Comparative evaluation of different carbohydrates as dietary energy source for the mud crab *Scylla serrata* megalopa

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Abstract. The study was conducted to determine the type of dietary carbohydrate source essential for the survival, molting, efficient growth and body protein content of the megalopa larvae of mud crab *Scylla serrata.* Following a completely randomize design, eighty (80) *S. serrata* megalopa larvae were fed diets containing different carbohydrate sources: (i) starch, (ii) sucrose, (iii) glucose and (iv) dextrin until they were able to molt into the crab instar I stage. The feeding trial results showed no significant difference ($p<0.05$) in the survival, weight gain and protein efficiency ratio of the megalopa receiving the diets with different carbohydrate sources. On the other hand, early occurrence of molting and higher body protein content was observed in megalopa fed with the starch containing diet. Crab larvae require carbohydrate as energy source and as an precursor of chitin formation in the shell. With respect to survival and growth performance, present results indicate that complex carbohydrate (starch) is the best form of carbohydrate to promote better survival, molting and growth performance of *S. serrata* megalopa. The present findings suggest the utilization of complex starch as an energy source in the formulation of diets for the megalopa larvae of mud crab, *Scylla serrata*.

Key Words: nutrition, polysaccharides, crustacea, molting.

Introduction. Carbohydrates are essential macronutrients in the diet together with proteins and lipids. They are considered as major sources of energy and are vital for the development of a well-balanced diet for aquatic animals (Radford et al 2007).

Eelier studies have shown that different carbohydrate sources of dietary ingredient for fish and crustaceans could elicits different physiological and growth responses (Kumar et al 2005; Simon & Jeffs 2013; Ochoa et al 2014; Traifalgar et al 2012). Further, these studies have shown that carbohydrate could be used as a cheap source of energy (Radford et al 2007) and has been regarded as a potential alternative to lipids as energy source to promote protein-sparing effects, efficient protein utilization and deposition in farmed aquatic animals (Kjaer et al 2008; Shiau & Pong 1991). Though most of the previous studies reported were promising but most of which were designed to assess the overall response of adult animals to dietary carbohydrate and there have been scarcity of information on the dietary value of carbohydrates on the early larvae of most aquatic animals.

Recently in the tropics the aquaculture of mud crab, *Scylla serrata* has received much attention due to the high demand in the market but the expansion of culture has been limited with the lack of appropriate hatchery technology for this species. The slow progress in the development of a stable and economically feasible hatchery for this species could be attributed to the lack of information regarding the larval biology and basic nutritional physiology. Like most other crustacean's knowledge on larval nutritional requirement have been limited and *S. serrata* carbohydrate utilization has been poorly-understood. There have been a good number of works related to the investigation of carbohydrate in the diets of *S. serrata* but all of which were designed for the juveniles or adult crabs (Catacutan 2002; Catacutan et al 2003; Bou et al 2014; Truong et al 2008; Niu et al 2012; Safari et al 2014).

Crustacean larvae is known to exhibit rapid growth associated with molting however this process also consumes nutrient reserves of the larvae. The larvae exuvia is made of chitin with the glucose amine as the main molecular backbone. The precursor of glucosamine in crustacean is glucose and protein (Akiyama et al 1992; Niu et al 2012; Safari et al 2014). These earlier studies suggest the importance of carbohydrate in the dietary requirement of crustaceans but information regarding the form of sugar or carbohydrate that the larvae are able to utilize efficiently has been limited. The present study evaluates the different forms of carbohydrate as a dietary source of energy for the megalopa larvae of *S. serrata* to attain optimum growth and survival.

Material and Method

Experimental animals and design. *S. serrata* megalopa were obtained from the UPV Multi-Species Hatchery, Miagao, Iloilo. Following a completely-randomized design, eighty (80) 250 mL containers were filled with water (25 ppt salinity), provided with ample aeration and each tank were stocked with a single megalopa. Daily water change, removal of organic debris and excess feeds were done to ensure optimal water condition. The water used was treated with 2 ppm oxygen tetracycline to make sure that any mortality in the experiment is not brought by diseases. Treatments consist of four diets incorporated with different carbohydrate sources, namely; (i) starch, (ii) sucrose, (iii) glucose, and (iv) dextrin. All containers were inspected daily for mortalities and molting of the experimental meagalopa larvae. Dead megalopa were immediately removed and molted ones recorded after detection.

