

Article

Feed Efficiency, Tissue Growth and Energy Budget Changes during the Molting Cycle of Juvenile Mud Crab, *Scylla Serrata*: Effects of Dietary Proteins, Fishmeal versus Soy Protein Concentrate

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Abstract: Three isoenergetic diets differing on their fishmeal/ soy protein concentrate (SPC) ratio were assessed on tissue growth and energy budget of juvenile crabs *Scylla serrata* in postmolt stages (PMolt) and in intermolt stages (IMolt). The average growth rate on dry matter basis, were $2.064 \pm 0.324\%$ and $0.492 \pm 0.08\%$ initial BW.day⁻¹ during PMolt and IMolt stages respectively. The efficiency in feed conversion (FCE, %), protein retention (PRE, %) and energy retention (ERE, %) were similar for the 3 experimental diets. However, FCE, PRE and ERE in PMolt stages were 4 to 5 times higher than in IMolt stages. Feed intake, energy and protein required for growth in PMolt stages were obviously higher than in IMolt stages. The energy budgets (%total energy intake) were marginally affected by diet but were significantly affected by the molt stage. Maintenance energy was lower in PMolt stages ($49.84 \pm 4.9\%$) than in IMolt stages ($83.33 \pm 2.45\%$). The excess of maintenance energy in IMolt stages represents the portion set aside for next molt: shell energy content ($4.97 \pm 0.31\%$) and energy for ecdysis ($\pm 28\%$). Conversely, recovery energy was significantly higher in PMolt stages ($34.39 \pm 0.99\%$) than in IMolt stages ($8.33 \pm 1.7\%$). In conclusion SPC sustained good tissue growth and good feed utilization and can be used as a main source of dietary protein for crab juveniles in captivity.

Keywords: fish meal; soy protein concentrate; feed efficiency; tissue growth; protein requirement; energy budget; juvenile crab; *Scylla serrata*

1. Introduction

The mud crab has been domesticated and farmed for many years in Asia, particularly in Vietnam where production is increasing quickly (www.shrimpnews.com). The mud crab of the genus *Scylla*, are being extensively cultured in the Asia-Pacific region due to their high market price [1,2]. However, the substantial crab farming operations which exist are still mainly based on trash fish which contaminates rearing water and leads to highly culture water pollution.

In New Caledonia, there is a political wish to diversify aquaculture, which has been based on blue shrimp (*Litopenaeus stylirostris*) farming. Among the different options available, the mud crab, the local specie *Scylla serrata* is regarded as having potential for aquaculture through its farming aptitudes and its high economical value. However, the possible development of crab farming in New Caledonia will necessarily involve the development of an artificial feed that can be produced from selected and controlled raw materials available on the international market. Therefore, it is essential to assess the nutritional requirements of *S. serrata*. A few studies have been conducted on this subject:

Sheen and Wu [3] showed better lipid utilization by the crab than shrimp by evaluating several amounts of a mixture of cod liver oil and corn oil on the growth of

juvenile crabs. The same authors have shown the importance of a dietary source of cholesterol and polyunsaturated fatty acids (22:6 n-3, 20:4 n-6, 18:3 n-3) for the healthy growth of juvenile crabs [4].

Furthermore, a series of studies has been conducted to measure the digestibility of several potential ingredients for formulated feed for crabs [5, 6]. 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 few studies [7-9]. These studies concluded that the optimal protein concentration (a mixture of fishmeal and soybean meal) in the feed for the best growth rate is between 30% and 47%.

Most of the previous studies have considered growth rate as gains in fresh weight. However, 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 [10]. Increase in tissue mass, on the other hand, is continuing process occurring through the molt cycle [9, 11]. The tissue growth can be measured as increase in dry weight, tissue lose water in direct proportion to their gain in dry mass [9, 12].

Our paper presents experimental results allowing to access the effects of two protein sources (fishmeal vs. soy protein concentrate) on voluntary feed intake, tissue growth, fecal production, nitrogenous excretion, and energy budget of juvenile crabs during postmolt and intermolt periods. Thus, our results provide better understanding of the effect of protein sources on the tissue growth and dietary energy utilization of juvenile crabs during a complete moulting cycle. This information is fundamental to adjust the feed formula and ration level for balance nutrition of captive aquaculture of juveniles *S. serrata*. Finally, based on the data collected during this study, our previous study [9] and the literature [10] we propose a schematic growth model for juvenile *S. serrata* over two moulting cycles as a function of the quantity of feed ingested.

2. Materials and Methods

2.1. Crabs and holding facilities

Juvenile crabs, *Scylla serrata*, were collected in the shallow intertidal zones associated to mangroves surrounding the experimental station (21°51'50" S, 166°3'0" E) in Boulouparis district, New Caledonia. Eighty crab juveniles were caught using a hand net and transferred to the laboratory and acclimated into 6 circular composite tanks (capacity 500 L) for two weeks prior to the beginning of the experiment. Next, sixty of these crabs were identified for their molt stages based on the criteria and methodology described by [13, 14]. These crabs were randomly, individually assigned to sixty experimental rectangular polyethylene tanks (30 x 20 x 30 cm) covered with black lids.

Two experimental crab groups, 30 individuals per each group, were considered: Postmolt (PMolt) and Intermolt (IMolt). Beginning experiment, the PMolt group was constituted of crabs which were about to molt (from D1 to D3 molt stages) and the IMolt group included crabs which were in early intermolt stages (C3 to C4).

During the trial, the PMolt crabs molted within the first two weeks and reached C2 or C3 stage, conversely the IMolt crabs did not molt and they attained D2 or D3 stage at the end of the experiment.

After being dried in soft paper, each crab was weighed to the nearest 0.01 g and its carapace width was measured to the nearest 0.01 mm.

Each experimental tank was continuously supplied with seawater running from a plastic reservoir (2000 liter capacity) at the rate of 0.19 liters.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 heater. The temperature in the experimental tanks was measured continuously (every 3 hours) using an automatic recording probe. During the

experimental period, temperature, salinity, pH, and dissolved oxygen in the water were maintained at 28.5 ± 1.5 °C, 34.0 ± 1.5 ‰, 7.87 ± 0.09 and 4.46 ± 0.16 mg L⁻¹ respectively.

2.2. Diet preparation and composition

The crabs were fed on experimental diets produced in the laboratory: the ingredients were ground up in a grinder (Retsch®) with a 1 mm screen. Next, the meal obtained was mixed with oil and water (30%) in a horizontal mixer (Mainca®) until the consistency was suitable for pelleting. The mixture was then extruded through a 3 mm die in a meat grinder. Pellets were then steamed for 15 min and stored at -20°C before use. The ingredient composition and proximate nutrient content of experimental diets are shown in Table 1.

Table 1. Proximal composition of ingredients and three experimental diets.

