THE EFFECT OF THREE COMMERCIAL FEEDS USED IN AQUACULTURE HATCHERIES ON PHYSIOLOGY OF THE PRAWN *Macrobrachium amazonicum* (DECAPODA, PALAEMONIDAE)

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ABSTRACT

We investigated the effect of three commercial feeds containing 32%, 35% (control), and 38% of crude protein on the physiology (metabolism, ammonia excretion, energy substrate and hepatosomatic index) of *Macrobrachium amazonicum*. The energetic content and energy/protein ratio of the feeds were quantified. The physiological aspects did not show differences among the animals fed with the three types of feeds concerning metabolism, ammonia excretion and energy substrate, which was kept as a proteins. However, we observed 120% higher hepatosomatic index in the animals fed with 38% of protein. This feed has also higher energetic value (4,700 ± 10.6 kcal kg^{-1}) and an energy/protein ratio (12.2:1). It is possible that these characteristics of the feed have contributed to higher hepatosomatic index. In this sense, the feed containing 38% of protein seems to be more appropriate because it may promote better storage of reserves in the hepatopancreas, a beneficial condition to reproduction and growth.

Keywords: freshwater prawn; metabolism; excretion; hepatopancreas; energy; protein

O EFEITO DE TRÊS RAÇÕES COMERCIAIS SOBRE A FISIOLOGIA DO CAMARÃO *Macrobrachium amazonicum* (DECAPODA, PALAEMONIDAE)

RESUMO

Investigou-se o efeito de três diferentes rações comerciais contendo 32%, 35% (controle) e 38% de proteína bruta sobre a fisiologia (metabolismo, excreção de amônia, substrato energético utilizado e índice hepatossomático) do camarão *Macrobrachium amazonicum*. O conteúdo energético e a relação energia/proteína das rações foram quantificados. Não houve diferenças entre os animais alimentados com os três tipos de rações no que tange ao metabolismo, excreção de amônia e substrato energético utilizado, que se manteve como proteínas. No entanto, foi observado índice hepatossomático 120% maior nos animais alimentados com a ração contendo 38% de proteína bruta. Essa ração também possui maior valor energético (4700 ± 10.6 kcal/kg) e relação energia:proteína de 12.2:1. É possível que essas características da ração possam ter contribuído para um maior índice hepatossomático e, consequentemente, melhor armazenamento de reservas no hepatopâncreas, condição benéfica para a reprodução, crescimento.

Palavras chave: camarão de água doce; metabolismo; excreção; hepatopâncreas; energia; proteína

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INTRODUCTION

The culture of freshwater prawn is growing within the worldwide aquaculture scenery (425% between 1998 and 2008), highlighting the genus *Macrobrachium* (FAO, 2012). In Brazil, the exotic species *Macrobrachium rosenbergii* is the only one cultured in commercial scale (FAO, 2012), although in other countries, the culture of native species have shown growth [*Macrobrachium malcolmsonii* in India and *Macrobrachium nipponense* in China (MIAO and GE, 2002)]. In Brazil, the native species *Macrobrachium amazonicum* rises as a potential one for aquaculture (NEW, 2005; MACIEL and VALENTI, 2009). It is an endemic species of South America with wide geographical distribution that comprises the hydrographic bays of the Amazon and Orinoco and their rivers (HOLTHUIS, 1952; COLLART and RABELO, 1996; MELO, 2003). They occupy from coast regions, in estuarine areas, up to continental waters, in rivers and lacustrine environments (MACIEL and VALENTI, 2009). *Macrobrachium amazonicum* stands out because of its tasty meat, well accepted by the consumer market, and its rusticity that favors its culture in captivity (VALENTI, 1993; MORAES-RIODADES and VALENTI, 2001). In the decade of 90, artisan exploration of this species represented about 90% of freshwater prawn fishing in Brazil (NEW *et al.,* 2000). However, a decline in its natural production has been detected and its culture emerges as an alternative to supply the market demand (MACIEL and VALENTI, 2009).

Since 2001, researchers from many region of Brazil have been developing technology for *M. amazonicum* commercial farming (NEW, 2005; MACIEL and VALENTI, 2009). Within this proposal, about 60 works have already been published involving aspects such as handling, morphology, reproduction, development, among others (AUGUSTO *et al.,* 2007; MACIEL and VALENTI, 2009; ANGER and HAYD, 2010; MACIEL *et al.* 2012; BOOCK *et al.* 2013; HAYD *et al.,* 2014). However, there is a lack of studies regarding the appropriate feeding for this species, including in relation to the effects on their physiology. The knowledge of the physiology of crustaceans of economic interest is a good indicator of food quality and several kinds of physiological answers can be used for this purpose like the evaluation of metabolism, hepatosomatic index, ammonia excretion, and the energy substrate used (GASTELÚ *et al.,* 2011; STUMPF *et al.,* 2011; JAMES *et al.*, 2013; XIE *et al.,* 2014).

The hepatosomatic index may be used to evaluate the nutritional conditions and the level of stress of the crustaceans (SIMON, 2009; STUMPF *et al.,* 2011; JAMES *et al.,* 2013; XIE *et al.,* 2014). The hepatopancreas is related to digestions, synthesis, and secretion of digestive enzymes, absorption, and stock of lipids derived from the food and carbohydrate metabolism (JAMES *et al.,* 2013; XIE *et al.,* 2014). The hepatopancreas also serves as the main energy reserve for the growth and molting and appears to be a much more important synthetic site yolk protein of the crustacean (LI *et al.,* 2006; DING *et al.,* 2010). A reduction in this index may occur during fasting, food shortage, and reduced food assimilation or during the ingestion of inappropriate diet because the nutrients stored in the hepatopancreas are mobilized to meet the energy demands (COMOGLIO *et al.,* 2005; JAMES *et al.,* 2013; XIE *et al.,* 2014). Metabolism, assessed by oxygen consumption, is a useful parameter to monitor the physiological conditions of the crustaceans because it may increase in adverse situations such as high temperatures and stressful salinities, fasting periods and ingestion of low nutritious food (COMOGLIO *et al.,* 2004; JENSEN *et al.,* 2013). The ammonia excreted though comes mainly from the catabolism of amino acids and it may present variations due to the metabolism of proteins and use of free amino acids as source of energy. In addition, the increase of ammonia excretion rate due to inappropriate feeding may result in elevation of ammonia concentration in the effluents of the aquaculture hatcheries. The type of energy substrate (protein, carbohydrate or lipids) predominantly oxidized to supply energy may be evaluated by the atomic ratio O:N (oxygen consumed to nitrogen excreted) and used to measure stress due to changes in the energy substrate used under various conditions (GONZÁLEZ-PEÑA and MOREIRA, 2003; BROWN, 2006; LEMOS *et al.,* 2006; AUGUSTO and MASUI, 2014). According to MAYZAUD and CONOVER (1988), the O:N ratio is associated with the availability of energy reserves and the

