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Growth of Fresh-Water Prawn *Macrobrachium tenellum* (Smith, 1871) Juveniles Fed Isoproteic Diets Substituting Fish Meal by Soya Bean Meal

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ABSTRACT

Growth parameters (standard length, weight, specific growth rate and daily weight gain) of prawn Macrobrachium tenellum juveniles fed 40% crude protein isoproteic diets substituting fish meal with soya bean meal at various levels (20, 40, 60, 80 and 100%) were evaluated for 45 days under laboratory conditions. Experimental diets were compared with a 100% fish meal based diet. Total survival was recorded for all the treatments at the end of the experiment. There were no significant differences ($p \geq 0.05$) for all the growth parameters among the dietary treatments. The initial mean weight (0.36 ± 0.10 g) increased almost three times (1.00 ± 0.13 g) after 45 days. The final specific growth fluctuated from 1.82% BW/d for the 60% soya bean meal inclusion diet, to 2.62% for the 100% fish meal diet. The mean final survival was 91.66%. Growth performance of M. tenellum juveniles was not affected by the dietary soya bean meal levels tested.

Key words: Growth, fresh water prawn, *Macrobrachium tenellum,* isoproteic diets, soya bean meal, aquaculture

INTRODUCTION

The fresh-water prawn *Macrobrachium tenellum* is distributed from Baja California, México, to the River Chira, in Peru, living in the rivers and estuaries along the Pacific coast (Holthius, 1980). This prawn represents a very important fishery resource and is strongly exploited by local communities (Cabrera-Jiménez et al., 1977). According to Ponce-Palafox et al. (2002) several biological characteristics favor its culture; for example, it is herbivorous, although also consumes small invertebrates and detritus (Barnes, 1977), tolerates wide variations for several water quality parameters such as dissolved oxygen (Cuevas, 1980), salinity (Aguilar et al., 1998) and

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temperature (Hernández et al., 1995). The prawns mate and spawn from August to November (Guzmán, 1987), and are non-aggressive compared with the other species (Sánchez, 1976). There are few reports on the hatchery production and on growing of the juveniles (Cabrera-Jiménez et al., 1979; Martínez et al., 1980; Ponce-Palafox, 1997), indicating that prawns with an average weight of 50-60 g can be harvested from the sixth month onwards (Ponce-Palafox and Arana, 1999).

There are no reports on a specific diet for *M. tenellum* and a wide variety of food ingredients are commonly given, including natural food and organic manure (Ponce-Palafox and Cabanillas, 1996). In some cases, the diets used have been formulated for other aquatic species (Ponce-Palafox and Arana, 1999; Rodríguez-González, 1998). Ponce-Palafox et al. (2002) indicated the need to develop a cost-effective and nutritionally balanced formulated diet, specific for *M. tenellum*, in order to optimize its production.

In the formulated practical diets in the aquaculture, protein is the most expensive energy component, and its quality represents a very important nutritional aspect. The use of alternative protein sources in the culture of some crustacean species of commercial importance to reduce feed costs has been reported (Akiyama, 1988; Piedad-Pascual et al., 1990; García-Ulloa et al., 2003).

It is accepted that soya bean meal is available worldwide and is cheaper than fish meal. Reigh et al. (1990) and Cabanillas et al. (2001) have proved that soya bean meal is a high quality supplement and is well digested by some crustacean. Campaña et al. (2000) demonstrated that soya bean meal was readily digested by the redclaw crayfish *Cherax quadricarinatus*, coinciding with the observations of Tidwell et al. (1993) for the Giant Malaysian prawn *Macrobrachium rosenbergii*. Since there is no information on the possible use of soya bean meal in the formulated diets for the growth of *M. tenellum* juveniles, the objective of the present work was to evaluate different dietary protein contents on its growth performance, replacing fish meal totally or partially with soya bean meal, as the main protein source.

