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Amino acid mobilization and growth of juvenile *Octopus maya* (Mollusca: Cephalopoda) under inanition and re-feeding

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ABSTRACT

Octopus maya is an endemic cephalopod from the Yucatán Peninsula in Mexico, with interest to develop their commercial culture. Like all cephalopods, protein is an important nutrient both for growing and energetic metabolism. This condition results in a high demand for protein and specifically for certain amino acids (AAs). In this study it is examined the effects of inanition and re-feeding on growth and AA content in soft tissue to detect the amino acid (AA) mobilization to identify the principal metabolic reserves in juvenile *O. maya*. After 25 days re-feeding of all starved groups, the octopuses were unable to reach the similar weight as the control group. However, SGR of some groups were greater than that of the controls, although the differences were not significant due to variability in the data. Therefore, it is assumed that the juveniles of *O. maya* would need a longer period of time to reach the control group. It is therefore demonstrated that juveniles of *O. maya* have a wide plasticity to tolerate, at least 10 days of food deprivation without any apparently physiological damage. Moreover, during inanition the juveniles of *O. maya* used preferentially Thr, Phe, Ile, Ala, Glu and Ser, suggesting a strong mobilization of both essential and non essential AA to maintain the homeostasis. Prove of that is that survival of the animals during fasting and re-feeding period was not affected by treatments.

1. Introduction

The octopus *Octopus maya* (Voss and Solís-Ramírez, 1966) is an endemic cephalopod from the Yucatan Peninsula in Mexico (CONAPESCA, 2008) being one of the most important resources in the fishery sector. Earlier studies have demonstrated that *O. maya* has a high potential for aquaculture due to their well adaptability to captivity conditions (Van Heukelem, 1977; Boletzky and Hanlon, 1983; Hanlon and Forsythe 1985; DeRusha et al., 1989) and direct development from hatching without a larval transition (Boletzky, 1974). Moreover, *O. maya* shows larger eggs than those observed in other cephalopods (up to 17 mm). They are able to accept dead preys or formulated diets even during the first life cycle stadiums (Aguila et al., 2007), resulting in high growth rates (up to 8% wet body weight, wBW, per day) as a result of high feeding rates (FR) and food conversion efficiencies (FC) (Van Heukelem, 1983; Hanlon and Forsythe, 1985; Domingues et al., 2007; Rosas et al., 2007).

Dependence on natural diets together with a long larval period has been the bottleneck in the cephalopod aquaculture (Domingues, 1999; Lee, 1994; Iglesias et al., 2000; Iglesias et al., 2007), due to the high cost associated to obtain the juveniles for the growth-out phase (Nabhitabhata, 1995). Therefore, several formulated diets have been tested in *O. maya*, efforts that have resulted in moderate growth rates, below to those achieved with natural diets (Aguila et al., 2007; Domingues et al., 2007; Rosas et al., 2007; Rosas et al., 2008). Similar results were found in cuttlefish (Castro et al., 1993; Castro and Lee, 1994) concluding that protein quality is an important factor to get high digestibility coefficients and better growth rates, being necessary to generate more information on the amino acid requirements to promote higher growth rates. Le Bihan et al., (2006) found that cuttlefish fed with surimi from the shrimp *Crangon crangon* soaked in fish silage resulted in better growth and conversion rates than those fed a similar diet without the fish silage. The difference between both diets was the peptides and AA present in the fish silage. However, it is not clear if the peptides and AA promoted a better assimilation or due to the solubility inherent to the soluble protein resulted in a higher ingestion rate due to a better feed attractability and palatability.

Like all cephalopods, *O. maya* is a carnivorous species and protein is an important nutrient for tissue accretion and energy source (Segawa and Hanlon, 1988; Rosas et al., 2007), therefore, cephalopods require high amounts of protein and amino acids (AAs) for optimum growth (Lee, 1994; Domingues et al., 2005; Solorzano et al., 2009). However, at present the protein and amino acid requirements have not been determined for *O. maya*.

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In rearing conditions octopuses are able to survive long periods without food due to several stress factors like system failures (pumping and power energy problems) or density stress (Domingues et al., 2010). After a period of food deprivation organisms are able to catch up their weight by increasing their growth rates as a compensatory growth. However when food deprivation is for longer periods the organisms are unable to catch up their weight compared to well feed siblings, calling this physiological phenomenon as the point of no return. Therefore when culturing organisms that undergo long periods of food deprivation it is important to know those limits to be able to catch up their lost weight without fatal consequences. Taking into consideration that octopuses have a low lipid reserves it is possible to think that during fasting condition octopuses will mobilize amino acids as the principal source of energy in attempt to maintain the homeostasis (Vidal et al., 2002; Villanueva et al., 2004; Iglesias et al., 2006; Vidal et al., 2006; Grigoriou and Richardson, 2009; Solorzano et al., 2009). Recently, Villanueva et al., (2004) determined that Leu, Lys and Arg represent almost half of the essential amino acids (EAAs) required by *Octopus vulgaris*, *Sepia officinalis* and *Loligo vulgaris* during early life stages. According to Akagi and Ohmori (2004) Thr could be the optimum substrate for D-lactate formation, suggesting that the principal biochemical pathway to obtain energy in cephalopods could be through gluconeogenesis, and therefore Thr may be limiting in the diet from these species as indicated by D'Mello (2003). Domingues et al. (2007) found similar results for the juvenile *O. maya*.

