



Growth response and fatty acid composition of juvenile red claw crayfish (*Cherax quadricarinatus*) fed different sources of dietary lipid

K.R. THOMPSON¹, T.J. BAILEY¹, L.S. METTS¹, Y.J. BRADY² & C.D. WEBSTER¹

¹ Aquaculture Research Center, Kentucky State University, Frankfort, KY, USA; ² Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA

(Correction added on 23 November 2009, after first online publication: T. J. Bailey was changed from third author to second author, and L. S. Metts from second author to third author.)

Abstract

A study was conducted to determine the effects of different sources of dietary lipid that differ in fatty acid (FA) composition on growth, feed conversion ratio (FCR), survival, and whole-body proximate and FA composition of juvenile red claw. Juvenile red claw (0.55 ± 0.02 g) were reared over a 12-week period. Five practical diets were supplemented with 70 g kg⁻¹ oil (by weight) from either linseed oil (LO), canola oil (CAO), corn oil (CO), beef tallow (BT) or menhaden oil (MO) and formulated to contain 400 g kg⁻¹ crude protein. Red claw were fed three times daily (800, 1200 and 1600) to controlled excess. At the conclusion of the experimental period, there were no significant differences ($P > 0.05$) found in percentage survival and FCR among dietary treatments and values averaged 85.4% and 3.4, respectively. However, final mean weight and specific growth rate of red claw fed Diet 1 (LO) was significantly higher (16.44 g and 3.95% day⁻¹, respectively) compared with that of red claw fed Diet 4 (BT; 12.24 g and 3.43% day⁻¹, respectively), but not different from red claw fed the other three diets. Likewise, red claw fed Diets 1 (LO) and 2 (CAO) had significantly higher percentage weight gain (2990 and 2880%, respectively) compared with the BT diet (2124%), but not different from red claw fed Diets 3 (CO) and 5 (MO). Whole-body fat and ash composition was significantly affected ($P < 0.05$) by source of added lipid, but no differences were found in whole-body moisture and protein. Moreover, whole-body FA composition showed differences among the varying oil sources and primarily reflected the FA in the diets. Results of the present study indicate that plant oils LO, CAO and CO rich in linolenic acid (18:3n-3), linoleic acid (18:2n-6) and oleic acid (18:1n-9) perform as well as MO containing high levels of n-3 highly unsaturated fatty acids eicosapentanoic acid (20:5n-3) and docosahexaenoic acid

(22:6n-3) for juvenile red claw crayfish grown indoors lacking natural food items. Further, red claw fed a diet containing BT, which has a high percentage of saturated FA, performed poorly compared with LO and CO in regards to weight gain. It appears that juvenile red claw can be fed diets containing less expensive plant-based oils with high levels of 18-carbon unsaturated fatty acids. This could reduce diet costs for producers and allow for profitability.

KEY WORDS: beef tallow, canola oil, *Cherax quadricarinatus*, corn oil, fatty acid, linseed oil, menhaden oil, n-3 highly unsaturated fatty acids, red claw

Received 10 October 2008, accepted 13 May 2009

Correspondence: Carl D. Webster, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601, USA. E-mail: cwebster@dkr.net

Introduction

Globally, the Australian red claw crayfish *Cherax quadricarinatus* has become a popular crustacean species in several countries around the world because of their potential large size and resemblance to high-priced American lobster *Homarus americanus*. However, production of red claw for aquaculture purposes in the USA continues to be scattered and small-scale principally because hatchery-grown, stocking-size juveniles are not readily available. Producers must purchase these individuals from hatcheries in other countries (Mexico or Australia) and costs can range from \$0.50 to \$0.60 (US) per juvenile (with transport costs).

Although there are obvious constraints of commercial culture of red claw in the USA, there are several favourable attributes that include: their tolerance to a wide range of water quality parameters; ease of spawning; ability to accept

a prepared diet; rapid growth rates (60–90 g) in a limited 3- to 4-month growing season in temperate-climate ponds; and their non-aggressive and non-burrowing behaviour which allows producers to stock juveniles in ponds at high densities with little or no cannibalism (Thompson *et al.* 2006; Metts *et al.* 2007).

In the USA, production of red claw may be intensive in nature (Thompson *et al.* 2005a). As diet costs can represent a large portion (up to 80%) of the total operating expenses of an aquaculture enterprise, and red claw grown intensively must rely on a prepared diet, it is important to keep diet costs as low as possible to help increase producers' profits. Currently, many producers of red claw utilize a modified marine shrimp diet to ensure adequate nutrition and/or use low-quality diets that do not meet all their nutritional requirements, thus compromising growth (Thompson *et al.* 2006).

Lipids are commonly known to play an important role as a concentrated source for dietary energy and offer other important nutritional functions such as a good source of essential fatty acids (EFA), sterols, phospholipids, fat-soluble vitamins, structural roles in cell membranes, components in hormones and precursors for prostaglandins and other eicosanoids (Deering *et al.* 1997; Lim *et al.* 1997; Sheen & Wu 1999). However, there is a need to diversify dietary lipid sources of either terrestrial plant and/or animal origin from both sustainability and economic standpoints (Huang *et al.* 2008). Fish oil is high in *n*-3 highly unsaturated fatty acids (HUFA), such as eicosapentanoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3), and is often utilized better than alternative vegetable oils and animal fats in some crustacean species (Guary *et al.* 1976; Kanazawa *et al.* 1977a; Catacutan 1991; Deering *et al.* 1997; Lim *et al.* 1997). However, the use of vegetable oils that are high in oleic acid (OA; 18:1*n*-9), linoleic acid (LA; 18:2*n*-6) and linolenic acid (LNA; 18:3*n*-3) and less expensive animal fats, such as beef tallow (BT), may serve to satisfy EFA requirements for red claw and thereby help reduce the use of marine fish oils.

Currently, the aquaculture industry is the largest consumer of marine fish oil worldwide because of its importance in diets for carnivorous fish and in shrimp diets. It has recently been reported that fish oil production is low, in line with declining fish meal (FM) production worldwide (FAO Globefish 2009). Presently, the price of marine fish oil was reported at US \$1000 t⁻¹ (FAO Globefish 2009). Thus, it is essential to find alternatives for marine fish oil for use in aquaculture diets.

While there have been recent studies on replacing FM with other, less expensive plant protein sources for juvenile red claw (Thompson *et al.* 2003a,b, 2005b, 2006; Muzinic *et al.* 2004), no data exist on evaluating various lipid sources that

may substitute for fish when grown in tanks with no natural food items present. The aim of the present study was to investigate the effects of different dietary lipid sources that differ in fatty acid (FA) composition on growth, feed conversion, survival, whole-body proximate and FA composition of juvenile red claw.

Materials and methods

Diet preparation

Dry ingredients (solvent-extracted menhaden FM, soybean meal, milo, wheat, wheat gluten, vitamin mix and mineral mix, dicalcium phosphate, choline chloride and vitamin C) were weighed (Mettler AT261 Delta Range; Mettler Instruments, Zürich, Switzerland) and prepared for mixing. The oil was extracted from the menhaden FM using 95% ethyl alcohol. Menhaden FM was extracted four times with a 2 : 1 (v/v) ethyl alcohol (95%) to FM for each extraction, followed by one extraction of 3 : 1 (v/v), and the final two extractions at 4 : 1 (v/v) ratio. During each extraction, FM was completely mixed with ethyl alcohol and allowed to settle for 10 min, followed by decanting of ethyl alcohol from the FM. After the final extraction, solvent-extracted FM was air-dried under a fume hood for 24 h and then stored in a freezer (–20 °C) until needed.

All dietary ingredients were mixed together for 1 h and water was added to obtain a 35% moisture level. Diets were then passed through an extruder with a 1.2-cm die two times to form 'spaghetti-like' strands and air-dried under a fume hood, convection oven (Grieve Corporation, Round Lake, IL, USA) and/or a counter-top using fans. After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills Inc., Clifton, NJ, USA). Diets were sieved (2 mm opening mesh) using a USA standard testing sieve (Fisher Scientific, Pittsburg, PA, USA). After sieving, oils were added to diets at 70 g kg⁻¹ of dry diet weight and mixed until all pellets were uniformly coated. Diets were stored in plastic containers in a freezer (–20 °C) until fed.