use of body protein. The authors suggest that pure protein catabolism will yield O:N ratios in the range of 3 to 16, approximately, whereas equal amounts of lipid and protein catabolism will yield values between 50 and 60.

Once the knowledge of the functioning aspects of the organism of the cultivable species constitutes an important tool for aquaculture, the present work assessed the effect of three different commercial feeds commonly used in culture systems on the physiology of the prawn *M. amazonicum*. The feeds were extruded and contained three different protein levels: 32, 35 (control) or 38% of crude protein. The present work aimed to evaluate the fast adjustment to physiological responses such as hepatosomatic index, oxygen consumption, ammonia excretion, and energy substrate predominantly oxidized. Energetic content, energy/protein ratio, and moisture content of the feeds were also verified.

MATERIAL AND METHODS

Macrobrachium amazonicum adult males belonging to Cinnamon Claw (CC) morphotype (MORAES-RIODADES and VALENTI, 2004) were collected from tanks of the Aquaculture Center of UNESP (CAUNESP), State of São Paulo, Brazil, using a trawl net of 6 mm. The animals were transferred to the UNESP - Campus Experimental do Litoral Paulista, in São Vicente - SP, in gallons of 60 L containing water from the collection site under constant aeration. In the Laboratory of Animal Physiology, the animals were acclimated for 10 days to laboratory conditions. Animals were weighted individually and randomly distributed (individual mass of 3.68 ± 0.592 g) in groups of three individuals in 16 tanks of de 25 L containing dechlorinated freshwater, oxygenation and artificial heating (26 °C) and controlled photoperiod of 12h:12h (light:dark). The tanks were daily siphoned and had about 30% of the water replaced. Total ammonia was daily monitored (Labcon kit). During this period, the animals were fed with Vannamei commercial extruded diet (Guabi Nutrição Animal Ltda.) containing 35% of protein. This was the feed offered to animals in the hatcheries of CAUNESP when the animals were collected. The feed was divided into two rations; about 30% was offered in the morning and 70% in the late afternoon, supplying 10% of the biomass of each tank. Biomass was estimated by the average of mass of the three animals from each tank.

After the period of acclimation to laboratory conditions, the animals were divided into three groups (T32, T35, and T38). Each group received daily one of the three types of extruded feeds containing different protein concentrations. Treatments below:

- T32 (Fri-Acqua Tilápia Crescimento; Fri-Ribe S.A.): feed formulated to tilapia; it contains 32% of crude protein; 4-6 mm of length;
- T35 (Vannamei 35; Guabi Ltda.): control; feed formulated to marine shrimp; it contains 35% of crude protein; diameter of 2.4 mm;
- T38 (Potimar Active 38; Guabi Ltda.): feed formulated to marine shrimp; it contains 38% of crude protei; diameter of 2.4 mm.

The composition of the three types of feeds, according to information given by the manufacturers, is presented in Table 1. To evaluate protein, carbohydrates and lipids concentration, food samples were weighed and homogenized with a pistil. Part of the homogenate was add in 1:1 of saline solution and centrifuged at 3,000 rpm for 10 min. Colorimetric analyses for total carbohydrate (phenol sulfuric acid method from DUBOIS *et al*., 1956) using glucose (Sigma) as control and total protein (BRADFORD, 1976) was determined using bovine serum albumin (BSA) (Sigma). Samples were lyophilized and weighted to determine moisture. Total lipid extraction were carried according to FOLCH *et al*. (1957), supernatant were recovered and lyophilized and weighed for total lipid determination.

Every treatment (T32, T35, and T38) was composed of three tanks of 25 L, each one containing three animals; during this period, animal handling was similar to the period of acclimation to laboratory conditions. Nine days after the exposure to different treatments, five animals of every treatment were selected for the evaluation of oxygen consumption, ammonia excretion, and hepatosomatic index. The animals that were in pre or post-molt were not used because they could not be representative of the inter-molt phase.

Table 1. Levels of guarantee per kg of the product, according to manufacturers. min. = minimum; max. = maximum.

	Tilápia Crescimento 32	Vannamei 35	Potimar Active 38
Crude Protein (min.; g)	320	350	380
Ether Extract (min.; g)	60	75	75
Moisture (max.; g)	120	100	100
Fibrous Matter (max.; g)	70	50	50
Mineral Matter (max.; g)	110	130	130
Calcium (max.; g)	30	30	25
Calcium (min.; g)	30	15	30
Phosphorus (min.; mg)	6000	14.5	14.5
Magnesium (max.; g)	0,03125	3	3
Vitamin A (min.; UI)	9000	4000	13000
Vitamin D3 (min.; UI)	3150	2000	2500
Vitamin E (min.; UI)	135	150	200
Vitamin B2 (min; mg.)	20.25	14	30
Vitamin B1 (min.; mg)	20.25	25	18
Vitamin C (min.; mg)	300	130	500
Niacin (min.; mg)	112.5	150	150
Vitamin B6 (min.; mg)	40	30	20.25
Biotin (min.; mg)	0.585	0.2	0.2600
Folic Acid (min.; mg)	5.4	6	8
Vitamin B12 (min.; mcg)	22.5	20	26
Choline (min.; mg)	800	1500	1500
Manganese (min.; mg)	62.5	20	20
Zinc (min.; mg)	100	100	100
Copper (min.; mg)	40	40	25
Cobalt (min.; mg)	0.6	0.5	0.5
Iodine (min.; mg)	1.25	1	1
Selenium (min.; mg)	0.25	0.3	0.45

