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Evaluation of practical diets containing various terrestrial protein sources on survival and growth parameters of redclaw crayfish *Cherax quadricarinatus*

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Abstract

Redclaw aquaculture has developed at a rapid pace during the past few years, yet no specialized diet for the species has been developed. The present study was designed to evaluate whether soybean-based diets containing either fishmeal (FM), poultry by-product meal (PBM), ground peameal (GPM) or distillers dried grains with solubles (DDGS) meal as a protein source are suitable for redclaw aquaculture. Juvenile redclaw crayfish *Cherax quadricarinatus* (0.125 ± 0.025 g) were stocked into 20 rectangular tanks at a stocking density of 12.5 m^{-2} for 8 weeks. Crayfish in four replicate tanks were maintained on one of five diets formulated to contain 35% crude protein and 7.1% lipids. Each 100 g of diet contained 25 g of protein from soybean meal (SBM) and 10 g of protein from the alternative protein sources mentioned above. A fifth treatment that did not receive feed was included to account for growth from natural productivity. There were no significant differences in survival (86–90%), growth (3.84–4.98 g animal^{-1}) or feed conversion ratio (2.10–2.79) of crayfish among the four treatments ($P > 0.05$). Survival and growth of crayfish in the treatment that received no supplementary feed were significantly less than those in treatments offered experimental diets. Results of the present experiment suggest that SBM-based diets with PBM, FM, DDGS or GPM have similar effects on growth performance and survival of juvenile redclaw, *C. quadricarinatus*.

Keywords: *Cherax quadricarinatus*, redclaw crayfish, nutrition, protein sources

Introduction

Redclaw crayfish, *Cherax quadricarinatus* (von Martens 1868), has a natural range that encompasses most of the drainages along northeastern Australia, the Gulf of Carpentaria and the Timor Sea (Merrick & Lambert 1991). The species is also found in Papua New Guinea (Austin 1986). Redclaw is an omnivorous gregarious species that tolerates a broad range of environmental conditions and relatively high stocking densities. Additionally, it has favourable reproductive characteristics, including moderate fecundity, a simple life cycle and maternal incubation of eggs and hatchlings through early juvenile stages (Jones 1995a, b; Masser & Rouse 1997; Jones & Ruscoe 2001). Redclaw has been cultured extensively (in earthen ponds) or semi-intensively (in large tanks) in Australia and other countries in southern Asia, North and South America and Africa (Lawrence & Jones 2002). Redclaw aquaculture has developed at a rapid pace during the past few years, yet development of specialized diets has not kept pace, although a successful aquaculture industry requires good diets for the species.

Feed accounts for a large percentage of total cost in aquaculture and protein is the most expensive component of aquaculture diets. Commercial aquatic feeds have traditionally been based on fishmeal (FM) as the main protein source because of FM's high protein content and balanced essential amino acid profile. Fishmeal is also a good source of essential fatty acids, digestible energy, minerals and vitamins. However, FM is relatively expensive, supply is limited and quality variable (Hardy 2006). Consequently, the leveling out of annual FM supplies, coupled with the increased demand for FM in feeds for livestock and poultry, is likely to reduce the dependence on FM in aquatic feeds (El-Sayed 1999). Furthermore, there are growing environmental concerns about the use of wild fish to produce FM. Hence, there is interest in replacing FM with less expensive protein sources.

To reduce feed costs and be environmentally friendly, aquaculturists need to replace FM with alternative protein sources (Akiyama 1992; Forster & Dominy 2006). Although FM is energy rich, has exceptional amino acid profiles, is very digestible and generates excellent attractability and palatability, it is one of the most expensive ingredients in formulated feeds. Natural climatic events such as El-Niño also affect the supply of FM, which affects price. Accordingly, predictions for future FM availability are uncertain (Hardy 2006) and replacement of FM in aquatic organism diets with ingredients of terrestrial origin has been and remains a high priority to ensure the development of sustainable and profitable aquaculture industries (Jones, DeSilva & Mitchell 1996). Redclaw today are offered penaeid feeds rich in FM, yet we do not know how much FM, if any, is necessary in redclaw diets.

