish ($P < 0.05$). Final mean weights of crayfish were highest for protein and lipid levels of 310 : 80, 310 : 120, 360 : 40, 360 : 80 and 360 : 120. Juvenile crayfish fed diets with the lowest protein and lipid levels (260 : 40, 260 : 80, 260 : 120 and 310 : 40) showed the poorest growth, lowest biomass and higher FCR among all treatments (Table 5). Crayfish fed the 310 : 80 diet had significantly higher weight gain (Table 5) and final biomass than treatments with lower protein level ($P < 0.05$).

A two-way ANOVA revealed a significant effect of protein level on most parameters, except feed intake; the lipid level had no significant effect on any parameter ($P < 0.05$).

The PER showed a significantly decreasing trend in relation to increasing dietary protein. Crayfish fed diets with the highest protein level used dietary protein less efficiently ($P < 0.05$) than those fed the lowest level, but protein intake increased with increasing protein levels in the diet. However, daily feed intake in the present study was not affected by dietary protein level (Table 5).

Polynomial regression analysis between DP and weight gain of the crayfish indicated a weight gain peak at dietary protein level of 270 g kg^{-1} ($y = -41.14 + 3.53 \times -0.064x^2$, $r^2 = 0.802$, $P < 0.05$) equivalent to 320 g kg^{-1} CP ($y = -27.629 + 2.14 \times -0.033x^2$, $r^2 = 0.802$, $P < 0.05$). This predicted equation is presented in Fig. 1.

Biochemical composition of muscle tissue of crayfish killed at the termination of the experiment is shown in Table 6. Protein, lipid and carbohydrate content in muscle were significantly affected by treatments. Protein and lipid content of crayfish fed the 310 : 80 diets was significantly ($P < 0.05$) higher than that of crayfish fed the 260 : 40 and 260 : 80 diets.

Table 5 Growth response and feed utilization of juvenile redclaw crayfish after 60 days of feeding on experimental diets containing different levels of protein and lipid (mean ± SD, $n = 3$)

Diet (protein : lipid)	SR[3] (%)	Final weight (g)	Biomass (g m^{-2})	SGR[4] % day^{-1})	PER[5]	Feed intake (g day^{-1} $animal^{-1}$)	Protein intake (g day^{-1} $animal^{-1}$)	FCR[6]
260 : 40	86.5 ± 3.5^{abc}	5.2 ± 0.22^{a}	287.4 ± 13.4^{a}	3.23 ± 0.04^{a}	2.58 ± 0.05^{ab}	0.110 ± 0.0^{a}	0.027 ± 0.0^{a}	1.55 ± 0.07^{cd}
260 : 80	84.4 ± 3.8^{abc}	5.8 ± 0.23^{ab}	295.4 ± 16.3^{a}	3.35 ± 0.10^{abc}	2.71 ± 0.11^{ab}	0.115 ± 0.0^{a}	0.028 ± 0.0^{a}	1.47 ± 0.16^{cd}
260 : 120	81.6 ± 3.3^{ab}	5.5 ± 0.25^{a}	307.0 ± 4.6^{a}	3.31 ± 0.12^{ab}	2.49 ± 0.16^{ab}	0.121 ± 0.0^{a}	0.030 ± 0.0^{a}	1.61 ± 0.25^{d}
310 : 40	89.9 ± 2.8^{bc}	5.8 ± 0.21^{ab}	325.5 ± 10.8^{ab}	3.36 ± 0.09^{abc}	2.43 ± 0.22^{ab}	0.109 ± 0.0^{a}	0.032 ± 0.0^{ab}	1.37 ± 0.23^{bcd}
310 : 80	91.1 ± 1.9^{c}	7.1 ± 0.25^{c}	370.2 ± 15.2^{c}	3.67 ± 0.17^{c}	3.08 ± 0.31^{b}	0.106 ± 0.0^{a}	0.031 ± 0.0^{ab}	1.08 ± 0.20^{a}
310 : 120	84.4 ± 3.8^{abc}	6.7 ± 0.20^{bc}	357.0 ± 4.9^{bc}	3.62 ± 0.09^{bc}	2.88 ± 0.11^{ab}	0.109 ± 0.0^{a}	0.032 ± 0.0^{ab}	1.14 ± 0.08^{ab}
360 : 40	80.0 ± 6.0^{a}	6.3 ± 0.21^{abc}	328.6 ± 9.7^{ab}	3.52 ± 0.04^{abc}	2.24 ± 0.10^{a}	0.113 ± 0.0^{a}	0.039 ± 0.0^{c}	1.27 ± 0.13^{abc}
360 : 80	81.6 ± 3.3^{ab}	6.4 ± 0.21^{abc}	345.6 ± 7.8^{bc}	3.52 ± 0.07^{bc}	2.27 ± 0.14^{a}	0.113 ± 0.0^{a}	0.039 ± 0.0^{c}	1.26 ± 0.17^{abc}
360 : 120	84.4 ± 3.8^{abc}	6.3 ± 0.27^{abc}	348.9 ± 10.6^{bc}	3.51 ± 0.04^{abc}	2.24 ± 0.09^{a}	0.111 ± 0.0^{a}	0.038 ± 0.0^{bc}	1.26 ± 0.11^{abc}
Probability[1]	0.003	0.036	0.009	0.038	0.011	0.537	0.000	0.001
Protein[2]	0.036	0.006	0.001	0.005	0.002	0.162	0.000	0.000
Lipid[2]	0.641	0.128	0.274	0.148	0.153	0.674	0.791	0.419
PxL[2]	0.431	0.561	0.461	0.613	0.315	0.662	0.788	0.297

