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Effect of Protein Source on Growth of Early Juvenile Redclaw Crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae)

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Abstract.— We examined the effect of dietary protein sources on survival and growth of early juvenile *C. quadricarinatus*. Five isoproteic (35%) and isocaloric diets containing various proportions of fish meal:soybean meal as protein sources were assayed. Fishmeal was replaced by soybean meal as a protein source at 0, 25, 50, 75 or 100% of the protein content. Survival and weight gain were determined after 90 days. The content of protein and lipids in the hepatopancreas and abdominal muscle of surviving animals were determined. The highest weight gain was observed with the replacement of 50% of fish meal by soybean meal, this weight gain was significantly higher than that of diets having only one protein source. There were no significant differences in protein content for either the hepatopancreas and muscle among all diets. Diets with 50% or 25% soy meal produced higher lipid levels in the hepatopancreas, while the replacement of fish meal by soy meal in the 50% and 75% diets showed the highest lipid levels in muscle. Based on results of this study, for culture purposes, and considering the high cost of fish meal, replacing 50% of the protein content of a fish meal diet with soybean meal would yield the best growth at the lowest cost. [*Keywords*.— *Cherax quadricarinatus*; diet; growth; juvenile; protein source].

INTRODUCTION

Among the decapod crustaceans used in aquaculture, several crayfish species are extensively cultured and have a high economic relevance. *Cherax quadricarinatus* (Von Martens), or redclaw, is a crayfish species native to the tropical region of Queensland, northern Australia, with high commercial potential. Production of this species on farms, either in Australia or other countries, has increased significantly during the last few decades, due to various traits that make it suitable for culture over other species (Medley et al. 1994). Culturing this species starts with an indoor growth stage of about 3 to 4 months. At the beginning of this period, recently independent (early) juveniles were maintained in the hatchery for several weeks, and were later transferred to larger tanks (nurseries) until they achieved a body weight of around 5 g. After that, juveniles were placed in earthen ponds until they reached a market weight of 50 g or higher.

Cherax quadricarinatus is a facultative omnivorous species; its diet includes plant and animal material, as well as detritus. Juveniles have a preference for food of animal origin, and zooplankton is a main food item in their natural environment (Jones 1997). Under culture conditions, however, the species accepts several types of food, including formulated diets. Several previous studies have focused on optimizing diets for growing redclaw in culture systems, either as juveniles (Cortés Jacinto et al. 2003, 2004; Thompson et al. 2004, 2005; Campaña Torres et al., 2005, 2006; among others) or adults (Rodríguez-González et al. 2006). Most of the diets in previous studies of juveniles only worked well with advanced juveniles (> 1 g body weight). Concurrent attempts were made in several other studies to minimize the cost of artificial diets during development.

Protein is usually the most expensive ingredient in diet formulations. When different protein levels were assayed with *C. quadricarinatus* juveniles, a 35% crude protein content was optimal for growth (Cortés Jacinto et al. 2003; Thompson et al. 2004, 2005), but a lower percentage protein (30%) diet was found to be satisfactory when fish meal was used as the main source of protein (Thompson et al. 2004). Fish meal is considered to be one of the more complete feed ingredients, containing much of the required proteins and fatty acids, and is also highly digestible. However, due to its increased cost, several assays with redclaw juveniles have focused on replacing fish meal, totally or partially, with other protein sources, especially those of plant origin (Campaña Torres et al. 2005, 2006). Soybean meal has been studied as a possible alternative to replace more expensive protein sources in crustacean food formulations (Alvarez et al. 2007; Muzinic et al. 2004; García- Ulloa et al. 2003). According to some studies (Muzinic et al. 2004) the total replacement of fish meal by soybean meal has been successful with advanced juvenile *C. quadricarinatus*, however, it has yielded negative results in other studies when using the same species (García-Ulloa et al. 2003).

Table 1. Ingredients (g kg^{-1}) of the experimental diets used in the first experiment.

