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Short Communication

Gross energy levels in practical diets for the Amazon river prawn postlarvae, *Macrobrachium amazonicum*

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ABSTRACT. The present study evaluated the effect of different energy levels (15.07, 15.91, 17.17, 18.00, and 19.68 kJ g^{-1} of diet) on isoproteic practical diets for *Macrobrachium amazonicum* during the nursery phase on the zootechnical performance and proximate composition of the whole body. Postlarvae with a mean weight of 0.043 ± 0.01 g were stored in 25 experimental units, at 150 animals per m^2, in a completely randomized design with five treatments and five replicates. The post-larvae were evaluated at the end of the experimental period according to the pre-established performance parameters. The analysis of variance for regression ($\alpha = 0.05$) allowed us to estimate that the maximum final weight was observed in the treatment with 16.74 kJ g^{-1}. In contrast, the lowest apparent feed conversion was observed at the 16.43 kJ g^{-1} level. The best values were found in 17.29 and 17.50 kJ g^{-1}, respectively, for the energy and protein retention rates. As the best fit of the equation was presented for the energy retention ratio ($R^2 = 0.63$), which approached the treatment of 17.17 kJ g^{-1}, this energy level is recommended in postlarvae diets.

Keywords: *Macrobrachium amazonicum*; freshwater prawn; nutrition; energy requirement; production performance; prawn farming

The Amazon river prawn, *Macrobrachium amazonicum*, is one of the main fishing resources exploited by artisanal fishing populations in the Amazon region (Bentes et al. 2012, Costa et al. 2016). In addition to being an important fishing resource, *M. amazonicum* has great potential for prawn farming with high survival rates (Maciel & Valenti 2009).

The farming technology for *M. amazonicum* was extensively revised by Moraes-Valenti & Valenti (2010), involving aspects of biology, reproduction, larviculture, and grow-out. About nutrition and feed, D'Abramo & New (2010) reviewed the main aspects related to the production of diets for species of farmed freshwater prawns in Brazil and worldwide. However, the determination of reference levels for the inclusion of nutrients necessary for formulating and producing practical diets (NRC 2011) remains to be discovered for *M. amazonicum* in the nursery phase.

The energy level in prawn diets should be determined for each species due to differences in the use of proteins, carbohydrates, and lipids as an energy source. Therefore, variations in the proportion of these nutrients are the main difficulty in determining energy levels and using them as a reference for different species (Sánchez-Paz et al. 2006).

As a result of unbalanced diets, the consumption of protein to supply the energy demand leads to poor feed conversion. It greatly increases the excretion of nitrogen compounds, deteriorating the water quality and environment due to increased ammonia levels and

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eutrophication. On the other hand, a diet with excess energy can lead to even worse zootechnical results since the animal can be satiated energetically without meeting the requirements of other nutrients fundamental to its development and health, leading to greater accumulation of body fat. Given the need to corroborate the development of diets for the species, the present study aimed to determine the best level of gross energy of practical diets by evaluating the performance of *M. amazonicum* in the nursery phase.

The study was carried out in the Laboratory of Prawn Culture and Nutrition of Aquatic Organisms of the Federal University of Paraná, Palotina sector, Brazil, for 45 days. Postlarvae of *M. amazonicum* were obtained from the Aquaculture Center of UNESP, Campus de Jaboticabal, São Paulo.

After the transport, they were acclimatized for 20 days in polyethylene tanks with a volume of 300 L and provided with biological filtration in a recirculating aquaculture system. During this period, the animals were fed commercial Guabi® 40-J feed (40% crude protein; CP) in the initial proportion of 30% biomass; the feed ratio was adjusted according to consumption. After this period, postlarvae were submitted to biometry and, afterward, randomly distributed in the experimental units.

The experimental units consisted of 25 aquariums (38×35×40 cm) with a useful individual volume of 47 L and a bottom area of 0.11 m^2, connected to a recirculation system (1,200 L h^{-1}) with mechanical and biological filtration, water temperature control by a thermostatic heater. The photoperiod adopted was 12:12 h light:dark, with an average intensity of 500 lux, as New et al. (2010) recommended.

The postlarvae of Amazon river prawns had a mean initial weight of 0.043 ± 0.01 g and an average total length of 17.99 ± 1.69 mm at the beginning of the experimental period and were stocked at a density equivalent to 150 postlarvae m^{-2} according to recommendations by Penteado et al. (2007), totaling 17 individuals in each aquarium.