[1]Probability by a one-way ANOVA between treatments. Values within columns with the same superscript letters are not significantly different (Duncan's test, $P = 0.05$).
[2]Two-way ANOVA *P*-level.
[3]Survival rate (SR) = (Final number of crayfish/initial number of crayfish) × 100.
[4]SGR (%/day) = [(ln W_x − ln W_i)/t] × 100, where ln W_x is the natural logarithm of weight at time x and ln W_i is the natural logarithm of the initial weight, and t is the time interval between i and x.
[5]Protein efficiency ratio (PER) = weight gain (*g*)/protein intake (*g*)
[6]Feed conversion ratio (FCR) = feed intake (g)/weight gain (g)

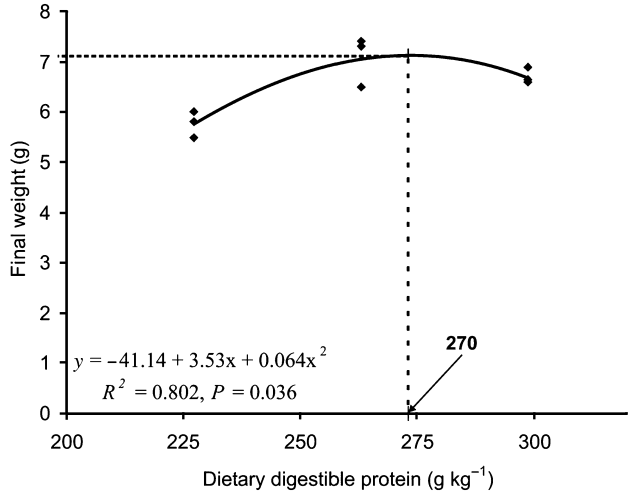

Figure 1 Effect of dietary digestible protein level on final mean weight of juvenile *C. quadricarinatus* ($n = 3$).

Table 6 Biochemical composition (mean ± SD $n = 5$) of muscle of juvenile redclaw crayfish (wet weight basis)

Diets (protein : lipid)	Muscle		
	Protein (mg g^{-1})	Lipid (mg g^{-1})	Carbohydrate (mg g^{-1})
260 : 40	158.9 ± 12.4^{a}	7.5 ± 1.2^{abc}	11.4 ± 2.6^{c}
260 : 80	154.2 ± 19.5^{a}	6.5 ± 0.6^{abc}	6.0 ± 1.9^{ab}
260 : 120	169.7 ± 18.7^{ab}	7.2 ± 2.1^{abc}	7.6 ± 2.0^{ab}
310 : 40	171.1 ± 13.0^{ab}	7.6 ± 1.4^{abc}	12.1 ± 5.1^{c}
310 : 80	193.1 ± 9.6^{b}	8.3 ± 1.1^{bc}	7.9 ± 0.8^{ab}
310 : 120	181.3 ± 17.0^{ab}	6.0 ± 1.1^{ab}	5.3 ± 1.8^{a}
360 : 40	190.5 ± 22.8^{b}	6.9 ± 3.1^{abc}	8.4 ± 1.8^{ab}
360 : 80	189.2 ± 12.5^{b}	8.5 ± .1.3^{c}	9.0 ± 0.5^{b}
360 : 120	178.9 ± 16.2^{ab}	5.6 ± 0.2^{a}	4.1 ± 0.8^{a}