Diet	Fish	Soy	Squid	Krill
Fish meal	510	100	–	–
Soybean meal	70	650	80	60
Squid meal (hydrolyzed)	–	–	630	–
Krill meal	–	–	–	550
Pre-jellified starch	190	170	190	190
Soybean oil	60	40	30	50
Bentonite	150	20	50	130
Mineral premix [a]	10	10	10	10
Vitamin premix [b]	10	10	10	10

[a] Mineral premix (mg kg^{-1}): $ZnSO_4$, 50; $MgSO_4$, 35; $MnSO_4$, 15; $CoSO_4$, 2.5; $CuSO_4$, 3; KI, 3.

[b] Vitamin premix (mg kg^{-1}, unless otherwise noted): A (retinol), 3000 UI kg^{-1}; D, 600 UI kg^{-1}; E (alpha tocopheryl acetate), 60; K, 5; C (ascorbic acid) , 150; B1 (thiamin), 10; B (riboflavin), 10; Vitamin B6 (piridoxin), 7; B12, 0.02; biotin, 0.4; pantothenic acid, 35; folic acid, 6; niacin, 80; choline, 500; inositol, 100.

Table 2. Proximate content of diets used in the first experiment. Each component is indicated as percentage of the total. NFE = nitrogen-free-extract.

Diet	Fish	Soy	Squid	Krill
Moisture	12.5	11.45	12.42	11.55
Protein	35.28	35.55	35.10	35.80
Lipid	10.63	9.75	9.68	10.38
Carbohydrates (NFE)	20.02	19.55	20.30	20.61
Ash	21.57	23.7	22.5	21.66

Table 3. Composition (g kg^{-1}) of the experimental diets used in the second experiment.

Diet	F100%	F75%	F50%	F25%	F0%
Fish floor	560	420	280	140	–
Soybean floor	0	190	390	590	780
Pre-gelled starch	190	190	190	190	170
Soybean oil	60	60	60	60	40
Bentonite	170	120	60	–	–
Mineral premix [a]	10	10	10	10	10
Vitamin premix [b]	10	10	10	10	10

[a] and [b]: same as stated in Table 1.

Table 4. Proximal analysis of diets used in the first experiment. Each component is indicated as percentage of the total. NFE=nitrogen-free-extract.

Diet	F100%	F75%	F50%	F25%	F0%
Moisture	11.8	12.1	12.35	12.41	13.4
Protein	35.28	35.01	35.19	35.37	35.1
Lipid	10.52	10.78	11.12	11.46	9.9
Carbohydrates (NFE)	19.84	20.36	20.86	21.35	19.87
Ash	22.56	21.75	20.48	19.41	21.73

The current study was aimed at evaluating several isoproteic and isocaloric diets prepared from different protein sources, as well as different combinations of two protein sources, on early juvenile *C. quadricarinatus*, in order to both maximize juvenile growth and minimize the cost of the diet.

MATERIALS AND METHODS

General Conditions

During the 90 day duration of each experiment, photoperiod was maintained at 14:10 (L:D) and temperature at 27 ± 1ºC. Each juvenile crayfish was housed individually in a plastic container (1 L capacity) with 150 mL of dechlorinated tap water (hardness: 80 mg L^{-1} as $CaCO_3$ equivalent, pH 7.8). Water was changed (100%) in all containers three times per week throughout the experiments. The crayfish were fed 5% of their mean body weight per day. All crayfish were weighed at the beginning of both experiments, and every 15 days thereafter, using an analytical balance (precision = 0.1 mg).