Various researchers report having maintained redclaw using diets developed for teleosts and crustaceans other than penaeid shrimp. Karplus, Barki, Cohen and Hulata (1995) used a commercial carp pellet with 25% protein and Pinto and Rouse (1996) used a commercial crayfish pellet with 25% protein. Jones and Ruscoe (2000, 2001) used a crayfish diet (Athmaize containing 17% protein), while Barki, Karplus, Manor, Parnes, Aflalo and Sagi (2006) used a 42% protein extruded fish pellet. Crude protein inclusion in redclaw diets is between 24% and 40% at lipid levels between 8% and 4% respectively (see Manomaitis 2001; Hernandez-Vergara, Rouse, Olvera-Novoa & Davis 2003; Thompson, Muzinic, Yancey, Webster, Rouse & Xiaon 2004; Thompson, Muzinic, Engler & Webster 2005; Thompson, Metts, Muzinic, Dasgupta & Webster 2006; Rodriguez-Gonzalez, Garcia-Ulloa, Hernández-Llamas & Villarreal 2006).

Plant proteins are often an economical source of nutrition because of their low price and consistent quality. However, due to potential problems associated with insufficient levels of essential amino acids (e.g. lysine and methionine), anti-nutrients and poor palatability, their use is often limited (Davis & Arnold 2000). The use of plant proteins such as soybean meal (SBM) (Lim & Dominy 1990; Tidwell, Webster, Yancey & D'Abramo 1993; Sudaryono, Hoxey, Kailis & Gand Evans 1995), solvent-extracted cottonseed meal (Lim 1996), various legumes (cowpea, green mungbean, rice bean) and leaf meals (Eusebio 1991; Eusebio & Coloso 1998), papaya or camote leaf meal (Penaflorida 1995) and co-extruded soybean poultry by-product meal (PBM) (Samocha, Davis, Saoud & DeBault 2004) have been evaluated as replacements of FM in shrimp feeds and many have been suitable at various inclusion levels. Sources of terrestrial animal protein, which are primarily rendered by-products such as meat and bone meal (Tan, Mai, Zheng, Zhou, Liu & Yu 2005) and PBM (Markey 2007), and a combination of soybean and PBM (Amaya, Davis & Rouse 2007) have also been evaluated to replace FM in shrimp feeds. Davis, Samocha, Bullis, Patnaik, Browdy, Stokes and Atwood (2004) demonstrated that FM can be removed from shrimp formulations if suitable alternative sources of protein and lipids are provided to meet the nutritional requirements of the animal. However, information about the use of alternative protein sources as replacement of FM in *C. quadricarinatus* diets is limited.

Webster, Thompson, Muzinic, Rouse and Manomaitis (2002), Muzinic, Thompson, Morris, Webster, Rouse and Manomaitis (2004), Thompson *et al.* (2005, 2006) and Saoud, Rodgers, Davis and Rouse (2008) reported that FM can be partially and even totally replaced by various plant proteins such as brewer's grains with yeast and SBM and terrestrial animal protein such as PBM without detrimental effects on redclaw survival and growth. The present study was designed to evaluate whether soybean-based diets, where FM was replaced by PBM, ground peameal (GPM) or distillers dried grains with solubles (DDGS) as alternative protein sources, are suitable redclaw diets.

Materials and methods

Juvenile redclaw crayfish *C. quadricarinatus* released during a 48-h period were collected and placed in an indoor nursery tank at the AGY Redclaw Hatchery, in Soto La Marina (Tamaulipas, Mexico). Juveniles were harvested and manually size sorted eliminating large and small outliers, separated into groups of 36 individuals and stocked into 20 rectangular tanks ($2.4 \times 1.2 \times 0.1$ m; $L \times W \times H$; 2.88 m^2 bottom area) resulting in a stocking density of 12.5 m^{-2}. Average initial weight of the juvenile redclaw at stocking was 0.125 ± 0.025 g (mean $\pm$

SD). Each tank received water continuously from an outside reservoir at a flow of 20 L h^{-1} for the duration of the experiment. Tanks contained 36 pieces of 5-cm-diameter PVC pipe used for refuge and four submerged air diffusers for aeration and water mixing. Juvenile crayfish were randomly assigned one of five dietary treatments with four replicate tanks per treatment.

Feeds used in the present experiment were produced by Rangen® (Angleton, TX, USA). All feeds were formulated to contain 35% crude protein and approximately 7.1% crude lipids (Table 1). Each diet contained 53.7–58 g 100 g^{-1} diet of solvent extracted SBM and 10 g 100 g^{-1} of the other primary protein sources. These included PBM, FM, DDGS and GPM (Table 1). Corn gluten was also used in diets three and four to help to balance the amino acid profiles of the feeds. Feeds were formulated to contain a minimum lysine and methionine plus cysteine content of 5% and 3% of total protein respectively. The amino acid profile of the four diets as determined by New Jersey Feed Laboratory (New Jersey, NY, USA) is presented in Table 2. A fifth treatment, which did not receive feed, was included to account for growth from natural productivity.