	Diets			
	F100	F50/S50	S100	
Composition (%)				
Fish meal	53.28	26.64		
Protein concentrate soy		28.70	57.39	
Crab meal	19.59	19.59	19.59	
Fish oil		1.96	3.92	
Wheat	16.65	13.42	10.77	
limestone	6.46	5.68	4.31	
Dicalcium phosphate	1.96	1.96	1.96	
Binder ¹	0.59	0.59	0.59	
Vitamin premix ²	0.29	0.29	0.29	
Vitamin C ³	0.88	0.88	0.88	
Trace elements	0.29	0.29	0.29	
Analysis (dry matter basis)				
Crude protein - P (%)	49.60	48.81	48.39	
Lipid (%)	4.98	4.69	4.19	
Ash (%)	23.82	21.73	16.22	
Gross energy - E (MJ.kg ⁻¹)	15.08	15.41	15.34	
P/E (g.Mj ⁻¹)	32.89	31.68	31.54	
Proximal analyze (dry matter basis)				
	Protein (%)	Lipid (%)	Ash (%)	Energy (MJ.kg ⁻¹)
Composition (%)				
Fish meal	73.73	11.18	12.40	22.47
Protein concentrate soy	64.02	2.47	7.00	23.53
Crab meal	42.23	1.90	46.47	11.47
Fish oil				39.27
Wheat	12.92	2.13	2.02	15.73

¹ Pegabind (R) synthetic resin from Bentoli Cie.

²Vitamin Premix for shrimp from BEC feed solutions PTY. LTD - ingredients Vitamin AD3 1000/200, Vitamin B1 98% Thiamine, Mononitrate, vitamin B2 Riboflavin 80%, Vitamin B3 99%, Niacin, Vitamin, B5 98% D-Calpan, Vitamin B6 98% Pyridoxine, Vitamin B9 97% Folic acid, Vitamin D3 500, Vitamin E 50 ADD, Vitamin K3 43.7%.

³Vitamine C: Rovimix Stay-C 35 from DSM Cie.

2.3. Experimental design

2.3.1. Growth trial

The Table 2(A) shows the experimental design of this study. Sixty (60) tanks with individual crabs were assigned to two groups of 30 individuals each: PMolt and IMolt. As each crab was separately raised, the experimental unit in this study was the individual. Each crab group was fed on three different experimental diets: F100; F50S50; and S100;

distributed ad libitum. Thus, 10 tanks of each crab group were randomly assigned to each diet (n = 10).

At the beginning of the experiment, the crabs were starved 48 hours and then weighed and measured. The initial average body weight and carapace size of crabs were 22.10 ± 8.46 g and 5.06 ± 0.70 cm respectively. The trial was conducted over 32 days. At the end of the experiment all crabs were left for 48 h starvation and then weighed, measured, dried at 80°C for 48 h and kept at -20°C before biochemical and energy content analysis.

The daily pre-weighed feed rations for each tank were delivered once a day at 6:00-7:00 am. Three hours after each meal the unconsumed feed was siphoned off, dried 24 hours at 80°C and weighed. The leaching rate (dry matter loss of the pellet in the water) was determined by measuring remaining dry matter of the diet after 3 hours immersion. Then the amount of ingested feed (dry matter basis) was obtained following this equation:

$$FI = dF - L - uF$$

Where: FI = ingested Feed; dF = distributed Feed; L = leaching after 3 hours and uF = unconsumed Feed.

Finally, the specific daily feed intake (ingested diet, %iBW d⁻¹) of each crab was determined.

During the experimental period only crabs from PMolt group molted: the date of ecdysis, carapace width and body weight were recorded for each crab after molting. The exuviae was collected and weighed before and after drying at 80°C for 24 h, then kept at -20°C for energy analysis.

2.3.2. Digestibility trial

Experimental design of this trial is presented in table 2(B). All diets contained 1% chromic oxide (Cr₂O₃) as an inert indicator to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED).

For each experimental diet, five crabs were fed ad libitum twice daily until approximately 1.0 g of fecal material (dry weight) was collected (Table 2(B)). Crab feces at the bottom of the tank were collected individually by Pasteur pipette and rinsed gently in distilled water. Fecal matter from five crabs in each experiment diet was pooled. All samples were kept at -20°C before freeze drying for analysis.

The chromium content of diets and fecal material used to calculate apparent digestibility values were determined using the method described by [15]. Apparent digestibility of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) were calculated using equations:

$$ADMD = 100 - 100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in feed} / \% \text{ Cr}_2\text{O}_3 \text{ in feces})$$

The digestibility of crude protein (ACPD) or gross energy (AGED) was determined using the formula ACPD or AGED = $100 - 100 \times (\% \text{ Cr}_2\text{O}_3 \text{ in feed} / \% \text{ Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{ protein or MJ kg}^{-1} \text{ energy in feces} / \% \text{ protein or MJ kg}^{-1} \text{ energy in feed})$.

2.3.3. Fecal production trial

Experimental design of this trial is presented in Table 2(C). Sixty tanks with individual crabs were assigned to PMolt and IMolt groups of 30 individuals each. Each group was randomly divided into 3 subgroups of 10 individuals, each subgroup receiving the three different experimental diets (F100; F50S50; and S100). Diets were distributed in 5 ration sizes (% of initial fresh body weight = % iBW): 0.5; 1; 1.5; 2 and ad libitum; each ration size was applied to 2 tanks for each diet treatment.

Fecal production was collected for each experimental unit three times a day during 10 days (Table 2 (C)). Then the feces were pooled for each diet treatment and whatever the ration size. At the end of the trial, the collected fecal matter was freeze-dried, weighed and stored at -20°C before biochemical and energy analysis.

Fifteen crabs were individually fed at five different ration sizes for each experimental diet (three replicates for one treatment): unfed; 0.5; 1; 1.5; and 2% (Table 2 (D)).

Immediately after the meal, each crab was transferred into another individual aquarium with fixed water volume (30 L). Ammonia-N excretion [16] in each aquarium was measured twice according the method described in [17]: before and 24 h after transferring the crab. Then, the ammonia-N value was converted into energy by multiplying by 24.83 kJ.g⁻¹ [18]. The potential loss of nitrogenous compounds through bacterial action or diffusion was quantified by setting control's aquarium without crabs. The values of ammonia-N concentration of this control treatment were used to adjust determined nitrogenous excretion.

Table 2. Experimental design of trials: growth (A), digestibility (B), fecal production (C) and nitrogenous excretion (D).

Trials	Experimental diets																
	F100					F50S50					S100						
(A) Growth																	
Ration size (% iBW d ⁻¹)	AL					AL					AL						
Number of PMolt crabs	10					10					10						
Number of IMolt crabs	10					10					10						
Feeding frequency	1/24h					1/24h					1/24h						
(B) Digestibility																	
Ration size (% iBW d ⁻¹)	Ad libitum					Ad libitum					Ad libitum						
Replicates (n)	5					5					5						
Feeding frequency	2/24h					2/24h					2/24h						
(C) Fecal production																	
Ration size (% iBW d ⁻¹)	0.5	1.0	1.5	2	AL	0.5	1.0	1.5	2	AL	0.5	1.0	1.5	2	AL		
Number of PMolt crabs	10					10					10						
Number of IMolt crabs	10					10					10						
Feeding frequency	1/24 h					1/24 h					1/24 h						
(D) Nitrogenous excretion																	
Ration size (%iBW d ⁻¹)	0.5	1.0	1.5	2	uf	0.5	1.0	1.5	2	uf	0.5	1.0	1.5	2	uf		
Replicates (n)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	

AL: *Ad libitum*; uf: unfed. Pmolt crabs comprised crab juveniles of premolt stage (from D1 to D3) at the beginning of the experiment. IMolt crabs included crab juveniles of early intermol stage (from C3 to C4) at the beginning of the experiment.