Experimental system

A 12-week feeding trial was conducted in 190 individual plastic-mesh culture units (12.7 cm × 12.7 cm × 12.7 cm; Plastic Window Breeder-Fine, Luster Products Company, Springfield, NJ, USA) and located within six rectangular, fibreglass tanks (325.5-L; 236.22 cm × 101.6 cm × 15.24 cm) at the Aquaculture Research Center, Kentucky State University, KY, USA. Dechlorinated city (tap) water was

recirculated through a 2000-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bio-balls (Red Ewald, Karnes City, TX, USA) and then passed through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, LA, USA) to remove waste solids and provide substrates for living *Nitrosomonas* and *Nitrobacter* bacteria. Each culture unit was equipped with an individual water tube and plastic aquarium pipe valve that supplied water at a rate of 0.8 L min⁻¹. Water temperature in the recirculating system was maintained at 27–30 °C through the use of water heaters. Continuous aeration in the culture system was provided by a blower and diffuser tubing inside the fibreglass tanks. Approximately 5% of the total water volume was replaced daily with dechlorinated city water. Lighting was provided by overhead fluorescent ceiling lights on a 12 : 12 h light : dark cycle. Sodium bicarbonate and crushed coral were added to the recirculating system to maintain alkalinity levels near 100 mg L⁻¹.

Each culture unit contained a 5-cm section of 2.54-cm diameter PVC pipe for shelter. All culture units were siphoned every other day to remove uneaten diet and wastes while leaving red claw molts. Water quality parameters were checked three times weekly. Dissolved oxygen, pH and water temperature were measured using the Hydrolab Quanta Water Quality Monitoring System: Model QD 02152 (Hydrolab Corporation, Loveland, CO, USA). Alkalinity and chloride were measured by titration method (HACH digital titrator, Loveland, CO, USA); total ammonia and nitrite were measured using an HACH DR 2800 spectrophotometer (HACH, Loveland, CO, USA).

Experimental animals

Juvenile red claw averaging 0.55 ± 0.02 g (SD), were obtained from a commercial supplier (Stick-Fins Inc., Elkton, FL, USA), and had been fed a 50/50 mixture of Big Red rabbit pellets (180 g kg⁻¹ crude protein (CP); Southern States Cooperative, Richmond, VA, USA) and Purina sinking catfish pellets (320 g kg⁻¹ CP; Purina Mills, St Louis, MO, USA) after hatching. Red claw juveniles were not fed for 2 days prior to shipment. After arrival in our laboratory, red claw were acclimated for 3 h to laboratory conditions and then individuals were stocked at random into each 190 plastic-mesh culture units by one individual. There were 38 red claw per treatment (diet) and each culture unit was considered a replication, respectively. Individual weights of each juvenile red claw were measured using an electronic scale (Mettler AT261 Delta Range; Mettler Instruments) prior to stocking. Mortalities were monitored daily and

replaced during the first week of the study, with no replacements thereafter.

Experimental diets and feeding

Red claw juveniles were fed 1 day after stocking their assigned experimental diets. Juveniles were fed three times daily (08:00, 12:00 and 16:00 h) to controlled excess (Thompson *et al.* 2003a,b, 2005b; Muzinic *et al.* 2004) for 12 weeks. Five practical diets were formulated to be isocaloric [17.1 kJ g⁻¹ of available energy (AE)] and isonitrogenous (400 g kg⁻¹ CP; as fed), containing protein primarily from soybean meal and solvent-extracted menhaden FM. Each diet was supplemented with 70 g kg⁻¹ oil (by weight)

Table 1 Ingredient and chemical composition (g kg⁻¹ dry weight) of the five practical diets containing five different lipid sources fed to red claw

	Diet
Ingredients	
Soybean meal (48%)	450
Menhaden FM (62%)	200
Milo, melick (10%)	100
Wheat, hard	100
Lipid ¹	70
Wheat gluten (72%)	40
Vitamin and mineral mix ²	25
Dicalcium phosphate	10
Choline chloride	3.0
Stay C (35% active) ³	2.0
Chemical analysis⁴	
Moisture	107.8
Crude protein ⁵	449.1
Crude lipid ⁵	107.3
Fibre ⁵	20.5
Ash ⁵	89.3
NFE	335.2
Available energy ⁶	17.1

Values in parentheses are percentage protein of ingredient. Proximate analysis values are mean values of two replications per diet. NFE, nitrogen-free extract (by difference).

¹ Lipid sources include linseed oil, canola oil, corn oil, beef tallow and menhaden oil.

² Vitamin mix was the Abernathy vitamin premix number 2 and supplied the following (mg or IU kg⁻¹ of diet): biotin, 0.60 mg; B₁₂, 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; B₆, 31 mg; riboflavin, 53 mg; thiamin, 43 mg; D₃, 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.

³ Vitamin C (Roche's Stay C at 35% active).

⁴ Values are mean values of diets 3 and 5.

⁵ Dry-matter basis.

⁶ Available energy was calculated as 16.7, 16.7 and 37.7 kJ g⁻¹ of protein, carbohydrate and lipid, respectively.

using lipids from various sources with different FA compositions (Table 1). These included linseed oil (LO; Diet 1), canola oil (CAO; Diet 2), corn oil (CO; Diet 3), BT (Diet 4) and menhaden oil (MO; Diet 5). Because of differences in proximate composition of the diet ingredients from tabular values (NRC 1993), diets varied somewhat in actual chemical analysis from calculated values. As shown in Table 2, the BT diet was highest in the saturated FA palmitic acid (PA; 16:0) and stearic acid (SA; 18:0) compared with all other oil diets. Plant oil diets mainly contained OA (18:1*n*-9), LA (18:2*n*-6) and LNA (18:3*n*-3) FA, while low in HUFA EPA (20:5*n*-3) and DHA (22:6*n*-3). MO diet was rich in HUFA with 120 g kg⁻¹ EPA and 50 g kg⁻¹ DHA in total FA.

Chemical analysis

Experimental diets were analysed to determine per cent moisture, protein, lipid, fibre and ash. Moisture was determined by the placement of a 2-g sample into a convection oven (135 °C) for 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 at (600 °C) for 2 h (AOAC 942.05 1995). The nitrogen-free extract (NFE) was determined by difference [NFE = 100 - (% protein + % lipid + % fibre + % ash)]. AE was calculated from 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; Thompson *et al.* 2006). Proximate composition (Table 1) and FA composition (Table 2) of diets were determined by a commercial analytical laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA).

Data collection

At the conclusion of the study, each red claw was individually weighed on an electric scale (Mettler AT261 Delta Range; Mettler Instruments) and sexed. Growth parameters of red claw, and by sex, were measured in terms of final individual weight (g), percentage weight gain and specific growth rate (SGR % day⁻¹), respectively. Feed conversion ratio (FCR) and percentage survival were also determined at harvest (Tables 3 & 4).

Growth parameters and feed efficiency were calculated as follows: SGR (% day⁻¹) = [(ln W_t - ln W_i)/ T] × 100, where W_t and W_i are the final weight and initial individual weight 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]. FCR = total diet fed (g)/total wet weight gain (g).

After weighing and sexing, four males and four females (40 total) were randomly sampled for each treatment, chill-killed using an ice-water bath, and analysed for body composition. After chill-killing, legs and claws were removed from the body, and the whole-body diced with a cleaver. Proximate analysis procedures were as described for the diets except for moisture (AOAC procedure 950.46 1995) and lipid which was determined by ether extraction (AOAC procedure 960.39 1995; Tables 5 & 6). There were two replicates per sex per treatment.