Based on previous work, mean weights of the crayfish were assumed to double weekly during the first 3 weeks and then grow by 1 g week^{-1}. Based on these expected growth rates and a feed conversion ratio (FCR) of 1.5, a feeding table was designed for the 8-week growth trial. Daily ration was weighed every afternoon and offered to the crayfish in the evening (~ 16:00 hours).

Dissolved oxygen concentrations and temperature were measured twice daily using a YSI 55 DO meter (Yellow Spring Instrument, Yellow Springs, OH, USA) and were 6.3 ± 0.5 mg L^{-1} (mean ± SD) and 27.8 ± 0.8 °C respectively. Total ammonia nitrogen was measured twice weekly using a LaMotte® Freshwater Aquaculture kit (LaMotte, Chestertown, MD, USA) and averaged 0.1 ± 0.1 ppm. These parameters were within acceptable limits for indoor production of redclaw crayfish (Masser & Rouse 1997). At the conclusion of the growth trial, crayfish were counted and individually weighed. Final weight, final biomass, per cent survival and FCR (FCR = weight of feed offered/wet weight gain of animal) were calculated.

Table 1 Ingredient composition (g 100 g^{-1}as is) of four practical soybean-based diets containing poultry by-product meal (PBM), fish meal (FM), distillers dried grain with solubles (DDGS) or ground pea meal (GPM) as protein source

Ingredient	PBM	FM	DDGS	GPM
Soybean meal (47% protein)	55.12	53.71	58.01	58.00
Milo	24.81	26.19	16.34	15.33
PBM 62% protein	9.99	–	–	–
GPM	–	–	–	10.00
DDGS	–	–	10.00	–
DDGS gluten	–	–	4.83	4.83
Menhaden FM, special select	–	10.01	–	–
Di-calcium phosphate	2.90	2.90	3.38	3.42
Fish oil (mixer)	5.08	5.09	2.83	5.822
Bentonite	1.50	1.50	1.50	1.50
Squid meal	–	–	0.50	0.50
Vitamin premix	0.33	0.33	0.33	0.33
Mould inhibitor	0.15	0.15	0.15	0.15
Trace mineral premix	0.08	0.09	0.09	0.09
Stay-C 35% (C)	0.02	0.02	0.02	0.02
Copper sulphate	0.01	0.01	0.01	0.01
Crude protein (%)	35.3	35.3	35.5	35.4
Crude lipid (%)	7.4	7.1	7.1	7.0
Crude fibre (%)	2.7	2.5	3.3	4.8
Ash (%)	9.6	9.7	8.8	8.6

Feeds were manufactured by Rangen ® (Buhle, ID, USA) under commercial conditions using their proprietary vitamin and mineral premixes.

Table 2 Amino acid profile of four practical soybean meal-based diets with 10% of the protein coming from poultry by-product meal (PBM), fishmeal (FM), distillers dried grain with solubles (DDGS) or ground pea meal (GPM)

	% of sample			
Amino acid	D1	FM	DDGS	GPM
Methionine	0.54	0.61	0.62	0.6
Cystine	0.53	0.54	0.62	0.61
Lysine	2.19	2.08	2.18	2.07
Phenylalanine	1.52	1.63	1.71	1.72
Leucine	2.65	2.78	2.91	2.99
Isoleucine	1.47	1.54	1.53	1.53
Threonine	1.31	1.33	1.35	1.34
Valine	1.27	1.33	1.3	1.29
Histidine	0.81	0.87	0.9	0.88
Arginine	2.42	2.41	2.54	2.51
Glycine	1.71	1.91	1.7	1.56
Aspartic acid	3.61	3.8	3.9	3.83
Serine	1.72	1.72	1.82	1.86
Glutamic acid	6.15	6.57	7.40	7.00
Proline	2.03	1.84	2.06	2.06
Hydroxyproline	0.24	0.15	0.15	0.07
Alanine	1.91	1.82	1.86	1.75
Tyrosine	1.08	1.1	1.2	1.21
Total	33.16	34.03	35.75	34.88

Analyses were conducted by New Jersey Feed Laboratory (New Jersey, NY, USA).

