A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868)

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**Abstract**

Redclaw crayfish (*Cherax quadricarinatus*, von Martens 1868) is a freshwater decapod crustacean with a number of biological and commercial attributes that make it an excellent aquaculture species. The redclaw aquaculture industry has been growing rapidly since the mid-1980s in tropical and subtropical regions of the world. Redclaw aquaculture is mostly in extensive pond systems, but interest in developing more intensive systems is increasing. To support continued intensification, development of high-quality practical diet formulations and information about redclaw nutrition requirements are necessary. A number of studies have determined optimum dietary protein and lipid requirements for juvenile redclaw. However, there is limited information on essential amino acid and fatty acid requirements. Several studies report the presence of various digestive enzymes that have been linked to the ability of the species to digest a wide range of dietary components. Furthermore, as in many other aquaculture species, there is a need to replace fishmeal with other protein sources. A number of studies explored the possibility of replacing fish meal with various terrestrial plant sources of protein and lipids and showed that redclaw can be offered diets containing low-cost, plant-based ingredients without compromising survival, growth and, to a certain extent, reproduction. Formulated diets containing less expensive, plant-based ingredients will contribute to a more profitable and environmentally sustainable redclaw aquaculture industry. Finally, there is also a paucity of information on vitamin and mineral requirements of redclaw and little information on nutrient requirements of broodstock. For the redclaw aquaculture industry to thrive, we need to have a better understanding of nutrient requirements at all life stages.

**KEY WORDS**: Cherax quadricarinatus, crayfish, diet, feed, nutrition, redclaw

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**Introduction**

Aquaculture of the Australian redclaw crayfish *Cherax quadricarinatus* (von Martens 1868) is developing rapidly in tropical and some temperate regions of the world. Webster et al. (2002) stated that aquaculture of the species was mainly restricted to North-Eastern Australia, but redclaw aquaculture has expanded into South-East Asia and Central/South America and production is no longer restricted to Oceania. The species grows well when offered diets developed for other crustaceans, but nutritional requirement data specific for redclaw have not been determined. As culture methodology shifts from extensive and semi-intensive ponds into more intensive systems and as hatchery production becomes more common, we will need to develop species-specific feed formulations (Huner et al. 1994; Medley et al. 1994; Webster et al. 1994, 2002; Curtis & Jones 1995). These diets should be less expensive than traditional shrimp feeds, offer a complete nutrient profile to the animal, be based on sustainable sources of raw ingredients and be available wherever the industry decides to grow. The present manuscript reviews known nutritional requirements of redclaw crayfish based on existing literature and the experience of the authors.

**Nutrition**

In natural ecosystems, crayfish have polytrophic feeding habits and have been described as predators, omnivores...
and/or detritivores (Momot et al. 1978; D’Abramo & Robinson 1989; Jones 1990; Brown 1995a; Momot 1995; Nystroém 2002; Garza de Yta et al. 2011), consuming a variety of macrophytes, benthic invertebrates, algae and detritus (Brown 1995a; Nystroém 2002). Jones (1990) suggested that in general Cherax species are primarily detritivores, a statement supported by the findings of Loya-Javellana et al. (1993) who reported that C. quadricarinatus demonstrates an ontogenetic shift from non-selective feeding on decayed plant material or zooplankton to a selective feeding on decayed plant material. Additionally, Jones (1995) observed that juvenile C. quadricarinatus grow better when feeding on fresh zooplankton than when offered formulated flake diets (400 g kg⁻¹ protein) but in both cases grew better when diets were supplemented with vegetal material. The feeding behaviour (omnivorous/detritivorous) of redclaw appears to allow for the incorporation of a broad range of animal- and plant-based ingredients into formulations of practical diets for aquaculture (Jones 1990; Campaña-Torres et al. 2005, 2006, 2008; Pavasovic et al. 2007a).

**Digestive system**

Loya-Javellana et al. (1994) described the ontogeny of redclaw foregut from embryonic stage to adult, while the embryonic development of the digestive system of was described by Meng et al. (2001). The digestive system of decapod crustaceans, including redclaw, can be divided into foregut, midgut and hindgut (Ceccaldi 1997; Meng et al. 2001). The foregut comprises the mouth (with associated mandibles), oesophagus and a large part of the cardiac stomach where the masticating parts are located. The oesophagus is a short, straight vertical structure that connects the mouth and the stomach. The cardiac stomach, an oval like sac, is dorsal in the cephalothorax and leads into the pyloric stomach (elliptically shaped), situated in a ventro-posterior position in relation to the cardiac stomach. The hepatopancreas (or midgut gland), a large, bilateral, multilobate diverticulum of the midgut with a basic unit called a blind tubule, occupies most of the cephalothoracic cavity. The hepatopancreas has diverse functions including absorption, storage of minerals, lipids and glycogen, and distribution of stored reserves during the intermoult period (Brown 1995a; Ceccaldi 1997; Verri et al. 2001). In most crustaceans, the digestive epithelium of the hepatopancreas is comprised of at least four different cell types: E, R, F and B, and in some crustaceans, an M-cell is found (Jacobs 1928; Gibson & Barker 1979; Ceccaldi 1997; Verri et al. 2001). E-cells (embryonic) arise by mitotic division at the distal tips of the each hepatopancreatic tubule and differentiate giving rise to R-cells and F-cells (Dall & Moriarty 1983; Ceccaldi 1997; Verri et al. 2001). R-cells have microvilli and also contain lipid droplets and glycogen, and their primary role is storage (Dall & Moriarty 1983; Ceccaldi 1997). F-cells (fibrillar cells), similar to R-cells, have microvilli that might contribute to absorption. These cells secrete and synthesize digestive enzymes and differentiate into B-cells (Dall & Moriarty 1983; Ceccaldi 1997). B-cells (blister cells) are associated with protein synthesis and enzyme secretion (Verri et al. 2001). Another type of cells found in some crustaceans is the M-cells (midget cells) that might be involved in nutrient absorption and storage (Ceccaldi 1997; Guillaume & Choubert 2001). The midgut, not lined by chitin, begins at the posterior end of the stomach and extends throughout the abdomen terminating at the anus. The hindgut is almost straight and impregnated with chitin, enlarging posteriorly into the rectum and terminates at the anus (see Ceccaldi 1997).

Loya-Javellana et al. (1995) measured the effect of animal size and feeding frequency on the foregut evacuation rates of redclaw. Results indicated that evacuation rates did not differ significantly between size groups (medium, large) nor between feeding frequency groups (fed daily, fed every second day). However, the model specifications differed between feeding frequencies, i.e. ingesta was evacuated linearly with time in the crayfish fed daily and according to a curvilinear pattern in those fed every second day, implying that crayfish are potentially capable of regulating their digestive processes according to food availability. Moreover, the return of appetite in redclaw is rapid; the average return of appetite increased to >50% of the satiation meal at 5–10 h postfeeding, when the residuum of the previous meal was ca. 60% or less. The authors reported that based on these results, redclaw can resume feeding before a considerable proportion of an earlier meal is processed in the foregut, suggesting that the species is capable of optimizing the frequency of feeding during active foraging periods.

A variety of digestive enzymes including proteases, lipases and carbohydrases are found in the midgut gland (hepatopancreas) and gastric fluid of crayfish (Zwilling & Neurath 1981; Brown 1995a; Hammer et al. 2000) including redclaw (Figueiredo et al. 2001). Digestive enzymes are synthesized and secreted into the digestive tract by F- and B-cells in the midgut gland (Ceccaldi 1997; Verri et al. 2001). The presence of a variety of enzymes in juvenile red-
claw has been linked to the ability of the species to digest a wide range of dietary components (Xue et al. 1999; Figueiredo et al. 2001; López-López et al. 2003, 2005; Pavasovic et al. 2007a). This complex digestive enzyme activity is affected by ontogeny (Figueiredo & Anderson 2003), moulting (Fernández et al. 1997; Vega-Villasante et al. 1999; Perera et al. 2008), diet composition (López-López et al. 2005; Pavasovic et al. 2007a), circadian rhythms, photoperiod and quality of light, temperature, stage of larval development, changes during vitellogenesis (Ceccaldi 1997), feeding habits and even habitat (Figueiredo & Anderson 2009).

Proteases, enzymes responsible for hydrolysis of peptide bonds in protein, are present in the gut of crustaceans in general. They include trypsin or a trypsin-like serine protease, astacin, chymotrypsin and exopeptidases [e.g. carboxypeptidases (A and B)] and aminopeptidases (New 1976; Vogt et al. 1989; Brown 1995a; Ceccaldi 1997; Guillaume 1997; Navarrete del Toro et al. 2006; Figueiredo & Anderson 2009). However, it is generally accepted that most crustaceans lack pepsin and stomach acid (see Brown 1995a; Guillaume 1997; Navarrete del Toro et al. 2006).