Table 2 Mean values (±SE) of the fatty acid composition (% relative) of practical diets fed to red claw crayfish

Fatty acids	Diets				
	LO	CAO	CO	BT	MO
16:0	9.98 ± 0.15	8.92 ± 0.01	13.90 ± 0.07	22.85 ± 0.16	20.24 ± 0.09
16:1	0.94 ± 0.07	0.91 ± 0.02	0.91 ± 0.01	2.39 ± 0.04	7.05 ± 0.11
18:0	4.32 ± 0.02	2.85 ± 0.0	3.21 ± 0.12	13.09 ± 0.12	4.28 ± 0.0
18:1 <i>n</i> -9	21.77 ± 0.08	49.10 ± 0.06	25.16 ± 0.02	31.99 ± 0.50	17.80 ± 1.67
18:2 <i>n</i> -6	22.09 ± 0.08	24.34 ± 0.13	49.76 ± 0.28	19.58 ± 0.35	13.58 ± 0.69
18:3 <i>n</i> -3	35.83 ± 0.15	8.00 ± 0.02	2.37 ± 0.02	2.45 ± 0.10	2.45 ± 0.17
20:4 <i>n</i> -6	0.13 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.27 ± 0.01	1.24 ± 0.04
20:5 <i>n</i> -3	0.97 ± 0.13	0.71 ± 0.01	0.72 ± 0.02	0.83 ± 0.05	11.98 ± 0.27
22:6 <i>n</i> -3	0.80 ± 0.06	0.67 ± 0.01	0.73 ± 0.01	0.81 ± 0.07	4.99 ± 0.11
Other	3.17	4.38	3.13	5.74	16.39
<i>n</i> -3 HUFA	1.77	1.38	1.45	1.64	16.97
<i>n</i> -6 HUFA	0.13	0.12	0.11	0.27	1.24
<i>n</i> -3 HUFA/ <i>n</i> -6 HUFA ratio	13.6	11.5	13.2	6.07	13.7
Σ <i>n</i> -3	38.1	9.81	4.15	4.6	23.5
Σ <i>n</i> -6	22.6	24.9	50.3	20.6	15.3

Values are means of two replications per diet.

Table 3 Overall mean values (\pm SE) of initial individual weight, final individual weight, per cent weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and percentage survival of red claw crayfish fed five practical diets containing different lipid sources

Parameter	Diets				
	LO	CAO	CO	BT	MO
Initial wt (g)	0.55 \pm 0.02	0.52 \pm 0.02	0.57 \pm 0.02	0.56 \pm 0.02	0.56 \pm 0.02
Final wt (g)	16.44 \pm 0.99 ^a	15.19 \pm 1.19 ^{ab}	14.41 \pm 1.23 ^{ab}	12.24 \pm 1.21 ^b	14.65 \pm 1.41 ^{ab}
Weight gain (%)	2990 \pm 238 ^a	2880 \pm 246 ^a	2522 \pm 233 ^{ab}	2124 \pm 213 ^b	2564 \pm 278 ^{ab}
SGR (% day ⁻¹)	3.95 \pm 0.12 ^a	3.82 \pm 0.15 ^{ab}	3.68 \pm 0.14 ^{ab}	3.43 \pm 0.17 ^b	3.66 \pm 0.15 ^{ab}
FCR	2.92 \pm 0.44	3.07 \pm 0.24	3.62 \pm 0.39	3.83 \pm 0.32	3.43 \pm 0.59
Survival (%) ¹	84.0	87.0	87.0	82.0	87.0

Mean values within a row having different superscripts are significantly different ($P < 0.05$).

¹ Chi-squared test showed no significant difference ($P > 0.05$) in survival with respect to diet type (χ^2 test statistic = 3.4898; critical value with 4 *df* = 9.488).

Table 4 Mean values (\pm SE) of initial individual weight, final individual weight, per cent weight gain and specific growth rate (SGR) of male (M) and female (F) red claw crayfish fed five practical diets containing different lipid sources

Parameter	Sex	Diet				
		LO	CAO	CO	BT	MO
Initial wt (g)	M	0.58 \pm 0.03	0.55 \pm 0.04	0.58 \pm 0.04	0.58 \pm 0.03	0.53 \pm 0.03
	F	0.54 \pm 0.02 ^{ab}	0.50 \pm 0.02 ^b	0.56 \pm 0.02 ^a	0.54 \pm 0.02 ^{ab}	0.57 \pm 0.02 ^a
Final wt (g)	M	15.69 \pm 1.5 ^{ab}	17.16 \pm 1.69 ^a	15.16 \pm 2.40 ^{ab}	12.57 \pm 1.97 ^{ab}	10.38 \pm 1.75 ^{b,y}
	F	17.02 \pm 1.33 ^a	14.06 \pm 1.57 ^{ab}	13.99 \pm 1.40 ^{ab}	12.00 \pm 1.58 ^{ab}	16.78 \pm 1.78 ^{a,x}
Weight gain (%)	M	2646 \pm 273 ^{ab}	3067 \pm 339 ^a	2574 \pm 366 ^{ab}	2082 \pm 308 ^b	1816 \pm 374 ^b
	F	3258 \pm 359 ^a	2773 \pm 339 ^{ab}	2492 \pm 306 ^{ab}	2153 \pm 298 ^b	2938 \pm 374 ^{ab}
SGR (% day ⁻¹)	M	3.80 \pm 0.20	4.03 \pm 0.14	3.73 \pm 0.23	3.43 \pm 0.25	3.39 \pm 0.18
	F	4.06 \pm 0.14 ^a	3.70 \pm 0.23 ^{ab}	3.65 \pm 0.19 ^{ab}	3.43 \pm 0.23 ^b	3.80 \pm 0.20 ^{ab}
Number of males		14	12	12	13	11
Number of females		18	21	21	18	22

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

Table 5 Mean values (\pm SE) of whole-body moisture, protein, fat and ash (wet-weight basis) of red claw crayfish fed five practical diets containing different lipid sources

Parameter	Diet				
	LO	CAO	CO	BT	MO
Moisture (g kg ⁻¹)	708.3 \pm 9.9	728.4 \pm 4.6	709.2 \pm 9.3	755.4 \pm 26.2	734.5 \pm 19.1
Protein (g kg ⁻¹)	148.9 \pm 6.5	131.5 \pm 3.6	144.0 \pm 2.3	134.8 \pm 15.1	132.3 \pm 7.0
Fat (g kg ⁻¹)	22.4 \pm 3.8 ^a	9.9 \pm 1.8 ^b	16.4 \pm 2.4 ^{ab}	10.5 \pm 4.6 ^b	11.4 \pm 3.5 ^b
Ash (g kg ⁻¹)	54.6 \pm 5.4 ^c	65.7 \pm 1.9 ^{bc}	73.8 \pm 7.2 ^{ab}	75.4 \pm 7.9 ^{ab}	89.4 \pm 4.5 ^a

Mean values in the same row with different letters are significantly different ($P < 0.05$). Values are means of four replications per diet.

Whole-body FA analysis (% relative) was conducted on juvenile red claw after 12 weeks. After weighing and sexing, four males and four females (40 total) were randomly chosen within each treatment, chill-killed using an ice-water bath and frozen in liquid nitrogen (-196°). After freezing, red claw legs, claws, and uropods were removed from the body. The remaining body was shattered into pieces and stored in glass vials with screw-top lids under nitrogen. The glass vials were

immediately cap-sealed with teflon tape and covered in aluminium foil to prevent photo-oxidation. Samples were stored in a freezer (-20°C) until lipid extraction. There were two replicates per sex per treatment.

Fatty acid composition (% relative) of cephalothorax and tail meat (TM) were analysed on four males and four females (40 total) for each organ within each treatment. There were two replicates per sex per treatment.