In the present study it was evaluated the inanition and re-feeding effects on the overall performance to detect the amino acid (AA) mobilization and overall performance for the juvenile *O. maya*.

2. Materials and methods

2.1. Broodstock collection and rearing of juvenile octopuses

Wild females from *O. maya* were caught on the coastal area from the Yucatan Peninsula (21° 9′ 55″ N, 90° 1′ 50″ W) using artisan lines with blue crabs *Callinectes* spp. as bait. Females were transported in 120 L circular tanks containing seawater to the UMDI-UNAM laboratory, situated at 300 m inland. In the laboratory, the females were also maintained in 250 L black tanks until the eggs were layed (Moguel et al., 2010). Hatchlings were fed for 15–20 days with living Artemia adults and crab paste until they passed 0.5 g living weight (Rosas et al., 2008; Avila-Poveda et al., 2009; Moguel et al., 2010).

2.2. Feeding acclimation of post-hatching

Juvenile *O. maya* of 32 days post-hatching (DPH) were weighed (wet body weight (wBW) mean value of 0.63 ± 0.10 SD g; N = 120 octopuses). Juveniles, dried with a paper towel, were individually weighed in an electronic balance (± 0.001 g), randomly individualized (1 L tanks), and distributed in six experimental groups (N = 20 animals per group). Juveniles were fed a single diet consisting in fresh crab meat mixed with 5% natural gelatin, fed at 30% of wBW (Rosas et al., 2008; Quintana et al., 2010) twice a day at 0900 and 1700 h. After 12 days all octopuses from the six groups were individually weighed again (wBW; mean value of 0.90 ± 0.21 SD g; N = 65 octopuses).

2.3. Inanition and re-feeding treatments

To determine the effect of food deprivation on the overall performance of juvenile *O. maya*, six groups (N = 20 per group) were deprived of food for 2, 4, 6, 8 and 10 days, while the sixth group was maintained with food as a control under a similar protocol as during the acclimation period. Inanition began at the same time for all octopuses from five experimental groups. At the end of each fasting period all octopuses from the six groups were weighed. After the inanition period, the octopuses from each treatment were re-feeding for 25 days, with the same diet and under similar protocol as explained above. The octopuses were weighed at the end of re-feeding period.

2.4. Growth rate

Growth rate (g day^{-1}) was determined as the difference between the initial and final weight for octopuses during acclimation period (days 0 and 12), at the end of each fasting periods (2, 4, 6, 8 and 10 days) and latest day of re-feeding period.

Specific growth rate (SGR, %day^{-1}) *was determined as:*

$$SGR = [(\ln W_2 - \ln W_1) / t] * 100$$

where W_2 and W_1 are the final and initial wet weights of the octopus (obtained during acclimation period, fasting periods or re-feeding period), ln is the natural logarithm, and t the duration of each time period (days).

2.5. Amino acid analysis

The amino acid content (g AA/100 g protein) of individual samples from juvenile *O. maya* arms (N = 3 octopus per treatment) from the acclimation period (control on day 12), and after the inanition period (on the 4 and 8 days food deprivation) was determined. Defatted tissue samples (20 mg) were hydrolyzed with 200 μL of 6 N HCl and 0.06% phenol in a closed vial and heated to 110 °C for 24 h. Amino acid profiles were determined following Waters AccQ-Tag™ procedure as follows: (1) Hydrolyzed samples were dried in a termic monoblock at 60 °C with nitrogen and rehydrated with 1 mL water HPLC grade. (2) Samples were then filtered (0.45 μm) and maintained at −20 °C until used. (3) Samples were derivatized using the Waters system AccQ-Tag™. (4) Samples were chromatographed through a reverse phase column (3.9 × 150 mm) 4 μm Nova Pak™ C-18, using the water-acetonitrile gradient recommended by the Waters AccQ-Tag™ system (Milford, MA, USA), in a Waters™ HPLC and a fluorescence detector (excitation and emission wavelength; 250 and 395 nm, respectively). (6) Analyses were conducted at a constant temperature of 39 °C. (7) HPLC signal calibration and standard curves were obtained by using an amino acid standard solution at three different concentrations containing from 18.75 to 150 pmol of each amino acid. Taking into account that Met and Cys were partially destroyed by acid hydrolysis, the results of both amino acids were taken with caution.

2.6. Statistical analysis

A one way analysis of variance was used to known the effect of fasting period on wet weight and growth rate (SGR, %day^{-1}), and when applied, a multiple comparison of means (Tuckey test) was used to known differences between groups. A significance level of $P<0.05$ was established. All statistical analyses were performed using the Statistica® program (Version 6.1).

3. Result

3.1. Acclimation period

No differences were detected among during the acclimation period. Mean values of 0.63 ± 0.02 g, 0.90 ± 0.04 g and 2.81 ± 0.4% day^{-1} were obtained for initial, final and specific growth rate (SGR) respectively, during acclimation period, (Table 1; Figs. 1 and 2).

3.2. Inanition effect on growth

After the food deprivation period, the final weight showed differences between treatments with 9% of wet weight lost in animals