Diets and feeding. Four semi purified, casein-gelatine based test diets were formulated which differ only in their carbohydrate component (Table 1). The diets were prepared and passed through a sieve of 100 µm, collected and stored in the refrigerator until used. Megalopa were fed with test diets containing different carbohydrate sources. Each megalopa were allotted feed equivalent to 30% of its body weight per day. Feeding was conducted twice daily and uneaten feeds collected 3 hours after the feed was given.

Carcass protein composition of the megalopa carcass was analysed using Biuret Test and the lipid was quantified following by bligh and dyer as described in the work of Janairo et al (2011).

Table 1

Feed formulation of the basal diet containing wheat flour (starch) as a carbohydrate source

Ingredients	*(g/100 g diet)*
Casein	39
Gelatin	31
[3]Wheat flour	15
Soybean lecithin	3
Fish oil	5
Cholesterol	1
[1]Vitamin mix	2
[2]Mineral mix	1
Agarose	3
Proximate analyses (%)	
Dry matter	90.0
Crude protein	60.0
Carbohydrate	18.0
Crude lipid	8.0
Ash	2.0

[1]Vitamin mix - Vitamin A: 1,200,000 IU kg^{-1}; Vitamin D3: 200,000 IU/kg; Vitamin E: 20,000 mg kg^{-1}; Vitamin B1: 40,000 mg kg^{-1}; Calcium pantothenate: 20,000mg kg^{-1}; Biotin: 40 mg kg^{-1}; Folic acid: 1,800 mg kg^{-1}; Ethoxyquin: 500 mg kg^{-1}.

[2]Mineral Mix – Fe: 40 000 mg kg^{-1}; Mn: 10,000 mg kg^{-1}; Zn: 40,000 mg kg^{-1}; Cu: 4000 mg kg^{-1}; I: 1800 mg kg^{-1}; Co: 20 mg kg^{-1}; Se: 200 mg kg^{-1}.

[3]Carbohydrate source. Other carbohydrate sources tested include glucose, sucrose and dextrin.

Statistical analysis. All data except for survival were analysed using one-way ANOVA. Differences among the means were tested for significance using Tukey's multiple range test and differences among the treatments were considered significant at the $p<0.05$ level. Results for survival were analysed by Chi-square test statistics.

Results. Influence of the different carbohydrate types on the successful molting of megalopa to crab instar 1 is presented in (Table 2). Significantly highest survival or successful metamorphosis to crab instar 1 has been found in treatment receiving the whole starch as the dietary carbohydrate source. The lowest survival obtained was observed in treatments receiving the Dextrin as the carbohydrate source but this is not statistically different ($\alpha=0.05$) from treatment with sucrose and glucose.

Table 2

Survival and mortality of megalopa along with the proportion that survived molting to crab instar I

Treatment	*Numbers of megalopa*			*Survival percentage (%)*
	Survived	Dead	Total	
I (starch)	8	12	20	40.0*
II (sucrose)	4	16	20	25.0
III (glucose)	3	17	20	15.0
IV (dextrin)	2	18	20	10.0
Total	17	63	80	-

* - indicate statistical significance among the other treatments.

With respect to molting, the intermolt duration of the *S. serrata* megalopa receiving the diets with different carbohydrate forms were observed to be asynchronous. Cumulative molting of the treatments with respect to time exhibited a pattern that the megalopa fed with diet containing starch and glucose as the carbohydrate sources were faster to metamorphose to the first crab instar I, as compared to the other treatments (Figure 1). With respect to the number of days it took for the megalopa to molt, the shortest duration was observed for treatments fed with starch and glucose. This was followed by megalopa fed diets containing sucrose and dextrin. Nevertheless, general pattern of molting showed that megalopa fed with starch both molted first and exhibited the shortest molting period.