Values within columns with different superscript letters are significantly different ($P < 0.05$).

Discussion

The physical, chemical and biological aspects of water influence the ability of crayfish to perform physiological functions, including growth. Culture conditions of the study were satisfactory and fell within the standards for nutritional evaluations in crustaceans (Akiyama & Chwang 1990; Meade & Watts 1995; Masser & Rouse 1997); and were similar to those reported by Hernández *et al.* (2002) in a nutritional trial with the species. Concentration of nitrite, nitrate and total ammonia nitrogen in the water remained under limits considered dangerous for cultured crayfish (Meade & Watts 1995; Masser & Rouse 1997). In the present experiment, good survival (≥80%) was obtained after 2 months of rearing. Jones *et al.* (1996a)), Webster *et al.* (1994) and Hernández-Vergara *et al.* (2003) report similar results for the species. A survival of more than 80% is considered good in crustacean studies (Cuzon & Guillaume 1997).

Protein quality depends upon palatability, essential amino acid composition and digestibility (Webster *et al.* 1994). Optimal dietary protein level for crayfish is influenced by dietary protein to energy balance, amino acid composition and digestibility of the protein in the diets. Although the amino acid contents of these protein ingredients was calculated, the relatively good FCR and PER values indicate that assimilation of amino acid was not a problem when compared with muscle amino acid composition for redclaw (Thompson *et al.* 2005). Theoretical amino acid levels in the diets were not limiting, with the exception of arginine for the 260 g kg^{-1} CP diets. This limitation could have resulted in a growth reduction in this trial. Mixture of animal and plant proteins are thought to provide better weight gain than either alone because the mixture will generally contain a complementary blend of amino acids which are more likely to meet or exceed the requirements (Lovell 1998). All diets were accepted equally well by the redclaw as there were no significant differences in feed intake. However, the diets were formulated using highly digestible ingredients (Campaña 2001), similar to those used on experimental diets designed for decapod crustaceans (Jones *et al.* 1996b; Cortés-Jacinto *et al.* 2003a). In practical diet formulations for crayfish, protein constitutes one of the major costs in terms of nutrients and ingredients. To minimize feed costs, it is important to optimize both dietary protein level and utilization by crayfish (Cortés-Jacinto *et al.* 2003a). Diets containing 310 and 360 g kg^{-1} CP with 80 and 120 g kg^{-1} CL showed the best results in terms of growth, biomass and FCR. In the present study, FCR of diet 310 : 80 was close to 1.08, and did not exceed 1.61 for any treatment. These values are similar to those found by Jones *et al.* (1996a) for juvenile *Cherax destructor* (0.95–1.21) and Hernández *et al.* (2002) for juvenile redclaw crayfish (1.03–1.32). Jones *et al.* (1996b) indicated that *C. destructor* fed nutritionally deficient diets, resulted in poor growth, while relatively high growth rates were observed for *C. albidus* when it was fed a high-protein diet. Although limited studies have been conducted to evaluate the influence of dietary protein on protein content of body tissue (Guillaume 1997), in this study, the highest mean protein content in the muscle was obtained when feeding the 360 : 80 diet. Dietary energy content (18.2 and18.7 kJ g^{-1}) was also in the range reported for better growth of juvenile *C. destructor* (Jones *et al.* 1996a) at 18.5 kJ g^{-1}, and similar to that used previously for juvenile redclaw crayfish (Cortés-Jacinto *et al.* 2003a) at 18.9 kJ g^{-1}.