Table 6 Mean values (\pm SE) of whole-body moisture, protein, fat and ash (wet-weight basis) of male (M) and female (F) red claw crayfish fed five practical diets containing different lipid sources

Parameter	Sex	Diet				
		LO	CAO	CO	BT	MO
Moisture (g kg ⁻¹)	M	718.9 \pm 2.6 ^{ab}	728.3 \pm 1.2 ^{ab}	697.8 \pm 15.7 ^b	789.0 \pm 38.0 ^a	716.1 \pm 9.5 ^b
	F	697.5 \pm 18.9	728.4 \pm 11.3	720.6 \pm 3.9	721.9 \pm 21.7	752.9 \pm 37.6
Protein (g kg ⁻¹)	M	150.6 \pm 9.1 ^a	136.0 \pm 3.4 ^{ab}	140.1 \pm 1.1 ^{ab}	112.4 \pm 19.0 ^b	141.4 \pm 6.0 ^{ab}
	F	147.3 \pm 12.9 ^{ab}	127.1 \pm 5.4 ^b	148.0 \pm 0.3 ^{ab}	157.3 \pm 2.3 ^a	123.3 \pm 9.9 ^b
Fat (g kg ⁻¹)	M	17.8 \pm 5.2 ^a	9.5 \pm 2.0 ^{ab}	13.2 \pm 3.4 ^{ab}	3.4 \pm 2.4 ^b	13.1 \pm 1.3 ^{ab}
	F	27.0 \pm 4.2	10.4 \pm 4.0	19.6 \pm 1.8	17.5 \pm 4.6	9.7 \pm 8.1
Ash (g kg ⁻¹)	M	55.2 \pm 13.0 ^b	68.9 \pm 0.1 ^{ab}	76.7 \pm 11.1 ^{ab}	84.1 \pm 2.1 ^{ab}	90.7 \pm 1.2 ^a
	F	54.0 \pm 1.8	62.5 \pm 0.5	70.9 \pm 13.0	66.8 \pm 14.8	88.1 \pm 10.7
Number of males		14	12	12	13	11
Number of females		18	21	21	18	22

Values are means of two replications per diet. Mean values within a row having different superscripts (a, b) are significantly different ($P < 0.05$) among dietary treatments. Mean values between males and females in the same column for each respective variable with different superscripts (x, y) were not significantly different ($P > 0.05$).

Whole-body, cephalothorax and TM FA analysis were determined by a commercial analytical laboratory (Eurofins Scientific, Inc.).

Statistical analysis

Data were analysed by ANOVA using the SAS General Linear Models procedure (SAS/STAT software version 9.1; SAS 2006; SAS Institute, Inc., Cary, NC, USA). Duncan's multiple range test was used to compare differences among individual means at the $P < 0.05$ probability level. A Chi-squared test was used to determine if survival was independent of diet type, and all percentage and ratio data were transformed to arc sine values prior to statistical analysis (Zar 1984).

Results

Water quality

Over the duration of the study, water quality parameters averaged (\pm SD): water temperature, 29.2 \pm 0.65 °C; dissolved oxygen, 5.59 \pm 0.62 mg L⁻¹; total ammonia nitrogen, 0.08 \pm 0.09 mg L⁻¹; nitrite, 0.02 \pm 0.01 mg L⁻¹; alkalinity, 123 \pm 30.8 mg L⁻¹; chlorides, 113 \pm 11.8 mg L⁻¹; pH, 8.48 \pm 0.15. These water quality values were within acceptable limits for indoor production of red claw crayfish (Masser & Rouse 1997).

Proximate composition (which includes per cent moisture, protein, lipid, fibre and ash) and NFE of two of the five practical diets are presented in Table 1 and FA composition of the diets are presented in Table 2.

Growth

There was no significant difference ($P > 0.05$) in initial weight among treatments and averaged 0.55 \pm 0.02 g. After 12 weeks, final mean weight and mean SGR of red claw fed the LO diet (Diet 1) were significantly higher ($P < 0.05$) (16.44 g and 3.95% day⁻¹, respectively) compared with that of red claw fed the BT diet (Diet 4; 12.24 g and 3.43% day⁻¹, respectively), but not different than red claw fed all other diets. Likewise, red claw fed the LO and CAO diets (Diets 1 and 2) had significantly higher ($P < 0.05$) percentage weight gain (2990 and 2880%, respectively) compared with red claw fed the BT diet (Diet 4; 2124%), but not different from red claw fed the CO diet (Diet 3) and MO diet (Diet 5). There were no significant differences ($P > 0.05$) in percentage survival and FCR among treatments and values averaged (85.4% and 3.4, respectively; Table 3).

Differences between males and females in growth performance

No significant differences ($P > 0.05$) were found in initial weight, percentage weight gain and SGR among diets, between male and female red claw crayfish with averages of 0.56 and 0.54 g (for males and females, respectively), 2437 and 2723% (for males and females, respectively) and 3.68 and 3.73% day⁻¹ (for males and females, respectively).

Mean final individual weight of female red claw fed the MO diet was significantly ($P < 0.05$) higher (16.78 g) compared with male red claw (10.38 g) fed the same diet (Table 4); however, there were no significant differences ($P > 0.05$) in final weight between the sexes among the other

four diets with averages of 15.15 and 14.28 g for males and females, respectively.

Differences between males in growth performance

When comparing males among dietary treatments, the final mean weight of red claw males fed the CO diet were significantly ($P < 0.05$) larger (17.16 g) than males fed the MO diet (10.38 g), but were not different ($P > 0.05$) among males fed the other three diets (Table 4). Percentage weight gain in males fed the CO diet was significantly ($P < 0.05$) higher (3067%) compared with males fed the BT diet (2082%) and the MO diet (1816%), but were not different ($P > 0.05$) among males fed the other two diets. Specific growth rate in males fed all five diets were not significantly ($P > 0.05$) different and averaged 3.68% day⁻¹.

Differences between females in growth performance

When comparing females among dietary treatments, the final mean weight of red claw females fed the LO and MO diets were significantly ($P < 0.05$) larger (17.02 and 16.78 g, respectively) than females fed the BT diet (12.0 g), but were not different ($P > 0.05$) among females fed the other two diets (Table 4). Percentage weight gain in females fed the LO diet was significantly ($P < 0.05$) higher (3258%) compared with females fed the BT diet (2153%), but were not different ($P > 0.05$) among females fed the other three diets. Specific growth rate in females fed the LO diet was significantly ($P < 0.05$) higher (4.06% day⁻¹) compared with females fed the BT diet (3.43% day⁻¹), but were not different ($P > 0.05$) among females fed the other three diets.

Proximate analysis of red claw

Whole-body proximate composition of red claw (wet-weight basis) is given in Table 5. No significant ($P > 0.05$) differences were found in moisture content and crude protein, which averaged 727.1 and 138.3 g kg⁻¹, respectively. However, red claw fed the LO diet was significantly higher in crude lipid (22.4 g kg⁻¹) compared with the CAO, BT and MO (9.9, 10.5 and 11.4 g kg⁻¹, respectively), but not different than red claw fed the CO diet (16.4 g kg⁻¹). Ash content was significantly higher in the MO diet (89.4 g kg⁻¹) compared with the LO and CAO diets (54.6 and 65.7 g kg⁻¹, respectively), but not different than red claw fed the CO and BT diets.

Whole-body proximate composition of red claw between males and females (wet-weight basis) is presented in Table 6.

There were no significant differences in overall proximate analysis of whole-body red claw between the sexes. Means of moisture content among diets for males was 730 g kg⁻¹ and for females was 724.3 g kg⁻¹; crude protein percentage among diets for males was 136.1 g kg⁻¹ and for females 140.6 g kg⁻¹; percentages of crude fat among diets for males was 11.4 g kg⁻¹ and for females 16.8 g kg⁻¹; and ash content among all diets for males was 75.1 g kg⁻¹ and 68.5 g kg⁻¹ for females, respectively.

When comparing males among dietary treatments, the crude protein percentage of red claw males fed the LO diet was significantly ($P < 0.05$) higher (150.6 g kg⁻¹) than males fed the BT diets (112.4 g kg⁻¹), but were not different ($P > 0.05$) among males fed the other three diets (Table 6). Crude fat in males fed the LO diet was significantly ($P < 0.05$) higher (17.8 g kg⁻¹) compared with males fed the BT diet (3.4 g kg⁻¹), but were not different ($P > 0.05$) among males fed the other three diets. Ash content in males fed the LO diet was significantly ($P < 0.05$) lower (55.2 g kg⁻¹) than males fed the MO diet (90.7 g kg⁻¹), but were not different ($P > 0.05$) among males fed the other three diets.