Oxygen consumption and ammonia excretion were measured in the last day of the experimental period (9th day). After this period, animals were placed in individual conical respirometric chambers containing 440 mL of filtered water, maintained in trays with water and electrical heater at 26 ^{o}C. Animals were kept in respirometric chambers for 30 min with aeration aiming at acclimation and reduction of the stress caused by manipulation. After this period, aeration was removed and oxygen concentration within the chamber was measured with the help of an oxymeter (monitor and probe YSI, Models 53 and 5905, respectively). After 60 min, oxygen concentration was measured again. Control chambers without animals were kept under the same experimental conditions. Variations in oxygen concentration were calculated by the difference between the values obtained in samples and controls (no animals). Oxygen consumption was expressed as individual rates (g ind^{-1} day^{-1}) and dry mass-specific (µg mg dry mass^{-1} h^{-1}).

Ammonia excretion [expressed as individual rates (mg ind^{-1} day^{-1}) and dry mass-specific (µg mg^{-1} dry mass h^{-1})] was measured in samples of 5 mL of water obtained from respirometric chambers in the beginning and in the end of the experiment to assess oxygen consumption. Ammonia concentration in water samples was determined in triplicate by colorimetry (KOROLEFF, 1976).

After the metabolism experiment, all animals were killed by freezing, the hepatopancreas was dissected, and oven dried at 60 ^{o}C for 48 h

and weighted in analytical scale. To calculate dry mass, we considered the animal's whole body, including the dissected hepatopancreas. The hepatosomatic index calculation (HIS) was performed according to equation: HSI(%) = Organ weight/Body weight x 100. Atomic ratio O:N was calculated by dividing the oxygen consumed (mol) by ammonia excreted (mol) (MAYZAUD and CONOVER, 1988).

Energetic content and hydration were assessed in six samples (1 gram each) of each type of feed. For the evaluation of the energetic content, the samples of the feeds were dried in oven at 60 ºC for 48 h, macerated, weighted, and the energetic content verified in calorimetric pump (IKA, C2000 Basic) (SANTOS, 2010). Values expressed as gross energy, in kcal kg^{-1}. The degree of hydration (%) was calculated as [(fresh weight - dry weight)/fresh weight] x 100. The ratio between energy and crude protein of the feeds was calculated by the division between the values of crude energy obtained and the protein concentration of the three diets determined.

Data obtained were evaluated regarding normality of distribution (Kolmogorov-Smirnov) and subjected to variance analysis (ANOVA). Posteriorly, data were subjected to the Student-Newman-Keuls multiple range test (SNK) to localize the statistically different ranges (ZAR, 2009). Analyses were done using Sigma Stat 1.0 program, applying minimal significance level of 5%. Graphs were performed in Slide Write Plus program. Data were presented as average ± standard error of average.

RESULTS

The composition of the three types of feeds is shown in Table 2. The concentration of proteins of the feeds was similar ($P>0.05$) to the values provided by the manufacturer. The concentration of total proteins was different ($P<0.05$) among the three types of feeds but total lipids and carbohydrates were similar. There was significant difference ($P<0.05$) among all treatments regarding energetic content; treatment T38 was the most energetic. Hydration content of the feeds was similar ($P>0.05$) among treatments T35 and T38; treatment T32 differed significantly ($P<0.05$), showing higher moisture. Energy:protein ratio in the feeds showed values of 7.3:1.0 for the treatment T32, 13.2:1.0 for T35 and 12.2:1.0 for T38.

Individual values of oxygen consumption and ammonia excretion did not present significant differences ($P>0.05$) among treatments (Table 3) as well as specific mass values (Figures 1 and 2). Atomic ratio O:N presents values which are in the interval between 8.7 and 14.8 (Table 3).

Table 2. Gross energy (kcal kg^{-1}), energy:protein ratio, total proteins (%), carbohydrates (%) and lipids (%), and hydration (%) of the feeds used in the three types of treatment (X ± SEM; N = 5)

	Treatment		
	T32	**T35**	**T38**
Gross Energy (kcal kg^{-a})	4340.00 ± 52.20^a	4580.00 ± 10.20^b	4700.00 ± 10.60^c
Energy:protein	7.3:1.0	13.2:1.0	12.2:1.0
Total Protein (%)	31.80 ± 1.22^c	34.73 ± 0.52^b	38.38 ± 0.64^a
Total Carbohydrate (%)	30.81 ± 6.34^a	25.83 ± 5.33^a	23.74 ± 7.34^a
Total Lipid (%)	6.30 ± 0.48^a	7.40 ± 1.03^a	7.54 ± 0.77^a
Hydration (%)	9.90 ± 1.10^a	5.70 ± 0.50^b	6.70 ± 0.20^b

T32 = feed containing 32% of crude protein; T35 = feed containing 35% of crude protein; T38 = feed containing 38% of crude protein. Distinct letters in the same row mean statistical differences among treatments (ANOVA; *P<0.05).*

The hepatosomatic index of the animals fed with the feed of treatment T38 (38% of crude protein) was higher (P<0.05) than the animals fed with the feeds of treatments T32 and T35 (32% and 35% of crude protein, respectively). There were no differences in the hepatosomatic index of the animals fed with the feeds containing 32 and 35% of crude protein (Table 3).

Table 3. Hepatosomatic index (%), oxygen consumption (g ind^{-1} day^{-1}), ammonia excretion (mg ind^{-1} day^{-1}) and atomic ratio O:N of the *Macrobrachium amazonicum* subjected to different treatments. (X ± SEM; N = 5).