MATERIALS AND METHODS

Experimental design

M. tenellum (n = 250) was collected from irrigation channels located at the coastal side of Colima State, Mexico, with a mean body weight of 0.36 ± 0.10 g, and transported to the laboratory in a 500-L fiber glass tank. After arrival, the prawns were held in two 400-L tanks and acclimate for one week to the laboratory conditions. Water temperature was maintained at 27 ± 1.2 °C. A 12 L:12 D daily photoperiod was adjusted throughout this period. Aerated fresh-water was supplied to each container and 50-80% of the total water volume was changed. Organisms were fed *ad libitum* two times daily (09:30 and 18:30 h) with a commercially pelleted shrimp diet (AS® Aceitera La Junta, S. A. de C. V., Jalisco, México) with 40% crude protein, and feces and food waste were siphoned. After acclimatizing to the laboratory conditions, 120 prawn were randomly selected from the original group and stocked in 10-L plastic containers (filled to 8-L with fresh-water) in a closed recirculation system (water flow rate = 0.25 l/min), as reported by García-Ulloa and Hernández-Garciabada (2003), at an initial density of five prawns per container with four repetition per diet. Five small PVC tubes (1/4“ diameter) were placed into each container. Before the study began, animals were fed *ad libitum* with the experimental diets for two weeks.

Diet formulation

Six isoproteic diets with 40% crude protein were formulated based on the calculations with the Pearson Square method (Houser and Akiyama, 1997) and using practical and semi-purified ingredients (Table 1 and 2). Dietary protein was supplied by the Peruvian fish meal, soya bean meal or an isoproteic mixture of soya bean and fish meal, to obtain graded levels of each protein, as follows: 100% fish protein (diet 1; 0%), 80% fish protein: 20% soya bean protein (diet 2; 20%), 60% fish protein: 40% soya bean protein (diet 3; 40%), 40% fish protein: 60% soya bean protein (diet 4; 60%), 20% fish protein: 80% soya bean protein (diet 5; 80%), and 100% soya bean protein (diet 6; 100%).

Feedstuffs were grounded and sieved through a 100-µ sieve so that all the ingredients were of uniform size. Then, ingredients were mixed in a domestic blender (MOULINEX®, México, D. F.) for 30 min, and oil was gradually added to the dry mix. Enough water was added to make stiff dough. The wet mixture was pressure pelleted through a meat grinder, steamed for 5 min, and dried in a draft oven at 60 °C overnight or until the pellets contained around 10% moisture. Finally, diets were kept in plastic bags and stored (5 °C) until used.

Table 1 - Proximate analysis of the dietary protein sources (%, dry weight basis).

Proximate analysis	**Soya bean meal**	**Peruvian fish meal**
Moisture	10.8	6.7
Crude protein	46.9	58.9
Ether extract	0.2	2.3
Fiber	5.0	2.5
Ash	6	20
Nitrogen-free extract	31	9.6

Table 2 - Formulation (% of total) and proximate composition of all experimental diets used in fish meal replacement study.

Ingredients (%)	**Diet (percentage replacement)**					
	1[1] (0%)	**2 (20%)**	**3 (40%)**	**4 (60%)**	**5 (80%)**	**6 (100%)**
Soya bean meal[2]		10.0	20.0	31.0	41.0	52.0
Fish meal[3]	52.0	42.0	32.0	21.0	11.0	
Rice bran[4]	19.3	19.3	19.3	19.3	19.3	19.3
Wheat meal[5]	19.5	19.5	19.5	19.5	19.5	19.5
Vitamin mix[6]	2.0	2.0	2.0	2.0	2.0	2.0
Fish oil[7]	6.0	6.0	6.0	6.0	6.0	6.0
Salt[8]	0.2	0.2	0.2	0.2	0.2	0.2
Binder[9]	1.0	1.0	1.0	1.0	1.0	1.0
	Proximate composition (%, dry weight basis)[10]					
Protein	40.8	39.8	39.5	40.3	39.6	39.4
Lipid	4.0	6.1	5.8	4.4	5.2	6.2
Fiber	4.9	1.6	1.5	1.2	1.6	5.9
Ash	10.9	8.7	7.9	7.3	6.2	5.2
N-free extract	33.2	37.0	39.8	43.5	42.3	41.1
Dry matter	93.7	93.2	94.5	96.7	94.9	94.8
Moisture	6.3	6.7	5.4	4.0	4.5	5.2