Total protease [two optimal pH peaks: 5.0 and 7.5 (gastric fluid) and 4.0 and 7.0 (midgut gland)], trypsin-like enzyme (EC 3.4.21.4), chymotrypsin-like enzyme (EC 3.4.21.1), carboxypeptidase A-like enzyme (EC 3.4.12.2), carboxypeptidase B-like enzyme (EC 3.4.12.3) and low levels of leucine aminopeptidase-like enzyme (EC 3.4.11.1) (Figueiredo et al. 2001) are all found in the gut of crayfish but might change in activity and concentration depending on age and diet. Ontogenetic changes in C. quadricarinatus cause total proteases, trypsin, leucine aminopeptidase and carboxypeptidases A and B to exhibit high activity in juveniles and to decrease as the species grows (Figueiredo & Anderson 2003).

Lipases are hydrolases that operate at the interface of emulsified lipid substances (Vogt 2002). They break down carboxyl ester bonds of triacylglycerols liberating carboxylic acids and glycerol. Figueiredo et al. (2001) reported lipase (EC 3.1.1.3) activity only in gastric fluid of adult C. quadricarinatus, whereas López-López et al. (2003) observed esterase–lipase activity in the hepatopancreas of juvenile redclaw.

Although aquatic animals in general are not efficient at utilizing carbohydrates as energy sources, some of the omnivorous crustaceans exhibit some carbohydrate digestion capabilities. Thus, some of the major carbohydrates (amylases, laminarinases, chitinases) are found in the digestive system of many crustaceans (Dall & Moriarty 1983; Ceccaldi 1997). The activity of some of these carbohydrases is age dependant (Figueiredo & Anderson 2003) and change with developmental stages of redclaw. For example, amylase and laminarinase activities are significantly greater in large C. quadricarinatus than at other stages, whereas protease activities decreased as the species grew. The carbohydrases detected in the midgut gland and gastric fluid of adult C. quadricarinatus also include α-amylase (EC 3.2.1.1), laminarinase (EC 3.2.1.6/EC 3.2.1.19), maltase (EC 3.2.1.20) and several para-nitrophenyl glycosidases (Figueiredo et al. 2001). Xylanase activity was also reported in the digestive system of redclaw crayfish (Xue 1998; Crawford et al. 2005). The presence of these carbohydrases would suggest that redclaw should be able to obtain a substantial amount of their metabolic energy needs from carbohydrates, yet research suggests that only a relatively small portion of their energetic needs are obtained from carbohydrates (see Pavasovic et al. 2006; Garza de Yta et al. 2012). Additional work on carbohydrate digestibility and assimilation by redclaw is warranted before definitive statements can be made.

Some crustaceans have been reported to possess cellulases (EC 3.2.1.4) (Yokoe & Yasumasu 1964; Kristensen 1972; Brown 1995a; Xue et al. 1999; Figueiredo & Anderson 2003, 2009). Cellulase activity is also present in all stages of growth in redclaw (Figueiredo & Anderson 2003), yet we have no definitive proof that redclaw can use cellulose nutritively. Enzymatic hydrolysis of cellulose to glucose generally requires the synergistic action of three distinct classes of cellulase enzymes: endoglucanases (endo-1,4-β-glucanases (EC 3.2.1.4) that cleave randomly internal β-1,4-glucosidic bonds; exoglucanases (exo-1,4-β-glucanases (EC 3.2.1.91) that cleave the disaccharide cellobiose from the non-reducing ends of the cellulose chains; and cellobio-

The occurrence of cellulase in the midgut gland and gastric fluid of redclaw (Byrne et al. 1999; Xue et al. 1999; Figueiredo et al. 2001; Figueiredo & Anderson 2003; Crawford et al. 2004; Pavasovic et al. 2006) is very interesting. Cellulose, the principal constituent of most plant cell walls, is known as the most abundant organic compound and renewable energy source on earth (Aspinall 1980; BeMiller 2008). Although the idea of using an abundant
and low-cost ingredient in aquafeeds is exciting (Byrne et al. 1999; Crawford et al. 2004; Pavasovic et al. 2007a), we believe that technological advances required to make cellulose a dietary energy source for aquatic organisms are yet to be described and might never be. Regardless, we will review current literature on the subject.

The catalytic activity of cellulase in redclaw digestive tracts is not markedly inhibited by antibiotic treatment, despite a significant decrease in the gut bacterial populations (up to 94%), suggesting that the activity is innate in the crayfish and not in microbial symbionts. Redclaw cellulase enzymes demonstrated broad substrate specificity, hydrolysing polysaccharides containing β-1,4 and mixed β-1,4 and β-1,3 glycosidic bonds but with a higher preference for soluble substrates (Xue et al. 1999). The occurrence and activity of cellulase in C. quadricarinatus is consistent with the feeding behaviour of redclaw, which consume significant amounts of plant materials and decomposing bacteria, fungi and animals (Byrne et al. 1999; Xue et al. 1999). Byrne et al. (1999) isolated an endo-1,4-beta-glucanase cDNA sequence (termed CqEG) from the hepatopancreas of redclaw, thus providing one of the first endogenous cellulase sequences in crustaceans. Crawford et al. (2004) complemented the study conducted by Byrne et al. (1999) by presenting the genomic organization of CqEG. According to the authors, the presence of an endogenous multigene glycosyl hydrolase family 9 in redclaw indicates that partial breakdown of plant cell polysaccharides is a significant evolutionary strategy for the species. Results of their study suggested the presence of two functional endoglucanase enzymes in redclaw that may be used to obtain energy (glucose) from soluble cellulose (see also Xue et al. 1999), a tool to allow access to other nutrients within plant cells (Béguin & Aubert 1994) or to reduce digestive viscosity of soluble polysaccharides leached from plant cell walls (Crawford et al. 2004). Crawford et al. (2005) reported that C. quadricarinatus has the capacity to liberate glucose from carboxymethyl cellulose, indicating that cellulose substrates can be a source of energy for crayfish. However, a study conducted by Pavasovic et al. (2006) indicated that the presence of cellulase (higher activity in gastric fluid than midgut gland) in the gut of redclaw is unlikely to hydrolyse α-cellulose into glucose and thus would not allow for the supply of energy to the species. Furthermore, the addition of α-cellulose to midgut gland extracts did not change solution viscosity, suggesting that insoluble non-starch polysaccharides do not increase viscosity of intestinal contents upon digestion, which in turn would slow the passage of materials through the gut (Pavasovic et al. 2006). The authors concluded that although cellulase activity is present in redclaw, there are no detectable nutritive benefits of including insoluble cellulose (α-cellulose) in diet formulations of the species.

In addition to proteases, lipases and carbohydrates, endonucleases probably also exist in redclaw. Endonuclease activity has been reported in the digestive tract of various other invertebrates including annelids, molluscs, echinoderms and arthropods (chelicerates, insects and crustaceans) (Yokoe & Yasumasu 1964; see also Watanabe & Tokuda 2001 and references therein; Linton et al. 2006) that are also probably members of the arsenal of digestive enzymes in redclaw guts, but have yet to be isolated.

Information derived from studies on biochemical composition and digestive enzyme activities on utilization of yolk during embryonic development may provide some clues of the nutrient requirements for the embryos and therefore can be used in understanding nutritional requirements of brood stock (Yao et al. 2006; Luo et al. 2008a). Luo et al. (2008a) studied five digestive enzymes (trypsin, pepsin, lipase, amylase and cellulase) in embryonic redclaw, and all showed changes in enzymatic activity closely correlated with morphogenesis, hydrolysing the yolk and providing construction substances and energy resources for formation of tissues, organs and various systems. The activities of the digestive enzymes were controlled by their genes and expressed sequentially during development. Specific activities of pepsin and trypsin increased during early stages of embryonic development, but pepsin activity decreased in later stages (stage VI), while trypsin remained at high level of activity (Luo et al. 2008b). Furthermore, chymotrypsin activity peaked in stage IV and then decreased significantly during the last stage of embryonic development. Low levels of lipase activity were also reported during embryonic development of redclaw (Luo et al. 2008a). Specific activity of amylase changed in a ‘V’ curve, increasing during later stages (stage VI). Cellulase activity during embryonic development in redclaw was relatively low (Luo et al. 2008a).