Table 3 Production parameters for juvenile redclaw, *Cherax quadricarinatus*, reared for 8 weeks and offered one of four soybean-based practical diets containing poultry by-product meal (PBM), fish meal (FM), distillers dried grain with solubles (DDGS) or ground pea meal (GPM)

Parameters	PBM	FM	DDGS	GPM	PSE	*P* value
Initial weight (g)	0.125	0.125	0.125	0.125		
Final weight (g)	4.04	3.85	4.20	4.98	0.449	0.339
Final biomass ($g\,tank^{-1}$)	132.3	119.3	135.1	158.0	14.690	0.352
FCR*	2.51	2.71	2.40	2.10	0.248	0.408
Survival (%)	90.3	86.1	90.3	88.2	0.035	0.803

Juvenile redclaw were stocked at an initial weight of 0.125 ± 0.025 g.
*FCR = (total feed offered)/(biomass increase).
FCR, feed conversion ratio; PSE, pooled standard error.

Data from the feeding trial were analysed using one-way analysis of variance (ANOVA) and Tukey's test to determine significant differences ($P<0.05$) among treatment means. All statistical analyses were performed using MINITAB software (version 15.1, MINITAB, State College, PA, USA).

Results

With the exception of the treatment that did not receive prepared feeds, there were no significant differences in survival and growth of crayfish among the various dietary treatments ($P>0.05$) (Table 3). Crayfish survival in the various treatments ranged from 86% to 90%, final individual weights ranged from 3.84 to 4.98 g, average final biomass ranged from 119.3 to 158 $g\,tank^{-1}$ and FCRs ranged from 2.10 to 2.70. Average final weight (0.54 g) and final biomass (15.1 $g\,tank^{-1}$) of crayfish in the treatment that received no supplementary feed were significantly less than the final weight, and the final biomass in the four treatments offered experimental diets ($P<0.05$), thus indicating negligible effect of natural productivity on growth.

Discussion

There is considerable interest in the use of alternative feed formulations in aquaculture, especially formulations that do not require marine animal protein. The present research evaluated a variety of open feed formulations using high inclusion rates of SBM in combination with various other protein sources. Results suggest that diets based on SBM with PBM, FM, DDGS or GPM have similar effects on growth performance and survival of juvenile redclaw, *C. quadricarinatus*. Similar results were observed by Roy, Bordinhon, Sookying, Davis, Brown and Whitis (2009) using the same diets used in the present experiment on the penaeid shrimp *Litopenaeus vannamei*.

Although it is difficult to compare growth rates, survivals and FCRs of crawfish from various production years or production systems and facilities, there is value in comparing data from other researchers using similar dietary protein levels and sources of protein. There are no reports of performance of redclaw crayfish offered diets in which FM was the sole protein source and therefore all comparisons are with diets using a mixture of plant and animal protein-rich ingredients. Survival and growth of redclaw in the present experiment were similar to results reported by other investigators (Jones 1995a, b; Manomaitis 2001; Hernandez-Vergara *et al*. 2003; Muzinic *et al*. 2004; Thompson *et al*. 2005; Saoud *et al*. 2008). Results were also similar to those observed during previous grow-out trials conducted at Megar S.A. de C.V. facilities under similar experimental conditions (Nabor Medina Vazquez, Megar S.A. de C.V., General Manager, pers. comm.) and offered commercial FM-based diets with 30% protein. Accordingly, we deduce that when essential amino acids are sufficient in the diets, a 30% protein, 7% lipid diet seems to be acceptable for redclaw growth, irrespective of whether the proteins are of animal, plant, terrestrial or marine sources.

Feed conversion ratios in the present work ranged between 2.1 and 2.7. These conversions are higher than expected and are due to the sloppy feeding method of the crayfish. The animals picked the pellets offered to them and withdrew into their refuges, so actual feeding was not observed and thus it was not possible to adjust feed quantity offered. Accordingly, growth rate of the crayfish in the present experiment might have been limited by feed quantity. Nevertheless, such results are more indicative of the suitability of plant protein sources as replacements for FM, because any negative effects of nutritive deficiency would be increased by feed limitation and we observed no negative effects in our animals.