[1]Fish protein/soya bean meal ratios: 0% = 100% fish meal, diet 1; 20% = 80% fish meal/20% soya bean meal, diet 2; 40% = 60% fish meal/40% soya bean meal, diet 3; 60% = 40% fish meal/60% soya bean meal, diet 4; 80% = 20% fish meal/80% soya bean meal, diet 5; 100% = 100% soya bean meal, diet 6.
[2]Source: soya bean meal (46.9 % protein), local vendor.
[3]Source: Peruvian fish meal (58.9% protein), Fondeport, Manzanillo, México.
[4]Source: rice bran (14% protein), Arrocera de Occidente, S. A. de C. V., Zapopan, Jalisco, México.
[5]Source: wheat flour (15% protein), Molino Central, S. A. de C. V., Guadalajara, Jalisco, México.
[6]Source: vitamin mix, Geymix® Plus, Novartis Salud Animal, S. A. de C. V., México, D. F., guaranteed analysis (per 500 g of mix) was: vitamin A (palmitate) 156,250 IU; vitamin D-3, 150,000 IU; vitamin E, 36 IU; riboflavin, 88 mg; d-pantothenic acid, 160 mg; niacin, 360 mg; cyanocobalamin, 0.5 mg; thiamine, 18.7 mg; menadione, 19.2 mg; folic acid, 3.0 mg; ascorbic acid, 25,000 mg; sacarose, 100 g.
[7]Source: fish oil, PULSE OMEGA 3, Seven Seas®, Health care Ltd., Scotland, UK.
[8]Source: unpurified salt, local vendor.
[9]Source: alginic acid, Bioxon de México, S. A. de C. V., Oaxaca, México. Dissolved in 35 parts hot (80°C) distilled water before mixing with diet.
[10]Represent the mean of three samples.

Three samples from each batch were pooled and analyzed for the proximal composition (Table 2). Crude protein was determined by using the Kjeldahl method (APHA, 1989), crude lipid was extracted with anhydrous ether in a Soxtec (Bligh and Dyer, 1959). Ash was determined by using a muffle furnace (AOAC, 1984) and crude fiber by the phenol-sulphuric acid method (Myklestad and Haug, 1972). During the experiment, a quantity of food equal to 10% of the animal biomass in each container was fed per day (twice daily, 09:00 and 17:00 hours). The relative quantity of food remaining in each container after the feeding was observed so that the ration size could be adjusted to minimize the waste. Diets seemed equally palatable as determined by visual inspection 1 h after the feeding.

Growth evaluation

Individual standard length (cm) and wet weight (g) were determined at the beginning of the experiment and each two weeks for 45 days. Prior to weighing, prawns were placed on absorbent paper to remove excess of water. Specific growth rate (SGR, % body weight/d) was calculated from SGR = 100 X (ln Wf – ln Wi)/t, where Wf = mean weight at the end of the period, Wi = mean weight at the beginning of the period, and t = time in days of the period (Ricker, 1979). Mean daily weight gain (MDWG, g/d) was calculated from MDWG = (Wf – Wi)/t. Food conversion ratio (FCR) was obtained from FCR = g feed consumed/g wet weight gained (Al Hafedh et al., 1999). Final survival per treatment was also recorded.

Statistical analysis

Statistical analysis was carried out according to Montgomery (1984). Data were tested for the normality and variance homogeneity (Kolgomorov-Smirnov’s test). One-way ANOVA was used to test data, followed by *post-hoc* Tukey’s test to separate significantly different mean values. A significance level of 0.05 was retained for each test. Arcsin transformations were applied to survival percentages before analysis (Sokal and Rohlf, 2000). Procedures available in Statgraphics Plus 5.0 were used to conduct analyses.

RESULTS

The data pertaining to mean body weight, length, SGR and survival are presented in Table 3. Prawns from all the treatments registered an increase in length and weight (Fig. 1).

Prawns in all the experimental groups grew at the rates that were consistent with the values routinely. Statistical differences in the mean final body weight (df =5; F = 6.95; $\alpha \leq 0.05$), mean weekly weight gain (df =5; F = 8.95; $\alpha \leq 0.05$) and specific growth rate (df =5; F = 3.85; $\alpha \leq 0.05$) among the treatments were detected after the fourth week of the feeding.