**Nutrient requirements**

Research on the nutritional requirements and practical diet formulations for redclaw increased rapidly as the culture of the species became established with further advances occurring in the 21st century. Dietary requirements of some nutrients have been determined for rapidly growing juveniles only, with limited information for larger redclaw approaching market weight or for broodstock. This is
probably because most broodstock are collected from extensively stocked farm ponds where the animals have access to primary productivity to supplement possible deficiencies in manufactured diets. Currently, diets for the commercial production of redclaw are based on formulations of other aquatic species, primarily penaeid shrimp feed but sometimes prawn and fish feed (Cortés-Jacinto et al. 2003, 2004, 2005; García-Ulloa et al. 2003; Thompson et al. 2003a, b). Redclaw have the capacity to adapt their digestive physiology in response to changes in their nutrient requirement or dietary profile (Pavasovic et al. 2007b) and consequently have been reared on a wide range of feed formulations. Redclaw diets could potentially be quite inexpensive to manufacture, considering that formulated diets with 200–300 g kg\(^{-1}\) crude protein and 50–100 g kg\(^{-1}\) lipids, based primarily on vegetable rather than animal ingredients, allow for good survival and growth of the species (Cortés-Jacinto et al. 2004).

Proteins and amino acids
Proteins and amino acids are essential nutrients required for maintenance, growth and reproduction in crustaceans as in other animals (Guillaume 1997). Protein requirements of crustaceans are affected by various factors including physiological stage and size, dietary characteristics of protein quantity and quality (e.g. digestibility), amount of non-protein energy in the feed, environmental factors (e.g. temperature) and methodology used for dietary protein determination (D’Abramo & Robinson 1989; D’Abramo & Sheen 1994; Guillaume 1997; Thompson et al. 2005, 2006; Rodríguez-González et al. 2006a). In general, a mixture of proteins of both animal and plant origin provide better growth than either alone because the mixture often contains a complementary blend of amino acids, which are more likely to meet or exceed the requirements (D’Abramo & Robinson 1989; Lovell 1998).

Protein
Most crayfish exhibit an ontogenetic diet shift where adult crayfish incorporate greater levels of detritus and plants in their diet as compared to juvenile crayfish that feed mostly on invertebrates (Mason 1975; Loya-Javellana et al. 1993; Lodge & Hill 1994; Momot 1995; Nyström 2002). Such differences in feeding habits between adult and juvenile crayfish have been attributed to slower growth of adult crayfish and therefore lower protein requirements than in faster growing juveniles (Lodge & Hill 1994). Several studies have attempted to determine protein requirements of juvenile and preadult C. quadricarinatus reared indoors or outdoors (see Table 1). Anson & Rouse (1996) evaluated growth response and survival of newly detached (0.01 g) redclaw offered various commercial feeds (shrimp feed, catfish feed with or without Artemia nauplii supplement) ranging in protein content from a 320 g kg\(^{-1}\) protein catfish diet to a 400 g kg\(^{-1}\) shrimp diet. The 400 g kg\(^{-1}\) shrimp diet resulted in best growth for the animals. D’Agaro et al. (2001) evaluated the dietary protein content (240 g kg\(^{-1}\) and 290 g kg\(^{-1}\); gross energy: 20.0–20.4 MJ kg\(^{-1}\)) on growth performance of juvenile C. quadricarinatus reared in a recirculating system. No significant differences in growth were reported among treatments, probably because of protein-sparing effects from other energy sources. Meade & Watts (1995) offered 0.01 g redclaw a number of commercially available formulated diets and found that a 300 g kg\(^{-1}\) crude protein, 100 g kg\(^{-1}\) fat feed provided best weight gain and survival as compared to all other treatments. However, the authors note that such feeds do not provide complete nutritional needs of crayfish.

Jones & Ruscoe (1996a) evaluated growth performance of juvenile redclaw in glass aquaria offered five formulated diets (four commercial formulations and one experimental reference formulation) and one natural diet containing crude protein ranging from 100–447 g kg\(^{-1}\). Growth was significantly greater in trials offered diets containing 365 g kg\(^{-1}\) protein (with fish meal as protein source) and 205 g kg\(^{-1}\) crude protein (entirely of non-animal material). The authors concluded that redclaw does not seem to have a specific requirement for high levels of proteins and that they can be successfully cultured on a diet primarily composed of material of plant origin. Similarly, Thompson et al. (2005) examined the growth performance of juvenile redclaw offered formulated practical diets containing increasing percentages of dietary protein (300, 350 and 400 g kg\(^{-1}\)). They found that juvenile redclaw can be offered a 350 g kg\(^{-1}\) protein formulated practical diet with a combination of plant-protein ingredients if fishmeal is excluded.

Natural food and forage can also supplement formulated diets and spare proteins in the prepared feed. Metts et al. (2007) reported that juvenile redclaw stocked semi-intensively and offered forage at a rate of 500 kg ha\(^{-1}\) month\(^{-1}\) may be able to utilize 130 g kg\(^{-1}\) protein diets. Thompson et al. (2006) reported that juvenile redclaw offered diets containing 280 g kg\(^{-1}\) crude protein with or without fish meal had significantly greater weight gain compared to redclaw offered 180 g kg\(^{-1}\) crude protein with or without fish.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Initial size (g)</th>
<th>Protein source</th>
<th>Protein levels tested</th>
<th>Optimal protein level (g kg⁻¹ of diet)</th>
<th>Culture type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Webster et al. (1994)</td>
<td>0.022</td>
<td>Mixture: Menhaden fish meal, soybean meal (SBM), shrimp head meal and ground corn</td>
<td>230-550</td>
<td>330 (recommended)</td>
<td>Aquaria, recirculating system</td>
</tr>
<tr>
<td>Keefe &amp; Rouse (1999)</td>
<td>0.02</td>
<td>Mixture: Corn, fish and SBMs</td>
<td>230-430</td>
<td>430</td>
<td>Individual containers, recirculating system</td>
</tr>
<tr>
<td>D'Agaro et al. (2001)</td>
<td>9.7</td>
<td>Mixture: Fish and SBMs and wheat middlings</td>
<td>240-294</td>
<td>240 (suggested)</td>
<td>Tanks, recirculating system</td>
</tr>
<tr>
<td>Hernandez et al. (2001)</td>
<td>0.2 and 8.52</td>
<td>Anchovy fish meal</td>
<td>250-500</td>
<td>300-350</td>
<td>Tanks, recirculating system</td>
</tr>
<tr>
<td>Manomaitis (2001)</td>
<td>0.07</td>
<td>Mixture: Menhaden fish meal (67%), SBM (46%), shrimp meal (44%) and wheat flour (11%)</td>
<td>250-400</td>
<td>400</td>
<td>Semi-recirculating system</td>
</tr>
<tr>
<td>Corte´s-Jacinto et al. (2003)</td>
<td>1.08</td>
<td>Mixture: Sardine, sorghum, soybean, red crab, squid meals, wheat meal and grenetine</td>
<td>200-550</td>
<td>310</td>
<td>Static experimental system</td>
</tr>
<tr>
<td>Corte´s-Jacinto et al. (2005)</td>
<td>0.71</td>
<td>Mixture: Sardine, sorghum, soybean, squid meals, wheat meal and grenetine</td>
<td>260-360</td>
<td>310</td>
<td>Static experimental tanks</td>
</tr>
<tr>
<td>Corte´s-Jacinto et al. (2009)</td>
<td>1.04</td>
<td>Mixture: Sardine, sorghum, soybean, red crab, squid meals, wheat meal and grenetine</td>
<td>280-400</td>
<td>350</td>
<td>Static experimental tanks</td>
</tr>
<tr>
<td>Díaz et al. (2006)</td>
<td>1-2</td>
<td>Rangen and Purina</td>
<td>320-350</td>
<td>350</td>
<td>Recirculating system</td>
</tr>
<tr>
<td>Rodríguez-González et al. (2006a)</td>
<td>23.0</td>
<td>Mixture: Sardine, sorghum, soybean, and squid meals, wheat meal and grenetine</td>
<td>220-370</td>
<td>320 (calculated: 300)</td>
<td>Tanks, static system</td>
</tr>
<tr>
<td>Rodríguez-González et al. (2008a)</td>
<td>25.5 females</td>
<td>Mixture: Sardine, sorghum, wheat, squid, red crab meals, soybean paste and grenetine</td>
<td>220-450</td>
<td>330 (recommended)</td>
<td>Tanks, static system</td>
</tr>
<tr>
<td>Thompson et al. (2004)</td>
<td>4.6</td>
<td>Mixture: Menhaden fish meal (67%), SBM (50%), Brewer's grains with yeast (35%), wheat gluten (41%) and wheat flour (14%)</td>
<td>220-420</td>
<td>220 (recommended)</td>
<td>Ponds</td>
</tr>
<tr>
<td>Thompson et al. (2005)</td>
<td>1.12</td>
<td>Mixture: Anchovy fish (65%) meal, SBM (48%), wheat flour (12.5%, milo (11.5%), BGY (35%) and wheat gluten (80%))</td>
<td>300-400</td>
<td>300 (150 g kg⁻¹ fish meal)</td>
<td>Tanks, recirculating system</td>
</tr>
<tr>
<td>Thompson et al. (2006)</td>
<td>5.75</td>
<td>Mixture: Menhaden fish meal (62%), SBM (48%), distillers' grains with solubles (28%), milo (10%) and wheat gluten (72%).</td>
<td>180-280</td>
<td>280 (0 g kg⁻¹ fish meal)</td>
<td>Ponds</td>
</tr>
<tr>
<td>Metts et al. (2007)</td>
<td>6.25</td>
<td>Mixture: Full-fat SBM, solvent-extracted SBM, wheat midds and whole wheat + alfalfa hay</td>
<td>130-280</td>
<td>130 (with or without alfalfa hay)</td>
<td>Ponds</td>
</tr>
<tr>
<td>Pavasovic et al. (2007b)</td>
<td>13.9</td>
<td>Fish meal, gelatin</td>
<td>130-320</td>
<td>250</td>
<td>Individual cages within tanks, recirculating system</td>
</tr>
<tr>
<td>Zenteno-Savin et al. (2008)</td>
<td>0.71</td>
<td>Same as Cortés-Jacinto et al. (2003) diets</td>
<td>260-360</td>
<td>310</td>
<td>Static experimental tanks</td>
</tr>
</tbody>
</table>
meal (778% and 799%, respectively). They concluded that pond-cultured redclaw performed well when offered diets with 280 g kg\(^{-1}\) protein inclusion even if devoid of fishmeal.

