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Feed intake, molt frequency, tissue growth, feed efficiency and energy budget during a molt cycle of mud crab juveniles, *Scylla serrata* (Forskål, 1775), fed on different practical diets with graded levels of soy protein concentrate as main source of protein

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ABSTRACT

There has been growing interest in the development of mud crab aquaculture in New Caledonia. However, for this to become established at a commercial level, a cost-effective formulated feed based on internationally-available ingredients needs to be developed. We have evaluated the optimal dietary protein content for juvenile crabs, *Scylla serrata* (Forskål, 1775), using a series of diets with a protein content ranging from 27 to 49% and soy protein concentrate (SPC) as the main protein source. For this purpose, 54 individually housed crabs were allocated to five dietary treatments (n = 10 or 11). The crabs were fed ad libitum, for 81 days with the allocated diets. The apparent digestibilities of dry mater, crude protein and energy were high (96.2–97.3%), irrespective of the diet. The voluntary feed intake (VFI) of crabs widely varied from 46 to 220 g kg^{-1} of fresh initial body weight per week (iBW $week^{-1}$) whatever the diet. However, SPC intake and protein intake increased significantly with dietary protein content up to the diet with 40% crude protein, but did not increase further with diets containing 44% and 49% crude protein. The cumulative molts were strongly affected by the VFI levels or energy intake and also, to a lesser extent, by the levels of SPC or protein in diets. Two phases in tissue gain were observed after ecdysis: an initial deposition phase lasting around 30 days followed by a plateau which lasted until the next molt. The daily tissue growth was 16.5% of dry body weight (dry BW) one day after ecdysis and dramatically decreased to 3.6% of dry BW over the first 10 days, then decreased more slowly to the minimum value of 1.3% of dry BW over the next 70 days. During the course of experiment, the best growth (tissue growth and molt frequency) and the best feed efficiency (FCR, PER, retention of proteins and lipids) were obtained with crabs fed on the diet with 40% crude protein. This result was confirmed by a bioenergetic study which showed significantly higher allocation of the energy intake for growth (RE) of crabs fed on diet 40% crude protein. Finally, under our experimental conditions, 1 kg of juvenile crabs required 6.5 ± 1.1 g of protein per day. This level was obtained with the diet SPC-42 that contained 40% of protein of which almost three quarters were derived from SPC. Two hypotheses are proposed to explain the negative effect of high level of SPC or protein on growth and feed efficiency for crabs fed on in diets containing 52% and 60% SPC.

1. Introduction

Mud crab aquaculture has been cultured for many years in Southeast Asia, based primarily on the capture and fattening of juvenile crabs from the wild, particularly in Vietnam where production is increasing quickly (Petersen et al., 2013). There is an unmet demand for mud crabs and this has led to over-exploitation of wild populations in many areas (Vega-Villasante et al., 2007). Difficulties in obtaining wild caught juveniles for farming operations, and concerns about further over-exploitation have led to major investments in the development of new hatchery techniques (Allan and Fielder, 2003). For the past few years, techniques for breeding in captivity and larval rearing have been developed allowing the production to triple in 5 years (from 10,000 t in 2004 to 30,000 t in 2009) (John and Paul, 2012). However, the substantial crab farming operations which now exist throughout Southeast Asia are still mainly based on wild caught crablets (Allan and Fielder, 2003) and animals in captivity are fed with excessive amounts of trash fish which contaminate the rearing water and lead to high mortality rates (Linder, 2005).

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In New Caledonia, there is a strong political wish to diversify aquaculture, which until recently, has been based on blue shrimp (*Litopenaeus stylirostris*) farming. Among the different options available, the mud crab, *Scylla serrata*, is regarded as having great potential for aquaculture through its farming characteristics and its high economic value (Shelley and Lovatelli, 2011). However, the possible development of crab farming in New Caledonia will require the development of an artificial feed that can be produced from selected and controlled raw materials that are available on the international market.

Therefore, it is essential to assess the nutritional requirements of *S. serrata*. A few studies have been carried out on this subject. Sheen and Wu (1999) showed better utilization of lipids by the crab than by shrimp through a study in which they measured the growth response of juvenile crabs that has been fed diets with a range of inclusion levels of a mixture of cod liver oil and corn oil. Later, Sheen (2000) indicated the importance of a dietary source of cholesterol and polyunsaturated fatty acids (22:6n−3, 20:4n−6, 18:3n−3) for the healthy growth of juvenile crabs. Furthermore, a series of studies have been carried out to measure the digestibility of several potential ingredients for formulated feed for crabs (Catacutan et al., 2003; Truong et al., 2009). These results showed that crabs were able to digest many different ingredients, in particular fiber and protein from plants. Finally, the optimal protein inclusion level in the diet has been the subject of two studies (Catacutan, 2002; Unnikrishnan and Paulraj, 2010). Both studies concluded that the optimal protein concentration (a mixture of fish meal and soybean meal) in the feed for the best growth rate is between 30% and 47%.