2.4. Biochemical analysis

The proximate compositions of diets, feces, initial and final crabs were estimated according to the method of [19] at *in vivo* laboratory, Vietnam. Moisture contents of crabs and feed were determined after oven-drying to a constant weight at 105 °C. Each sample was combusted at high temperature in pure oxygen then the nitrogen content was measured by thermal conductivity detection and converted to equivalent protein by an appropriate numerical factor, crude protein % = % N x 6.25. Crude lipid content was measured by solvent extraction with petroleum ether. Ash content was determined by a muffle furnace at 550 °C for 8-10 h. Energy contents of diets, fecal material, exuviae and crabs were determined by calorimetric bomb (Parr® 6200, USA, calibrated by benzoic acid) at Ifremer laboratory, New Caledonia.

2.5. Definition, calculation and statistics

The energy partitioning of the budget suggested by the National Research Council [20] was adopted in this study with minor modification in order to comply with mud crab growth through molting. The energy budget of growing crabs is expressed in our study as:

IE = FE + UE + SE + RE +HEm (1)

Where:

IE is the energy intake which was obtained for each crab by calculating the difference between the feed distributed and the feed leached and uneaten.

FE is energy lost from the animal through the feces for each crab, FE was calculated from the model linking the feed intake (energy intake) and fecal production.

UE is energy lost from the animal through the total ammonia excretion which was calculated for each crab from the model linking the feed intake (energy intake) and nitrogenous excretion.

SE is surface energy losses which is exuvia lost at ecdysis for the PMolt crab group.

RE is the recovered energy which is channeled into growth. RE was estimated considering the initial crab energy content calculated from the relationship defined between dry and fresh weight ($Y = 0.3229 X + 0.4246$; $R^2 = 0.96$, $n = 30$). It is calculated for each crab through the dry weight gain converted into energy.

HEm is the maintenance energy calculated for each crab from the equation (1):

$$\text{HEm} = \text{IE} - (\text{FE} + \text{UE} + \text{SE} + \text{RE}) \quad (2)$$

Specific tissue growth rate (SGRd , % dry iBW.day⁻¹) = $100 \times (\text{dry fBW} - \text{dry iBW}) \times \text{dry iBW}^{-1} \cdot \text{day}^{-1}$; where fBW, iBW are final and initial body weight, respectively.

Initial dry body weight (dry iBW) was estimated through equation: $Y = 0.3229 X + 0.4246$ ($R^2 = 0.96$, $n = 30$), where Y is dry body weight, X is fresh body weight. This equation was obtained from our extra sample assessment for crabs in intermolt (IMolt group). In our experiment, we showed in our extra sample assessment that dry body weight of individuals from PMolt group did not change significantly before and after ecdysis. Therefore in our study, for PMolt crabs we used the body weight after ecdysis as the initial body weight (iBW). Consequently, for PMolt crab group, growth period study lasted between ecdysis and the end of the experiment with total duration of 18 to 22 days while for IMolt crabs the growth period was the same with the experimental duration of 32 days.

The efficiency in feed conversion (FCE), protein retention (PRE) and energy retention (ERE) is the ratio between weight gain in dry matter, protein and energy with the amount of feed (dry matter basic), protein or energy ingested, respectively [20-24].

Nitrogenous excretion (UE), fecal production (FE), recovered energy (RE), and maintenance energy (HEm) were analyzed using ANCOVA analysis with feed intakes as the covariate. For these parameters, the adjusted individual data and mean were calculated for a same amount of ingested feed.

Optimum growth is defined as the best increase in dry body weight for the least feed intake [25]. Therefore, the feed intake corresponding to the highest values of FCE was determined as the optimum intake, then converted into protein and energy required for optimal tissue growth of individual crab.

The statistical analyses were performed on SPSS 16.0 for windows. There are significant differences among the various treatments when $P < 0.05$. Shapiro-Wilk and Levene's test were used for checking normality and homogeneity of variances, respectively. Percentage data were transformed into arcsine to get normality. The Two way ANOVA fixed model was used to test the effects of diet and crab group (PMolt or IMolt) on body biochemical composition, energy content and each energy budget component (FE, UE, HEm, RE). Analyses of Covariance (ANCOVA) were used to investigate the effect of different experimental diets on nitrogenous excretion, fecal production, and specific growth rate across amounts of feed ingested. Linear function between nitrogenous excretion and feed intake was applied for ANCOVA analysis while its best relationship was exponential. Data were Log transformed to get a linear relation between growth rate and feed intake for IMolt crabs. Digestibility coefficients of crabs fed on different experimental diets were compared by one way ANOVA.

3. Results

3.1. Body chemical composition and energy content

The contents of protein, lipid, ash and energy in the body of PMolt and IMolt crabs juveniles for three different experimental diets are presented in Table 3. Whatever the crab

group, PMolt or IMolt, no significant effect of the diet was shown on the biochemical composition and energy content of the animal ($P = 0.29$; 0.31 ; 0.74 and 0.13 for protein, lipid, ash and energy, respectively).

Whenever, the body lipid content was significantly higher for IMolt than for PMolt crabs ($P = 0.003$) for the 3 diets tested.

Table 3. Crab body proximal composition (%) and energy content (kJ.g^{-1} of dry body weight) on dry weight basis of PMolt and IMolt crab groups fed *ad libitum*.

Group	Diet	Protein	Lipid	Ash	Energy
PMolt	F100 (n = 3)	38.7 ± 1.1^a	1.5 ± 0.1^a	46.2 ± 2.0^a	10.4 ± 1.1^a
	F50S50 (n = 3)	37.6 ± 2.5^a	1.3 ± 0.8^a	46.3 ± 4.3^a	9.9 ± 1.1^a
	S100 (n = 3)	41.9 ± 4.2^a	1.9 ± 0.4^a	42.9 ± 3.4^a	11.2 ± 0.7^a
IMolt	F100 (n = 3)	39.9 ± 4.7^a	2.8 ± 0.8^b	42.2 ± 3.3^a	10.1 ± 0.1^a
	F50S50 (n = 3)	39.7 ± 1.3^a	2.4 ± 0.1^b	42.5 ± 0.9^a	10.0 ± 0.3^a
	S100 (n = 2)	42.3 ± 1.5^a	3.1 ± 0.4^b	40.0 ± 0.3^a	11.5 ± 1.1^a

Values are means \pm SD (n = replicates per treatment), within the same column, means with different letters are significantly different ($P < 0.05$).

3.2. Diet digestibility

Apparent digestibility for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of the three experimental diets are given in Table 4. No significant difference in ADMD, ACPD and AGED was found between diets ($P = 0.43$; 0.59 and 0.19 respectively). The averages of ADMD, ACPD and AGED were high for each diet: $90.34 \pm 1.79\%$; $97.72 \pm 0.44\%$; 90.42 ± 1.82 respectively.

3.3. Fecal production and nitrogenous excretion

The fecal production of juvenile crabs, whatever experimental crab group considered, PMolt or IMolt, increased significantly with feed intake ($P < 0.01$) for all three experimental diets. The relationship between fecal production and feed intake was described as a simple linear function (Figure 1). The diet composition did not affect the fecal production ($P = 0.46$).

Nitrogenous excretion of juvenile crabs increased with feed intake. Exponential functions were used to describe this relationship for all three experimental diets (Figure 2). Feed type and feed intake significantly affected nitrogenous excretion ($P < 0.01$). The later was significantly lower for F100, intermediate for S100 and higher for F50S50 (pairwise comparisons, $P < 0.01$).