The completely randomized design was adopted with five treatments and five random replicates. The treatments consisted of five practical diets with variable crude energy levels of 15.07 to 19.68 kJ g^{-1} of diet, isoproteics with 37% CP (Table 1), as recommended by Santos et al. (2017) for *M. amazonicum* juveniles. The formulation of the diets was carried out based on the references for crustaceans (NRC 2011).

The diets were processed according to Coyle et al. (2010) to avoid the eventual selection of particles or ingredients by prawns. The ingredients used had the standard granulometry in a hammer mill with a 1.0 mm diameter sieve, mixed, and pelleted to obtain 1.5 mm diameter granules. Then, each diet was dehydrated in an air recirculation oven at a temperature of 55°C for 24 h. After drying, they were packed and stored (-4°C) until the moment of their use.

The diets were initially supplied in the proportion of 30% of the initial biomass divided into four daily feeds (07:30, 11:30, 15:30, and 19:30 h), as recommended by Araujo & Valenti (2007). Then the amount was adjusted according to the consumption, so there were no leftovers. The recirculation system was paralyzed daily and before the first feeding, and each experimental unit was siphoned to remove the solid waste (feces and unconsumed feed).

The water quality parameters: dissolved oxygen, pH, and water temperature, were monitored daily with an Oximeter AT-170 (Alfakit®), pH meter AT-315 (Alfakit®), and thermometer (Incoterm®), respectively. The total ammonia, nitrite, and nitrate concentrations were determined weekly by colorimetric reaction using the digital photocolorimeter AT100-PB (Alfakit®), and the total alkalinity and hardness were determined by titration according to APHA (2005).

The values obtained during the experiment for dissolved oxygen (8.23 ± 0.05 mg L^{-1}), pH (8.44 ± 0.01), temperature (29.46 ± 0.02°C), total ammonia (0.005 ± 0.007 mg L^{-1}), nitrite (0.005 ± 0.003 mg L^{-1}), nitrate (1.124 ± 0.346 mg L^{-1}), alkalinity (83.90 ± 7.18 mg of $CaCO_3$ L^{-1}) and hardness (43.60 ± 6.74 mg of $CaCO_3$ L^{-1}) are adequate for freshwater prawn culture (Valenti et al. 2010).

At the end of the experimental period, all the prawns from each experimental unit were counted for the evaluation of the survival rate (survival (%) = total number of live prawns / total number of initial prawns × 100) and the individual weight was measured in analytical scale (AY220 Martel®) for determination of weight gain (weight gain (mg) = final weight (mg) - initial weight (mg)), apparent feed conversion ratio (FCR = total feed intake (g) / total weight gain of prawns (g)), feed efficiency ratio (FER = total weight of prawns (g) / total intake (g)), energy retention ratio (ERR (%) = 100 × [(final weight × final body's gross energy) - (initial weight × initial body's gross energy)] / energy intake (consumed)) and protein retention ratio (PRR (%) = 100 × [(final weight × final body's crude protein) - (initial weight × initial body's crude protein)] / protein intake (consumed)), as described by NRC (2011) and Seenivasan et al. (2012).

Table 1. Composition of experimental diet for *Macrobrachium amazonicum.* [1]Composition per kg of the product: vit. A 16.875 UI; vit. D_3 3.375 UI; vit. E 200 UI; vit. K_3 6.7 mg; vit. B1 20 mg; vit. B_2 36 mg; vit. B_6 25.5 mg; vit. B_{12} 45 mcg; vit. C 1.200 mg; folic acid 11.2 mg; pantothenic calcium 67.5 mg; nicotinic acid 170.00 mg; biotin 1.68 mg; inositol 265 mg; Ir 65 mg. Cu 13.8 mg; Zn 150 mg; Mn 85 mg; Co 0.35 mg; I 1.3 mg and Se 0.4 mg; [2]BHT: butylated hydroxytoluene; [3]protein: energy ratio.

