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Research Article

Effects of different protein and carbohydrate contents on growth and survival of juveniles of southern Chilean freshwater crayfish, *Samastacus spinifrons*

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ABSTRACT. In cultivated aquatic organisms nutritional requirements are critical, not only for their impact on production techniques, but also, for their high incidence on production costs. There is limited knowledge on some species such as the southern Chilean freshwater crayfish, *Samastacus spinifrons*. In order to generate practical knowledge, a study was carried out to determine protein and carbohydrate content requirements. These factors were evaluated upon their effects on growth and survival of juveniles. For this purpose, individual weight, biomass gain, survival, and feed conversion parameters were measured. The assay was carried out in 42 days. It was conducted in a flow through system, using 21 plastic tanks of 10.6 L capacity. Each tank was seeded with 20 juveniles weighing 50 mg average each. A 3x2 factorial design was proposed with three protein contents (20, 30, 40%) and two carbohydrate contents (low: from 16.3 to 23.5% and high: from 34.6 to 35.8%). Six treatments and three replicates were performed. Individuals were fed on apparent satiation once a day. The diets formulated with 30% of protein and the two carbohydrate contents resulted in higher biomass increases, food conversion efficiencies over 26%, and specific growth rate of 0.78%, all displaying significant differences. Survival showed highly significant differences; in all diets were superior to 60%, however the diets with 30% of protein surpassed 90%.

Keywords: *Samastacus spinifrons*, Crustacea, diets, protein-carbohydrate content, southern Chile.

Efecto de diferentes niveles de proteína y carbohidratos sobre el crecimiento y sobrevivencia de juveniles del camarón de río del sur de Chile, *Samastacus spinifrons*

RESUMEN. En organismos cultivados, los requerimientos nutricionales, resultan ser de suma importancia, no sólo por sus impactos en las técnicas de producción, sino también por su alta incidencia en los costos de producción. Este conocimiento es escaso en algunas especies como el camarón de río del sur de Chile, *Samastacus spinifrons*. A fin de generar un conocimiento práctico, se definieron los requerimientos de proteínas y de carbohidratos y se evaluaron sus efectos en el crecimiento y supervivencia de juveniles. Para esto, se midió peso individual, biomasa ganada, supervivencia y parámetros de conversión alimenticia. La parte experimental se realizó en un sistema de flujo abierto, conformado por 21 estanques de plástico con capacidades de 10,6 L, durante un periodo de 42 días. Se sembraron 20 individuos en cada estanque con pesos individuales de 50 mg en promedio cada uno. Se propuso un diseño factorial de 3x2 con tres niveles de proteína (20, 30 y 40%) y dos niveles de carbohidrato (bajo: 16,3 a 23,5% y alto: 34,6 a 35,8%). Se desarrollaron seis tratamientos con tres réplicas. Los individuos fueron alimentados a aparente saciedad, una vez al día. Las dietas con 30% de contenido proteínico con ambos niveles de carbohidratos ofrecieron incrementos de biomasa más altos, eficiencia de conversión alimenticia de 26% y tasa de crecimiento específico de 0,78%, todos ellos con diferencias significativas. La supervivencia presentó diferencias altamente significativas, si bien en todas las dietas fue superior al 60%, en las dietas con 30% de proteína superó el 90%.

Palabras clave: *Samastacus spinifrons*, Crustacea, dietas, niveles de proteínas y carbohidratos, sur de Chile.

INTRODUCTION

In the Astacidea field, several investigations on the cultivation of species have been performed, the *Astacus*

and *Austropotamobius* in Europe, the *Pacifastacus* and *Procambarus* in North America, and the *Cherax* in Australia and South America (Celada *et al.*, 1989; Ackefors *et al.*, 1992; Webster *et al.*, 1994; Jones, 1995; Ghiasvand *et al.*, 2012; Xu *et al.*, 2013).