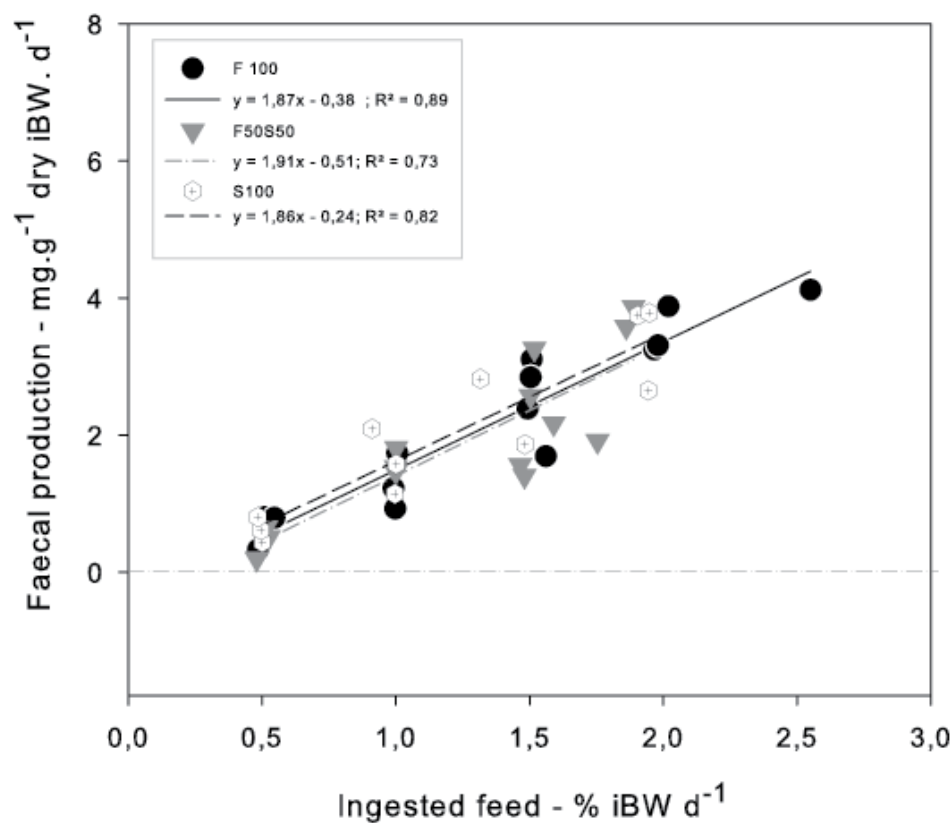


Figure 1. Relationships between faecal production of juvenile crabs and ingested feed for the three experimental diets. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

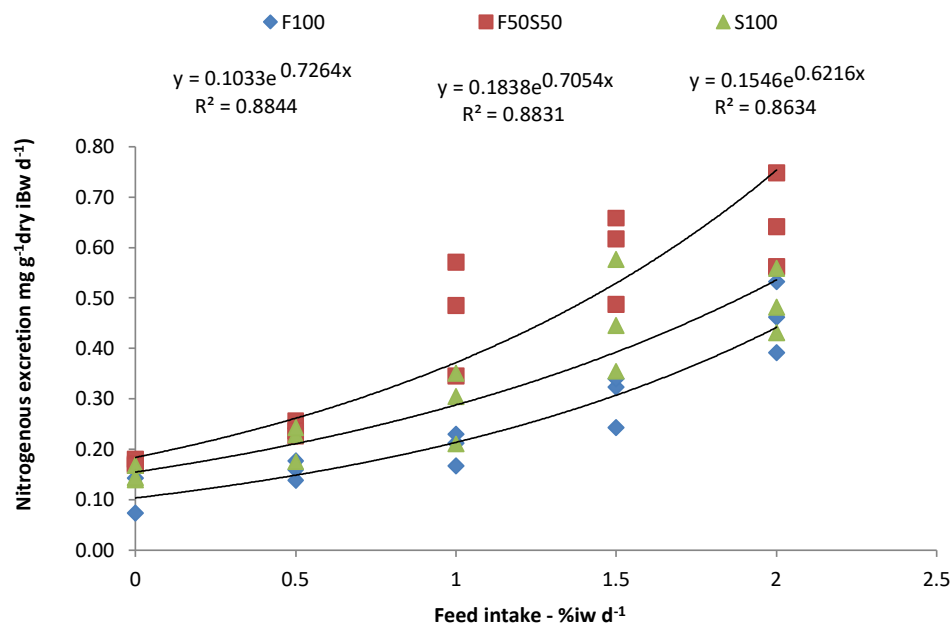


Figure 2. Relationships between faecal production of juvenile crabs and ingested feed for the three experimental diets. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

3.4. Growth and feed utilization

Crabs from PMolt group molted within the first two weeks of the experiment and no crabs molted in IMolt group: at the end of experiment, crabs were recorded in C2 to C3 and D2 to D3 of molt stages for PMolt and IMolt groups, respectively.

The specific growth rate in dry weight or tissue growth (SGRd) of juvenile crabs from PMolt and IMolt groups increased with feed intake ($P < 0.01$) whatever the experimental diets. Linear and logarithmic functions best fit the “tissue growth - feed intake” relationship of PMolt and IMolt crabs, respectively (Figure 3 and Figure 4).

ANCOVA analyze with feed intake as covariate allowed us to compare the adjusted means of tissue growth (SGRd) (Figure 5). For PMolt crab group, tissue growth was not significantly affected by diet type ($P = 0.43$). For IMolt crabs, it was significantly higher on diet S100 compared to F100 ($P = 0.02$) and F50S50 ($P = 0.04$), but no difference was observed between diet F100 and diet F50S50 ($P = 0.98$).

Data presented in Figure 5 shows that, during a molt cycle, 80% of the tissue growth occurred during postmolt (PMolt crabs) and only 20% during intermolt (IMolt crabs).

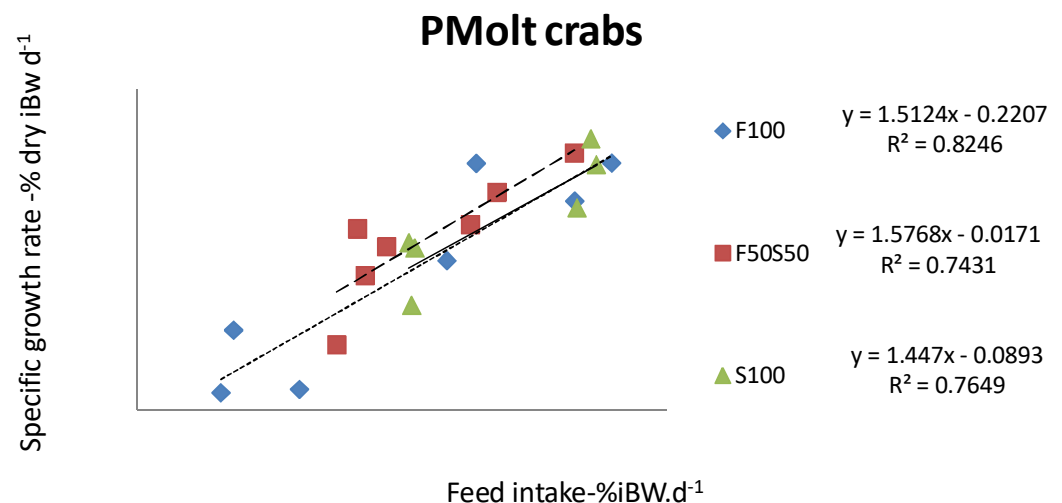


Figure 3. Relationship between specific growth rate (SGRd) and feed intake of juvenile crabs for the 3 experimental diets for PMolt crabs. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