Ingredients (%)	Crude energy levels (kJ g^{-1})				
	15.07	15.91	17.17	18.00	19.68
Soybean meal	23.00	23.00	23.00	24.00	22.50
Fish meal	37.00	37.00	37.00	37.50	40.00
Corn grain	9.40	7.00	5.00	4.00	1.00
Wheat meal	16.20	12.70	10.00	6.00	2.00
Corn starch	3.40	8.90	12.00	12.50	10.50
Fish oil	0.50	0.75	1.50	3.00	7.00
Soybean oil	0.50	0.75	1.50	3.00	7.00
Dicalcium phosphate	4.80	4.80	4.80	4.80	4.80
Calcarium	2.00	2.00	2.00	2.00	2.00
Ascorbic acid	0.20	0.20	0.20	0.20	0.20
Mineral and vitamin mix[1]	2.00	2.00	2.00	2.00	2.00
BHT[2]	1.00	1.00	1.00	1.00	1.00
Chemical composition (%)					
Dry matter	89.51	90.25	89.19	89.82	90.02
Crude protein	37.00	37.00	37.00	37.00	37.00
Mineral matter	28.88	24.76	20.15	19.00	15.54
Ether extract	6.55	7.14	11.78	13.23	19.61
P:E mg CP kJ^{-1} [3]	24.55	23.26	21.55	20.55	18.80

Afterward, all the prawns were euthanized in cold water to perform the full body chemical analysis: percentage of dry matter, ash, crude protein, and crude energy (AOAC 2005).

After the tests of the assumptions of normality and homoscedasticity in the data obtained, the univariate analysis of variance was used to determine significant differences ($P < 0.05$) for the different water quality parameters, performance and survival parameters, and body chemical analysis, followed by regression analysis. The statistical software used was Statistica 7.0® (Statsoft 2004).

The values of survival (%), final weight (g), FCR, FER (%), PRR, and EER of *M. amazonicum* postlarvae are shown (Table 2).

All treatments presented high survival for the species, above 80%, and did not present significantly different values between treatments ($P > 0.05$). The energy level of the diet influenced the mean final weight ($P < 0.05$), and from the regression model was estimated as the highest final weight, i.e. the maximum point of the regression, the gross energy value in the diet of 16.74 kJ g^{-1}.

The FCR data also presented a significant regression analysis for the quadratic relation ($P < 0.05$), where estimating the minimum point of the equation, the value of 16.43 kJ g^{-1} was determined as the quantity of energy that provided the best FCR.

The FER was affected by the increasing energy levels in the diet, presenting an increasing quadratic behavior ($P < 0.05$). In estimating the maximum efficiency by the regression, the value of 16.74 kJ g^{-1} was obtained.

The results of the ERR and PRR in the carcasses in this experiment were better, with maximum points of 17.29 and 17.51 kJ g^{-1} for ERR and PRR, respectively.

The results of the body chemical analysis of *M. amazonicum* postlarvae for dry matter, ash, CP, and gross energy are presented in Table 3. The use of increasing energy levels in the diet but keeping it isoproteic did not interfere with the body composition of the animals for the variables analyzed ($P > 0.05$).

The survival rate obtained in this experiment followed the recommendations of D'Abramo & Castell (1997). The reduction of the mean weight to levels higher than 16.74 kJ g^{-1} may be related to the diet's high protein: energy (P:E) ratio, with energy being a limitation of daily feed intake (D'Abramo & New 2010). Thus, lower feed intake due to early satiety leads to lower nutrient intake. Consequently, reduced muscle tissue formation can be observed by lower protein deposition in the carcass at the highest energy level

Table 2. Performance and survival of *Macrobrachium amazonicum* postlarvae fed experimental diets with increasing energy and isoproteic levels during the nursery phase. [1]Quadratic effect, $y = -1\times10^{-7}x^2 + 0.0008x - 1.475$; $R^2 = 0.2070$, $P = 0.03$. [2]Quadratic effect; $y = 6\times10^{-6}x^2 - 0.0471x + 101.925$; $R^2 = 0.2215$; $P = 0.028$. [3]Quadratic effect; $y = -2\times10^{-5}x^2 + 0.16x - 322.26$; $R^2 = 0.2351$; $P = 0.021$. [4]Quadratic effect; $y = -4.6\times10^{-6}x^2 + 0.038x - 73.692$; $R^2 = 0.2238$; $P = 0.031$. [5]Quadratic effect; $y = -14.11\times10^{-6}x^2 + 0.118x - 235.529$; $R^2 = 0.6287$; $P = 0.011$. CV: coefficient variation.