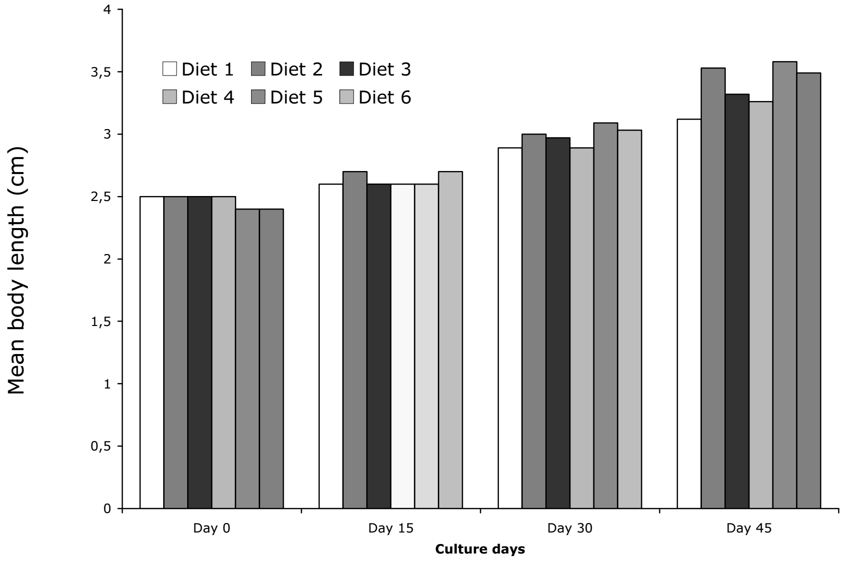
Table 3 -. Fresh-water prawn (*M. tenellum*) growth and feed performance in an indoor flow-through culture system over 45 experimental days, and fed on isoproteic diets containing various fish meal and soya bean meal levels. Values within a row sharing a common superscript are not significantly different (Tukey’s test; $P \leq 0.05$).

Dietary treatment	1[1] (0%)	2 (20%)	3 (40%)	4 (60%)	5 (80%)	6 (100%)
Mean initial body weight (g)	0.37^{a}	0.35^{a}	0.32^{a}	0.37^{a}	0.39^{a}	0.38^{a}
	(0.11)	(0.08)	(0.09)	(0.11)	(0.10)	(0.11)
Mean final body weight (g)	0.95^{a}	$1.09^{a,b}$	0.87^{a}	0.80^{a}	$1.15^{a,b}$	$1.14^{a,b}$
	(0.27)	(0.49)	(0.51)	(0.50)	(0.60)	(0.73)
Mean initial body length (cm)	2.52^{a}	2.54^{a}	2.50^{a}	2.54^{a}	2.42^{a}	2.39^{a}
	(0.29)	(0.22)	(0.26)	(0.27)	(0.24)	(0.21)
Mean final body length (cm)	3.10^{a}	3.53^{a}	3.32^{a}	3.26^{a}	3.60^{a}	3.50^{a}
	(0.35)	(0.56)	(0.50)	(0.63)	(0.57)	(0.76)
Mean weekly weight gain (g/week)	$0.098^{a,b}$	$0.112^{a,b}$	0.077^{a}	0.011^{a}	$0.119^{a,b}$	$0.133^{a,b}$
	(0.014)	(0.007)	(0.035)	(0.028)	(0.014)	(0.063)
Specific growth rate (%/d)	2.21^{a}	$2.44^{a,b}$	1.82^{a}	1.88^{a}	$2.54^{a,b}$	$2.62^{a,b}$
	(0.26)	(0.12)	(0.67)	(0.54)	(0.23)	(0.78)
Feed conversion ratio	1.32^{a}	1.41^{a}	1.47^{a}	1.51^{a}	1.34^{a}	1.37^{a}
	(0.12)	(0.21)	(0.18)	(0.25)	(0.23)	(0.23)
Survival (%)	90^{a}	95^{a}	95^{a}	85^{a}	95^{a}	90^{a}
	(10.0)	(8.66)	(8.66)	(8.66)	(8.66)	(10.0)