First Experiment: Protein Sources

Ten to fifteen juvenile *C. quadricarinatus* hatched from three different females (blocking factor), were randomly assigned to each of four treatment groups, resulting in a total of 41 juveniles per group. Mean initial weight of the crayfish (± standard error) was 23.8 ± 0.1 mg (N = 164). The four treatment groups were defined according to the main protein source used to prepare the diets: 1) F: fish diet, 2) SOY: soybean diet, 3) S: squid diet, and 4) K: krill diet. The first three diets were prepared from commercial meal obtained from Conarpesa S.A. (Argentina), while the krill diet was prepared from krill meal obtained from Aker Seafoods Antartic (Norway). The ingredients and relative composition of each diet are shown in Table 1, while the corresponding proximal analysis (conducted according to the methodology of AOAC, 1990) is detailed in Table 2. All the assayed diets were isoproteic and isocaloric.

Second Experiment: Different Combinations of Protein Sources

The two protein sources (fish and soy meal) tested in the first experiment that yielded the best results for growth and survival of juveniles were selected and combined in different proportions (Tables 3 and 4). The resulting five experimental isoproteic and isocaloric diets were: 1) F100% (fish meal was the only protein source), 2) F75% (75% of protein content provided by fish meal, 25 % provided by soybean meal), 3) F50% (50% of protein content provided by fish meal, 50 % provided by soybean meal), 4) F25% (25% of protein content provided by fish meal, 75% provided by soybean meal), 5) F0% (soybean meal was the only protein source). All the assayed diets were isoproteic and isocaloric.

Fifteen juvenile *C. quadricarinatus* hatched from three different females (blocking factor), were randomly assigned to each of the five treatment groups, resulting in a total of 45 juveniles per group. Mean initial weight of all crayfish (± standard error) was 22.5 ± 0.1 mg (N = 225).

Measured Variables

Mortality was expressed as the cumulative percentage of the crayfish that died in each of the two experiments. Body weight (± 0.1 mg) was determined every 15 days and weight gain (WG) was calculated as follows: $WG = ((Wx - Wi) / Wi) \times 100$, where Wx is the body weight measured every 15 days, from the beginning of the experiment, and Wi is the initial body weight. At the end of each experiment, the post-orbital length (measured from the posterior end of the eye orbit to the end of the tail) was measured with a micrometric caliper (± 0.01 mm). Molt percentage was estimated as (Nm/ Ni) x 100, where Nm is the number of crayfish that molted at least once during the experiment, and Ni is the initial number of animals, for each experimental group.

At the end of each experiment, all crayfish were dissected (after euthanasias in an ice-water bath) and the hepatopancreas and abdominal muscle were quickly extracted and frozen at –70°C until analysis. Glycogen was extracted by the Van Handel (1965) method; free glucose content was estimated with a Labtest kit, (glucose oxidase method), after acid hydrolysis with HCl followed by neutralization with Na_2CO_3. Total proteins were quantified according to Lowry et al. (1951), using bovine albumin as standard. Total lipids were extracted by the method of Folch et al. (1957) and quantified by the method of Frings and Dunn (1970). Total cholesterol was extracted following Folch et al. (1957) and quantified by means of a colorimetric kit (Labtest).

Statistical Analysis

Growth and size measurements, as well as levels of protein, carbohydrates and lipids, were compared among experimental groups by means of a two-way ANOVA (factors considered were experimental groups and blocks), followed by Tukey's post-hoc test for multiple comparisons (Sokal and Rohlf 1981). Logarithmic transformations were performed to obtain a homogeneous variance within groups. Percentages of mortality and molting were compared by means of the Fisher exact test (Sokal and Rohlf 1981). In all cases, the significance level was 5%.

Water Quality Determination

Levels of ammonia, nitrite, alkalinity and hardness were measured, both before and after replacing water in the individual containers. Ammonia was determined by means of a colorimetric method (Wiener kit), after hydrolysis with urease. Colorimetric kits of low detection range (Acuanalítica S.A.) were used for the remaining water quality parameters.

RESULTS

First Experiment

Water quality was not affected by diets during the experiment, with the exception of the squid (S) diet. This diet produced levels of ammonia (0.734 ± 0.126 mg L^{-1}, N=5) and nitrite (0.148 ± 0.001 mg L^{-1}, N=5) exceeding recommended levels (Jones 1997; Boyd 1982). Hardness and alkalinity were both within recommended levels for crayfish (Jones 1997; Boyd 1982).