Variables	Crude energy levels (kJ g^{-1})					CV
	15.07	15.91	17.17	18.00	19.68	(%)
Survival (%)	85.9 ± 8.9	91.8 ± 8.9	90.6 ± 6.7	88.2 ± 5.9	80.9 ± 8.3	9.46
Final weight (g)[1]	0.15 ± 0.02	0.16 ± 0.02	0.19 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	12.68
Feed conversion ratio[2]	6.0 ± 1.8	5.0 ± 0.9	4.0 ± 0.8	5.5 ± 1.2	6.5 ± 2.1	27.57
Feed efficiency ratio[3]	17.6 ± 4.2	20.5 ± 3.8	25.9 ± 5.1	18.8 ± 3.6	16.6 ± 4.8	25.13
Protein retention ratio (%)[4]	5.9 ± 0.9	7.6 ± 1.2	8.1 ± 1.3	10.2 ± 1.4	4.7 ± 1.2	30.25
Energy retention ratio (%)[5]	5.0 ± 0.8	5.3 ± 0.9	7.7 ± 1.1	5.3 ± 0.8	4.9 ± 1.2	24.06

Table 3. Whole-body chemical composition of *Macrobrachium amazonicum* postlarvae fed diets with increasing levels of gross energy and isoproteic. CV: coefficient variation.

Variable	Crude energy levels (kJ g^{-1})					CV
	15.07	15.91	17.17	18.00	19.68	(%)
Moisture (%)	73.67	72.92	73.10	72.74	72.55	0.43
Ash (%)	4.23	4.31	4.74	4.66	4.71	5.35
Crude protein (%)	18.51	20.57	21.67	20.45	18.36	7.16
Gross energy (kcal kg^{-1})	4471	4443	4548	4584	4576	1.40

tested (19.68 kJ g^{-1}), although not significant ($P < 0.05$) (Shiau & Lan 1991).

FCR values were higher in all treatments than those found by Seenivasan et al. (2012), which recorded values between 2.47 and 3.60 for postlarvae of *M. rosenbergii* supplemented with *Bacillus subtilis*, with a mean weight of 0.21 g. However, these animals were larger than those used in the present study. Sampaio et al. (2004) reported mean FCR values for *M. amazonicum* prawn of 2.03 in postlarvae of 0.28 g and 20.60 mm fed with purified diets and vitamin and selenium supplementation. Araujo & Valenti (2007), when studying feeding frequencies for this species, obtained FCR values close to those found in this study, with values ranging from 3 to 10, without significant differences, when they tested up to eight daily feeds at a rate of 40% of the biomass, with postlarvae of 0.009 mg.

These data make it possible to infer that the mechanisms by which feed conversion is affected still need to be studied and should be carefully considered. Especially when the feed offered to post-larvae is quantified, and the feed ingested needs to be quantified, not directly reported to the physiological phenomena of ingestion, digestion, absorption, and deposition of prawn.

According to Bureau et al. (2002) and Glencross et al. (2013), growth gains are highly related to increased crustacean ingestion capacity; therefore, additional gains promoted by genetic characteristics in energy efficiency, protein efficiency, and reducing the need for maintenance of energy should not be discarded. D'Abramo & New (2010) further concluded that the best efficiency of dietary use is also dependent on energy and dietary protein ratio, as the amount of non-protein energy affects daily feed consumption.

Choosing the PRR, since it was the parameter that presented the regression with the best fit ($R^2 = 0.63$) and defining the treatment with 17.16 kJ g^{-1} as superior to the others; we found P:E between 21.55 mg CP kJ^{-1} as the best relation. This relationship is superior to the best relationship found by Pezzato et al. (2003), Goda (2008), Zhang et al. (2017), and Méndez-Martínez et al. (2018). Pezzato et al. (2003) determined that the best relationship was 10.28 mg CP kJ^{-1} for the Amazon river prawn postlarvae fed with purified diets, with 30% CP and 15.07 kJ $g^{-1}$of crude energy. Goda (2008) recommended a dietary P:E ratio of 17 mg CP kJ^{-1} to stimulate growth performance and nutrient utilization efficiency of *M. rosenbergii* postlarvae. Méndez-Martínez et al. (2018) verified that the best ratio of dietary P:E for growth in prawn (*M. americanum*) was 350 g kg^{-1} protein with P:E of 18 mg CP kJ^{-1}.