When comparing females among dietary treatments, the crude protein percentage of red claw females fed the BT diet was significantly ($P < 0.05$) higher (157.3 g kg⁻¹) than females fed the CAO (127.1 g kg⁻¹) and MO (123.3 g kg⁻¹) diets, but were not different ($P > 0.05$) among females fed the other two diets (Table 6). Crude fat and ash content in females fed all diets were not significantly ($P > 0.05$) different, averaging 16.8 and 68.5 g kg⁻¹, respectively.

Fatty acid composition of whole-body red claw crayfish

When both sexes are combined, red claw fed the LO diet was significantly higher ($P < 0.05$) in LNA, 18:3n-3 (20.09%); red claw fed the CAO diet was significantly higher ($P < 0.05$) in OA, 18:1n-9 (45.94%); red claw fed the CO diet was significantly higher ($P < 0.05$) in LA, 18:2n-6 (36.44%); red claw fed the BT diet was significantly higher ($P < 0.05$) in PA, 16:0 (20.51%) and SA, 18:0 (8.61%); while red claw fed the MO diet was significantly higher in palmitoleic acid, 16:1 (POA; 7.29%), EPA, 20:5n-3 (10.71%) and DHA, 22:6n-3 (2.50%) compared with all other diets, respectively. Data also indicates that red claw fed the CO, BT and MO diets were significantly higher ($P < 0.05$) in arachidonic acid, 20:4n-6 (AA; averaging 1.97%) than red claw fed the LO and CAO diets, respectively (Table 7).

When FA data was analysed between the sexes, female red claw had significantly ($P < 0.05$) higher values in the diets

Table 7 Mean values (\pm SE) of the fatty acid composition (% relative) of the whole-body of red claw crayfish

Fatty acid	Diet				
	LO	CAO	CO	BT	MO
16:0	14.08 \pm 0.21 ^d	13.01 \pm 0.13 ^a	15.30 \pm 0.33 ^c	20.51 \pm 0.22 ^a	19.13 \pm 0.38 ^b
16:1	2.46 \pm 0.17 ^c	2.57 \pm 0.24 ^c	2.02 \pm 0.18 ^c	3.57 \pm 0.22 ^b	7.29 \pm 0.45 ^a
18:0	5.90 \pm 0.17 ^c	4.25 \pm 0.13 ^e	4.97 \pm 0.10 ^d	8.61 \pm 0.10 ^a	6.57 \pm 0.39 ^b
18:1 <i>n</i> -9	25.70 \pm 0.19 ^c	45.94 \pm 0.23 ^a	25.92 \pm 0.19 ^c	35.58 \pm 0.22 ^b	22.09 \pm 0.25 ^d
18:2 <i>n</i> -6	18.66 \pm 0.19 ^b	18.78 \pm 0.20 ^b	36.44 \pm 0.27 ^a	16.57 \pm 0.40 ^c	11.86 \pm 0.25 ^d
18:3 <i>n</i> -3	20.09 \pm 1.03 ^a	4.68 \pm 0.07 ^b	2.00 \pm 0.07 ^c	1.78 \pm 0.01 ^c	1.91 \pm 0.09 ^c
20:4 <i>n</i> -6	1.41 \pm 0.22 ^{bc}	1.12 \pm 0.07 ^c	1.81 \pm 0.16 ^{ab}	1.92 \pm 0.11 ^{ab}	2.18 \pm 0.26 ^a
20:5 <i>n</i> -3	2.04 \pm 0.17 ^b	1.72 \pm 0.11 ^b	2.11 \pm 0.17 ^b	2.20 \pm 0.20 ^b	10.71 \pm 0.38 ^a
22:6 <i>n</i> -3	0.88 \pm 0.05 ^b	0.82 \pm 0.05 ^b	0.96 \pm 0.04 ^b	0.92 \pm 0.08 ^b	2.50 \pm 0.07 ^a
Other	8.78	7.11	8.47	8.34	15.76
<i>n</i> -3 HUFA	2.92	2.54	3.07	3.12	13.21
<i>n</i> -6 HUFA	1.41	1.12	1.81	1.92	2.18
<i>n</i> -3 HUFA/ <i>n</i> -6 HUFA ratio	2.07	2.27	1.70	1.63	6.10
Σ <i>n</i> -3	23.4	7.60	5.41	5.40	16.86
Σ <i>n</i> -6	22.6	21.2	40.7	20.0	15.7

Values are means of four replications per diet. Mean values in the same row for each fatty acid with different superscripts are significantly different ($P < 0.05$).

LO (14.43%) and CAO (13.23%) for PA than males (13.73 and 12.79%). Likewise, females had a significantly higher value of MO (22.51%) for OA than males (21.67%). However, there were no other significant differences in all other FA analysed between the sexes (Table 8).

When comparing FA data among male red claw, males fed the BT diet contained significantly ($P < 0.05$) higher PA (20.55%) than males fed the other four diets (Table 8). OA in males fed the CAO diet was significantly ($P < 0.05$) higher (46.25%) than males fed the other four diets. LA in males fed the CO diet was significantly ($P < 0.05$) higher (36.61%) than males fed the other four diets. LNA in males fed the LO diet was significantly ($P < 0.05$) higher (19.76%) than males fed the other four diets. EPA and DHA in males fed the MO diet was significantly ($P < 0.05$) higher (10.88 and 2.54%, respectively) than males fed the other four diets. The remaining three FA (POA, SA, AA) also showed significant ($P < 0.05$) differences among dietary treatments for male red claw.

When comparing FA data among female red claw, females fed the BT diet contained significantly ($P < 0.05$) higher PA (20.47%) than females fed the other four diets (Table 8). OA in females fed the CAO diet was significantly ($P < 0.05$) higher (44.63%) than females fed the other four diets. LA in females fed the CO diet was significantly ($P < 0.05$) higher (36.28%) than females fed the other four diets. LNA in females fed the LO diet was significantly ($P < 0.05$) higher (20.42%) than females fed the other four diets. EPA and DHA in females fed the MO diet was significantly ($P < 0.05$) higher (10.55 and 2.47%, respectively) than females fed the

other four diets. The remaining three FA (POA, SA, AA) also showed significant ($P < 0.05$) differences among dietary treatments for female red claw.

When comparing FA data of the cephalothorax (Ceph.) and TM regions, results show that Ceph were significantly ($P < 0.05$) higher in PA (16.70%), POA (4.20%), OA (31.29%), LA (21.54%), LNA (6.05%) and DHA (0.53%) compared with TM, regardless of diet and gender. The inverse comparison was true for the remaining FA analysed (Table 9).

When comparing FA data between males and females, male red claw were significantly ($P < 0.05$) higher in eicosadienoic acid, 20:2 (4.61%) and DHA (0.94%) compared with females (2.54 and 0.44%, respectively), regardless of diet and organ. However, all other FA analysed were not significantly ($P > 0.05$) different among sexes.

Discussion

To date, no research has been conducted on the utilization of alternative lipid sources to replace marine fish oil in practical diets for juvenile red claw. Results from the present study indicate that growth and survival of juvenile red claw fed diets containing LO, CAO or CO were similar to that of red claw fed a diet containing MO when grown indoors having no natural food items present for consumption. While the MO diet contained high levels of the *n*-3 HUFA EPA, 20:5*n*-3 and DHA, 22:6*n*-3, data from the present study suggest that the three dietary vegetable oils containing mainly OA, 18:1*n*-9; LA, 18:2*n*-6 or LNA, 18:3*n*-3 were of good

Table 8 Mean values (\pm SE) of the fatty acid composition (% relative) of the whole-body of male (M) and female (F) red claw crayfish fed five practical diets containing different lipid sources