Feed for aquaculture generally requires large quantities of ingredients from the sea and this sector is the largest consumer of fish meal among the animal husbandry subsectors (Shepherd and Jackson, 2013). Indeed, fish meal is the primary source of protein and energy in most aquafeeds and accounts for up to 75% of the mass of total aquaculture feed (Tacon and Dominy, 1999). In 1989, fish meal used in aquaculture accounted for only 10% of global production (FAO, 2000), this had dramatically increased to 73% by 2010 (Shepherd and Jackson, 2013). Production of carnivorous species, promoted in aquaculture, particularly marine crustaceans, marine finfish and salmonids, requires a lot of fish meal (Rana et al., 2009). The cost of fish meal is increasing over time as a result of the increased competition for available supplies (Sookying et al., 2013). Hammersmith Marketing Ltd (2008) indicated that the price of fish meal increased by about 62.9% from August 2005 to June 2008. In these conditions, feed can account for as much as 40–60% of the cost in aquaculture production (Hertrampf and Piedad-Pascual, 2000). In this framework, partially or completely shifting from a fish meal protein source to more sustainable protein sources in formulated feeds is a priority for sustainable mud crab aquaculture development (Christensen et al., 2004; Tuan et al., 2006).

Many plant-based ingredients are used as a source of proteins in aquafeeds. Among them, soybean meal (SBM) is well known for its relatively high protein content, well balanced amino acid profile, and stable market supply, as well as its reasonable cost (Amaya et al., 2007a, b; Davis and Arnold, 2000). Chen et al. (1994) reported that up to 33% fish meal protein could be replaced by soybean cake for juvenile mitten crab without reducing growth. Recently, Luo et al. (2011) found that 30% inclusion of soybean meal and rapeseed meal mixture (1:1 ratio) could replace 40% fish meal in diets for Chinese mitten crab without impairing growth performance and feed utilization. However, the inclusion of SBM in fish diets has been limited by relatively high levels of heat stable antinutritional and antigenic factors including protease inhibitors, oligosaccharides (e.g., stachyose, raffinose), saponins, isoflavones, phytate, and tannins (Francis et al., 2001). Although heat and enzyme treatments can neutralize some of these compounds, they are still a significant problem when including SBM in aquaculture feeds (Gatlin et al., 2007). Soy protein concentrate (SPC), although more expensive than SBM, does not contain the alcohol-soluble fraction present in SBM and has a higher essential amino acid concentration. It also has greater nutrient digestibility compared with SBM and can be included at much higher concentrations in diets of piscivorous marine species (Bureau et al., 1998; USSEC, 2008). Recently we showed that SPC is well digested and able to replace the fishmeal as the main source protein for crab juveniles *S. serrata* (unpublished).

Most of the previous studies have considered growth rate as gains in fresh weight. Fresh weight changes follow a basic pattern through the molt cycle, i.e. large and abrupt increases associated with rapid water uptake at ecdysis; further moderate gains associated with carapace mineralization and tissue growth during postmolt and relative stabilization of fresh weight during intermolt until the onset of the successive ecdysis (Heasman, 1980). An increase in tissue mass, on the other hand, is a continuous process occurring through the molt cycle (Freeman, 1990). The tissue growth can be measured as the increase in dry weight as tissues lose water in direct proportion to their gain in dry mass (Passano, 1960). In this paper we report on the response of juvenile crabs *S. serrata* to graded levels of soy protein concentrate and respective dietary proteins on growth (somatic growth and molt frequency), feed efficiency (feed conversion efficiency-FCR; protein efficiency ratio-PER; retentions of protein, lipid and energy) and energy budget.

2. Materials and methods

2.1. Crabs and holding facilities

All the juvenile crabs, *S. serrata*, using for our dietary trials, were collected from the mangrove habitat surrounding the experimental station (21°51′50″ S, 166°3′0″ E) in the Boulouparis district, New Caledonia. Seventy juvenile crabs were caught using a hand net racket and transferred to the laboratory and acclimated into 8 circular composite tanks (capacity 500 L) for one week prior to the beginning of the experiment. The crabs were examined to identify their molt stage using the criteria and methodology described by Drach and Tchernigovtzeff (1967), Freeman et al. (1987) and Heasman (1980). Fifty four of the crabs were identified as being in intermolt stage and were selected for the experiment. These crabs were randomly and individually assigned to fifty four experimental rectangular polyethylene tanks (30 × 20 × 30 cm) covered with black lids. After being dried in soft paper and cotton cloth, each crab was weighed to the nearest 0.01 g by the Scientech®, SL 3000 balance and its carapace width was measured to the nearest 0.01 mm.

Each experimental tank was continuously supplied with seawater running from a polyethylene reservoir (2000 L capacity) at the rate of 0.19 L min^{-1}. The water pumped from the lagoon was filtered through a 25 μm net bag into the reservoir. The temperature was automatically controlled using a 300 W, French heater. The temperature in the experimental tanks was measured every 3 h using an automatic recording probe. During the experimental period, water environmental parameters were maintained with: T°C = 21.5 ± 2.5, S ‰ = 34.0 ± 1.5, pH = 7.97 ± 0.09 and O_2 = 4.48 ± 0.16 mg L^{-1}.

2.2. Diet preparation and composition

The crabs were fed on five different experimental diets (SPC-12; SPC-32; SPC-42; SPC-52; and SPC-60) formulated with graded levels of SPC as a main source of protein (Table 1). The diets were produced in the laboratory using the following procedure: the dry ingredients were ground up in a grinder (Retsch® SR200, Gemany) with a 1 mm screen. The meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca® RM-90-135, USA) until the consistency was suitable for pelleting. The mixture was then extruded through a 3 mm die in a meat grinder. Pellets were steamed for 15 min and stored at −20 °C before use (Truong et al., 2008; Unnikrishnan and Paulraj, 2010). The ingredient compositions and proximate nutrient contents of experimental diets are shown in Table 1.