Chile is a country located in South America, sustaining one of the most intensive fish farming activities for salmon, and sharing with Norway the first places in salmon production (FAO, 2014). However, the country's intention is to diversify its aquaculture activities towards other potential species. Thus, Chile has implemented a National Aquaculture Policy to fulfill this purpose. Among the species, with possibilities to diversify the aquaculture, four species of the family Parastacidae reported along the Chilean coast are identified. However, most of the attention has been drawn towards the southern Chilean crawfish, *Samastacus spinifrons*, characterized by its good flavor and corporal size. The existing data on *S. spinifrons*, is mostly on its biology (Rudolph, 2002, 2015) and on some preliminary production tests done by Fundación Chile (Augsburger, 2003). However, in order to reach an effective development of the cultured species, it is necessary to have the base of seed production to assure the following grow-out phase. A fundamental aspect to succeed with its culture is the knowledge of juvenile nutritional requirements (Saoud *et al.*, 2012), from weaning to juvenile size, that allow them to be placed under growing techniques. *S. spinifrons* passes the larval period in the abdominal cavity of gravid females (Rudolph, 2002) as any other astacids (Jones, 1995) which is an advantage for cultural purposes. Protein will then be relevant to consider in for further stages, to generate information on their protein requirements. Probably in a similar way done for most practical shrimp diets based upon cost and growth response (Lim, 1997). Both elements are imperative to evaluate different protein dietary content and determine the optimal content to sustain maximum growth.

Taking into account the above-mentioned considerations, and the nutritional importance in culturing production of aquatic species for juvenile *S. spinifrons*, the present study was performed to establish a protein content requirement, by setting up a factorial design (3x2) formulating diets with three different protein contents (20, 30 and 40%) and two carbohydrate contents (low and high).

MATERIALS AND METHODS

The assay was carried out at Universidad Católica de Temuco (UC Temuco) Aquaculture Department, and was conducted in a flow through system, using 21 rectangular plastic tanks of 10.6 L capacity with an open flow (Q) of 0.12 L min⁻¹ with constant aeration. PVC pipe pieces of 1.27 cm diameter and length of 4 cm were placed into each tank as shelter elements. Twenty juveniles weighing 49.6 ± 4.82 mg were seeded into each tank and were maintained for 42 days. Six

diets were formulated to contain three contents of protein and two relative carbohydrate contents. A 3×2 factorial design with three replicates per dietary treatment was used in this study. Diets were prepared with the ingredients indicated in Table 1. The feed formulation model was developed using an Excel software (Table 2) with the following factors and contents: three protein contents (20, 30 and 40%) and two carbohydrate contents (low: from 16.3 to 23.5% referred to as: "C low" and high: from 34.6 to 35.8%, named as: "C high") (Table 3). Selected ingredients and prepared diets were analyzed at UC Temuco Aquaculture Department in the Nutritional Laboratory according to the Official Methods of Analysis (AOAC, 1998).

For the food preparation, each grounded ingredient was passed through a 300 µm mesh size and weighed the recommended quantity according to the formulation. In the manufacture process of the experimental diets, dry ingredients, except wheat flour, were mixed during 40 min in a blender machine (Kitchen aid; model K5SS). Separately, wheat flour was hydrated and put under a cooking process for 15 min. The resulting gelatinized wheat was mixed with fish oil and a capsule of vitamin E (antioxidant). Finally, the oily gelatinized wheat was combined with other dry ingredients for further 45 min.

A meat grinder machine RCA (1 HP) with a matrix screen of 1.5 mm orifices was used to make different pelleted diets. The pellets were dried during 36 h at 55°C and later stored under freezing conditions (-20°C). The juveniles were fed once per day at same time and mortality was checked and removed.

The amount of food provided in each diet was registered on a daily basis. The uneaten diet in each tank was removed every day before feeding with a suction tube and stored in separate recipients into the refrigerator, depending of the experimental unit. After seven days, the removed uneaten diet was defrosted and dried in a 55°C oven for 24 h. The removed uneaten diet was discounted from the food placed into each tank and then, the actual food consumption for each tank was determined.

Individual weight was measured at the beginning and at the end of the experiment (day 42) with a Sartorius analytical scale (capacity of 217 g and precision of 0.0001 g).

Growth parameters were calculated and evaluated: individual weight gain (mg), specific growth rate (SGR), biomass gain (%), and survival (%). Nutritional parameters were also evaluated: feed conversion ratio and feed conversion efficiency (%), according to De la Higuera (1987) and Bureau *et al.* (2002).

Table 1. Proximate composition (%) of ingredients used in the formulation of diets tested on *Samastacus spinifrons* juveniles.