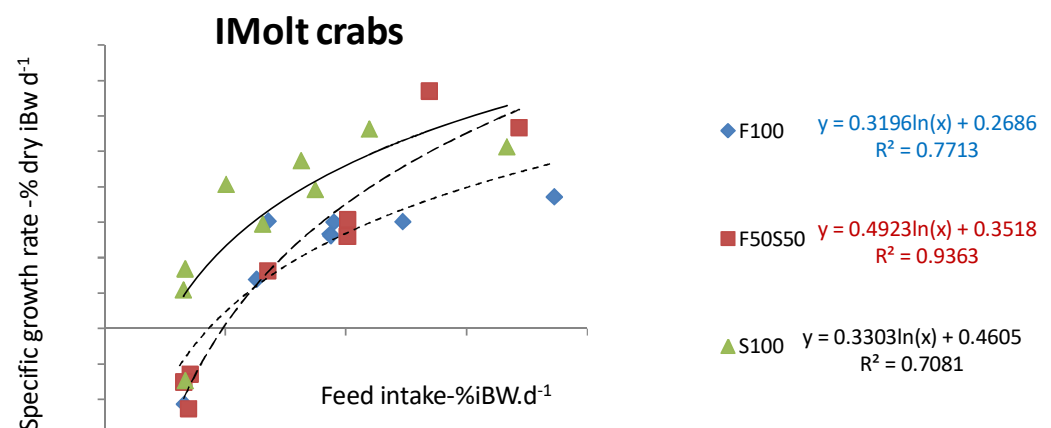


Figure 4. Relationship between specific growth rate (SGRd) and feed intake of juvenile crabs for the 3 experimental diets at IMolt crabs. Amount of feed intake is percentage of initial fresh body weight (iBW) per day.

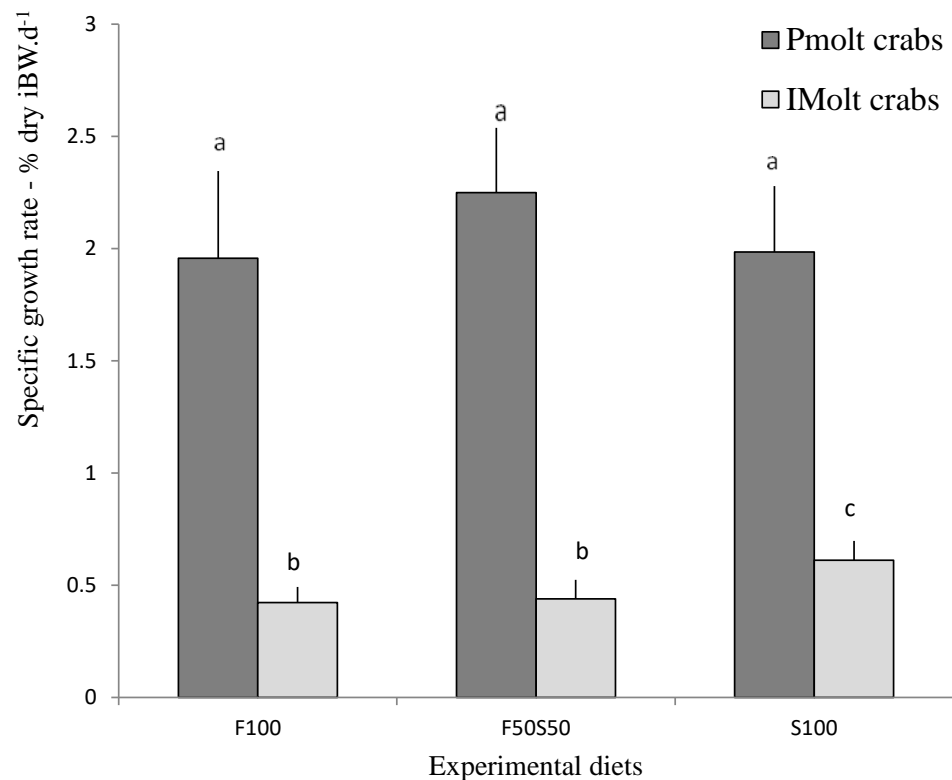


Figure 5. Adjusted mean specific growth rates (\pm SD) for average feed intakes of PMolt and IMolt crabs. Means with different superscript letters differ significantly ($P < 0.05$).

Feed intake values (in dry matter, energy and protein) for optimal tissue growth (the best growth rate for the minimum feed intake) of PMolt and IMolt crabs were calculated from the polynomial functions linking feed conversion efficiency (FCE) and feed intakes:

(i) For PMolt crabs, as FCE was not affected by the experimental diets, only one polynomial equation was defined by mixing the data ($n = 16$) from all 3 diets:

$$y = -36.296x^2 + 114.26x - 33.494 \quad (R^2 = 0.65) \quad (3).$$

The calculated feed intake for optimum growth is 4.57 % dry iBW. d⁻¹ and the derived optimum values of energy and protein intakes are 0.70 kJ. dry body g⁻¹. d⁻¹ and 0.022 g. dry body g⁻¹. d⁻¹, respectively.

(ii) For the IMolt crab group, as FCE was significantly higher in diet S100, two polynomial equations were defined: one for diet S100 treatment and one by pooling data ($n=14$) for diets F100 and F50S50.

$$\text{Diet S100 (n = 7): } y = -12.362x^2 + 22.008x + 8.4093 \quad (R^2 = 0.67) \quad (4)$$

$$\text{Diets [F100+F50S50] (n=14): } y = -20.679x^2 + 54.56x - 22.728 \quad (R^2 = 0.76) \quad (5)$$

Values of feed intake for optimal growth are 2.72% and 4.24% dry iBW.d⁻¹ respectively for S100 and [F100+F50S50] diets. The corresponding values of energy and protein intakes are 0.42 kJ.dry body.g⁻¹.d⁻¹ and 0.013 g.dry g⁻¹.d⁻¹ for diet S100 and 0.68 kJ.dry body g⁻¹.d⁻¹ and 0.021 g.dry body.g⁻¹.d⁻¹ for diet [F100+F50S50].

Estimated feed intake values for optimal growth reveal two trends: firstly, feed intake is obviously higher in the PMolt crab compared to IMolt group, and secondly consumption of diet S100 was lower than that of diets F100 and F50S50 for the IMolt crab group.

Adjusted mean values, for the optimum feed intake, of feed conversion efficiency (FCE), protein retention efficiency (PRE) and energy retention efficiency (ERE) were calculated from equations (3); (4); and (5) for PMolt and IMolt crab groups fed on three different diets (Table 5). FCE, PRE and ERE values for PMolt crabs were respectively: 49.86

$\pm 7.01\%$, $39.29 \pm 5.52\%$, $35.34 \pm 4.27\%$ and obviously 4-5 times higher than IMolt crabs with respective values of $11.37 \pm 1.85\%$; $9.1 \pm 1.48\%$; $7.84 \pm 1.27\%$.