	Treatment		
	T32	**T35**	**T38**
Hepatosomatic index (%)	1.90 ± 0.30^{a}	1.30 ± 0.10^{a}	3.50 ± 0.40^{b}
Oxygen consumption (g ind^{-1} day^{-1})	0.02 ± 0.001^{a}	0.02 ± 0.002^{a}	0.02 ± 0.002^{a}
Ammonia excretion (mg ind^{-1} day^{-1})	1.66 ± 0.19^{a}	1.16 ± 0.11^{a}	1.52 ± 0.05^{a}
O:N	8.70 ± 2.30^{a}	14.8 ± 1.00^{a}	10.0 ±1.30^{a}

T32 = 32% of crude protein; T35 = 35% of crude protein; T38 = 38% of crude protein. Distinct letters in the same row mean statistical differences among treatments (ANOVA; P<0.05).

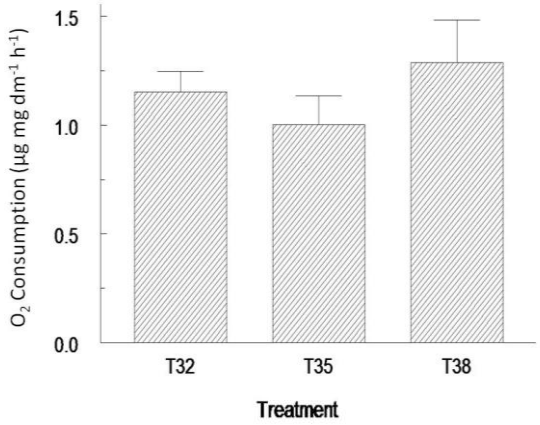

Figure 1. Oxygen consumption rate (µg mg dm^{-1} h^{-1}) of the *Macrobrachium amazonicum* males fed with different feeds. T32 = 32% of crude protein; T35 = 35% of crude protein; T38 = 38% of crude protein. (X ± SEM, N = 5).

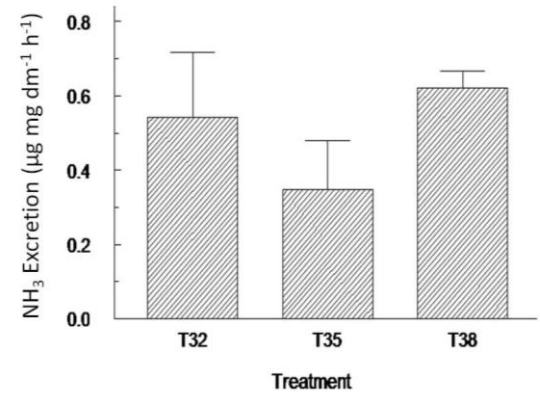

Figure 2. Ammonia excretion (µg mg dm^{-1} h^{-1}) of the *Macrobrachium amazonicum* fed with different feeds. T32 = 32% of crude protein; T35 = 35% of crude protein; T38 = 38% of crude protein (X ± SEM, N = 5).

DISCUSSION

The food given to cultivable species may influence the aspects of their physiology and in consequence reproduction, growth rate, energy stock, immunity to diseases (KUBITZA, 1999; GARCÍA-ORTEGA *et al*., 2000; VALENTI and NEW 2000; HARTNOLL, 2001). The physiological aspects of the prawn *M. amazonicum* evaluated here showed higher hepatosomatic index in the animals fed with the feed containing 38% of crude protein. However, there were no differences among the animals fed with the three types of feeds tested with respect to metabolism, ammonia excretion, and the energy substrate used. The physiological parameters evaluated here normally alter in a short period of time (fast adjustment) (COMOGLIO *et al*., 2008; DISSANAYAKE *et al*., 2008; WEI-JIAN *et al*., 2013), what can be proved by the elevated increase in the hepatosomatic index of the *M. amazonicum* fed with feed containing 38% of protein.

The hepatopancreas of crustaceans is the site for secretion of digestive enzymes, absorption of nutrients and reserve, mainly of lipids, supplying energy to growth, metabolism, and reproduction (SIMON, 2009; STUMPF *et al*., 2011; JAMES *et al*., 2013; XIE *et al*., 2014). When the nutrients ingested are not enough to meet the metabolic demand, crustaceans can turn to their reserve stored in the hepatopancreas (NAVARRO *et al*., 2006). In the marine penaeid *Marsupenaeus japonicus* subjected to a fasting period of 7 to 28 days, it was verified a reduction in the hepatosomatic index of 3% to 2% (CUZON *et al*., 2000). In *Litopenaeus vannamei* fasting for 15 days, hepatosomatic index suffers a reduction of 4% to 2% (COMOGLIO *et al*., 2004).

In the present work, the hepatosomatic index 120% higher in the animals fed with the feed containing 38% of crude protein may be related to higher levels of crude protein. In addition, the analyses conducted here show that this feed also have energy:protein ratio of 12.2:1; this characteristic may have contributed to higher hepatosomatic index.

The amount of crude protein indicated to decapod crustaceans is approximately 30 to 60% (WICKINS and LEE, 2002; HOLME *et al.*, 2004; MITRA *et al.*, 2005). In Asian countries, protein contents between 24 and 40% have been indicated during the growth phase of the freshwater prawn *M. rosenbergii* (MITRA *et al.*, 2005; HABASHY, 2009; DAVASSI, 2011). Protein is a limiting growth factor; it is needed in higher proportions compared to carbohydrates and lipids. The energetic content and the energy:protein ratio are also important factors to be considered in the diet of cultivable species because if on one hand the nourishment very rich in protein may lead to the increase of the ingestion rate, the increase of energy content may reduce food consumption before the protein needs are obtained (PEZZATO *et al.*, 2003). It is suggested that digestible energy of the feeds for crustaceans displays levels between 3,100 and 4,060 kcal kg^{-1} (CUZON and GUILLAUME, 1997). Thus, while the levels of crude energy of 3,200 kcal kg^{-1} are enough to the growth of *M. rosenbergii* breeders (MITRA *et al.*, 2005), *M. amazonicum* postlarvae show better responses of growth and survival when fed with diets containing 3,600 kcal kg^{-1} of crude energy (PEZZATO *et al.*, 2003). Although the energetic content of the feeds analyzed here varied between 4,340 kcal kg^{-1} (feed containing 32% of protein) and 4,700 kcal kg^{-1} (feed containing 38% of protein) and exceeded the values recommended for crustaceans, it reflects the energetic content of the feeds that have been used in hatcheries of *M. amazonicum* in Brazil. The feed containing 38% of crude protein also has an energy:protein ratio of the 12.2:1; similar value was verified by PEZZATO *et al*. (2003) (12.0 : 1.0) as the best for post larvae of *M. amazonicum*. This relation is so important that even feeding *M. amazonicum* with feed containing higher protein concentration in T35, their hepatosomatic index was similar to the animals from treatment T32, which were fed with lower protein feed, apart from the highest energetic content. It is possible that the elevated energy:protein ratio of T35 (control) might have generated lower ingestion rate of the feed.