Ingredients	Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash	Water
Fishmeal	73.59	7.25	1.35	1.19	16.62	10.34
Blood meal	87.53	1.71	2.59	1.92	6.25	5.44
Lupine meal	49.23	10.59	32.73	3.64	3.81	9.33
Lupine fiber meal	3.00	0.70	35.40	50.10	2.60	8.20
Carrot meal	12.56	2.51	55.23	13.74	15.96	16.19
Kelp meal	14.89	0.65	56.27	5.75	22.44	23.25
Wheat meal	11.27	1.93	83.36	2.30	1.14	12.65
Fish oil		100.00				

Table 2. Composition of experimental diets (%), according to ingredient participation. CP: crude protein, C: carbohydrate.

Ingredients	Diets					
	20%CP		30%CP		40%CP	
	C low	C high	C low	C high	C low	C high
Fish meal	24.8	2.1	23.0	4.0	23.1	1.0
Blood meal	1.0	2.0	14.0	3.9	23.0	21.0
Lupine meal	1.0	30.4	2.1	47.4	8.0	41.5
Lupine fiber meal	38.0	29.6	36.0	14.5	30.5	0.1
Carrot meal	5.0	5.0	5.0	5.0	5.0	5.0
Kelp meal	4.0	4.0	4.0	4.0	4.0	4.0
Wheat meal	2.5	15.6	2.6	16.0	2.6	24.8
Fish oil	7.8	6.3	4.7	1.6	2.0	0.1
Vitamins and minerals	1.5	1.5	1.5	1.5	1.5	1.5
Inorganic	14.4	3.5	7.1	2.1	0.3	1.0

Normality, homoscedasticity and independence were tested previously on recollected data, then a two-way analysis of variance was applied to determine its interactions. If significant ($P < 0.05$) differences were found, Tukey tests were used to compare averages between treatments. In each carbohydrate content, protein diet contents were compared by one-way ANOVA followed by a Tukey test. All values were calculated using Minitab statistical software (version 16.1, Minitab State College, PA, USA).

Electric heaters kept the water temperature at approximately 18°C. Dissolved oxygen (5.2 ± 0.6 and pH (7.0 ± 0.2) were measured with YSI-85 equipment.

RESULTS

Growth parameters such as individual weight gain (mg), SGR, biomass gain (%), food conversion rate (FCR), food conversion efficiency (FCE-%) and survival rate (%) of *S. spinifrons* juveniles are shown in Table 4. No significant interactions ($P > 0.05$) were found for individual weight gain and SGR. Biomass gain and FCR were significantly high for diets with

30% CP followed by 20% CP in interactions with low carbohydrate concentration contents. 30% CP+C low exhibited significantly higher FCE than other combinations. Survival for 30% CP + C low was the highest value (95%) however, differences only showed to be significant with 20% CP + C low.

Protein content behavior under relative low carbohydrate content, offered better growth values than relative high carbohydrate content, not only in biomass gain (Fig. 1), but also in all the other parameters measured (individual weight gain, SGR, FCR, and FCE). Survival had a punctual significant exception in 20% CP with C low (62.5%). Highest values were significantly recorded at 30% CP for biomass gain, FCR and FCE. For SGR, FCR and FCE, 30% CP was significantly different to 20% and 40% CP. For survival at C low significant differences were presented in all CP content, being 30% CP the best. At relative high carbohydrate content, there were no significant differences between protein contents. Curves for relative high carbohydrate content showed more flattened tendency form than relative low carbohydrate content.

Table 3. Proximate composition analysis (%) of experimental diets. CP: crude protein, C: carbohydrate.

Diets	Crude protein	Ether extract	Nitrogen-free extract	Crude fiber	Ash	Water
20% CP+C low	20.8	6.7	23.5	21.4	23.6	4.1
20% CP+C high	19.4	6.8	34.6	18.9	7.3	13.0
30% CP+C low	30.5	9.5	22.2	19.8	14.2	3.8
30% CP+C high	29.5	4.4	34.9	11.2	6.3	13.7
40% CP+C low	41.1	5.3	16.3	18.6	7.4	11.4
40% CP+C high	41.0	2.3	35.8	3.5	5.5	11.9

Table 4. *Samastacus spinifrons*. Growth and survival of juveniles after 42 days of feeding on experimental diets containing different crude protein (20%, 30%, 40%), and relative carbohydrate contents (C high, C low) in diets. Different letters indicate significant difference ($P < 0.05$). SGR: specific growth rate, FCR: food conversion rate, FCE: food conversion efficiency.