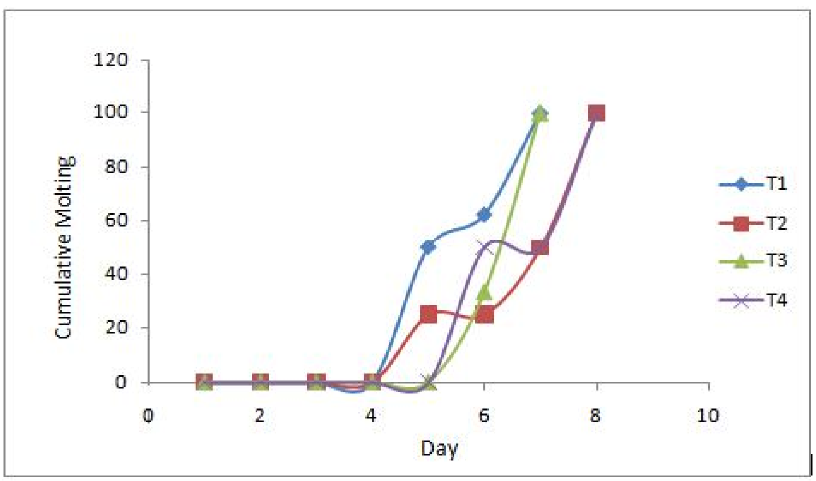

Figure 1. Cumulative molting of megalopa (%) to crab instar 1 with respect to time (days). T1 = starch, T2 = sucrose, T3 = glucose, T4 = dextrin.

Results on weight gain (Figure 2) and protein efficiency ratio (Figure 3) showed that the treatments have no significant ($\alpha=0.05$) influence on these biological parameters.

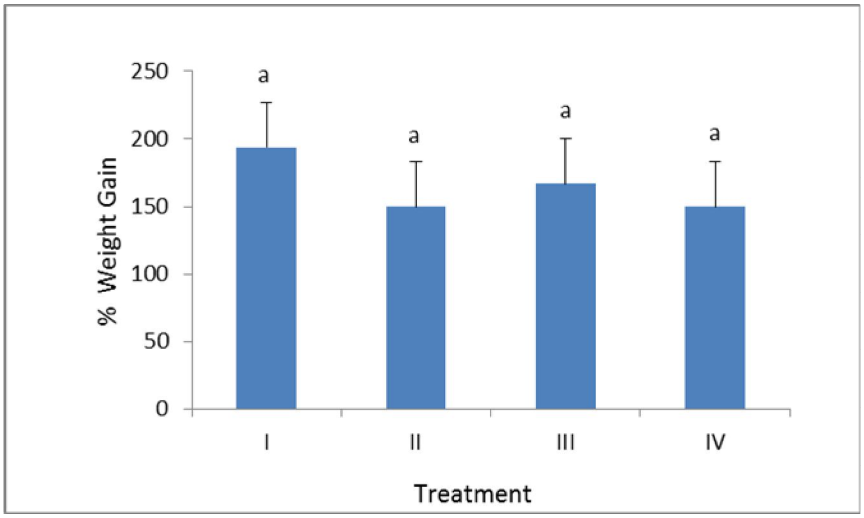

Figure 2. Mean Percent Weight Gain of Megalopa fed with different carbohydrate sources. Treatments are represented by the different carbohydrate sources used: I = starch, II = sucrose, III = glucose, IV = dextrin. Bars with similar superscripts are not significantly different at $p<0.05$.