Within the PMolt crab group, experimental diets did not significantly influence FCE ($P = 0.73$), PRE ($P = 0.72$) and ERE ($P = 0.19$). In contrast, for the IMolt crab group FCE, PRE and ERE were significantly higher with diet S100 than with diet F100 and F50S50 ($P < 0.01$).

Table 5. Adjusted mean values of efficiency in feed conversion (FCE), protein retention (PRE) and energy retention (ERE) at the optimal feed intake for crab groups, PMolt and IMolt, fed on three different diets.

Group	Diet	Feed efficiency		
		FCE	PRE	ERE
PMolt	F100 (n = 7)	47.57 ± 7.97^a	37.49 ± 6.28^a	34.29 ± 4.31^a
	F50S50 (n = 7)	50.39 ± 5.29^a	39.72 ± 4.17^a	33.03 ± 3.41^a
	S100 (n = 7)	51.61 ± 7.77^a	40.68 ± 6.13^a	38.70 ± 5.10^a
IMolt	F100 (n = 5)	7.55 ± 0.87^b	6.04 ± 0.70^b	5.21 ± 0.60^b
	F50S50 (n = 5)	10.15 ± 3.38^b	8.12 ± 2.70^b	7.00 ± 2.33^b
	S100 (n = 6)	16.43 ± 1.29^c	13.14 ± 1.03^c	11.33 ± 0.89^c

FCE: $100 \times (\text{dry final body weight} - \text{dry initial body weight}) / \text{feed intake}$; PRE: $100 \times [(\text{dry final body weight} \times \text{final protein content}) - (\text{dry initial body weight} \times \text{initial protein content})] / (\text{feed intake} \times \text{protein content})$; ERE: $100 \times [(\text{dry final body weight} \times \text{final energy content}) - (\text{dry initial body weight} \times \text{initial energy content})] / (\text{feed intake} \times \text{energy content})$.

Values are means \pm SD, within the same column; means with different letters are significantly different ($P < 0.05$).

3.5. Energy budget

Energy budgets were built from the adjusted means calculated for averaged feed intakes for each crab group (PMolt and IMolt) fed on the 3 diet treatments (Table 6). Average feed intakes were $0.65 \text{ kJ.dry iBW g}^{-1}.\text{d}^{-1}$ and $0.45 \text{ kJ.dry iBW g}^{-1}.\text{d}^{-1}$ for PMolt and IMolt crabs respectively. On average, the PMolt crab group consumed 30% more food than the IMolt group. The higher feed intake in the PMolt crab group led to obviously higher energy loss in feces (FE) and excretion (UE). Lost energy in ecdysis (SE) only concerned the PMolt crab group. Energy allocated for tissue growth (RE) in the PMolt group was 80 to 90% higher than for the IMolt crab group, even though feed intake was only 30% more for the PMolt group. Finally, maintenance energy (HE_m) was 16 to 21% lower in the PMolt than the IMolt crabs.

Table 6. Adjusted mean values of energy budgets for PMolt and IMolt crabs fed on 3 different experimental diets.

Group	Diet	IE	Partition of energy (kJ.g ⁻¹ of iBW.d ⁻¹)				
			FE	UE	SE	RE	HE _m
PMolt	F100 (n = 7)	0.65	0.075 ± 0.004^a	0.018 ± 0.003^a	0.031 ± 0.000^a	0.213 ± 0.044^a	0.308 ± 0.042^a
	F50S50 (n = 7)	0.65	0.068 ± 0.002^b	0.022 ± 0.003^b	0.032 ± 0.000^a	0.204 ± 0.035^a	0.320 ± 0.034^a
	S100 (n = 7)	0.65	0.067 ± 0.002^b	0.016 ± 0.001^a	0.032 ± 0.000^a	0.246 ± 0.052^a	0.282 ± 0.050^a
IMolt	F100 (n = 5)	0.65	0.052 ± 0.004^c	0.009 ± 0.002^c		0.024 ± 0.004^b	0.367 ± 0.009^b
	F50S50 (n = 5)	0.65	0.043 ± 0.003^d	0.013 ± 0.002^d		0.033 ± 0.014^b	0.376 ± 0.011^b
	S100 (n = 6)	0.65	0.047 ± 0.003^d	0.010 ± 0.001^c		0.056 ± 0.006^c	0.345 ± 0.012^c

IE: Energy intake (kJ.g⁻¹ of iBW.d⁻¹); FE: Feces; UE: excretion; SE: ecdysis; RE: growth; HE_m: maintenance.

Values are means \pm SD. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets within each crab group.

Whatever the crab group considered, the diet treatments had little effect on the energetic balance, except for FE which was significantly higher for treatment F100 ($P < 0.01$), and for UE which was significantly higher for treatment F50S50 ($P < 0.01$).

Furthermore, in the IMolt crab group, energy allocated to tissue growth was significantly higher for animals fed on the diet S100 ($P < 0.01$). The main differences in the energy budget calculated as % of energy intake between IMolt and PMolt crabs presented in Table 7 were observed for SE (Ecdysis), RE (Growth) and HEm (Maintenance). Only the PMolt crab group was affected by SE which represents about 5% of total energy intake. Moreover, RE and HEm were respectively 4 times higher and 1.7 times lower in the PMolt than the IMolt crab group.

Diets had a significant effect on FE, EU, RE and HEm. Energy lost in Feces (FE) and excretion (UE) was respectively higher for crabs fed diets F100 and F50S50 ($P < 0.01$). However, those diets marginally affected the global energy budget because FE and EU represent less than 15% of the total energy intake.

The percentage of energy allocated to growth (RE) was 5 - 7% higher ($P < 0.01$) for crabs fed on S100 than F100 and F50S50 for both PMolt and IMolt crab groups. In contrast, the percentage of energy allocated to maintenance (HEm) of crabs fed the S100 diet is 4-6% lower than F100 and F50S50 diets for both PMolt and IMolt crabs.

Finally, the diets treatment had no significant effect on energy lost in exuvia (SE).

Table 7. Energy budgets in proportion of energy intake (IE): fecal production (FE), excretion (UE), ecdysis (SE), growth (RE) and maintenance (HEm) for PMolt and IMolt crab groups fed on three diet types: F100, F50S50, and S100.

Group	Diet	%IE				
		FE	UE	SE	RE	HEm
PMolt	F100 (n = 7)	11.74 ± 0.52 ^a	2.83 ± 0.52 ^a	4.93 ± 0.44 ^a	33.17 ± 6.98 ^a	47.90 ± 6.58 ^{ae}
	F50S50 (n = 7)	10.59 ± 0.38 ^b	3.39 ± 0.45 ^b	5.01 ± 0.27 ^a	31.74 ± 5.65 ^a	49.84 ± 4.90 ^a
	S100 (n = 7)	10.44 ± 0.48 ^b	2.48 ± 0.13 ^a	4.98 ± 0.23 ^a	38.27 ± 7.57 ^b	44.16 ± 8.80 ^{be}
IMolt	F100 (n = 5)	11.53 ± 0.79 ^a	2.03 ± 0.4 ^{ce}		5.31 ± 0.79 ^c	81.49 ± 2.02 ^{cf}
	F50S50 (n = 5)	9.62 ± 0.58 ^c	2.78 ± 0.35 ^d		7.36 ± 3.05 ^c	83.33 ± 2.45 ^c
	S100 (n = 6)	10.43 ± 0.70 ^{cb}	2.33 ± 0.33 ^{ae}		12.33 ± 1.27 ^d	76.45 ± 2.68 ^{df}

Values are means ± SD. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets within each crab group.