Clearly the commercial feeds evaluated here possess some differences in pellet size and granulometry what might have influenced the ingestion rate because the milling degree of feeds can alter physical properties, stability, hardness, and selectiveness by shrimp (SMITH *et al*., 1985; CUZON *et al*., 1994). The milling degree of the feed can even reduce the size of particles, exposing a larger area to the action of digestive enzymes, increasing the digestion and absorption of nutrients. The feeds also have chemical attractants such as amino acids and unsaturated fatty acids that make easier for prawn to find them. Thus, the lowest hepatosomatic indices observed in the prawn fed with commercial feeds containing 32 and 35% of protein may be related to the lowest ingestion rate and/or leaching of nutrients and consequently, lower accumulation of reserves or production of digestive enzymes. Other factors could alter ingestion rate such as age of the animals, gender, temperature and oxygen dissolved in water, and seasonality. However, these diversities were eliminated once the animals studied came from a single collection and the experiments were conducted under controlled laboratory conditions.

Future works which assess ingestion rate and growth of animals in hatcheries fed with feeds formulated in laboratory containing protein levels and energy:protein ratio here evaluated might deliver further information with respect to the advantages of this feed once an increase in the ingestion rate might also be related to higher hepatosomatic index.

Metabolism refers the total sum of all chemical reactions that occur in an organism and may vary due to diet, growth, reproduction, body mass, activity patters, seasons of the year, age, sex, etc. Oxygen consumption (individual and dry mass-specific) did not vary due to the three types of feeds given to prawn *M. amazonicum*. Values obtained for metabolic rate are in accordance with the one found for the species, between 1 and 3 (µg mg MS h^{-1}) (DALL and SMITH, 1986; ZANDERS and RODRIGUEZ, 1992; AUGUSTO and MASUI, 2014; MAZARELLI *et al*., 2015). Some authors have found variations in the metabolic

rate of crustaceans fed with different kinds of diet. For instance, in *L. vannamei* shrimp fed with feed containing different protein levels, it was verified high oxygen consumption in animals fed with 15% of protein, in comparison to those fed with 5 and 40% (PASCUAL *et al.*, 2004). Nevertheless, in *Litopenaeus stylirostris* fed for 50 days with six experimental diets, ranging from 25 to 58% crude protein, the basal metabolism did not change when shrimp were fed any of experimental feeds (GAUQUELIN *et al.*, 2007). It is possible that *M. amazonicum*, similar to *L. stylirostris*, do not alter catabolism and anabolism due to the three types of feeds offered, what could have as consequence alterations in growth, activity pattern and gonadal development. Increases in the metabolism of species fed with lower protein levels, for instance, could indicate that this feed is metabolically less effective because the animals need to use more energy to keep their metabolic routine. In this sense, the results obtained here with *M. amazonicum* are interesting, because the animals were fed not only with feed for marine shrimp but also with tilapia feed, option occasionally offered to the animals in the hatcheries that has lower costs. However, further studies must examine this response not only in a short-term basis as it has been investigated here, but also during chronic exposure of about 30 days to check if this response pattern is kept in long-term period.

Ammonia is the main nitrogen compound resulting from the protein metabolism of aquatic animals, including crustaceans. Variations in the excreted ammonia can result from both the increase and reduction of the metabolism of proteins due to diet alterations regarding the use of free amino acids as energy source and osmotic effectors of osmoregulation (WANG *et al.*, 2003; AUGUSTO *et al.*, 2007). Although the ammonia excretion rate by crustaceans is highly variable and dependent on diverse factors such as salinity, pH, ontogenetic stage, and especially on the feeding rate and type of diet, data verified here did not vary due to the feed offered to the animals. The values found (between 0.3 and 0.6 µg mg MS^{-1} h^{-1}) are similar to the ones verified for the CC morphotype of *M. amazonicum* (MAZARELLI *et al.*, 2015) and for *L. vannamei* (COMOGLIO *et al.*, 2004). Therefore, considering a period of 9 days under observation in laboratory, independently of the type of feed given, the animals do not alter their ammonia release.

The atomic ratio between the amount of oxygen consumed and the amount of nitrogen excreted (O:N) is used in the literature to indicate the energy substrate preferentially used by aquatic animals, it means, proteins, carbohydrates, and/or lipids. Many factors may influence the type of energy substrate predominantly used by crustaceans, including the diet. CHEN and NAN (1994) observed that different species of marine shrimp fed with the same food use different energy substrates: *Penaeus japonicus* and *Metapenaeus ensis* use proteins (O:N = ≈ 20) and *P. monodon*, *P. penicillatus* and *P. chinensis* use lipids (respectively, O:N = 27, 30 and 42). In *M. rosenbergii* fed with diets containing different levels of fibers, the O:N ratio varied from 10 up to 50 (GONZÁLEZ-PEÑA and MOREIRA, 2003). ROSAS *et al.* (2002) showed that sea shrimp *L. vannamei* use proteins (O:N = ≈ 10) as a source of energy and have the capacity to synthesize dietary carbohydrates through the gluconeogenic pathway. In a study with the marine lobster *Homarus americanus*, fed with diet rich in protein, it was observed the use of proteins (O:N = ≈ 13) (BROWN, 2006). The atomic ratio O:N evaluated in the present work also indicated the preferential use of proteins as energy substrate in all treatments. AUGUSTO and MASUI (2014) also verified the predominant use of proteins by *M. amazonicum* males and females fed with commercial feed for marine shrimp containing 30% of protein. Proteins are not totally combusted, eliminating by urine derivatives of protein metabolism that still have the capacity of releasing energy. The feeds formulated for shrimp aim at both proper nutritional balance and lower financial costs by using carbohydrates as energy source, directing the proteins for the construction of new tissues. The use of proteins as energy substrate may be related to the differential composition of amino acids in the feed, that when in excess or in unbalanced amounts may be channeled to energy supply (MAYZAUD and CONOVER, 1988). Commercial shrimp feeds are produced based on scientific information on marine shrimp, but the protein needs of freshwater species may be different. On the other hand, ROSAS *et al*. (2002) showed that *L. vannamei* is well adapted to use protein as a source of