Parameters	20%		30%		40%	
	C low	C high	C low	C high	C low	C high
Individual weight gain (mg)	18.15 ± 0.91	16.30 ± 3.39	20.25 ± 0.63	18.05 ± 0.77	13.90 ± 1.69	16.90 ± 2.68
SGR	0.67 ± 0.01	0.63 ± 0.09	0.83 ± 0.01	0.72 ± 0.09	0.57 ± 0.04	0.65 ± 0.07
Biomass gain (%)	28.15 ± 3.18 ^{ab}	19.25 ± 0.91 ^b	36.00 ± 1.83 ^a	22.70 ± 1.98 ^b	22.25 ± 1.20 ^b	19.3 ± 1.41 ^b
FCR	3.18 ± 0.07 ^{ab}	3.34 ± 0.35 ^b	2.32 ± 0.14 ^a	3.74 ± 0.06 ^b	3.73 ± 0.30 ^b	3.98 ± 0.37 ^b
FCE (%)	31.44 ± 0.69 ^b	30.09 ± 3.16 ^b	43.15 ± 2.63 ^a	26.72 ± 0.43 ^b	26.86 ± 2.19 ^b	25.2 ± 2.39 ^b
Survival (%)	62.5 ± 3.54 ^b	85.0 ± 0.00 ^a	95.0 ± 0.00 ^a	92.5 ± 3.53 ^a	82.5 ± 3.54 ^a	85.0 ± 0.0 ^a

In both carbohydrate cases (relative low and high contents), growth parameters increased their performance from 20% up to 30% CP and then descended to 40% CP. Mean biomass gain data was fitted to a quadratic model to estimate the protein requirement according to the dose-response relationship. Optimum and maximum protein requirements were obtained by applying the following quadratic model: $y = -1080x^2 + 618.6x - 52.35$ (determination coefficient $R^2 = 0.927$). Calculations indicate the corresponding optimum protein content to be 28.6% CP at low carbohydrate content (Fig. 1), other parameters modelled followed the same pathway. Each curve models for corresponding parameters can be seen in Figure 1.

DISCUSSION

In the present study, 30% CP + C low, showed significantly better results in evaluating the growth parameters such as biomass gain, FCR and FCE. Individual weight gain and SGR showed similar values with no significant differences. Survival was higher than 82% for most combinations except for 20% CP+C low (62%).

Dietary protein content have been studied for other crustacean species such as *Macrobrachium rosenbergii*, ranging from 150 to 400 g kg⁻¹ (Balazs & Ross, 1976; New *et al.*, 1980; D'Abramo & Reed, 1988; Mitra *et*

al., 2005; Teshima *et al.*, 2006). High dietary protein content (500 g kg⁻¹) reported maximum growth of *M. rosenbergii* post larvae (Heinen & Mansi, 1991). If the dietary protein content are too low (diet with 230 g kg⁻¹ CP) the growth rate will be reduced (Balazs & Ross, 1976); Boonyaratpalin & New (1982) and Bartlett & Enkerlin (1983) (diet with 150 g kg⁻¹ CP) and Zaki *et al.* (2002) (diet with 150-250 g kg⁻¹ CP). This may be due to the utilization of protein by *M. rosenbergii* muscle tissue to maintain other vital functions (Goda, 2008). On the other side, growth depression was reported in prawn fed diets exceeding their protein requirement due to the excess dietary protein being metabolized by prawns as a source of energy and nitrogen excreted as ammonia (Burford *et al.*, 2004). Hari & Kurup (2003) suggested that further increase over 300 g kg⁻¹ CP in dietary protein does not have any added advantage in *M. rosenbergii*. Moreover, for 400-500 g kg⁻¹ CP resulted in growth depression as it can also be seen in the present work.