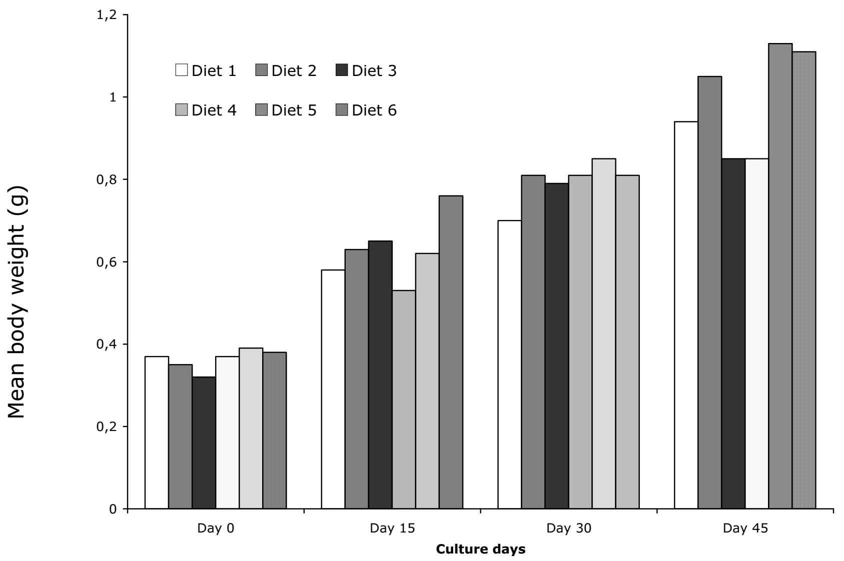
[1]Fish protein/soya bean meal ratios: 0% = 100% fish meal, diet 1; 20% = 80% fish meal/20% soya bean meal, diet 2; 40% = 60% fish meal/40% soya bean meal, diet 3; 60% = 40%fish meal/60% soya bean meal, diet 4; 80% = 20% fish meal/80% soya bean meal, diet 5; 100% = 100% soya bean meal, diet 6.

n = 5 prawns for each diet with four repetitions (20 per diet).

Values in parentheses indicate ± standard deviation.



A)



B)

Figure 1 - Mean final length (cm, A) and weight (g, B) of *M. tenellum* juveniles fed isoproteic diets substituting fish meal with soya bean meal. Fish protein/soya bean meal ratios: 0% = 100% fish meal, diet 1; 20% = 80% fish meal/20% soya bean meal, diet 2; 40% = 60% fish meal/40% soya bean meal, diet 3; 60% = 40% fish meal/60% soya bean meal, diet 4; 80% = 20% fish meal/80% soya bean meal, diet 5; 100% = 100% soya bean meal, diet 6. Mean initial length and wet weight = 2.48 ± 0.24 cm and 0.36 ± 0.10 g, respectively.

No significant differences in the mean initial body weight (df =5; F = 0.44, $\alpha \geq 0.05$), mean initial and final body length (df =5; F = 0.35, $\alpha \geq 0.05$ and df =5; F = 0.36; $\alpha \geq 0.05$ respectively), Feed conversion factor (df =5; F = 0.36, $\alpha \geq 0.05$) and survival (df =5; F = 0.76, $\alpha \leq 0.05$) were observed. Although the overall growth response of the diets 5 (80%) and 6 (100%) were higher than those obtained for the rest of the diets, these differences were not significant. The SGR ranged from 1.82 ±

0.67% BW/d for the diet 3 (40%), to 2.62 ± 0.78 for the diet 6 (100%). Prawns fed with the diet 6 (100%) obtained the highest weekly growth (0.133 ± 0.063 g). FCR among the diets detected at the end of the experiment was on average 1.4 ± 0.27 for all the treatments. Final survival fluctuated from 85% for the diet 4 (60%), to 95% for the diets 2 (20%), 3 (40%) and 5 (80%).

DISCUSSION

After failed attempts for developing a Giant Malaysian prawn *M. rosenbergii* industry in México (Ponce-Palafox et al., 2002; García-Ulloa, 1997), the culture of native prawn species in the farms turned an important activity for the local aquaculture industry. According to Ponce-Palafox et al. (2002), *M. americanum* and *M. tenellum* are native fresh water prawns species with aquaculture potential in the Mexican Pacific coast. For *M. tenellum*, there are some studies on physiology (Signoret et al., 1997; Hernandez et al., 1995; 1996), biology (Román-Contreras, 1979) and reproduction (Rodríguez-González, 1998), but information about nutrition is scarce.