Hernandez et al. (2001) studied the effect of eighteen isocaloric (417.4–422.8 kcal 100 g\(^{-1}\)) diets containing six levels of protein inclusion (250, 300, 350, 400, 450 and 500 g kg\(^{-1}\)) each at lipid levels of 40, 80 and 120 g kg\(^{-1}\), on growth and survival of hatchling and juvenile redclaw reared under controlled conditions. The authors concluded that diets containing 300–350 g kg\(^{-1}\) protein (40–80 g kg\(^{-1}\) lipid) result in best growth performance for both size classes. Manomaitis (2001) offered juvenile redclaw diets with various protein inclusion levels (250–400%) for 7 weeks. Final weight, specific growth rate (SGR) and percentage weight gain of the juveniles were positively correlated with increasing protein levels in the diet. However, a second similar trial with larger juveniles resulted in no effect of dietary protein level on all test factors. The author concluded that a diet of at least 400 g kg\(^{-1}\) crude protein should be offered to newly released redclaw, whereas for larger juveniles, a diet containing 300 g kg\(^{-1}\) protein is sufficient.

Cortés-Jacinto et al. (2003) evaluated the response of juvenile redclaw offered experimental diets containing seven levels (200, 250, 310, 370, 430, 490 and 550 g kg\(^{-1}\)) of dietary protein and with 18.73–21.45 kJ g\(^{-1}\) gross energy (protein to energy ratio: 10.7–25.6 mg kJ\(^{-1}\)). Results showed that highest mean weight (9.6 g) and SGR (3.64% day\(^{-1}\)) were achieved by offering a diet containing 310 g kg\(^{-1}\) crude protein. The optimum dietary protein requirement, calculated from using a second-order polynomial \((y = 1.142 + 0.484 - 0.0071x^2, \ r^2 = 0.952)\), was 342 g kg\(^{-1}\). Similar results were achieved by a later study conducted by Cortés-Jacinto et al. (2005) determining the effect of various protein (260, 310 and 360 g kg\(^{-1}\)) and lipid (40, 80 and 120 g kg\(^{-1}\)) levels, with gross energy content of 17.5–19.4 kJ g\(^{-1}\), on growth of juvenile \(C. \quad quadricarinatus\). Best growth was observed when using dietary protein inclusion of 310 g kg\(^{-1}\) (80 g kg\(^{-1}\) crude lipid) with gross dietary energy content of 19.69 kJ g\(^{-1}\). Similar results were observed by Díaz et al. (2006).

Dietary protein also appears to have an effect on redclaw health. Zenteno-Savín et al. (2008) reported that diets containing 310 g kg\(^{-1}\) crude protein satisfy nutritional requirements for optimal growth, while preventing diet-induced oxidative stress and protecting the integrity of the immune response in juvenile redclaw. Similarly, Cortés-Jacinto et al. (2009) reported that a 350 g kg\(^{-1}\) protein diet stimulates antioxidant response of superoxide dismutase (SOD) (SOD is a cytosolic enzyme specific for scavenging superoxide radicals and is involved in protective mechanisms within injured tissues following oxidative processes and phagocytosis) of juvenile redclaw.

For earthen pond culture, it is not necessary to supply high dietary protein because redclaw supposedly obtain a substantial proportion of their nutrient requirements from natural food materials in the pond (Jones 1990; Jones & Ruscoe 1996b). Jones & Ruscoe (1996b) stocked juvenile redclaw in cages in a pond and offered diets containing crude protein ranging from 100 to 447 g kg\(^{-1}\). Although crayfish offered a reference crayfish diet (205 g kg\(^{-1}\) crude protein) grew better than crayfish offered all other diets, the authors suggested that the crayfish did not have a direct use of the feed offered but obtained the bulk of their nutrition from natural productivity of the pond benthos. In a similar experiment, Thompson et al. (2004) found that 220 g kg\(^{-1}\) dietary protein was sufficient for redclaw culture.

In other experiments, Pavasovic et al. (2007b) reported maximum growth of subadult redclaw offered diets containing 250 g kg\(^{-1}\) crude protein with a strong positive correlation between dietary protein and protein content in the tail. However, other researchers did not observe a significant effect of dietary protein on percentage protein in redclaw tail muscle or even total body protein (Muzinic et al. 2004; Thompson et al. 2004).

A summary of the literature thus suggests that diets with 250 g kg\(^{-1}\) or greater protein inclusion are suitable for redclaw growout in ponds with natural productivity. Diets with 350 g kg\(^{-1}\) protein inclusion or greater are recommended for redclaw grown in closed recirculation systems. All diets should have a gross energy content of 18 kJ g\(^{-1}\), minimum. These suggestions are supported by Cortés-Jacinto et al. (2004) who propose a minimum protein inclusion in redclaw diets of 220 g kg\(^{-1}\) with 15.21 kJ g\(^{-1}\) of digestible energy.

No discussion of aquatic animal nutrition is complete without mentioning broodstock diets. Broodstock nutrition is of high importance for successful reproduction and egg quality; adequate nutrients and energy in broodstock diets are necessary for the onset of gonadal maturation, because maternal nutrient intake during ovarian development is critical and influences the composition of ovaries and the nutritional status of eggs. Crustacean embryos rely exclusively on the nutrients and energy supplied by the egg (yolk) (Harrison 1997). In decapod crustaceans, protein is a structural, functional and energy constituent of tissues and plays an important role in spawning, fertilization and
normal development of embryos (Harrison 1990; Wouters et al. 2001; García-Guerrero et al. 2003; Rodríguez-González et al. 2006a). Asgari (2004) reported that based on spawning rate, fecundity, hatchability and egg size, a diet containing 400–450 g kg\(^{-1}\) crude protein and 16.72 kJ g\(^{-1}\) energy is optimal for redclaw crayfish broodstock, yet Rodríguez-González et al. (2006a) tested diets with lesser protein inclusion and found no differences in survival, final weight and fecundity of female broodstock. However, regression analysis indicated that maximum spawning was from females offered a 300 g kg\(^{-1}\) crude protein diet and dietary protein levels of 320 g kg\(^{-1}\) had a significant effect on egg quality but not on biochemical composition of the eggs. Such findings were recently corroborated by Li et al. (2010) who found that using diets with higher protein content improves redclaw female spawning, especially when gonadosomatic index is >1.6. Rodríguez-González et al. (2009a) separated maturation from gonadal development as they relate to female broodstock diets and found that diets with 220–450 g kg\(^{-1}\) crude protein result in maturation of female redclaw, but a dietary protein range from 284 to 355 g kg\(^{-1}\) improved gonadal development and resulted in more protein production in the hepatopancreas. In previous work, Rodríguez-González et al. (2006b) had observed that external sources of protein and energy were vital for nutrient accumulation in the gonad. Additionally, protein contents in the gonad were correlated with gonadosomatic index; at mature stages, higher protein concentration was observed. These gonadal proteins were a result of an active mobilization of energy reserves from exogenous sources, incorporated into the oocytes by endocytosis (Abdu et al. 2000). Based on current knowledge, we suggest that broodstock females be offered diets with 350 g kg\(^{-1}\) protein and a minimum of 18 kJ g\(^{-1}\) gross energy, a part of which comes from fish oil to supply the necessary omega-3 HU-FAs.