Lowest mortality was obtained with fish (F) and krill (K) diets (Table 5), which both resulted in a mortality percentages of only 32% by the end of the experiment. These values were significantly lower than that of the SOY diet, while the mortality percentage in the S diet was significantly higher than that observed in the other three diets.

From day 30 until the end of the experiment (day 90), the F diet yielded the highest weight gain followed by SOY and K diet (Figure 1), which did not differ between themselves. Weight gain observed with the S diet was significantly lower than the

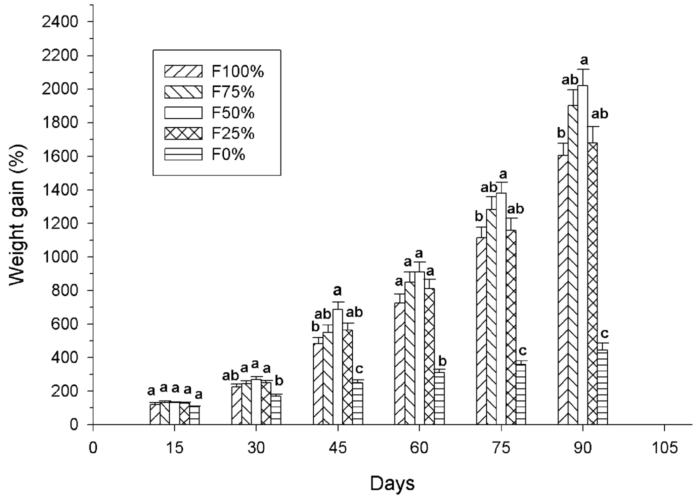

Figure 1. Weight gain of *C. quadricarinatus* juveniles fed diets prepared with different protein sources (first experiment). For each 15-day weight period, different letters indicate significant differences ($P < 0.05$) among treatments.

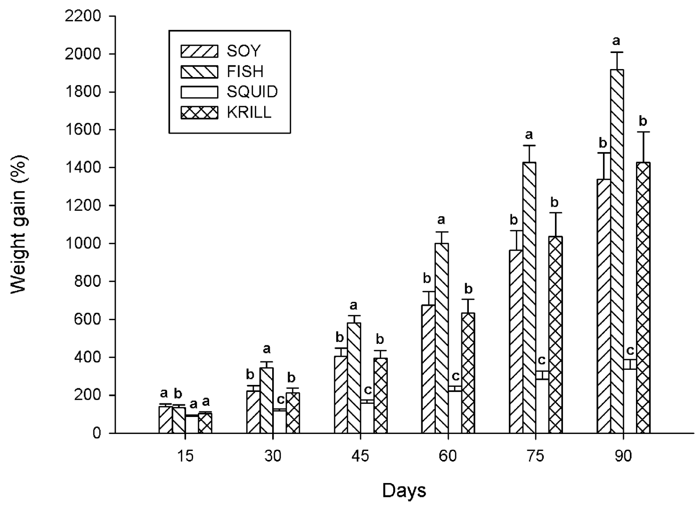

Figure 2. Weight gain of *C. quadricarinatus* juveniles fed diets prepared with different proportions of fish meal (F) and soybean meal as protein sources (second experiment). For each 15-day period, different letters indicate significant differences ($P < 0.05$) among treatments.

other diets, (measured from the beginning of the experiment). Comparison of final weights (Table 5) showed a similar pattern. Post-orbital length was significantly greater with both F and K diets, while the S diet produced the lowest value (Table 5). In all cases the differences among diets were not statistically significant (females).