energy and that have the capacity to synthesize dietary carbohydrates through the gluconeogenic pathway. In the present study, O:N ratio was lower in animals fed with tilapia feed what may suggest even higher unbalance in terms of composition of amino acids once the value found (O:N = 8.70 ± 2.30) strongly suggests the use of protein as energy substrate.

In the present work, the values of the hepatosomatic index suggest greater accumulation of reserves in the animals fed with the feed containing higher level of crude protein (38%) and energy:protein ratio of 12.2:1.0. Since the results do not demonstrate differences in the type of energy substrate used, oxygen consumption, and ammonia excretion, it is possible that the animals have kept stable metabolism independent of feeding, but they were able to store reserves when fed with diet containing higher level of crude protein. The data presented here offer an insight for future works that investigate physiological effects, as well as growth and reproduction of *M. amazonicum* fed with formulated feeds; these parameters must also be evaluated in long term. Information about an appropriate feeding for *M. amazonicum* is still scarce and this work adds information about the physiological aspects of this specie as a result of the feed given.

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REFERENCES

ANGER, K. and HAYAD, L. 2010 Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil. *Aquatic Biology*, *9*: 251–261.

AUGUSTO, A.S.; GREENE, L.J.; LAURE, H.; McNAMARA, J.C. 2007 The ontogeny of isosmotic intracellular regulation in the diadromous, freshwater Palaemonid shrimps, *Macrobrachium amazonicum* and *M. olfersii* (Crustacea, Decapoda). *Journal of Crustacean Biology*, *27*(4): 626-634.

AUGUSTO, A and MASUI D.C, 2014 Sex and reproductive stage differences in the growth, metabolism, feed, fecal production, excretion and energy budget of the Amazon River prawn (*Macrobrachium amazonicum*). *Marine and Freshwater Behavior and Physiology*, *47*(6): 373-388.

BROWN, A.C. 2006 Effect of natural and laboratory diet on O:N ratio in juvenile lobsters (*Homarus americanus*). *Comparative Biochemistry and Physiology*, *144A*(1): 93-97.

BOOCK, M.V.; MARQUES, H.L.A.; BARROS, H.P.; MALLASEN, M.; SANTOS, M.R.; MORAES-VALENTI, P. 2013 Growing amazon river prawns in rice fields in Brazil. *World Aquaculture Magazine*, 44(2): 32-35.

BRADFORD, M.M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

CHEN, J.C. and NAN, F.H. 1994 Comparisons of oxygen-consumption and ammnonia excretion of 5 penaeids. *Journal of Crustacean Biology*, *14*(2): 289-294.

COLLART, O.O. and RABELO, H. 1996 Variation in egg size of the freshwater prawn *Macrobrachium amazonicum* (Decapoda, palaemonidae). *Journal of Crustacean Biology*, *16*(4): 684-688.

COMOGLIO, L.; GAXIOLA, G.; ROQUE, A.; CUZON, G.; AMIN. O. 2004 The effect of starvation on refeeding, digestive enzyme activity, oxygen consumption, and ammonia excretion in juvenile white shrimp *Litopenaeus vannamei. Journal of Selfish Research, 23*(1): 234-249.

COMOGLIO, L.; SMOLKO, L.; AMIN, O. 2005 Effects of starvation on oxygen consumption, ammonia, excretion and biochemical composition of the hepatopâncreas on adult males of the False Southern King crab *Paralomis granulosa* (Crustacea, Decapoda). *Comparative Biochemistry and Physiology B - Biochemistry and Molecular Biology*, *140*(3): 411-416.

COMOGLIO, L, GOLDSMIT, J and AMIN, O. 2008 Starvation effects on physiological parameters and biochemical composition of the hepatopancreas of the southern king crab Lithodes santolla (Molina, 1782). *Revista de Biología Marina y Oceanografía*, 43(2): 345-353

CUZON, G.; GUILLAUME, J.; CAHU, C. 1994 Composition, preparation and utilization of feeds for Crustacea. *Aquaculture*, 124(1-4): 253-267.

CUZON, G. and GUILLAUME, J. 1997 Energy and protein: energy ratio. In: D'ABRAMO, L.R.; CONKLIN, D.E.; AKIYAMA, D.M. *Crustacean nutrition - Advances in world aquaculture*. Baton Rouge, EUA: WAS, 1997. Cap.3. p.51-70.

CUZON, G.; ROSAS, C.; GAXIOLA, G.; TABOADA, G.; VAN WORMHOUDT, A. 2000 Utilization of carbohydrates by shrimp. In: CRUZ-SUAREZ, L.E.; RICQUE MARIE, D.; TAPIA-SALAZAR, M.; OLVERA-NOVOA, M.A.; CIVERA-CERECEDO, R. (eds) *Avances en Nutrición Acuícola V*. Memorias del V Simposium Internacional de Nutrición Acuícola. Mérida, Yucatán. p.328-339.

DALL, W. and SMITH, D.M. 1986 Changes in protein-bound and free amino acids in the muscle of the tiger prawn *Penaeus esculentus* during starvation. *Marine Biology*, *95*(4): 509-520.