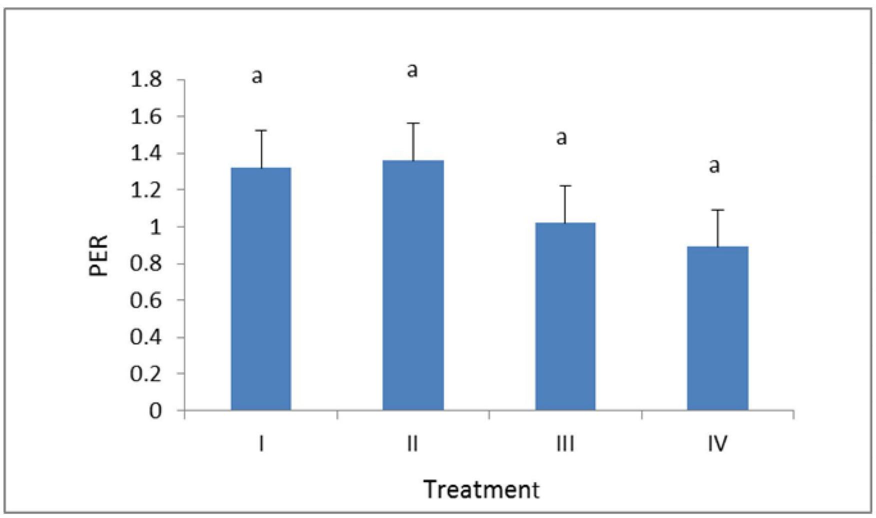

Figure 3. Protein Efficiency Ratio (PER) of Megalopa fed with different carbohydrate sources: I = starch, II = sucrose, III =glucose, IV =dextrin.

Analysis of the body protein showed that megalopa fed with diet containing starch, a complex carbohydrate has significantly higher protein content than the other groups fed with simpler carbohydrate sources (Figure 4). Further, the results showed that growth and PER were not influenced by different dietary carbohydrate sources.

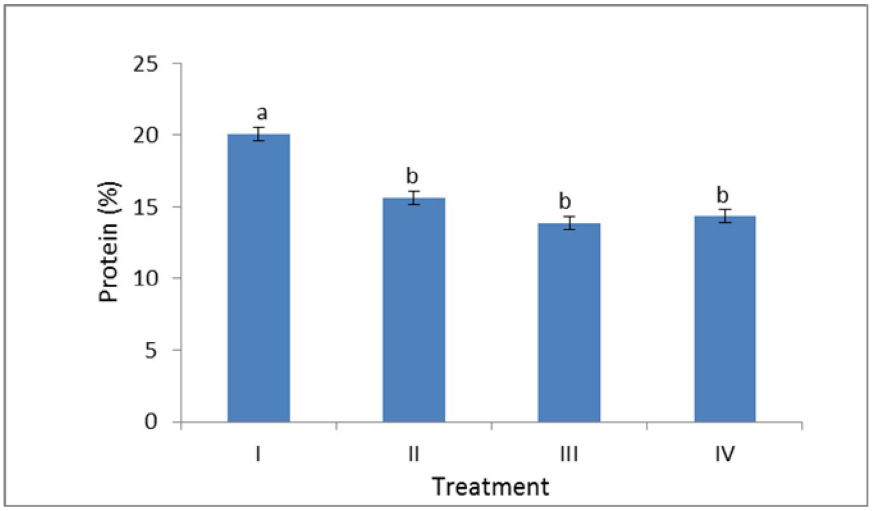

Figure 4. Body protein content of megalopa fed with diets containing different carbohydrate sources.