Mean values of tissue composition in both hepatopancreas and abdominal muscle are shown in Table 6 for the Soy, K and F diets. The highest protein levels in the hepatopancreas were observed with K and SOY diets, followed by the F diet (Table 6). Cholesterol level was highest with the SOY diet but only significantly higher than that of the F diet. Lipids levels in the hepatopancreas were significantly higher for both SOY and F diets, compared to the K diet. No statistically significant differences were observed in glycogen levels of the hepatopancreas among diets. In abdominal muscle, the SOY diet showed significantly higher levels of cholesterol, lipids and glycogen when compared to the other diets

Table 5. Initial and final weights (mean ± SE, in mg), and final post-orbital length (mean ± SE. in mm) of *C. quadricarinatus* juveniles. Number of surviving animals (Ns) and mortality percentages (%M) are also indicated. Initial numbers of juveniles per diet were 41 and 45 for the first and second experiment respectively. Different superscript letters indicate significant differences ($P < 0.05$) within columns within experiment. ND = not determined.

	Diet	Initial weight	Final weight	Final post-orbital length	Ns	%M
First experiment	Soy (SOY)	23.85 ± 0.17 [a]	339.76 ± 31.67 [a]	8.32 ± 0.32 [a]	20	51 [a]
	Fish (F)	23.70 ± 0.17 [a]	477.11 ± 21.46 [b]	9.72 ± 0.19 [b]	28	32 [b]
	Squid (S)	23.84 ± 0.18 [a]	103.13 ± 11.13 [c]	6.24 ± 0.18 [c]	11	73 [c]
	Krill (K)	23.79 ± 0.15 [a]	363.35 ± 38.13 [a]	9.24 ± 0.25 [b]	28	32 [b]
Second experiment	F0%	22.30 ± 0.17 [a]	122.06 ± 15.18 [a]	ND	23	49 [a]
	F25%	22.60 ± 0.16 [a]	402.96 ± 18.74 [b]	9.27 ± 0.14 [ab]	36	20 [a]
	F50%	22.55 ± 0.17 [a]	478.96 ± 22.20 [c]	9.53 ± 0.19 [a]	32	29 [a]
	F75%	22.50 ± 0.24 [a]	447.95 ± 21.76 [bc]	9.40 ± 0.19 [a]	27	40 [a]
	F100%	22.50 ± 0.19 [a]	380.18 ± 9.76 [b]	8.94 ± 0.14 [b]	33	27 [a]

(Table 6). Because of both the high mortality and the low size attained by the juveniles fed the squid diet, no tissue composition data for the S group could be obtained.

Second Experiment

Although the differences in mortality among the experimental groups were not statistically significant, mortality in the group fed diet F0% was higher compared to the remaining groups (Table 5). Water quality parameters were also similar among experimental groups, although some fluctuations were noted in nitrites and ammonia, All values for water quality were within the acceptable range for growing crayfish (Jones 1997; Boyd 1982).

A clear trend of the F50% diet producing a higher weight gain than the other diets throughout the experiment is evident in Figure 2. Although this difference was not statistically significant when compared to diets F25% and F75%, it was significantly different from both F100% and F0%, the latter yielding a weight gain markedly and significantly lower than the other four diets. No statistically significant differences among blocks (females) were noted. Statistical comparisons of final body weight (Table 5) resulted in a pattern similar to weight gain, showing that diets F50% and F75% had the highest values. A similar trend was observed for post-orbital length. Due to the extremely small size of the juveniles fed the F0% diet, no post-orbital length was recorded, and this group was excluded from the analysis of tissue content.

Table 6. Tissue analyses of hepatopancreas (HP) and abdominal muscle (AM) of *C. quadricarinatus* juveniles at the end of the first experiment, for three of the four diets Mean ± SE (mg g^{-1} of tissue) is indicated. Different letters indicate significant differences ($P < 0.05$) among diets within rows.