### Amino acids

Determination of the exact amino acid requirements in crustaceans is difficult (Shiau 1998), and this is probably the reason for the paucity of reports on the specific amino acid requirements of redclaw. In general, the essential amino acid requirements for most crustaceans include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (D’Abramo & Robinson 1989; Brown 1995b; Guillaume 1997) plus asparagine for crayfish (Brown 1995b). Tyrosine and cysteine are considered semi-essential in the diet as they potentially spare the requirement of phenylalanine and methionine, respectively (Guillaume 1997). There is a significant correlation between the dietary amino acid requirements of a species and the pattern of amino acids in whole body tissue (Cowey & Tacon 1983; Wilson & Poe 1985). Consequently, dietary amino acid requirements of growing animals are often assumed to be similar to the amino acid composition of the tissue proteins formed during growth. Mitchell (1950) suggested that an animal’s amino acid requirements might first be deduced from the amino acid composition of its tissues. However, our experience suggests that when using body composition as reference of requirement, one would overestimate dietary requirement of essential amino acids and underestimate requirement of other protein components.

Muzinic et al. (2004) evaluated the amino acid composition of practical diets containing various levels of soybean meal (SBM) and brewer’s grains with yeast as replacements for fish meal, and results suggested that the amino acid levels in a 400 g kg\(^{-1}\) crude protein diet were adequate for good growth and survival of juvenile redclaw crayfish whichever protein source was used. Similarly, Thompson et al. (2005) noted that a complementary blend of SBM and other plant-protein sources used to replace FM in a 350 g kg\(^{-1}\) protein diet appeared to provide sufficient levels of essential amino acids to meet requirements of redclaw. In pond-cultured redclaw, diets containing 280 g kg\(^{-1}\) crude protein with or without fish meal may sufficiently satisfy the requirements of essential amino acids of male and female redclaw (Thompson et al. 2006) probably because natural productivity supplements the formulated feeds being offered. Consequently, and based on their response to diets without fishmeal, one may assume that methionine and lysine requirements of redclaw are relatively low. Such assumptions are yet to be empirically tested.

### Protein to energy requirements

Knowledge of energetic utilization of farmed organisms is necessary for the development of cost-effective diets. Energy from non-protein sources (lipids, carbohydrates) relative to protein levels must be supplied into diets in sufficient amounts to insure that protein is used for tissue synthesis as protein is considered the most expensive major component of crustacean diets (D’Abramo & Robinson 1989; Cuzon & Guillaume et al. 1997; Cho et al. 2005). If the non-protein energy to protein ratio is insufficient, dietary protein may be catabolized and used as an energy source to satisfy maintenance before somatic growth. Con-
versely, if dietary energy to protein ratio is in excess, feed consumption may be reduced, resulting in a decrease in protein intake and other essential nutrients required for maximum growth. Excessively high ratios of energy to nutrients can also lead to deposition of large amounts of body fat (Cuzon & Guillaume 1997).

A review of various published results concerning dietary protein to energy requirements of redclaw suggests that optimal growth is obtained when the animals are offered feed with a protein to energy content between 16 and 20 mg kJ⁻¹ and a crude protein content between 310 and 350 g kg⁻¹ of the diet by weight (D’Agaro et al. 2001; Cortés-Jacinto et al. 2003, 2005, 2009). D’Agaro et al. (2001) found no significant differences in growth of redclaw when offered diets containing protein to energy ratio of 50 and 60 mg kcal⁻¹ (240 and 294 g kg⁻¹ protein, respectively) and attributed their results to the protein-sparing capacity of the energy in the diets. This protein-sparing effect was also observed by Hernandez et al. (2001). Values for protein to energy ratio for optimal reproductive activity and gonadal development in redclaw were 18 ± 2 mg kJ⁻¹ (Rodríguez-González et al. 2011) and 17.16 mg kJ⁻¹ (Rodríguez-González et al. 2006a), respectively, within the range observed as necessary for juvenile growth.

**Lipids and essential fatty acids (EFAs)**

**Lipid**

Dietary lipids play an important role in crustacean nutrition as they provide energy and essential fatty acids (EFAs), sterols, phospholipids and fat-soluble vitamins necessary for proper functioning of physiological processes and maintenance of biological structure and function of cell membranes (D’Abramo & Robinson 1989; Sargent et al. 1989; D’Abramo 1997; Teshima 1997). Lipid used as energy source can also spare dietary proteins and reduce nitrogenous waste production (D’Abramo & Robinson 1989; Lim & Sessa 1995; Cho & Bureau 2001). However, high dietary lipid levels can cause significant reductions in growth rate, feed consumption and also might reduce the utilization of other nutrients resulting in reduced growth (D’Abramo 1997). Additionally, an increase in dietary lipid levels was linked to increases in the lipid content of midgut glands (hepatopancreas) (D’Abramo 1997).

In general, nutritional studies with crustaceans indicate that lipid content of formulated diets should range between 50 and 80 g kg⁻¹ of feed by weight to ensure optimal growth and survival (D’Abramo 1997). The lipid level required for optimal growth is influenced by several factors including quality and quantity of protein, availability, quantity and quality of other sources of energy and adequate provision of EFAs (D’Abramo 1997) as well as the ability of the organism to digest carbohydrates and use glucose in its metabolism. Lipids are often supplemented in excess of minimal requirements to spare protein for somatic growth. Such a protein-sparing effect of lipids was reported in hatchling and juvenile redclaw offered diets containing 40–80 g kg⁻¹ lipid (300–350 g kg⁻¹ protein) (Hernandez et al. 2001), suggesting that this range of lipid inclusion to redclaw diets is suitable.

A few studies investigated dietary lipid requirements of redclaw under laboratory conditions (Hernandez et al. 2001; Cortés-Jacinto et al. 2005; Zenteno-Savin et al. 2008), and all seem to agree that a diet containing 80 g kg⁻¹ dietary lipid with approximately 300 g kg⁻¹ protein and gross energy 17.5–19.1 kJ g⁻¹ is suitable for good growth performance of juvenile C. quadricarinatus while preventing diet-induced oxidative stress and protecting the integrity of the immune function.

Although lipids are necessary in redclaw diets, it appears that natural productivity can replace dietary lipids to a certain extent. Hernández-Vergara et al. (2003) evaluated the effect of different dietary lipid levels (42, 82 and 123 g kg⁻¹) on growth, survival and proximate composition of juvenile redclaw reared semi-intensively in outdoors tanks and observed no effect of treatment on the various parameters. Accordingly, it seems that in the presence of some natural productivity, a diet containing 42 g kg⁻¹ dietary lipid (17.58 kJ g⁻¹, 300 g kg⁻¹ crude protein) is sufficient for growth and survival of juvenile redclaw.

Differences in lipid metabolic routes between sexes where females have higher carcass lipid content than males are often reported (e.g. Hernández-Vergara et al. 2003). This is generally attributed to storage of lipids for ova development or vitellogenesis. Yet, studies on developing adequate diets for maturation of broodstock redclaw are rare (Rodríguez-González et al. 2006a,b, 2009a,b). Considering that lipids are the main energy sources during ontogeny of crustaceans and also structural components of cell membranes (Holland 1978; Harrison 1997), lack of research on the subject seems surprising. However, when one considers the ease of collecting egg-bearing females from ponds, one understands the lack of interest in broodstock maintenance. Nonetheless, as the industry grows and biosecurity issues become more important and infectious diseases appear, indoor closed system hatcheries will become necessary and with them special broodstock diets.
According to Rodríguez-González et al. (2006b), the lipid requirements for the developing gonad of female redclaw mainly originates from the diet. Rodríguez-González et al. (2009b) studied the effect of dietary lipid levels (40, 80 and 120 g kg⁻¹) on female redclaw crayfish and their eggs. Results indicated no significant differences in survival, final weight or fecundity. However, dietary lipid content influenced size and weight of eggs, with greatest egg weight obtained from females offered the 87 g kg⁻¹ lipid diet. In a similar study on the effects of dietary lipids on female redclaw reproduction, Li et al. (2010) observed a significant correlation between lipid transportation in the hepatopancreas and the ovaries, but it appeared that the lipid reserves in the hepatopancreas could not meet the requirements of ovaries. The authors concluded that the lipid requirements of gonads come only partly from the diet.