Fatty acid	Sex	Diet				
		LO	CAO	CO	BT	MO
16:0	M	13.73 \pm 0.05 ^{cd,y}	12.79 \pm 0.50 ^{d,y}	15.34 \pm 0.78 ^c	20.55 \pm 0.55 ^a	18.59 \pm 0.43 ^b
	F	14.43 \pm 0.15 ^{d,x}	13.23 \pm 0.06 ^{a,x}	15.26 \pm 0.15 ^c	20.47 \pm 0.05 ^a	19.69 \pm 0.29 ^b
16:1	M	2.21 \pm 0.07 ^b	2.21 \pm 0.06 ^b	1.88 \pm 0.39 ^b	3.39 \pm 0.41 ^b	6.70 \pm 0.72 ^a
	F	2.71 \pm 0.19 ^{cd}	2.93 \pm 0.32 ^c	2.16 \pm 0.11 ^d	3.76 \pm 0.21 ^b	7.89 \pm 0.09 ^a
18:0	M	6.03 \pm 0.35 ^{bc}	4.38 \pm 0.18 ^d	4.98 \pm 0.21 ^{cd}	8.70 \pm 0.06 ^a	7.05 \pm 0.60 ^b
	F	5.76 \pm 0.09 ^b	4.14 \pm 0.21 ^d	4.96 \pm 0.11 ^c	8.53 \pm 0.21 ^a	6.10 \pm 0.36 ^b
18:1n-9	M	25.91 \pm 0.36 ^c	46.25 \pm 0.28 ^a	26.02 \pm 0.01 ^c	35.39 \pm 0.03 ^b	21.67 \pm 0.03 ^{d,y}
	F	25.49 \pm 0.06 ^c	45.63 \pm 0.24 ^a	25.83 \pm 0.44 ^c	35.78 \pm 0.47 ^b	22.51 \pm 0.18 ^{d,x}
18:2n-6	M	18.51 \pm 0.38 ^b	18.78 \pm 0.45 ^b	36.61 \pm 0.25 ^a	16.17 \pm 0.78 ^c	11.81 \pm 0.10 ^d
	F	18.80 \pm 0.18 ^b	18.79 \pm 0.21 ^b	36.28 \pm 0.58 ^a	16.96 \pm 0.16 ^c	11.91 \pm 0.60 ^d
18:3n-3	M	19.76 \pm 2.18 ^a	4.60 \pm 0.07 ^b	2.08 \pm 0.06 ^b	1.77 \pm 0.01 ^b	2.06 \pm 0.07 ^b
	F	20.42 \pm 1.16 ^a	4.76 \pm 0.12 ^b	1.92 \pm 0.13 ^c	1.80 \pm 0.01 ^c	1.76 \pm 0.06 ^c
20:4n-6	M	1.59 \pm 0.45 ^{ab}	1.24 \pm 0.02 ^b	1.82 \pm 0.35 ^{ab}	2.08 \pm 0.14 ^{ab}	2.57 \pm 0.29 ^a
	F	1.22 \pm 0.12 ^b	1.01 \pm 0.08 ^b	1.81 \pm 0.20 ^a	1.76 \pm 0.07 ^a	1.79 \pm 0.07 ^a
20:5n-3	M	2.18 \pm 0.32 ^b	1.81 \pm 0.21 ^b	2.06 \pm 0.35 ^b	2.43 \pm 0.32 ^b	10.88 \pm 0.74 ^a
	F	1.91 \pm 0.18 ^b	1.63 \pm 0.14 ^b	2.16 \pm 0.22 ^b	1.97 \pm 0.19 ^b	10.55 \pm 0.53 ^a
22:6n-3	M	0.94 \pm 0.07 ^b	0.84 \pm 0.08 ^b	1.01 \pm 0.06 ^b	1.01 \pm 0.09 ^b	2.54 \pm 0.12 ^a
	F	0.83 \pm 0.05 ^b	0.79 \pm 0.08 ^b	0.91 \pm 0.05 ^b	0.83 \pm 0.13 ^b	2.47 \pm 0.13 ^a

Values are means of two replications per diet. Mean values within a row having different superscripts (a, b) are significantly different ($P < 0.05$) among dietary treatments. Mean values between males and females in the same column for each respective variable with different superscripts (x, y) are significantly different ($P < 0.05$).

Table 9 Mean values (\pm SE) of fatty acid composition (% relative) of comparing cephalothorax (Ceph.) and tail meat (TM) excluding effects by treatment (diet) and sex

Fatty acid	Organ	
	Ceph.	TM
16:0	16.70 \pm 1.04 ^a	11.41 \pm 0.71 ^b
16:1	4.20 \pm 0.60 ^a	2.04 \pm 0.36 ^b
18:0	4.95 \pm 0.31 ^b	6.49 \pm 0.43 ^a
18:1n-9	31.29 \pm 2.19 ^a	20.01 \pm 1.58 ^b
18:2n-6	21.54 \pm 2.04	17.40 \pm 1.43
18:3n-3	6.05 \pm 1.66	3.11 \pm 0.69
20:0	0.35 \pm 0.03 ^b	0.69 \pm 0.10 ^a
20:2	1.81 \pm 0.34 ^b	5.35 \pm 0.74 ^a
20:4n-6	0.86 \pm 0.16 ^b	2.74 \pm 0.55 ^a
20:5n-3	2.02 \pm 0.65 ^b	6.58 \pm 1.44 ^a
22:6n-3	0.53 \pm 0.15	0.84 \pm 0.16

Values are means of two replications per diet. Mean values between Ceph. and TM in the same row for each fatty acid with different superscripts are significantly different ($P < 0.05$).

nutritional value and may have satisfied the EFA requirements based upon growth performance, FCR and percentage survival of red claw in the present study. Specific growth rate and per cent weight gain values in the present study (ranging from 3.43 to 3.95% day⁻¹ and 2124 to 2990%, respectively) were similar to previous published reports of juvenile red claw (Webster *et al.* 1994; Garcia-Ulloa *et al.* 2003;

Thompson *et al.* 2003a,b, 2005b; Hernandez *et al.* 2004; Muzilic *et al.* 2004; Jacinto *et al.* 2005).

Research studies on freshwater crustacean diets have reported similar results to the present study in which plant oils have been utilized to completely replace marine fish oils (Joseph & Williams 1975; Castell & Covey 1976; Sandifer & Joseph 1976). D'Abramo & Sheen (1993) observed that addition of either a mixture of HUFA, or with AA, 20:4n-6 or containing solely DHA in diets fed to juvenile *Macrobrachium rosenbergii* significantly increased weight gain relative to prawn fed diets containing equivalent levels of LNA or LA. Hernandez-Vergara *et al.* (2003) reported that growth of juvenile red claw fed a diet containing a mixture of MO and CO did not significantly differ to red claw fed a diet containing only CO. However, their study was conducted in an outdoor flow-through culture system and natural productivity contributed approximately 26% of the growth. Thus, natural foods may have supplied EFA. The present study was conducted in an indoor recirculating system where natural foods were not present.

Results from the present study are in contrast to study results on various marine shrimp species where addition of marine oils, such as sardine, pollack, cod liver and short-necked clam, ensured superior nutritional value and promoted better growth versus vegetable oils or animal fats (Gitary *et al.* 1976; Kanazawa *et al.* 1977a; Catacutan 1991; Decring *et al.* 1997). Lim *et al.* (1997) reported that MO was

better utilized by juvenile white shrimp (*Penaeus vannamei*) than LO, soybean oil, CO, safflower oil or coconut oil because of the presence of HUFA, especially EPA and DHA. Kanazawa *et al.* (1978, 1979) and Read (1981) also reported better growth-promoting effect of MO in Kuruma prawn (*P. japonicus*) and juvenile Indian white shrimp (*P. indicus*) largely because of the presence of EPA and DHA. Kumaraguru Vasagam *et al.* (2005) evaluated three different vegetable oils (sunflower oil, peanut oil and palm oil) and one fish oil (sardine oil) in diets for juvenile black tiger shrimp (*P. monodon*) and reported that shrimp fed vegetable oil had significantly lower growth and nutrient digestibility comparable to that of shrimp fed diets with sardine oil.