Our results indicated that juvenile redclaw crayfish required 270 g kg^{-1} DP (equivalent to 320 g kg^{-1} dietary CP) and 67 g kg^{-1} DL for optimum growth. The findings of this study are also consistent with results of Hernández *et al.* (2002), where diets containing 300 and 350 g kg^{-1} CP and a lipid level between 40 and 80 g kg^{-1} produced the best growth in the same species, and Webster *et al.* (1994) which recommended 330 g kg^{-1} CP for redclaw. Nevertheless, these authors did not report optimal DP requirements. As CP of meals is variable in quality, results in terms of DP facilitate comparisons with other studies. Protein requirements for crayfish found in this research are similar to the range for other decapods. Some researchers (Davis & Robinson 1986; Ackefors *et al.* 1992; Hernández *et al.* 2002) found that high lipid content (120 g kg^{-1}) reduced crayfish growth. Davis & Robinson (1986) demonstrated that the optimum lipid level for *P. acutus acutus* was 60 g kg^{-1}. Webster *et al.* (1994) suggest a lipid level of around 100 g kg^{-1} for adequate growth of juvenile redclaw crayfish. Hernández-Vergara *et al.* (2003), on the other hand reported that juvenile redclaw do not require lipids at levels above of 40 g kg^{-1} when natural food is present in the culture system. In our study, 80 g kg^{-1} lipids were optimum for the species in clear water systems.

Diets 310 : 40 and 360 : 120 did not differ in growth response and feed utilization. Redclaw juveniles fed with a 310 : 40 diet probably did not have enough available energy to spare all protein for tissue formation, thus resulting in growth reduction. A decrease in weight gain beyond the optimum DP : DE ratio has been previously observed in some studies with shrimp (Sedgwick 1979; Hajra *et al.* 1988), where the reduction in the retention of synthesized protein appears to be a consequence of excess dietary protein over dietary energy (Carter & Houlihan 2001; Ward *et al.* 2003). In crustaceans this imbalance results in higher protein degradation and lower retention of synthesized protein (Hewitt 1992). However, Lawrence & Jones (2002) have reported that diets composed of primarily of vegetable ingredients, rather than animal meals, result in better growth of redclaw crayfish. In juveniles fed the 360 : 120 diet, excess fishmeal as a protein source could have reduced growth rate, as was previously reported by Cortés-Jacinto *et al.* (2003a). Furthermore, it has been stated that high lipid levels could reduce growth in redclaw. This is similar to the report by Ward *et al.* (2003) where an increase in lipid level did not result in a higher growth rate, suggesting that the extra lipid was not efficiently used as an energy source for protein sparing. Lack of differences in growth between 310 : 40 and 360 : 120 treatments could also be related to the fact that carbohydrates have been shown to spare protein more efficiently than lipids in crustaceans (Bautista 1986; López-López *et al.* 2005). Additional carbohydrates in the 310 : 40 diet may have spared relatively more dietary protein with respect to the 360 : 120 diet.

Juvenile *C. albidus* and *C. destructor* fed diets containing 300 g kg^{-1} CP produced SGRs of 2.3% day^{-1} and 1.9% day^{-1} respectively (Jones *et al.* 1996b). In our study, juvenile redclaw generated SGRs between 3.2 and 3.6% day^{-1}, which agree with the work of Thompson *et al.* (2005), and show that *C. quadricarinatus* has a higher bioenergetic efficiency than other decapods.

Optimal dietary protein level is moderately dependent upon size or age (Guillaume 1997; Campaña 2001; Gutiérrez-Yurrita & Montes 2001). Cortes-Jacinto *et al.* (2003), defined the requirements for juvenile redclaw crayfish (1 g) to be 310 g kg^{-1} CP with 18.9 kJ g^{-1} gross energy. For juvenile crayfish, the optimum DP : DE ratio was 18.4 mg protein kJ g^{-1} with 270 g kg^{-1} DP level, 75 g kg^{-1} DL, and 0.031 g of protein intake per animal per day, based on performance, survival, SGR, FCR and PER. In the present study, the absence of interaction implies that the effect of the protein level on growth was the same for different lipid levels. Therefore, interaction among factors is an effect on the treatments that is in addition to the sum of the effects of each factor considered separately. However, further research is still needed.

Results of the current research contribute to the development of nutritionally balanced and practical diets that will optimize production of redclaw crayfish.

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