4. Discussion

Feed for aquaculture requires large quantities of ingredients from the sea and is the main consumer of fishmeal among the animal husbandry subsectors [26, 27]. Indeed, fishmeal is the primary source of protein and energy in most aquafeeds and accounted in 2006 for 68.2% total global fish meal production [28]. In 1989, fishmeal used in aquaculture accounted for only 10% [29], this part had dramatically increased to 73% by 2010 [26].

Production of carnivorous species, promoted in aquaculture, particularly marine crustaceans, marine finfish and salmonids, requires a lot of fishmeal [30]. The cost of fishmeal is increasing over time as a result of the uncertainty of availability [31]. [32] indicated that the price of fishmeal increased about 62.9% from August 2005 to June 2008. In such conditions, aquafeed can account for as much as 40-60% of the production cost [33]. In this framework, partially or completely shifting from fishmeal protein source to plant protein sources in formulated diet is a priority for sustainable mud crab aquaculture development [34, 35].

In our study, tissue growth of the crabs was considered on the dry weigh basis. This approach is quite important as water content of crustaceans is high and varies dramatically through their molt cycles. Indeed, in crab *S. serrata*, free water content reached 88% in postmolt and decreased to 66 % in intermolt and premolt stage [10]. During a molt cycle of the juvenile crab *S. serrata*, two tissue growth periods were identified [9] and confirmed in this study. Thus, in our experimental conditions, 2 phases of tissue growth were observed: a postmolt period which lasted between 18 and 22 days and an intermolt period which lasted 32 days of the experiment. Most of tissue growth occurred following crab molting until early intermolt stage and the remaining during intermolt stage. Indeed, the proportion of organic matter and soluble mineral salt rapidly

increased by 80% during the postmolt stage (from ecdysis to C3 stage), and the other 20% was gained during the remaining period (from C4 to D4 stage). This result is consistent with findings of [10] on growth study of mud crab juveniles (4.25-7.25 cm carapace width) and confirms our previous results [9]. From our data and those [10] we propose schematic growth models of juvenile crab *S. serrata* over two molt cycles where we can distinguish a discontinuous live weight gain at ecdysis and a continuous tissue growth separated in two successive phases during a molt cycle (Figure 6). In contrast, we noted an extremely rapid increase of mineral salts corresponding to mineralization of the cuticle after ecdysis, which is disconnected from tissue growth. Interestingly, we also observed that the feed consumption or voluntary feed intake (VFI) appears to be correlated with tissue growth rate. The VFI is maximum during two weeks following the ecdysis when tissue growth rate is high then decreases in a parallel to the tissue growth rate until a minimum value shortly before the next molt (I). In this condition 50% of the feed is consumed during the first third of the molt cycle when the tissue growth rate is the highest.

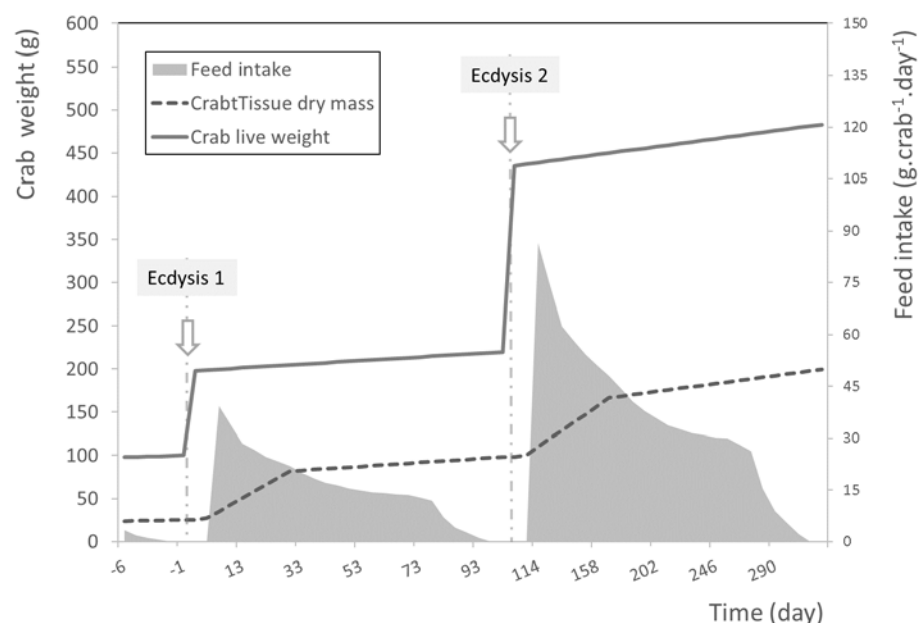


Figure 6. Schematic growth models and voluntary feed intake (VFI) of juvenile crab *S. serrata* over two molt cycles. The duration of the second molt cycle is 1.24 times (+24%) that of the first molt cycle (based on [10]).

In order to compare our results with other studies we had to transform the data of the later in order to express them in dry weight. The study [8] on juvenile crabs *S. serrata* (iBW = 0.25 g) found that the optimum daily protein ($0.065 \text{ g.dry body g}^{-1}.\text{d}^{-1}$) and energy ($2.351 \text{ kJ.dry body g}^{-1}.\text{d}^{-1}$) intakes were three times higher than that of the present study. These differences could be partly explained by the difference in size of the crabs used which was 10 times larger in our study. Indeed, it is well admitted that protein and energy requirements per weight unit mass of the animal decrease during its growth: for instance, 2 g and 10 g shrimps *L. vannamei* required daily respectively 0.014 and 0.007 g.g^{-1} of digested protein and 0.558 and 0.298 $\text{kJ.g}^{-1}.\text{d}^{-1}$ of digestible energy [36].

Many plant-based ingredients are used as a source of proteins for aquafeed. 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 [37-39]. [40] reported that up to 33% fishmeal protein could be replaced by soybean cake for juvenile mitten crab without reducing growth. [41] found that 30% of soybean meal and rapeseed meal mixture (1:1 ratio) could replace 40% FM for Chinese mitten crab without impairing growth performance and feed utilization. More recently, [42] determined the

optimum dietary replacement of fish meal with soy protein concentrate to be 51.49% for juvenile swimming crab *Portunus trituberculatus*.

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 [43]. Although heat and enzyme treatments can neutralize some of these compounds, they are still a significant problem when including SBM in aquaculture feeds [44]. While, soy protein concentrate (SPC), although more expensive, 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 [45, 46].

In our study, the juvenile mud crabs digested soybean protein concentrate as well as fishmeal. Moreover, the two ingredients exhibited high digestibility coefficients for dry matter, protein and energy. These results are consistent with previous studies on mud crabs reporting high soybean meal digestibility at an inclusion level of up to 45% with ADC values for ADMD, ACPD and AGED ranging from 80.4-95.7%; 91.7-97.1% and 88.6-97.9%, respectively [5, 6, 35, 47]. Furthermore, [47] showed that crude protein from soybean meal was better digested by mud crabs than the other plant ingredients tested (canola, lupin and cotton seed meal).