In the present study, red claw fed a diet containing LO with high levels of LNA (36%), had a higher weight gain and final individual weight numerically than red claw fed all other lipid sources and was statistically higher than red claw fed a diet containing BT. A similar observation was made by Guary *et al.* (1976) and Kanazawa *et al.* (1977b) for juvenile Kuruma prawns and Lim *et al.* (1997) for juvenile white shrimp as they demonstrated that among plant oils, oils rich in LNA (such as LO) promoted better growth than oils rich in LA (soybean oil and CO). Data suggest that the BT diet containing a high level of saturated FA PA (16:0) and SA (18:0) performed poorly compared with the LO and CAO diets with respect to weight gain, which is in agreement with data from leader prawn (Deering *et al.* 1997).

Results indicate that percentage survival was not adversely affected by a lower inclusion of *n*-3 HUFA when fed the three vegetable oil diets compared with red claw fed MO. Percentage survival in the present study ranged from 82 to 87% and was similar to or higher than other published reports for red claw (Webster *et al.* 1994; Jacinto *et al.* 2003; Thompson *et al.* 2003a,b, 2005b; Hernandez *et al.* 2004; Muzinic *et al.* 2004; Pavasovic *et al.* 2007).

Results indicate that whole-body protein was not influenced or related to the inclusion of different dietary lipid sources in the present study as no statistical differences were found. However, it appears that carcass lipid may have been affected by the nutritional value of dietary lipid as red claw fed the LO and CO diets had the highest fat content and not red claw fed the MO and/or BT diets. These results are conflicting with previous reports on marine shrimp which have shown that carcass lipid is increased by the addition of fish oil when compared with that of plant oils (Colvin 1976; Catacutan 1991; Lim *et al.* 1997).

After 12 weeks of feeding, results showed that individual FA patterns of whole-body red claw mirrored the FA in the diets. This has been demonstrated in various studies with

marine shrimp species (Colvin 1976; Guary *et al.* 1976; Kanazawa *et al.* 1977a, 1979; Deshimaru *et al.* 1979; Bottino *et al.* 1980; Catacutan 1991; Lim *et al.* 1997). In the present study, FA concentrations of OA in the LO, CO, BT and MO diets were slightly higher in whole-body red claw than those of the dietary lipids. Moreover, red claw fed the LO, CAO, CO and BT diets also contained higher amounts of EPA and DHA in the whole-body compared with the dietary lipids. We did not include baseline FA composition data of red claw juveniles prior to the initiation of the present study. As FA composition is dependent upon the diet fed, it was felt that FA composition of red claw at the conclusion of the present study would be reflective to the diets fed during the 12-week feeding trial and not of the diets fed from the commercial supplier prior to the study. Further, as different commercial suppliers feed different diets to red claw, baseline values would only be reflective of these particular red claw, not those from another supplier. Thus, baseline values were not included. This is in agreement with other crustacean and fish feeding trials (Deering *et al.* 1997; Maina *et al.* 2003; Shapawi *et al.* 2008) evaluating growth and FA composition fed different sources of dietary lipid.

While red claw were fed to controlled excess, FCR values in the present study (2.92–3.83) were much lower than Thompson *et al.* (2005b) who fed red claw similarly to the present study. Pavasovic *et al.* (2007) also reported higher FCR values that ranged from 5.84 to 9.31 for sub-adult red claw (13.9 g) and fed experimental diets at a rate of 3% of body weight per day. However, FCR values in the present study were higher than other reports (Manomaitis 2001; Jacinto *et al.* 2003). 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 purposely present in the culture units and explains why FCR values were higher than in some other studies. It is important to refrain from withholding food from organisms during a feeding trial, and overfeeding is more desirable than underfeeding (Tacon & Cowey 1985; Thompson *et al.* 2005b). 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 (Thompson *et al.* 2005b).

Male and female red claw crayfish were separated to compare growth performance, whole-body proximate composition and FA composition. While the experimental animals tested were of juvenile stage rather than mature stage, it was decided to evaluate sexes separately to determine if lipids were utilized differently. With respect to growth performance, Thompson *et al.* (2003a) found that female red

claw had significantly higher per cent weight gain and SGR compared with male red claw. Those results were consistent with Manomaitis (2001) who also stated that female red claw had higher weight gain compared with male red claw when fed all treatments. In the present study, female red claw fed a diet containing MO had higher individual final weight values compared with males fed the same diet. However, it is not clear why females fed a diet with MO had higher final weight values than males as no significant differences in final weight were found among the other four diets between sexes. Further, there was no significant difference in red claw initial weights, per cent weight gain and SGR between males and females.

Results from the present study indicate that the FA composition of whole-body red claw between the sexes was similar. Further, when comparing each sex separately among the dietary treatments, there was a similar trend among the sexes in the FA composition of the whole-body and mirrored the FA in the diets. Interestingly, when comparing the differences between the FA composition of the cephalothorax (Ceph.) and TM portion of juvenile red claw and excluding effects by diet and sex, data showed that Ceph. was significantly higher in PA (16:0), POA (16:1) and OA levels than TM; while TM had significantly higher levels of SA (18:0) and all the 20-carbon FA, except DHA, than Ceph.

We conclude that diets for juvenile red claw can be formulated to replace MO with plant oils LO, CAO or CO; however, red claw fed the BT diet, with higher percentages of saturated FA, performed poorly compared with LO and CO in regards to weight gain. Addition of marine fish oil may not be essential for the growth of red claw as the plant-derived oils used in the present study appear sufficient for juvenile red claw.

Further studies should be conducted to elucidate the EFA requirements and metabolism of this species and possibly conduct specific quantitative EFA requirement studies using purified or semi-purified diets to confirm the present study results.

Acknowledgements

The authors thank N. Ann, K.C., K.N. Dee, B.R. Lee, E.M. Maa, Cathy Rhin, B. Rett, M.S. Tee, D.R. Wynne and Sam Wise for technical assistance. This research project was partially funded by a USDA 1890 Institution Capacity Building Grant awarded to Kentucky State University and a USDA grant under agreement KYX-80-05-13A to Kentucky State University. This manuscript is in partial fulfillment of Master's of Science degree in Aquaculture of T.J. Bailey, at Kentucky State University.

References

- AOAC (1995) *Official Methods of the Analysis*, 16th edn. Association of Official Analytical Chemists, Arlington, VA.
- Boillibo, N., Gennity, J., Lilly, M., Simmons, E. & Finne, G. (1980) Seasonal and nutritional effects of the fatty acids of three species of shrimp, *Penaeus setiferus*, *P. aztecus* and *P. duorarum*. *Aquaculture*, **19**, 139–148.
- Castell, J.D. & Covey, J.F. (1976) Dietary lipid requirements of adult lobsters, *Homarus americanus*. *J. Nutr.*, **106**, 1159–1165.
- Catacutan, M.R. (1991) Growth and fatty acid composition of *Penaeus monodon* juveniles fed various lipids. *Isaeral J. Aquac.*, **43**, 47–56.
- Colvill, P.M. (1976) The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus*. *Aquaculture*, **8**, 81–89.
- D'Abramo, L.R. & Sheen, S.S. (1993) Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, **115**, 63–86.
- Deerling, M.J., Fielder, D.R. & Hewitt, M.J. (1997) Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. *Aquaculture*, **151**, 131–141.
- Deshihharu, O., Kuroki, K. & Yone, Y. (1979) The composition and level of dietary lipid appropriate for growth of prawn. *Bull. Jpn. Soc. Sci. Fish.*, **45**, 591–594.
- FAO (Food and Agricultural Organization) (2009) Fish oil market report – 2009. Available at: <http://www.globefish.org/index.php?id=4661> (accessed on 26 February 2009).
- García-Ulloa, G.M., Lopez-Chavarin, H.M., Rodríguez-Gonzalez, H. & Colmenares, H.V. (2003) Growth of redclaw crayfish *Cherax quadricarinatus* (Von Martens 1868) (Decapoda: Parastacidae) juveniles fed isoproteic diets with partial or total substitution of fish meal by soya bean meal: preliminary study. *Aquac. Nutr.*, **9**, 25–31.
- Garling, D.L. & Wilson, R.P. (1977) Effect of dietary carbohydrate on growth and body composition of fingerling channel catfish. *Prog. Fish Cult.*, **39**, 43–47.
- Gualty, J.C., Kayama, M., Murakami, Y. & Ceccaldi, H.J. (1976) The effects of a fat-free diet and compounded diets supplemented with various oils on moult, growth and fatty acid composition of prawn, *Penaeus japonicus* Bate. *Aquaculture*, **7**, 245–254.
- Hernández, P.V., Olvera-Novoa, M.A. & Rouse, D.B. (2004) Effect of dietary cholesterol on growth and survival of juvenile redclaw crayfish *Cherax quadricarinatus* under laboratory conditions. *Aquaculture*, **236**, 405–411.
- Hernández-Vergara, M.P., Rouse, D.B., Olvera-Novoa, M.A. & Davis, D.A. (2003) Effects of dietary lipid level and source on growth and proximate composition of juvenile redclaw (*Cherax quadricarinatus*) reared under semi-intensive culture conditions. *Aquaculture*, **223**, 107–115.
- Hudg, S.S.Y., Fu, C.H.L., Higgs, D.A., Balfry, S.K., Schulte, P.M. & Bfauner, C.J. (2008) Effects of dietary canola oil level on growth performance, fatty acid composition and ionoregulatory development of spring chinook salmon parr, *Oncorhynchus tshawytscha*. *Aquaculture*, **274**, 109–117.
- Jacinto, E.C., Colmenares, H.V., Cerecedo, R.C. & Cordova, R.M. (2003) Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquac. Nutr.*, **9**, 207–213.
- Jacinto, E.C., Colmenares, H.V., Cruz-Suarez, L.E., Cerecedo, R.C., Nolasco-Soria, H. & Hernandez-Llamas, A. (2005) Effect of different dietary protein and lipid levels on growth and survival of juvenile Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquac. Nutr.*, **11**, 283–291.