In practical feed formulation, the P:E ratio of the diet should be used together with the absolute amount of dietary protein and lipid/carbohydrate. For juveniles of *M. nipponense*, the ideal feed formulation would be 330 g kg^{-1} of protein in the diet and 140 g kg^{-1} of lipid, with a P:E ratio of the diet of 16.49 mg CP kJ^{-1} (Zhang et al. 2017), being considered by the authors a high energy requirement. These values infer that the protein and energy levels and, consequently, their balance influence the performance responses of prawns.

The lowest P:E ratio in the treatment of 15.07 kJ g^{-1} (P:E 10.28 mg CP kJ^{-1}) may be responsible for the worst FCR and FER, probably resulting in protein catabolism as a way of supplying energy to vital processes, causing lower retention and protein efficiency, as well as lower growth. The highest ratio, in the treatment of 19.68 kJ g^{-1} when providing a P:E ratio of 13.43 mg CP kJ^{-1}, may have compromised feed intake, in which energy was a limitation of feed consumption, compromising the ingestion of other nutrients in quantities. This observation can be confirmed by the PRR and ERR data, which has a lower index with P:E extremes.

Energetic and dietary protein assessments are essential to optimize performance results and are related to energy intake, utilization efficiency, and metabolic demands to maintain vital processes (Glencross et al. 2013). Pezzato et al. (2003) evaluated two levels of energy (13.40 and 15.07 kJ g^{-1}) in purified diets; these authors observed that weight gain and feed conversion were influenced by the protein and energy contained in the diet, identifying a quadratic effect with a better result for *M. amazonicum* postlarvae fed with purified diets containing 35% CP and 15.07 kJ g^{-1} corresponding to the protein;energy (P:E) ratio of 10.28 when using postlarvae with a mean weight of 0.280 ± 0.032 g and 259.0 ± 32.0 mm of total length.

When analyzing the whole *M. amazonicum* body chemical composition obtained in a natural environment, weighing between 0.9 and 1.2 g, Furuya et al. (2006) recorded moisture, ash, and CP values of 70.3, 1.5, and 24.8%, respectively. Portella et al. (2013) presented, for *M. amazonicum* postlarvae with 0.01 g of weight, values of moisture and CP of 76.5 and 21.5%, respectively, in which only prawn muscle was used (cephalothorax, legs, and abdominal skeleton were removed). The results of the present study show similarities with those demonstrated by Portella et al. (2013); however, they differ from those presented by Furuya et al. (2006), which can be explained by the use of larger animals captured in the natural environment by the last authors.

In a study with *M. rosenbergii*, Seenivasan et al. (2012) tested different levels of inclusion of *Bacillus subtilis*. They obtained whole body with moisture variations between 75.1 and 76%, CP between 13.00 and 15.66%, and ash of 3.84 to 4.50%. These values presented are different from those obtained in the present study, especially when referring to CP. However, animals of different species were used, with larger sizes and weights than in this experiment. According to Furuya et al. (2006), variations in the bromatological results for the variables analyzed can be attributed to the availability and type of feed consumed by the animals (farming or natural environment) and to the regions of the crustacean body included in the analysis (whole animal, abdominal or cephalic region and presence or absence of carapace).

The analysis of lipids in the whole body was not performed in this experiment due to the small sample content, which was insufficient for laboratory determination. However, the literature cites values around 1.5 to 12.4%. It identifies *M. amazonicum* as a potential source of fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid, which can be used directly in human diet or incorporated into fish diets, aiming to improve its composition in fatty acids for later human consumption (Furuya et al. 2006, Seenivasan et al. 2012).

There is little information about nutritional requirements for *M. amazonicum*, especially on the qualitative and quantitative aspects of dietary protein, energy requirement, and energy: protein ratio for maximum zootechnical response (Pezzato et al. 2003). Such information is essential for developing efficient diets since the energy must be supplemented sufficiently. The protein is used almost exclusively for synthesizing tissues, and the main energy source must come from carbohydrates and lipids. The results demonstrate how much the energy of the diet interferes with the zootechnical data, even though the adequate P:E ratio maximizes the performance of *M. amazonicum* postlarvae.

Dietary energy may interfere with the performance of *M. amazonicum*, revealing the importance of its balance with the protein to maximize growth. Considering the performance achieved among the different treatments, it was concluded that 17.17 kJ g^{-1} of crude energy in diets with 37% CP, with P:E of 21.55 mg CP kJ^{-1} g^{-1} promoted the best performance for *M. amazonicum* postlarvae.

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