These protein contents are within 30-35% CP requirements for juvenile *M. rosenbergii* (D'Abramo & New, 2000). Ackefors *et al.* (1992) suggested that commercial diets for juvenile *Astacus astacus* may contain approximately 30-35% protein, 20-25% carbohydrate and not more than 10% lipids for the best growth rate. Ghiasvand *et al.* (2012) expressed that *Astacus leptodactylus* can be fed a practical diet containing 30% protein content. Celada *et al.* (1989)

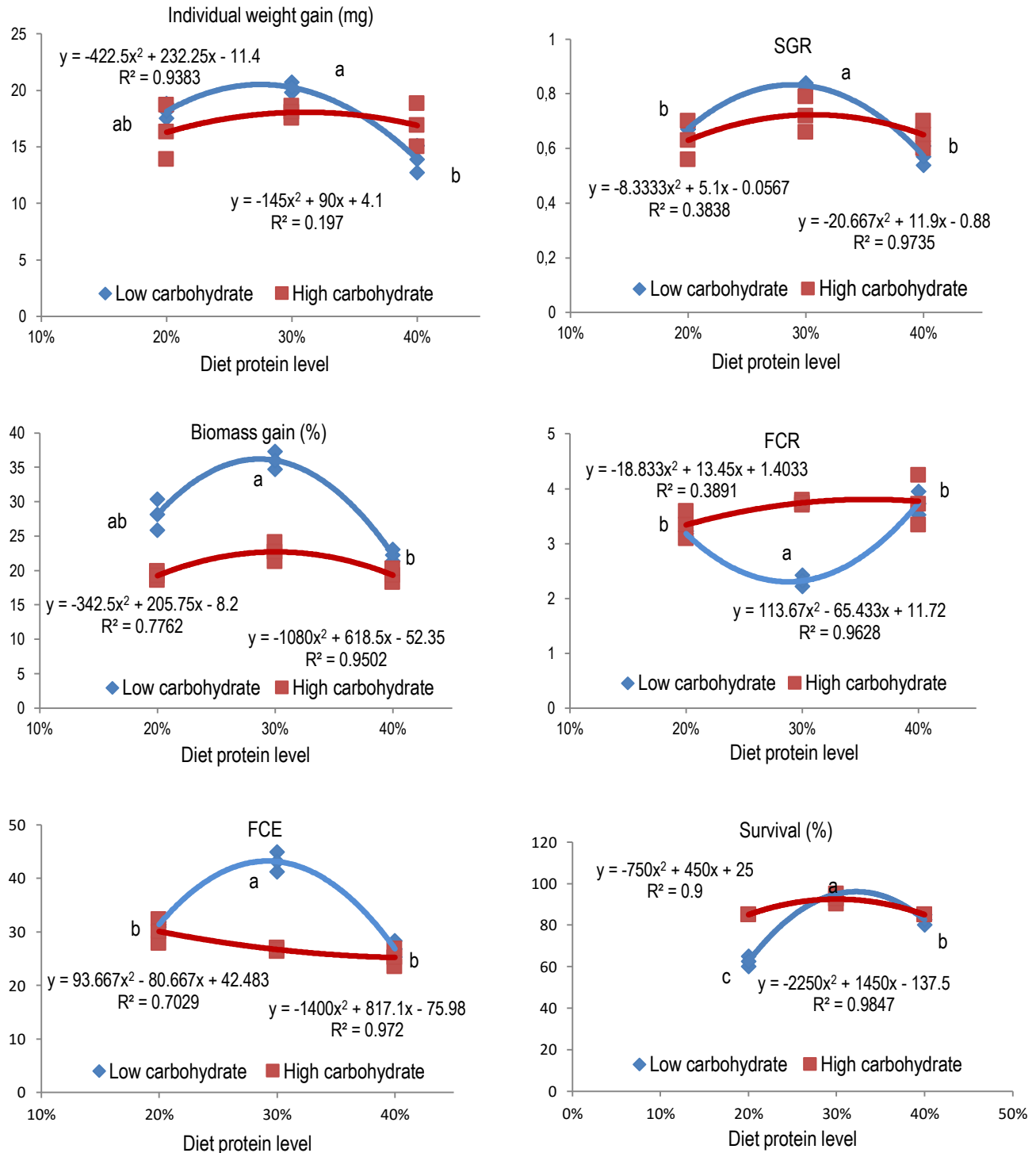


Figure 1. Protein content curves behavior of growth parameters and survival under low and high carbohydrate content in juveniles of *Samastacus spinifrons*. Same letters above points at low carbohydrate curve mean no significant differences. High carbohydrate curves did not present significant differences.

reported that the best growth of juvenile *Pacifastacus leniusculus* was obtained with diets containing 27-33% protein from several fresh and artificially compounded

diets and Gonzalez *et al.* (2012) expressed that a 40% of protein can be suitable in commercial dry diets for this species. For *Cherax quadricarinatus*, Webster *et*

al. (1994) indicated that a diet formulated with 33% protein appeared to be adequate for juvenile phase, whereas Zenteno-Savin *et al.* (2008) stated that 31% of protein is the content to satisfy nutritional requirements for optimal growth. Xu *et al.* (2013) reported that 27-30% protein diet provided the same growth efficiency for *Procambarus clarkia*, *P. leniusculus* and *P. clarkia*. These last species seem to be closer to *S. spinifrons* in protein requirements than *Cherax*, which could be explained due temperature dependence as *Cherax* are more tropical than other colder species.