- Joseph, J.D. & Williams, J.E. (1975) Shrimp head oil: a potential feed additive for mariculture. *Proc. World Maricult. Soc.*, **6**, 147–155.
- Kanazawa, A., Teshima, S. & Tokiwa, S. (1977a) Nutritional requirements of prawn. Effect of dietary lipid in growth. *Bull. Jpn. Soc. Sci. Fish.*, **43**, 849–856.
- Kanazawa, A., Tokiwa, S., Kayama, M. & Hirata, M. (1977b) Essential fatty acids in the diet of prawn. I. Effect of linoleic and linolenic acids on growth. *Bull. Jpn. Soc. Sci. Fish.*, **43**, 1111–1114.
- Kanazawa, A., Teshima, S., Endo, M. & Kayama, M. (1978) Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus*. *Mem. Fac. Fish., Kagoshima Univ.*, **27**, 35–40.
- Kanazawa, A., Teshima, S. & Endo, M. (1979) Requirements of prawn, *Penaeus japonicus* for essential fatty acids. *Mem. Fac. Fish., Kagoshima Univ.*, **28**, 27–33.
- Kumaraguru Vasagam, K.P., Ramesh, S. & Balasubramanian, T. (2005) Dietary value of different vegetable oil in black tiger shrimp *Penaeus monodon* in the presence and absence of soy lecithin supplementation: Effect on growth, nutrient digestibility and body composition. *Aquaculture*, **250**, 317–327.
- Lim, C., Ako, H., Brown, C.L. & Hahn, K. (1997) Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. *Aquaculture*, **151**, 143–253.
- Maina, J.G., Beames, R.M., Higgs, D., Mbugua, P.N., Iwama, G. & Kisia, S.M. (2003) Partial replacement of fishmeal with sunflower cake and corn oil in diets for tilapia *Oreochromis niloticus* (Linn): effect on whole body fatty acids. *Aquac. Res.*, **34**, 601–608.
- Manomaitis, L. (2001) *Assessment of the Crude Protein Requirement of Juvenile Red Claw Crayfish (Cherax quadricarinatus)*. MSc Thesis, Auburn University, Auburn, AL.
- Masser, M.P. & Rouse, D.B. (1997) *Australian Red Claw Crayfish*. SRAC, SRAC Publication No. 244, Stoneville, Mississippi, USA.
- Metts, L.S., Thompson, K.R., Xiong, Y., Kong, B., Webster, C.D. & Brady, Y. (2007) Use of alfalfa hay, compared to feeding practical diets containing two protein levels, on growth, survival, body composition, and processing traits of Australian red claw crayfish *Cherax quadricarinatus*, grown in ponds. *J. World Aquac. Soc.*, **38**, 218–230.
- Muzinic, L.A., Thompson, K.R., Morris, A., Webster, C.D., Rouse, D.B. & Manomaitis, L. (2004) Partial and total replacement of fish meal with soybean meal and brewer's grain with yeast in practical diets for Australian red claw crayfish *Cherax quadricarinatus*. *Aquaculture*, **230**, 359–376.
- NRC (National Research Council) (1993) *Nutrient requirements of warmwater fish*. National Academy Press, Washington, DC, pp. 102.
- Pavasovic, A., Anderson, A.J., Mather, P.B. & Richardson, N.A. (2007) Influence of dietary protein on digestive enzyme activity, growth and tail muscle composition in redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquac. Res.*, **38**, 644–652.
- Read, G.H.L. (1981) The response of *Penaeus indicus* (Crustacea: Penaeidea) to purified and compounded diets of varying fatty acid composition. *Aquaculture*, **24**, 245–256.
- Sandifer, P.A. & Joseph, J.D. (1976) Growth responses and fatty acid composition of juvenile prawns (*Macrobrachium rosenbergii*) fed a prepared ration augmented with shrimp head oil. *Aquaculture*, **8**, 129–138.
- SAS (2006) *SAS/STAT, version 9.1*. SAS Institute, Cary, NC.
- Shapawi, R., Mustafa, S. & Ng, W.K. (2008) Effects of dietary fish oil replacement with vegetable oils on growth and tissue fatty acid composition of humpback grouper, *Cromileptes altivelis* (Valenciennes). *Aquac. Res.*, **39**, 315–323.
- Sheen, S. & Wu, S. (1999) The effects of dietary lipid levels on the growth response of juvenile mud crab *Scylla serrata*. *Aquaculture*, **175**, 143–153.
- Tacon, A. & Cowey, C.B. (1985) Protein and amino acid requirements. In: *Fish Energetics-New Perspectives* (Tyler, P. & Calow, P. eds), pp. 155–183. Croom Helm, London.
- Thompson, K.R., Muzinic, L.A., Christian, T.D., Webster, C.D., Manomaitis, L. & Rouse, D.B. (2003a) Lecithin requirements of juvenile Australian Red claw crayfish *Cherax quadricarinatus*. *Aquac. Nutr.*, **9**, 223–230.
- Thompson, K.R., Muzinic, L.A., Christian, T.D., Webster, C.D., Manomaitis, L. & Rouse, D.B. (2003b) Effect on growth, survival, and fatty acid composition of Australian red claw crayfish *Cherax quadricarinatus* fed practical diets with and without supplemental lecithin and/or cholesterol. *J. World Aquac. Soc.*, **24**, 1–10.
- Thompson, K.R., Muzinic, L.A., Engler, L.S. & Webster, C.D. (2005a) Recent status on the nutrition and feeds for Australian red claw crayfish. *Aquac. Mag.*, **31**, 28–36.
- Thompson, K.R., Muzinic, L.A., Engler, L.S. & Webster, C.D. (2005b) Evaluation of practical diets containing different protein levels, with or without fish meal, for juveniles Australian red claw crayfish (*Cherax quadricarinatus*). *Aquaculture*, **244**, 241–249.
- Thompson, K.R., Metts, L.S., Muzinic, L.A., Dasgupta, S. & Webster, C.D. (2006) 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. *Aquac. Nutr.*, **12**, 227–238.
- Webster, C.D., Goodgame-Tiu, L.S., Tidwell, J.H. & Rouse, D.B. (1994) Evaluation of practical feed formulations with different protein levels for juvenile red claw crayfish, *Cherax quadricarinatus*. *Trans. Ky. Acad. Sci.*, **55**, 108–112.
- Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A.L. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops* × *M. saxatilis* fed practical diets. *J. World Aquac. Soc.*, **30**, 443–453.
- Zar, J.H. (1984) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliff, NJ.