**Essential fatty acids**

Polyunsaturated fatty acids (PUFA) of the C18 series (linolenic (18:3n-3) and linoleic (18:2n-6) acids) and n-3 and n-6 highly unsaturated fatty acids [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ArA)] are considered essential in crustacean diets (see D’Abramo & Robinson 1989; D’Abramo 1997; Venero et al. 2008). No studies on specific EFA requirements of redclaw were found; however, a few studies evaluated diets containing various levels of fatty acids on growth performance of the species. Thompson et al. (2003a) reported that a mixture of 5% cod liver oil and 1% corn oil added to the diets probably met the EFA requirements of juvenile redclaw. The blend of these oils provides a mix of PUFA such as linoleic (18:2n-6), linolenic (18:3n-3), oleic (18:1n-9) acids and highly unsaturated fatty acids such as eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids, that is sufficient for redclaw survival and growth. Similarly, Thompson et al. (2003b) evaluated practical diets with and without supplemental lecithin and/or cholesterol offered to juvenile redclaw. The authors indicated that diets with 0 g kg⁻¹ supplemental lecithin and/or cholesterol contained a combination of PUFA and HUFA in the diet, which satisfied the EFA requirements of juvenile redclaw. Thompson et al. (2010) examined the effect of different sources of lipids (linseed oil, canola oil, corn oil, beef tallow or menhaden oil) that differ in fatty acid profile on growth response of juvenile redclaws. Results showed that whole-body fatty acid composition of redclaw differed among animals offered the various lipid sources, generally reflecting the fatty acid composition of the diets. Plant oils rich in α-linolenic acid (18:3n-3), linoleic acid (18:2n-6) and oleic acid (18:1n-9) perform as well as menhaden oil containing high levels of n-3 HUFA when offered to juvenile redclaw reared indoors and lacking natural productivity. The authors concluded that redclaw can be fed diets containing plant-based oils with high levels of 18-carbon unsaturated fatty acids. Muninic et al. (2004) reported that practical diets containing various levels of SBM and brewer’s grains with yeast, as replacements for fish meal, have both n-6 and n-3 highly unsaturated fatty acids such as linoleic (18:2n-6), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids that may satisfy the EFA requirements of juvenile redclaw.

The fatty acid profile during early embryonic development of redclaw shows that the major fatty acids, oleic/ vaccenic (18:1), palmitic (16:0), linoleic (18:2n-6) and palmitoleic (16:1n-7) remain major during later developmental stages and are required in larger quantities than other fatty acids (Alimon et al. 2003). Monounsaturated fatty acids constituted the major moiety of the fatty acid profile, and the PUFA were dominated by linoleic (n-6) series (low n-3 to n-6 ratio) (Alimon et al. 2003). Luo et al. (2008a) reported that the predominant fatty acids of both neutral and polar lipids of redclaw during embryonic development were C16:0, C18:0, C18:1n-9 and C18:3n-3.

Saturated fatty acids (16:0 and 18:0) and monounsaturated fatty acids (16:1n-7 and 18:1n-9) are generally used for energetic purposes, whereas PUFA (20:5n-3 and 22:6n-3) are important as structural components of cell membranes and in the development of the central nervous system (Luo et al. 2008a). However, even during vitellogenesis, there are high proportions of monounsaturated fatty acids in the ovaries and hepatopancreas, suggesting their use as major sources of energy (Li et al. 2010). Such information would suggest that broodstock diets could be formulated to contain more vegetable oils to be used for energy during vitellogenesis without compromising development of eggs, which require some n-3 HUFAIs found in expensive but necessary fish oils.

**Lipid classes**

**Phospholipids**

Phospholipids are added to the diet of crustaceans for various reasons such as a source of energy; a major component of cell membranes; emulsification of lipid aggregates during digestion and absorption; and because they play a major role in lipid transportation in the haemolymph (Coutteau...
et al. 1997; Teshima 1997). Thompson et al. (2003a) evaluated the effect of different levels of supplemental soybean lecithin on growth and survival of juvenile redclaw crayfish and found no effect. The authors suggested that diets containing enough lipids and 5 g kg⁻¹ choline chloride by weight may be sufficient for redclaw crayfish growth. Similar observations were made by Thompson et al. (2003b) in a study examining growth performance of juvenile redclaw reared over an 8-week period and offered diets with and without supplemental lecithin and/or cholesterol.

Sterols

It is generally accepted that crustaceans are unable to synthesize sterols de novo and require an exogenous dietary source for growth, development and/or survival (see Teshima 1997; Kanazawa 2001). Cholesterol is the major essential sterol in crustacean nutrition with an important role as a cell constituent, metabolic precursor of steroid hormones and moulting hormones (see Brown 1995b; Teshima 1997; Shiau 1998). Dietary cholesterol requirement for optimal growth performance in various crustacean species ranges from 1.2 to 20 g kg⁻¹ of the dry weight of a diet (Teshima 1997; Kanazawa 2001).

Studies on cholesterol requirements of *C. quadricarinatus* are few. Hernández et al. (2004) evaluated the effect of dietary cholesterol on growth and survival of redclaw and observed no significant differences among treatments of various cholesterol supplantations but noticed slight growth increase in redclaw offered a diet with 5 g kg⁻¹ cholesterol inclusion. A growth study by Thompson et al. (2003b) whereby they offered *C. quadricarinatus* juveniles (0.2 g) practical diets with or without supplemental lecithin and/or cholesterol showed no significant differences in weight gain among treatments. The authors interpreted their results to suggest that redclaw could be farmed using feeds less expensive than traditional marine shrimp feeds, currently used in redclaw culture.

Carotenoids

Carotenoids, a family of over 600 natural lipid-soluble pigments, are the most universally widespread (e.g. bacteria, algae, plants and animals) and structurally diverse pigmentsing agents. They are synthesized through the isoprenoid pathway, which also produces diverse compounds such as EFAs, steroids, sterols and vitamins A, D, E and K (see Meyers & Latscha 1997; Liñán-Cabello et al. 2002). Crustaceans are unable to synthesize carotenoids de novo and must obtain them from the diet (Meyers & Latscha 1997). Yet, most crustaceans contain and utilize carotenoid pigments; mainly in the carapace, eyes, blood eggs, midgut gland and ovaries (Sagi et al. 1995; Meyers & Latscha 1997). Functions of carotenoids include pigmentation, sources of provitamin A, antioxidants, positive effects on development, growth, maturation, reproduction and enhancement of immunity (see Meyers & Latscha 1997). The most common pigments, derived from diets or from metabolic transformation of precursor carotenoids, are astaxanthin, β-carotene, echinenone and canthaxanthin (Meyers & Latscha 1997). Astaxanthin has been described as the most frequent end product of carotenoid metabolism in crustacean (Meyers & Latscha 1997).

Rouse & Rash (1999) reported that astaxanthin added to diets offered to juvenile redclaw resulted in an increase in survival and growth by 20%. However, in a study by Harpaz et al. (1998) where the effect of three carotenoid sources (dried alga cells prepared from *Dunaliella salina* (source of β-carotene), synthetic astaxanthin and alfalfa meal) on growth and pigmentation of juvenile *C. quadricarinatus* was evaluated, no significant differences were found on growth and survival. Redclaws receiving carotenoid-enriched diets exhibited better pigmentation than those receiving carotenoid-free diets. The authors suggested however that adding alfalfa meal and artificial astaxanthin to redclaw diets produce desired body coloration.

Carotenoids play an essential role before and after gonadal maturation processes (reviewed by Liñán-Cabello et al. 2002). Sagi et al. (1995) noted that target tissues for carotenoid accumulation in *C. quadricarinatus* are the ovary and cuticle. In a study by Liñán-Cabello et al. (2004), the effects of carotenoid (β-carotene and astaxathin) and vitamin A injections were correlated with the ontogenic development of oocytes in female redclaw. Their results showed that retinol palmitate had the greatest inductive effect on the primary vitellogenic phase and on the indicators of ontogenic oocyte development. Accordingly, we deduce that carotenoids and retinols are essential nutritive additives during gonadic maturation of redclaw and help giving adults a coloration that would help in marketing. Additional work should evaluate the effects of dietary carotenoid supplementation on immune responses and general physiology of redclaw.

Carbohydrates

Carbohydrates are generally an inexpensive source of energy for crustacean feeds (Shiau 1997). Although carbo-
hydrates are not considered essential nutrients, they are incorporated into feeds to reduce costs, for their binding activity during feed manufacturing and possibly for protein-sparing effects (D’Abramo & Robinson 1989; Guillaume & Choubert 2001; Wouters et al. 2001; Pillay & Kutty 2005). Additionally, carbohydrates play several roles in crustacean metabolism including glycogen storage, chitin synthesis and the formation of steroids and fatty acids (Parvathy 1971; Dall et al. 1990; Ali 1993; Sánchez-Paz et al. 2006).

Crustaceans are able to utilize more complex carbohydrates (e.g. starch, chitin) than simple sugars (e.g. glucose) (Shiau 1997), yet it is generally accepted that freshwater crayfish have poor polysaccharide digestibility and are unlikely to obtain substantial nutrition from fibrous material (Shiau 1997; Pavasovic et al. 2006). However, dietary fibres, water-soluble and water-insoluble polysaccharides delay stomach emptying by increasing viscosity of the diet (see Shiau 1997 and references therein), thus assisting in digestion.