DAVASSI, L.A. 2011 Survival and growth of the freshwater prawn *Macrobrachium rosenbergii* in relation to different nutrients composition. *Journal of Fisheries and Aquatic Science*, *6*(6): 649-654.

DING, X.; NAGARAJU, G.P.C.; NOVOTNEY, D.; LOVETT, D.L.; BORST, D.W. 2010 Yolk protein expression in the green crab, *Carcinus maenas*. *Aquaculture*, *298*(3-4): 325-331.

DISSANAYAKE, A.; GALLOWAY, T.S.; JONES, M.B. 2008 Nutritional status of *Carcinus maenas* (Crustacea: Decapoda) influences susceptibility to contaminant exposure. *Aquatic Toxicology*, *89*(1): 40-46.

DUBOIS, M.; GILLES, K.A.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. 1956 Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3): 350-356.

FAO - FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2012 *FIGIS – Fisheries Statistics – Aquaculture*. [on line] URL: <www.fao.org>.

FOLCH, J.; LEES, M. and STANLEY, G.H.S. 1957 A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, *226*(1): 497-509.

GARCÍA-ORTEGA, A.; KOUSSOULAKI, A.; BOER, H.; VERRETH, J. 2000 In vitro protein digestibility of *Artemia* decapsulated cysts and nauplii, and of microbound diets for larval fish. *Aquaculture Research*, *31*(5): 475-477.

GASTELÚ, J.C.; OLIVEIRA, J.; BRITO, L.O.; GALVEZ, A.O.; MOREIRA, M.G. 2011 Efeito da temperatura e dos alimentos protéico e lipídico nos estádios de maturação ovariana e estágios de muda do *Macrobrachium acanthurus* (WIEGMANN, 1836). *Ciência Animal Brasileira*, *12*(3): 443-455.

GAUQUELIN, F.; CUZON, G.; GAXIOLA, G.; ROSAS, C.; ARENA, L.; BUREAU, D.P. AND COCHARD, J.C. 2007 Effect of dietary protein level on growth and energy utilization by *Litopenaeus stylirostris* under laboratory conditions. *Aquaculture, 271*(1-4): 439-448.

GONZÁLEZ-PEÑA, M.C.G. and MOREIRA, M.G.S. 2003 Effect of dietary cellulose level on specific dynamic action and ammonia excretion of the prawn *Macrobrachium rosenbergii* (De Man 1879). *Aquaculture Research*, *34*(10): 821-827.

HABASHY, M.M. 2009 Growth and body composition of juvenile freshwater prawn, *Macrobrachium rosenbergii* fed different dietary protein/starch ratios. *Global Veterinaria*, *3*(1): 45-50.

HARTNOLL, R. 2001 Growth in Crustacea - twenty years on. *Hydrobiologia*, *449(1-3)*: 111-122.

HAYD, L.A.; LEMOS, D.; VALENTI, W.C. 2014 Effects of ambient nitrite on Amazon river prawn, *Macrobrachium amazonicum,* larvae. *Journal of the World Aquaculture Society, 45*(1): 55-64.

HOLME, M.H.; ZENG, C.; SOUTHGATE, P.C. 2004 A review of recent progress toward development of a formulated microbound diet for mud crab, *Scylla serrata*, larvae and their nutritional requirements. *Aquaculture, 286*(3-4): 164-175.

HOLTHUIS, L.B. 1952 *A general revision of the Palaemonidae (Crustacea, Decapoda, Natantia) of the Americas*. II. The subfamily Palaenominae. *Occasional Papers of the Allan Hancock Foundation*, *12*: 1-339.

JAMES, P.; VASILYEV, R.; SIIKAVUOPIO, S.; KOVATCHEVA, N.; SAMUELSEN, T.A.; MUNDHEIM, H.; CARLEHÖG, M. 2013 The

effects of varying the percentage of herring versus salmon protein in manufactures diets on the survival, meat content, hepatosomatic index and meat sensory quality of adult red king crab *Paralithodes camtschaticus* held in captivity. *Aquaculture*, *416-417*: 390-395.

JENSEN, M.; FITZGIBBON, Q.; CARTER, C.G.; ADAMS, L.R. 2013 Effect of body mass and activity on the metabolic rate and ammonia-N excretion of the spiny lobster *Sagmariasus verreauxi* during ontogeny. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology*, *166*(1): 191-198.

KOROLEFF, F. 1976 Determination of ammonia. In: GRASSHOFF, K.; EHRHARDT, M.; KREMLING, K. (eds) *Methods of seawater analysis*. *2*: 150-157.

KUBITZA, F. 2009 Manejo na produção de peixes. *Panorama da Aquicultura*, *19*(111): 14-27.

LEMOS, D.; NETTO, B.; GERMANO, A. 2006 Energy budget of juvenile fat snook *Centropomus parallelus* fed live food. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, *144*(1): 33-40.

LI, K.; CHEN, L.; ZHOU, Z.; LI, E.; ZHAO, X; GUO, H. 2006 The site of vitellogenin synthesis in Chinese mitten-handed crab *Eriocheir sinensis*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *143*(4): 453-458.

MACIEL, C.R. and VALENTI, W.C. 2009. Biology, fisheries, and aquaculture of the amazon river prawn *Macrobrachium amazonicum*: a review. *Nauplius*, *17*(2): 61-79.

MACIEL, C.R.; NEW, M.B.; VALENTI, W.C. 2012 The predation of *Artemia* nauplii by the larvae of the Amazon river prawn *Macrobrachium amazonicum* (Heller, 1862) is affected by prey density, time of day, and ontogenetic development. *Journal of the World Aquaculture Society*, *43*(5): 659-669.

MAYZAUD, P. and CONOVER, R.J. 1988 O:N ratio as a tool to describe zooplankton metabolism. *Marine Ecology Progress Series*, *45*(3): 289-302.

MAZARELLI, C.; SANTOS, M; AMORIM, R.; AUGUSTO, A. 2015 Effect of salinity on the metabolism and osmoregulation of selected ontogenetic stages of an amazon population of *Macrobrachium amazonicum* shrimp (decapoda, palaemonidae)**.** *Brazilian Journal of Biology*, *75*(2): 372-379.