		Diet		
		Soy	Fish	Krill
HP	Protein	114.60 ± 5.11 [ab]	109.97 ± 6.71 [a]	133.92 ± 7.64 [b]
	Lipid	143.54 ± 17.10 [a]	111.97 ± 13.29 [a]	64.23 ± 6.17 [b]
	Cholesterol	123.41 ± 15.96 [a]	64.24 ± 1.50 [b]	100.64 ± 19.76 [ab]
	Glycogen	3.10 ± 0.21 [a]	2.98 ± 0.24 [a]	2.88 ± 0.11 [a]
AM	Protein	165.41 ± 6.47 [a]	117.22 ± 12.75 [a]	114.88 ± 11.25 [a]
	Lipid	32.92 ± 6.22 [a]	8.62 ± 1.20 [b]	23.22 ± 3.70 [a]
	Cholesterol	39.05 ± 1.50 [a]	25.83 ± 4.93 [ab]	16.77 ± 3.22 [b]
	Glycogen	2.83 ± 0.14 [a]	2.10 ± 0.17 [b]	2.30 ± 0.33 [ab]

The F75% diet produced the highest lipid accumulation in hepatopancreas, although the level was not statistically different from the F50% and F100% diets (Table 7). In the abdominal muscle, higher lipid levels were detected with diets F25% and F50%, although the latter was not significantly different from F75% and F100%. No significant differences in protein content of either tissue were detected among the dietary treatment groups (Table 7).

DISCUSSION

From day 30 until the end of the first experiment, the fastest growth was evident in juveniles fed the fish meal diet. Post-orbital length was also largest with this diet. The convenience of feeding fish meal has been clearly documented for crustaceans. For example, Thompson et al. (2005) found that in *C. quadricarinatus* juveniles, a diet of 35% crude protein could be reduced to 30% if half of the protein content was provided by fish meal. However, at crude protein percentages lower than 30%, growth of juvenile redclaw was reduced, regardless of the protein source used (Thompson et al. 2006).

Both krill and soybean diets used in the current study resulted in similar weight gains. However, according to Sclabos and Toro (2003) a krill diet resulted in the highest weight gain. Nevertheless, other studies have documented high digestibility of soybean-based diets. For example, Campaña-Torres et al. (2005, 2006) evaluated *C. quadricarinatus* juveniles (3 – 4 g body weight), fed diets based either on plant (soy paste, wheat, sorghum), or animal (sardine, squid, crab) material. They found digestibility of lipids and carbohydrates to be higher with the plant-derived diets, while the highest final body weight was observed with both sardine and soy paste diets.

With regard to tissue composition, the soybean diet yielded the highest levels of protein, cholesterol and glycogen, both in hepatopancreas and muscle. Although the fish and krill diets showed protein levels similar to those observed in the soybean diet, levels of lipids, cholesterol and/or glycogen in this tissue

Table 7. Tissue analyses of hepatopancreas and abdominal muscle of *C. quadricarinatus* juveniles, at the end of the second experiment (four of the five diets). Mean ± SE (mg g^{-1} of tissue) is indicated. Different letters indicate significant differences ($P < 0.05$) among diets within rows.

		Diet			
		F25%	**F50%**	**F75%**	**F100%**
Hepatopancreas	Protein	30.72 ± 5.18 ^{a}	31.45 ± 4.10 ^{a}	37.36 ± 5.11 ^{a}	50.34 ± 12.27 ^{a}
	Lipid	71.22 ± 12.59 ^{a}	112.33 ± 16.57 ^{ab}	123.34 ± 11.66 ^{b}	100.92 ± 10.56 ^{ab}
Abdominal muscle	Protein	84.35 ± 13.72 ^{a}	93.68 ± 6.71 ^{a}	73.86 ± 4.83 ^{a}	82.67 ± 7.39 ^{a}
	Lipid	9.04 ± 1.13 ^{a}	7.74 ± 1.68 ^{ab}	4.74 ± 0.70 ^{b}	5.54 ± 0.48 ^{b}

did not always reach the levels obtained with the soybean diet. This indicates that soybean meal may have a high digestibility for both carbohydrates and proteins. López-López et al. (2005) determined the activity of hepatopancreatic enzymes in advanced *C. quadricarinatus* juveniles fed over 30 d with diets prepared with different protein sources (squid, crab, fish, sorghum, soy and wheat). Their results indicated that amylase activity was higher in juveniles fed soy or wheat, while protease activity was higher with both fish and soy meal. Our current results are in accord with these previous results.