Few protein sources other than FM have an amino acid profile that completely meets the needs of aquatic organisms. Accordingly, more than one source of protein is typically utilized in feed formulations. Various dietary formulations that include SBM and other protein sources have been successfully formulated. One of the most important FM substitutes has been PBM because of its availability and low cost. A combination of co-extruded SBM and PBM was used to replace FM in *L. vannamei* diets by Samocha *et al.* (2004). Davis and Arnold (2000) also used PBM without any SBM for the replacement of FM in *L. vannamei* diets. However, it should be mentioned that Davis and Arnold (2000) did not test diets with 100% replacement of FM with PBM. Saoud *et al.* (2008) found no adverse effects on production parameters when FM was partially or totally replaced with PBM in practical diets for *C. quadricarinatus*. An argument made by Saoud *et al.* (2008) was that redclaw are an omnivorous species that probably supplement artificial diets with natural productivity in ponds, thus reducing requirements for essential amino acids in the diet. However, the present work was performed in tanks with limited primary productivity and thus it seems that well-formulated diets that offer a balanced amino acid profile are sufficient for redclaw culture, irrespective of the ingredients used. This bodes well for people hoping to go into intensive redclaw aquaculture where natural productivity is not a dependable source of nutrition, even in earthen ponds.

Soybean meal is the most thoroughly evaluated and most frequently used dietary FM replacement in commercial aquaculture diets. Several attempts were successful in partially or totally replacing FM with SBM in practical diets of crustaceans (Lim & Dominy 1990; Piedad-Pascual, Cruz & Sumalangcay A Jr 1990; Tidwell *et al.* 1993; Sudaryono *et al.* 1995; Davis & Arnold 2000; Muzinic *et al.* 2004; Samocha *et al.* 2004; Thompson *et al.* 2006). However, SBM alone cannot meet all essential dietary requirements of organisms and thus needs to be supplemented with other dietary ingredients. Peameal has long been used in livestock feeds as a source of energy and amino acid source to complement SBM, but has only recently been evaluated in feeds for aquatic species. Although data are limited, feed-grade peas have been evaluated in diets for *Penaeus monodon* (Smith, Tabrett & Sarac 1999; Bautista-Tereul, Eusebio & Welsh 2003), *Litopenaeus stylirostris* (Cruz-Suarez, Ricque-Marie, Tapia-Salazar, Mccallum & Hichling 2001) and *L. vannamei* (Davis, Arnold & McCallum 2002). Davis *et al.* (2002) concluded that when peas are properly processed (extruded or micronized) and supplemented to diets up till 20% inclusion levels, their protein and energy are highly digestible with no apparent adverse effects on *L. vannamei* growth, survival or feed efficiency. The fact that penaeids are carnivorous scavengers while *C. quadricarinatus* is an omnivore suggests that redclaw might be better able to digest peameal and greater inclusion levels should be tested simultaneously with digestibility studies.

Distillers dried grain with solubles was recently analysed by Tidwell *et al.* (1993) as another source of energy for crustaceans. They found that growth, survival and pond yield of freshwater prawn (*Macrobrachium rosenbergii*) were not affected by the replacement of FM with SBM and DDGS. Also, Thompson *et al.* (2006) completely replaced FM with DDGS and found no significant differences in FCR, survival and total yield of *C. quadricarinatus*. The utilization of DDGS is an interesting and suitable option for replacing FM because its availability will increase as corn-based ethanol production increases.

Results of the present study show that *C. quadricarinatus* survival and growth are not affected when FM in the diet is replaced by a terrestrial plant–animal protein combination (PBM and SBM) or all-plant protein alternatives such as peameal and DDGS in combination with corn gluten meal at 35% crude protein inclusion level. However, it is important to mention that although natural productivity contributed very little to actual growth, it might have contributed enough nutrition to supplement minor deficiencies in essential amino acids. Such questions can only be answered by repeating the experiment using semi-purified diets and flow-through holding systems where the incoming water is filtered and treated so as to contain no external nutritive source.

The omnivorous capacity of redclaw observed in the present work seems obvious in retrospect, considering that *C. quadricarinatus* is both a plant and animal scavenger in nature. However, the potential cost reduction resulting from replacing FM with other ingredients should be evaluated. Cost and quality of substituting FM by any other protein source will vary depending on location. Availability, quality and cost of ingredients vary among regions and countries, causing feed formulations and price to vary concomitantly. Dependability in supply and quality of feed ingredients are also important factors in deciding what protein sources to use. The present work suggests that various terrestrial protein sources can be used in redclaw diets and feed manufacturers can use least cost analysis to produce inexpensive but suitable diets. Furthermore, even when plant protein sources are expensive, the development of all-plant protein diets could provide a niche market for crustacean producers, as some segments of the market are willing to pay more for organisms produced 'sustainably' or at least without the use of animal by-products (Samocha *et al*. 2004).

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