MELO, A.G. 2003 *Manual de identificação dos Crustacea Decapoda de água doce do Brasil*. São Paulo, Edições Loyola/Museu de Zoologia, USP. 430p.

MIAO, W. and GE, X. 2002 Freshwater prawn culture in China: an overview. *Aquaculture Asia*, *VII*(1): 9-12.

MITRA, G.; MUKHOPADHYAY, P.K.; CHATTOPADHYAY D.N. 2005 Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming. *Aquatic Feeds: Formulation and Beyond*, *2*(1): 17-19.

MORAES-RIODADES, P.M.C. and VALENTI, W.C. 2001 Freshwater prawn farming in Brazilian Amazonia shows potential for economic and social development. *Global Aquaculture Advocate, 4*(5): 73–74.

MORAES-RIODADES, P.M.C. and VALENTI, W.C. 2004 Morphotypes in male Amazon River Prawns, *Macrobrachium amazonicum*. *Aquaculture*, *236*(1-4): 297-307.

NAVARRO, R.D.; DA SILVA, R.F.; RIBEIRO-FILHO, O.P.; CALADO, L.L.; REZENDE, F.P. SILVA, S.S.; SANTOS, L.C. 2006 Morphometric comparison and somatic indexes of lambari prata (*Astayanax scabripinnis* Jerenyns, 1842) in diferente culture systems. *Zootecnia Tropical, 24*(2): 165-176.

NEW, M.B. 2005 Freshwater prawn farming: global status, recent research and a glance at the future. *Aquaculture Research*, *36*(3): 210-230.

NEW, M.B.; D'ABRAMO L.R.; VALENTI W.C.; SINGHOLKA S. 2000 Sustainability of freshwater prawn culture. In: NEW, M.B. and VALENTI, W.C. (eds) *Freshwater prawn farming: the farming of Macrobrachium rosenbergii*. Oxford, UK: Blackwell Science. p.429–443.

PASCUAL, C.; ZENTENO, E.; CUZON, G.; SANCHEZ, A.; GAXIOLA, G.; TABOADA, G.; SUAREZ, J.; MALDONADO, T. and ROSAS, C. 2004 *Litopenaeus vannamei* juveniles energetic balance and immunological response to dietary protein. *Aquaculture*, *236*(1-4): 431-450.

PEZZATO, L.E.; BARROS, M.M.; SAMPAIO, F.G.; FALCON, D.R.; GONÇALVES, G.S.; HISANO H. 2003 Relação energia:proteína dietária para pós-larvas de *Macrobrachium amazonicum*

(Crustacea, Decapoda). *Acta Scientiarum. Animal Sciences, 25*(2): 235-241.

ROSAS, C.; CUZON, G.; GAXIOLA, G.; PASCUAL, C.; TABOADA, G.; ARENA, L.; VAN WORMHOUDT, A. 2002 A energetic conceptual model of the physiological role of dietary carbohydrate and salinity on *L. vannamei* juveniles. *Journal of Experimental Marine Biology and Ecology*, *268*(1): 47-67.

SANTOS, R.C. 2010 O valor energético dos alimentos. Exemplo de uma determinação experimental, usando calorimetria de combustão. *Química Nova*, *33*(1): 220-224.

SIMON, C.J. 2009 Digestive enzyme response to natural and formulated diets in cultured juvenile spiny lobster, *Jasus edwardsii*. *Aquaculture*, *294*(3-4): 271-281.

SMITH, L.L.; LEE, P.G.; LAWRENCE, A.L.; STRAWN, K. 1985 Growth and digestibility by three sizes of *Penaeus vannamei* Boone: Effects of dietary protein level and protein source. *Aquaculture*, *46*(2):85-96.

STUMPF, L.; CALVO, N.S.; DÍAS, F.C.; VALENTI, W.C.; LÓPEZ GRECO, L.S. 2011 Effect of intermittent feeding on growth in early juveniles of the crayfish *Cherax quadricarinatus*. *Aquaculture, 319*(1-2): 98-104.

VALENTI, W.C. 1993 Freshwater prawn culture in Brazil. *World Aquaculture*, *24*(1): 29–34.

VALENTI, W.C. and NEW, M.B. 2000 Grow-out systems - Monoculture. In: NEW, M.B. and VALENTI, W.C. (eds*) Freshwater Prawn Culture: The farming of* ***Macrobrachium rosenbergii***. Oxford. Blackwell Science. p.157-176.

WANG, A-L.; WANG, W-N.; WANG, Y.; SHANG, L-X.; LIU, Y.; SUN, R-Y. 2003 Effect of dietary vitamin C supplementation on the oxygen consumption, ammonia-N excretion and Na^+/K^+ ATPase of *Macrobrachium nipponense* exposed to ambient ammonia. *Aquaculture*, *220*(1-4): 833-841.

WEI-JIAN, Q.; MIN-DONG, C.; YU-ZHI, S.; JUNVING, Z.; ZHENG-JUN, S. 2013 Acute toxicity of two pestides to *Macrobrachium nipponense. Journal of Ecology and Rural Environment*, *29*(5): 676-680.

WICKINS, J.C. and LEE, D.O'C. 2002 *Crustacean Farming: Ranching and Culture*. Wiley-Blackwell. 2ª ed. 464p.

XIE, S.; TIAN, L.; JIN, Y.; YANG, H.; LIANG, G.; LIU, Y. 2014 Effect of glycine supplementation on growth performance, body composition and salinity stress of juvenile Pacific white shrimp, *Litopenaeus vannamei* fed low fishmeal diet. *Aquaculture*, *418-419*: 159–164.

ZANDERS, I.P. and RODRIGUEZ, J.M. 1992 Effect of temperature and salinity stress on osmoionic regulation in adult and on oxygen consumption in larvae and adult of *Macrobrachium amazonicum* (Decapoda, Palaemonidae). *Comparative Biochemestry and Physiology*, *101*(3): 505-509.

ZAR, J.H. 2009 *Biostatistical analysis*. . 5ª ed. Prentice Hall. 960p.