Some polysaccharides such as carboxymethyl cellulose cannot be digested even though some cellulase activity was detected in the gut of redclaw. Pavasovic et al. (2006) demonstrated cellulase activity in redclaw digestive systems, yet observed no detectable nutritive benefits of including insoluble cellulose (α-cellulose) in diet formulations for the species. Dietary inclusions of α-cellulose above 120 g kg⁻¹ significantly reduced survival rate, feed conversion efficiencies and general growth performance of redclaw. Jones & Ruscoe (1996c) assessed growth performance of redclaw maintained in earthen ponds and offered five diets containing various carbohydrate sources (maize, wheat, rice, sorghum, lupin and barley). No significant differences in survival and growth were observed among treatments. This suggests that the source of carbohydrate is not of particular importance in redclaw nutrition. In a recent experiment in Mexico, we assessed the effect of stargrass hay supplementation to formulated feed diets on redclaw growth and found no nutritive value beyond what the animal obtains from formulated feed. Dietary carbohydrates also do not appear to affect or improve gonadal maturation (Rodríguez-González et al. 2006b). Nevertheless, carbohydrates will always constitute a good portion of formulated redclaw diets because they are an inexpensive source of energy and filler.

Vitamins and minerals

Vitamins and minerals are essential micronutrients necessary for normal life processes in crustaceans. Deficiencies in vitamins and/or minerals lead to slower growth, negatively affect reproduction and/or eventual mortality in crustaceans (Conklin 1997; Davis & Lawrance 1997). Information on specific vitamin and mineral requirements of redclaw is scarce to non-existent. It is assumed that vitamin and mineral requirements are similar to those of other crustaceans (D’Abramo & Robinson 1989). However, gonadal maturation was shown to be affected by vitamin levels. Liñán-Cabello et al. (2004) reported that retinol has a significant effect in oocyte maturation of *C. quadricarinatus* and is an essential nutritive additive for gonadal maturation. Luo et al. (2004) found that excessive vitamin E affected reproduction of *C. quadricarinatus* and that optimal content of vitamin E was 192 mg kg⁻¹. Additionally, the authors speculated that vitamin E could protect C20:5n-3 and C22:6n-3, necessary for the development of the nervous system, from oxidizing and improved the accumulation of important amino acids and fatty acids in fertilized eggs.

Feed ingredients

**Ingredient digestibility**

The nutritive value of a feed ingredient is based on its chemical composition and on an animal’s capacity to digest, absorb and utilize it. Digestibility is the quantity of the nutrient or energy in the ingested feedstuff that is not excreted in the faeces (NRC 1993; Lee & Lawrence 1997; Guillaume & Choubert 2001). In general, freshwater crustaceans have higher apparent digestibility efficiency (ADE) and apparent crude protein digestibility (ACPD) values for high carbohydrate feeds than marine crustaceans, and both have high ACPD values for animal meals and purified proteins (Lee & Lawrence 1997).

Campana-Torres et al. (2005) evaluated dry matter and protein digestibility of juvenile *C. quadricarinatus* offered diets supplemented with 150 g kg⁻¹ of three plant-derived (soy paste, textured wheat and sorghum meal) and four animal-derived (two sardine meals, squid meal and red crab meal) ingredients. They found that plant-derived ingredients and corresponding diets had better digestibility than animal ingredients (see Table 2). In a subsequent study, Campaña-Torres et al. (2006) reported that mean carbohydrate and lipid digestibilities of vegetal ingredients and corresponding diets were better than carbohydrate and lipid digestibilities of animal ingredients although some of the animal ingredients (e.g. red crab) had high lipid digestibility (see Table 2). The authors concluded that redclaw are able...
Table 2 Apparent dry matter digestibility (ADMD) coefficients for protein (APD), carbohydrates (ACD), lipids (ALD) and gross energy (AGED) of the various nutrient sources for redclaw (*C. quadricarinatus*).

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>% ADMD</th>
<th>% APD</th>
<th>% ACD</th>
<th>% ALD</th>
<th>% AGED</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardine meal (580 g kg(^{-1}) crude protein)</td>
<td>62.6</td>
<td>47.4</td>
<td>18.7/41.1(^{3})</td>
<td>42.0/60.6(^{1})</td>
<td>–</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Sardine meal (670 g kg(^{-1}) crude protein)</td>
<td>83.2</td>
<td>72.4</td>
<td>28.0/12.3(^{3})</td>
<td>83.5/84.5(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Red crab meal</td>
<td>79.0</td>
<td>53.8</td>
<td>32.6/34.1(^{3})</td>
<td>92.1/97.5(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Squid meal</td>
<td>80.1</td>
<td>70.8</td>
<td>18.6/15.6(^{3})</td>
<td>84.6/60.6(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Textured wheat</td>
<td>88.9</td>
<td>90.5</td>
<td>87.5/83.9(^{3})</td>
<td>95.0/96.4(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Soy paste</td>
<td>90.8</td>
<td>91.8</td>
<td>88.5/81.6(^{3})</td>
<td>93.6/95.1(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Sorghum</td>
<td>90.8</td>
<td>89.6</td>
<td>94.4/93.6(^{3})</td>
<td>85.6/77.3(^{3})</td>
<td>–</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>Fish meal</td>
<td>80.2</td>
<td>89.8</td>
<td>–</td>
<td>–</td>
<td>86.9</td>
<td>4</td>
</tr>
<tr>
<td>Meat and bone meal (MBM)</td>
<td>65.3</td>
<td>83.4</td>
<td>–</td>
<td>–</td>
<td>74.5</td>
<td>4</td>
</tr>
<tr>
<td>Poultry meal (PM)</td>
<td>77.9</td>
<td>87.2</td>
<td>–</td>
<td>–</td>
<td>84.9</td>
<td>4</td>
</tr>
<tr>
<td>Soybean meal (SBM)</td>
<td>89.3</td>
<td>94.6</td>
<td>–</td>
<td>–</td>
<td>93.8</td>
<td>4</td>
</tr>
<tr>
<td>Canola meal (CM)</td>
<td>74.5</td>
<td>91.0</td>
<td>–</td>
<td>–</td>
<td>80.9</td>
<td>4</td>
</tr>
<tr>
<td>Lupin meal (LM)</td>
<td>83.8</td>
<td>94.6</td>
<td>–</td>
<td>–</td>
<td>89.6</td>
<td>4</td>
</tr>
<tr>
<td>Brewer’s yeast (BY)</td>
<td>85.6</td>
<td>92.6</td>
<td>–</td>
<td>–</td>
<td>86.6</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Campaña-Torres et al. 2005 (using juveniles: 3.6 ± 1.3 g).
2 Campaña-Torres et al. 2006 (using juveniles: 3.62 ± 1.3 g).
3 Campaña-Torres et al. 2006 (using preadults: 10 ± 0.8 g).
4 Pavasovic et al. 2007a (using adults: 94.5 ± 3.5 g).

To efficiently consume diets containing a variety of plant- and animal-derived ingredients, with better digestion efficiency of plant-derived ingredients (see also Campaña-Torres et al. 2008). Similarly, Pavasovic et al. (2007a) evaluated the potential use of several ingredients (fish meal, meat and bone meal, poultry meal, SBM, canola meal, lupin meal and brewer’s yeast) in dietary formulations for adult redclaw. Results showed that apparent digestibility of dry matter, crude protein and gross energy was better for SBM diets than diets containing meat and bone meal (Table 2). Accordingly, it seems that redclaw has the capacity to utilize a broad range of dietary ingredients including animal, single cell and plant matter in their diet. However, protein digestibility seems to be affected by other dietary ingredients. Supplementation of diets with either 300 g kg\(^{-1}\) α-cellulose or Fuller’s earth significantly reduces apparent dry matter digestibility (ADMD) and apparent protein digestibility (APD) of redclaw (Pavasovic et al. 2006).

Redclaw have the ability to modify their digestive enzyme secretions in response to different ingredients in the diet over time (López-López et al. 2005; Pavasovic et al. 2007a). Therefore, as they age and their food changes, they adapt to the new dietary sources, particularly starches (López-López et al. 2005). However, digestibility of nutrients is slightly better in juveniles than in preadults (Campana-Torres 2001; Campaña-Torres et al. 2006, 2008). This deterioration of digestibility with age might be because of faster metabolism in early stages of development (Guillaumé 1997).