Since plant protein meals are cheaper than animal protein sources, much research has been focused in substituting animal protein with the plant protein meals (Akiyama, 1988; Kikuchi, 1999). However, it has been proved that the dietary inclusion of plant protein meal, generally reduce growth and feed efficiency in fish and shellfish species (Cowey et al., 1971; Akiyama et al., 1989). In this study, growth performance and survival of *M. tenellum* juveniles fed with the soya bean meal based diets, were similar to the values found for the 100% fish meal diet, suggesting this prawn was capable to efficiently utilize soya bean meal in the formulated diets under the laboratory conditions. However, the source and quality of the soya bean meal should be taken into consideration when a formulated diet is elaborated. Piedad-Pascual et al. (1990), Tidwell et al. (1993) and Reigh et al. (1993) reported the ability to utilize soya bean meal as dietary protein supplement for some crustaceans, but in those cases, growth performance was not improved in comparison with fish meal diets, possibly due to the presence of active antimetabolites in soya bean that had been incompletely heat-processed (García-Ulloa, 1998), trypsin inhibitors (Ayet et al., 1996), or the amino acid deficiencies in soya bean (Reed and D'Abramo, 1989).

The mean SGR in the present study ranged from 1.82 ± 0.67% BW/d for the diet 3 (40%), to 2.62 ± 0.78 for the diet 6 (100%) respectively, being similar to those observed by Jones (1988), and Meade and Watts (1995) for crayfish juveniles fed formulated diet, but lower than that obtained by García-Ulloa et al. (2003) for *Cherax quadricarinatus* juveniles, including various soya bean meal levels in the diet. After 45 days, organisms displayed external traits (Cabrera-Jiménez, 1983) and mating behavior, possibly affecting the somatic growth of prawns (mainly in females), which could partially explain the low MDWG obtained. Although there were no differences among the diets, the FCR's in all treatments were higher (>1.3) compared with those reported by Jones et al. (1996) and Cortés-Jacinto et al. (2003), working with juvenile crayfish fed with balanced feeds. Since there was no information related to the *M. tenellum* nutritional requirements, the results might not be properly compared with the above-mentioned studies due to differences in species, diets and culture conditions used.

The mean final survival averaged 91.66% for all the treatments. For other fresh water crustaceans (Jones, 1995; Rouse et al., 1991), the use of artificial refuges such as the small PVC tubes placed into the culture containers, offered protection to the prawns during the moulting process, probably contributing to the high survival obtained.

In conclusion, results indicate that the growth of *M. tenellum* juveniles was not affected with the dietary inclusion of soya bean meal at the levels tested. However, further trials would be necessary to define the adequate inclusion level in its diet in order to develop a cost-effective and nutritionally balanced formulated diet for the commercial prawn aquaculture. In addition, the natural food contribution under pond-culture conditions should be also considered.

RESUMO

Os parâmetros do crescimento (comprimento padrão, peso, taxa de crescimento específica e ganho diário do peso) de juveniles do *Macrobrachium tenellum* alimentaram a 40% a proteína crua as dietas isoproteic que substituem a

refeição de peixes com feijão de soya a refeição nos vários níveis (20, 40, 60, 80 e 100%) foi avaliada por 45 dias sob condições do laboratório. As dietas experimentais foram comparadas com uma dieta baseada da refeição de peixes de 100%. A sobrevivência total foi gravada para todos os tratamentos no fim da experiência. Não havia nenhuma diferença significativa ($p \geq 0.05$) para todos os parâmetros do crescimento entre os tratamentos dietéticos. O peso médio inicial (0.36 ± 0.10 g) aumentado quase três vezes (1.00 ± 0.13 g) após 45 dias da cultura. O crescimento específico final flutuou de 1.82% BW/d para a dieta do inclusion da refeição do feijão de soya de 60%, a 2.62% obtidos para a dieta da refeição de peixes de 100%. A sobrevivência final média foi de 91.66%. O desempenho do crescimento de juveniles do *M. tenellum* não foi afetado pelos níveis dietéticos da refeição do feijão de soya testados.

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