Besides the high protein digestibility of SPC for the mud crab, our study showed that this protein source can also totally replace fishmeal in the diet without affecting the tissue growth of juvenile *S. serrata*. The SPC diet, without fishmeal, even led to a better growth rate during intermolt (IMolt crab group) and higher efficiency values of feed conversion, energy retention and protein retention. These results are supported by other authors who worked on crustaceans like shrimps; such as, [48] showed that shifting from 80% fishmeal (FM) to soybean and canola meal did not affect the growth rate of *L. vannamei*. Previously, [49] replaced FM by SPC in diets for *L. vannamei* and suggested that SPC, with a methionine supplement, can substitute up to 75% of fishmeal. [50] showed that the replacement of all the fishmeal by alternative ingredients, soy protein concentrate and microbial floc, did not significantly affect growth, feed conversion ratio, specific growth rate, protein efficiency ratio or survival of the shrimp *L. vannamei*. On this same shrimp species, [51] have shown that SPC could replace up to 50% of the FM without a significant negative effect on growth performance, haemolymph parameters and immune responses.

Opposite trends have also been observed. [52] reported that a maximum of 40% of marine protein sources (fishmeal, shrimp head meal and squid meal) could be replaced by soybean meal in diets, but that higher FM replacement levels resulted in lower growth rates of the shrimp *L. vannamei*. Similarly, [53] showed that the maximum FM replacement levels by SPC without affecting the growth rate of the shrimp *Penaeus monodon* was 50%. These authors also reported that high concentrations of soy-based products in feed negatively affected the palatability of the diets for shrimps. However, in our study, no influence of SPC level was noted on feed intake. Indeed, all experimental diets contained the same amount (20%) of crustacean meal which is highly palatable for crabs.

The energy budgets of juvenile crabs determined in the present study are based on the average energy intake (IE) for crabs whose tissue growth was studied during postmolt period (from ecdysis to C2-3 molt stages, PMolt) and intermolt period (from C3-4 to D1-3 molt stages, IMolt). Adjusted values, for the corresponding average energy intake, were determined for energy lost in feces (FE), excretion (UE), exuviae (SE), and energy retained as growth (RE).

As the experimental unit in this study was the individual crab, it has been possible to establish the energy budget for each individual adjusted for an average amount of energy intake. In the energy budgets of both PMolt and IMolt crab groups, the proportions of energy intake lost in feces and ammonia excretion were small and similar for the three feed types tested. Generally, the level of nitrogenous excretion is of little importance in the bio-energetic flow for crustaceans [54]. Furthermore, excretion contributed to no more than 3% of the energy budget of fish [55, 56] which is similar to our result on crabs. The

small proportion of FE and UE in the energy balance of the crabs in our study corresponds to those found by other authors for fishes and shrimps [22, 54, 57-59].

Based on [20], HEm is defined as the amount of maintenance energy required for an animal to maintain zero energy for growth or an equilibrium between IE and energy expenditure (FE, UE, SE, RE). In the present study, we calculated HEm in two ways: the first resolving the energy balance equation and the second from the model linking SGR and feed intake when growth is null. Resolving the energy balance equation, HEm values were 0.304 and 0.358 $\text{kJ} \cdot \text{dry iBWg}^{-1} \cdot \text{d}^{-1}$ for PMolt and IMolt crabs, respectively. These HEm values included i) the heat increment of feeding (HiE), ii) the formation and excretion of metabolic wastes; iii) the transformation and inter-conversion of the substrates and their retention in tissues; and iv) the molting process [20].

The average HEm value for PMolt and IMolt crabs estimated from the model linking tissue growth, (SGRd), and feed intake for zero growth (RE=0), was $0.179 \pm 0.003 \text{ kJ} \cdot \text{dry iBWg}^{-1} \cdot \text{d}^{-1}$ which is about half the value of the HEm estimated above by the energy balance equation. This difference is explained by the size of the meals which was smaller (0.012 g. dry iBWg⁻¹.d⁻¹) in the case of estimation by model linking SGR and feed intake compared to that obtained by the estimate from the energy balance (0.03 g and 0.042 g. dry iBWg⁻¹.d⁻¹ for PMolt IMolt crabs respectively); indeed, the heat increment of feeding increases with the size of the meal [60].

The maintenance energy value of the shrimp *Litopenaeus vannamei* (345 $\text{kJ} \cdot \text{wet BWkg}^{-1} \cdot \text{d}^{-1}$) determined from the growth-meal size model [36] was about 6 times higher than the HEm value for crabs in our study. This difference could be explained by the fact that the mud crab is much less active than the shrimp, particularly in captivity.

Mud crab juvenile consumed a larger part of energy for maintenance than for growth, regardless of growth period during the molt cycle and experimental diets. This result is consistent with other studies on fish: Nile tilapia [57], Cobia [22, 58], yellow grouper [59]. However, other studies on crayfish showed a higher proportion of intake energy transferred to growth than to maintenance. Thus, *Cherax tenuimanus* and *C. destructor* could convert respectively 41.8-57.5% and 50% of ingested energy into growth [54, 61].

One striking result of our study on juvenile mud crabs is the estimated portion of energy intake channeled to molt. Indeed, the energy for molting included energy lost in exoskeleton (SE) plus the energy required for molting which in our study was a portion of the maintenance energy (HEm). Our study showed that crabs during intermolt exhibit a higher maintenance energy ratio (up to $83.33 \pm 2.45\%$) than crabs during postmolt period (up to $49.84 \pm 4.9\%$). The difference between the maintenance energy ratios of the crabs from the two growth periods showed that 33% of energy intake during intermolt period is stored and required for molting. This proportion included 5% lost with the exoskeleton and the remaining 28% for extra metabolism cost during ecdysis. This result is confirmed by the biochemical analysis indicating significantly higher lipid content in crabs during intermolt period. Lipid accumulation is done to anticipate ecdysis which requires energy, notably for osmoregulation during molting [62, 63]. Similarly, another study on crab (*Eriocheir sinensis*) showed that lipids were accumulated in a digestive gland before molting, reaching a peak at late premolt and then decreasing during postmolt [63]. A study on the shrimp *Penaeus semisulcatus* showed the same phenomenon, with lipid drops accumulating in hepatopancreatic R-cells from the intermolt to the premolt stage. Conversely only a few lipid drops were observed during the postmolt stage [64].

The proportion of energy intake channeled to tissue growth (RE) was lower during intermolt ($12.32 \pm 0.31\%$) than postmolt period ($37.39 \pm 6.73\%$). Thus, two interrelated but antagonist physiological phenomena occurred: ecdysis is necessary for crab growth but it occurs at its expense.

5. Conclusions

Our study on the juvenile crab *S. serrata* led to the following conclusions:

- Voluntary feed intake varies with the rate of tissue growth rate which is maximum two weeks after ecdysis.
- 33% of the energy intake during intermolt period are stored for molting.
- When compared with fishmeal, soy protein concentrate did not affect the global energy balance of the crab juveniles.
- Soy protein concentrate can be used as a main source of dietary protein for crab juveniles in captivity.
- Soy protein concentrate sustained good tissue growth and good feed utilization by the crab juveniles, in some cases better than fish meal.

Our results provide useful information on feed utilization of the crab during a molt cycle. We may also suggest the benefit to study nutritional requirement of mud crabs on the dry weight basis which we believe more accurate regarding the huge free water change during a molt cycle of this species. Finally, the methodology proposed will allow to carry out some specific nutritional experiments in shorter period without the necessity to wait for several molt cycles.

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