Discussion. Dietary carbohydrate utilization of mudcrab *S. serrata* megalopa is still unknown and like most other crustaceans, they have been considered as aquatic species having no particular requirement for carbohydrates (Fegan 2004). However, several studies have shown that mud crabs are able to digest and utilize a wide range of carbohydrate sources including plant-based materials (Catacutan et al 2003; Anderson et al 2003). Further evidence suggests that *S. serrata* larvae and adult are equipped with the digestive capacity to digest carbohydrates in feeds (Serrano & Traifalgar 2012). Currently the increasing global interest to reduce feed prices and reliance on protein-rich feed ingredients, research on carbohydrates as dietary energy sources to promote efficient protein utilization in crustacean has gain much attention. The present study, evaluates the effecicacy of different forms of dietary carbohydrates as a dietary energy source for *S. serrata* megalopa larvae. To the best of our knowledge, this is the first time that the carbohydrate nutrition of megalopa larvae of *S. serrata* was evaluated. Results of the feeding trail suggest that among the different forms of dietary carbohydrate the starch as a complex form of carbohydrate elicited better survival for the megalopa larvae. This indicates that the larvae are not able to tolerate digested form of sugars that include glucose, dextrin and sucrose. Also, this suggests that at the megalopa stage *S. serrata* has already the capacity to digest and utilize complex form of carbohydrate. This finding conforms to the previous report of Johnston (2003) showing that earlier stages of crustaceans have higher carbohydrases and cellulase activities. The capacity of *S. serrata* megalopa larvae to digest complex carbohaydrate as evidenced by the presence of high gut amylase activity has been also reported by Serrano & Traifalgar (2012). Similar to the present results, Niu et al (2012) reported that complex carbohydrate, wheat starch promoted higher survival in shrimp *Penaeus monodon* compared to those fed with glucose and dextrin diets.

Similar trend on the influence of dietary carbohydrate types on molting and metamorphosis of *S. serrata* megalopa were also observed. Metamorphosis time to the crab instar was found to be faster in individuals fed with starch as compared to those fed with simpler carbohydrate sources. Megalopa fed diets containing simple sugars exhibited significantly slower development. The early occurrence of molting in individuals fed with dietary starch can be attributed to the high apparent digestibility of this complex carbohydrate source as reported in previous studies (Catacutan et al 2003; Truong et al 2008; Simon 2009; Genodepa et al 2004). In addition, the higher survival after metamorphosis and the faster time to molt suggest that the complex carbohydrate has sustained the energy needs of the larvae to undergo molting. Metamorphosis requires a significant amount of energy and if not satisfied this could result to death. The low survival upon metamorphosis in treatments with simple dietary sugars suggests that these sugars cannot support the energy requirement of the megalopa larvae. Similar findings were also observed by Niu et al (2012) in *P. monodon* that molting mortalities were found associated with the diets containing glucose as the dietary carbohydrate source. Better performance of megalopa fed the starch as dietary carbohydrate could be attributed to the low glycemic index of complex carbohydrate. Complex carbohydrates are able to delay the time for glucose to reach its maximum concentration, thus prolonging glucose efflux rate in the body, ensuring high availability of glucose to the tissues for efficient metabolic energy utilization (Deng et al 2001). In contrast simple sugars in the diet could result to a rapid rise in available glucose that could block cellular transport receptors thus in effect lowering the availability of glucose to the cell.

In terms body protein, the present results showed higher protein content in individuals fed with starch as compared to the other treatments receiving the simple sugars in the diet. This finding is comparable to those found by Niu et al (2012) with shrimp *P. monodon* showing highest body and muscle protein content in treatments fed with wheat starch containing diets. The higher body protein found on crustaceans fed with complex carbohydrate sources is being attributed to the ability of this compound to spare protein for energy production in the body (Erfanulla & Jafri 1995; Lin et al 1997; Niu et al 2012). Complex carbohydrates are slowly but continually digested to form glucose resulting to a higher availability for metabolic energy production thus sparing protein for energy generation (Bages & Sloae 1981; Catacutan 2002; Young et al 2006). In contrast, simple sugars in diet are known to flood the cellular transport receptors and compete with the absorption of several amino acids in the intestinal absorption sites (Andrews et al 1972; Shiau & Pong 1991) resulting to poor utilization of both sugar and amino acid to support growth. Overall these earlier findings are in agreement with the present findings, suggesting the importance of complex starch as a better dietary energy source for efficient metamorphosis and survival of *S. serrata* megalopa.

Conclusions. The present findings indicate that *S. serrata* megalopa could utilize efficiently complex carbohydrate (starch) as compared to simple sugars (monosaccharides and disaccharides, dextrin) as dietary energy source to attain higher metamorphic survival to the crab instar stage.

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