Fish meal and fish oil replacement in redclaw diets

Diet constitutes a major expenditure in aquaculture. Fish meal and other marine meals (krill, shrimp, squid and scallop meals) are used as protein sources in many finfish and crustacean diets, as they are considered excellent sources of high-quality proteins, highly unsaturated fatty acids, vitamins, minerals and attractants (Tacon & Akiyama 1997; Webster et al. 2008). Fish oils have been used as a dietary lipid source in commercial aquafeeds because of their ready availability, fair price and abundance of EFAs (Turchini et al. 2009). However, owing to aquaculture expansion, competition from other agricultural sectors, uncertainty in long-term availability (e.g. overfishing), yearly fluctuations in supply, quality and potential price variation, there has been considerable interest in partial or total replacement of fish meal and other marine meals and fish oil with less expensive plant-protein meals and oils (see Naylor et al. 2000, 2009; Venero et al. 2008; Webster et al. 2008).

Fishmeal replacement Few studies have evaluated the replacement of fish meal in redclaw diets. SBM replacement of fishmeal at various levels resulted in marginal growth differences among redclaw juveniles, but organisms offered a fishmeal-based diet had better growth and more frequent molting than those offered graded levels of SBM (García-Ulloa et al. 2003; Muzinic et al. 2004). Similarly, Gutierrez & Rodriguez (2010) examined the effect of protein source (SBM) on growth of juvenile *C. quadricarinatus* reared in...
individual containers. Based on their results, best growth was obtained with a mixture of 50% fish meal and 50% SBM.

Saoud et al. (2008) evaluated the response of juvenile redclaw offered six practical diets (260 g kg⁻¹ crude protein, 70 g kg⁻¹ crude lipid) replacing fishmeal with poultry by-product meal at various inclusion levels. Yuniarti et al. (2011) performed a similar experiment but substituted golden apple snail (Pomacea canuculata) meal for fishmeal. No significant differences in survival and growth were detected among treatments in both experiments. The authors concluded that poultry by-product meal and golden apple snail meal are potential candidates for fish meal replacement in redclaw diets. Similar results were reported by Garza de Yta et al. (2012) who evaluated growth response of juvenile C. quadricarinatus, reared in tanks, offered soybean-based diets (350 g kg⁻¹ crude protein, 71 g kg⁻¹ lipids) containing either fish meal, poultry by-product meal, ground pea meal or distillers' dried grains with solubles (DDGS) meal as protein source. No significant differences were found in survival, growth or feed conversion ratio (FCR) of redclaw crayfish. This might be because natural productivity contributed to supplementation of minor deficiencies in essential amino acids. Thompson et al. (2005) reported that juvenile redclaw reared in a recirculating system can be offered practical diets containing 350 g kg⁻¹ crude protein with no fish meal if a combination of less expensive plant-protein ingredients such as SBM, wheat, brewer’s grains with yeast is added to the diet. In pond culture of juvenile redclaw, practical diets containing 280 g kg⁻¹ crude protein with no fish meal but containing a combination of plant-protein ingredients (SBM, distillers' DDGS and milo) was adequate for good growth (Thompson et al. 2006).

Forage (detrital) crops (e.g. rice, hay) are often used in freshwater crayfish cultivation (Ackefors 2000; Salame & Rouse 2000; Jones et al. 2002) for presumed benefits such as supplementation of direct and indirect sources of food and supplying protective cover for moulting crayfish as they seek refuge from predators. Fletcher & Warburton (1997) tested fresh and decomposed duckweed (Spirodela sp.) as feed for juvenile crayfish redclaw and found that decomposed duckweed supported crayfish growth as well as commercial pellets did. The authors suggested that preparation of diets using detrital aquatic plants may be a cost-effective method of increasing redclaw production. Salame & Rouse (2000) evaluated forage-based feeding strategies for redclaw reared in earthen ponds. Juvenile redclaw stocked at a density of 4 m⁻² were offered two feeding regimes: manufactured pellets and pellets + forage (stargrass (Cynodon plectostachyum) and janeiro grass (Eriochloa polystachya)) at a rate of 100 kg ha⁻¹ month⁻¹. Survival and yield were greater in ponds receiving pellets and forage than survival and yield in ponds receiving pellets only. However, Metts et al. (2007) performed similar experiments but found no benefit from forage supplementation. Generally, results of the majority of studies that have been performed do not support using forage in redclaw aquaculture.

**Fish oil replacement** Fish oils rich in HUFA are traditionally used in aquatic animal feeds. Aquaculturists would like to replace them with terrestrial plant oils such as linseed oil, canola oil and corn oil, rich in linolenic acid (18:3n-3), linoleic acid (18:2n-6) and oleic acid (18:1n-9). Thompson et al. (2010) evaluated growth response and fatty acid composition of juvenile redclaw crayfish offered diets containing various lipid sources such as linseed oil, canola oil, corn oil, beef tallow or menhaden oil. Crayfish offered plant-derived oils performed as well as those offered feeds based on menhaden oil containing high levels of n-3 HUFA. The diet containing beef tallow had a higher percentage of saturated fatty acids and resulted in poor growth. The authors concluded that menhaden oil can be replaced by plant-based oils with high levels of 18-carbon unsaturated fatty acids in diets of juvenile redclaw, thus reducing costs for producers. We suggest further research before definitive conclusions are made but believe that because redclaw are freshwater organisms, they probably can perform well without dietary EPA and DHA inclusion. It is possible that they can elongate and desaturate α-linolenic acid.

**Binders and water stability**

Crustaceans exhibit relatively slow and intermittent feeding activity that has an impact on food acquisition and processing (Loya-Javellana et al. 1995; Houser & Akiyama 1997). These behavioural characteristics affect physical properties including water stability and durability of the pellets (Meyers & Zein-Eldin 1975; Lim & Cuzon 1994; Houser & Akiyama 1997; Obaldo et al. 2002). Pellets need to be firmly bound to avoid breaking up into small particles that results in leaching of nutrients into water, reduction in water quality, poor animal growth, inefficient feed conversion and low survival (Lim & Cuzon 1994; Houser & Akiyama 1997; Obaldo et al. 2002). Binders affect pellet stability in three ways: they reduce void spaces resulting in a more compact and durable pellet; act as adhesives stick-
ing particles together; and exert a chemical action on the ingredients and alter the nature of the feed resulting in a more durable pellet (Lim & Cuzon 1994; DeSilva & Anderson 1995). Presently, diets of redclaw consist of steam pressed highly conglomerated pellets of about 5 mm in diameter (see Ruscoe et al. 2005). Redclaw can only ingest large grain fragments; fine granules are propelled forward and away from the mouth by currents induced by the scaphognathites as water is passed through the gill chamber (Ruscoe 2002). Moreover, redclaw move out of shelter when food is offered, grasp large food items and return to the relative safety of the artificial shelter where consumption and digestion can be completed without fear of predation (Loya-Javellana et al. 1993). This means that many pellets will lay on the pond bottom for a while before redclaws come searching for a second helping. Such feeding behaviour requires a very water durable pellet to ensure optimal FCR and growth.

Ruscoe et al. (2005) assessed the water stability of practical crayfish research diets manufactured using various binders (agar, gelatin, carboxymethylcellulose and carrageenan). They found that rate of dry matter loss decreased over time and that carrageenan and carboxymethylcellulose are better binders than agar and gelatin. The 50 g kg\(^{-1}\) binder concentration slowed the decay rate by as much as 62% as compared with 30 g kg\(^{-1}\) binder concentration. Additionally, 10% moisture alginate-bound pellets are more stable than 50% binder-bound pellets. Growth and survival are not influenced by diet moisture although slightly better growth was noted with moist diets offered to redclaw crayfish (Ruscoe et al. 2000). According to Ruscoe et al. (2002), moist diets allow the manipulation and ingestion of appropriately sized pieces as determined by the animal, without the losses associated with abrasion, rasping and subsequent fragmentation of hard-pellet diets. This is in agreement with the morphological evidence suggesting that the mouthparts of juvenile redclaw are well suited to the ingestion of soft, moist foods (Loya-Javellana & Fielder 1997), where pappo-serrate setae on the labrum allow for gentle prey manipulation by pushing prey down in front of the mouth opening (Garm 2004).

**Conclusion**

Currently, there are few if any commercial feeds specifically formulated and manufactured for redclaw crayfish. Progress has been made over the past decade, but there are still knowledge gaps in relation to nutrient requirements for redclaw. Some of the areas that require further research include essential amino acid requirements, vitamin and mineral requirements, pelleting technology to produce a dry but malleable pellet, an estimation of optimal feed regimens, broodstock nutrient requirements and formulations of diets using regionally available ingredients with least cost formulations. Presently, we recommend that semi-intensive farms use sinking diets containing 350 g kg\(^{-1}\) crude protein, 60 g kg\(^{-1}\) lipids, 18–20 MJ kg\(^{-1}\) digestible energy with crustacean vitamin and mineral premix supplement and a water stability of at least 30 min. Broodstock diets should contain fish oil and carotenoid pigments. We believe redclaw aquaculture has reached critical mass and is growing. With appropriate feeds, nursery technology and growout protocols, production is set to increase in the near future.

**References**