Several statistical methods can be used to estimate nutrient requirements in dose-response experiments based on mathematical and biological assumptions and principles (Shearer, 2000). Estimates of requirements for maximum growth were obtained by fitting dose-response data to a quadratic model. The results that *S. spinifrons* juveniles required between 28 and 29% dietary crude protein for optimum growth as a result to apply a model found ($y = -1080x^2 + 618.6x - 52.35$). In this sense, Cortes *et al.* (2003) expressed protein requirement of 31%, calculated for *C. quadricarinatus* from using the same second order polynomial model than this present study ($y = -0.0071x^2 + 0.484x + 1.142$).

From a nutritional point of view, protein utilization indexes can be considered as good indicators of "protein sparing effect" (Goda, 2008). In the present study, the diet containing a protein content of 30% CP + C low, provided the highest growth indexes and dietary protein utilization efficiency compared with the higher dietary carbohydrate content. These results suggest that *S. spinifrons* could be using dietary carbohydrate as main energy source at lower contents to spare dietary protein for maximum growth. Similar results were obtained by D'Abramo & New (2000), and also by Goda (2008) for *M. rosenbergii*.

Best FCR was presented at 30% CP + C low (2.32), less than those obtained by Jones *et al.* (1996) for *C. destructor* and Cortes *et al.* (2003) for *C. quadricarinatus*. Hari & Kurup (2003) reported their better FCR (2.84 and 2.89 for 300 and 350 g kg⁻¹ CP diet, respectively) for *M. rosenbergii* juveniles, comparable with results obtained by Millikin *et al.* (1980), Ashmore *et al.* (1985) and Gomez *et al.* (1988). The decreased growth rate showed by juveniles over 40% CP can be attributed to the high rate of protein catabolism (Hari & Kurup, 2003). Growth depression was already reported in prawn fed diets exceeding their protein requirement as a result of toxicity (Zein-Eldin & Corliss, 1976).

Regarding carbohydrate utilization, Bautista & Subosa (1997) mentioned that protein contents in diets improve growth when combined with carbohydrate

contents. This means that carbohydrate is used as an economical energy source. In this study, two relative contents of carbohydrate were used: low content (16.3 to 23.5%) and high content (34.6 to 35.8%). Results showed that low relative content is enough to keep better growth parameters. In this sense, carbohydrate is used to build chitin (main component of exoskeleton) and also a component of fatty acids and sterols (Clifford & Brick, 1978; Kucharski & Da Silva, 1991; Cruz-Suarez, 1996). Diaz-Herrera *et al.* (1992) reported carbohydrates as main energy sources for metabolic requirements in post larvae and juveniles of *M. rosenbergii*; later supported by Luna *et al.* (2007). Rosas *et al.* (2000) stated the possibility to reduce protein contents in diets by carbohydrates included as starch ingredient.

Survival for CP + Carbohydrate combinations showed values higher than 82%, except in 20% CP + C low (62.5%). They can be compared with others. Survival values during a period up to 94 days ranged between 65-85% for *C. destructor* (Jones *et al.*, 1996). For *C. quadricarinatus*, Ponce *et al.* (1998) 60-95% of survival rate; Webster *et al.* (1994) 50-71%; Thompson *et al.* (2003a) 56-80%; Thompson *et al.* (2003b) 95-100%; Jacinto *et al.* (2003) 65-89%; and Muzinic *et al.* (2004) 79-98%. Mortality may be associated to nutrition variations in the diet and experimental conditions. A survival rate above 50% between stocking and harvesting has been considered to be acceptable by New & Singholka (1985) and Valenti (1990); but Cuzon & Guillaume (1997) reported that survival rates greater than 80% was considered good in crustacean studies.

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