Survival at the end of both experiments averaged 60%, exceeding that reported by Jones (1997). It is important to note that mortality of early redclaw juveniles is normally high and decreases significantly after they reach about 1 g of body weight (Jones 1997; authors' own observations). Water quality parameters in all experimental groups remained within an acceptable range, with the exception of the squid diet, where nitrites and ammonia increased to critical levels, probably causing the relatively high mortality and low growth in that group. The hydrolyzed squid preparation used for preparing that diet contained a high proportion of short amino acid chains that can be degraded faster than longer chains; therefore, accelerating pellet decomposition. Pellet stability for crayfish diets was previously studied by Jussila et al. (1998) with *Cherax tenuimanus* (Smith), who found that the rate of weight gain increased when more stable pellets were used. Similar results were reported by Coccia et al. (2010) for juvenile *Charax albidus* Clarke.

For aquaculture, a basic cost-benefit criterion is to develop a diet with minimum protein content while maximizing growth response. This diet composition does not only minimize costs, but also helps to prevent an excess of nitrogen excretion by animals, which degrades water quality. All diets used in the current study were formulated for a 35% crude protein content. Keefe and Rouse (1999), as well as Manomaitis et al. (2001), claimed that the first independent stage for *C. quadricarinatus* juveniles (0.02 g) would require at least 40% crude protein in the diet. Webster et al. (1994) established that 33% crude protein was optimal for *C. quadricarinatus* juveniles of the same size. Most reports on diet formulation for redclaw juveniles state that the optimum percentage of protein (for juveniles between 1 and 4 g body weight) is reported to be around 35%, but could be reduced at larger sizes during the grow-out period (Monomaitis et al. 2001; Cortés Jacinto et al. 2003; Thompson et al. 2004, 2005, 2006).

The second experiment, tested different proportions of the two best protein sources observed in the first experiment. This second experiment demonstrated that best growth was obtained with the mixture of 50% fish and 50% soybean meal, relative to protein content, followed by those containing 75%, 25% and 100% crude protein from fish meal. Moreover, the 50% fish meal diet produced the highest lipid accumulation in both hepatopancreas and abdominal muscle. Similar results were obtained with juveniles of the white shrimp *Litopenaeus schmitti* (Burkenroad) (Álvarez et al. 2007). In that study, growth significantly increased with diets having up to 75% replacement (w/w) of fish meal with soybean meal, which corresponded to a replacement of near 60% of fish protein. However, in a previous study with advanced *C. quadricarinatus* juveniles fed mixtures containing different proportions of fish and soybean meal (García-Ulloa et al. 2003), growth was slower when fish meal was replaced by soy meal in any proportion. In contrast, Muzinic et al. (2004) reported no difference in growth of redclaw juveniles (mostly advanced) with replacement of fish by soybean meal at any level tested.

In the current study, we found that recently independent juveniles (0.02 g) respond very well to mixtures of proteins and other nutrients provided by both fish and soybean meal. From an economic point of view, although both kinds of meals have increased in price by a factor of 2.8 on the international market during the last ten years; fish meal is currently 2.7-fold more expensive than soybean meal (Index Mundi, 2009). Therefore, the partial replacement of fish meal, as protein source, by soybean meal would significantly reduce the cost of the ingredients needed to formulate an optimal diet for growth of early *C. quadricarinatus* juveniles. This point should be taken into account by diet manufacturers for preparing a starting feed (i.e., for early juveniles) that maximizes growth while reducing costs. Further studies of other nutritional components such as vitamins, growth factors and carotenoids, among others, should be carried out to improve the efficiency of formulated pellets on growth and survival of early juvenile crayfish.

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