
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 matter, 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.

Highlights

► There were two tissue growth periods of crabs during a molt cycle: FTG (Fast tissue growth) corresponding to postmolt period and STG (Slow tissue growth) corresponding to intermolt & premolt periods. ► Protein, lipid and ash gain during one molt cycle correlated to tissue growth model. ► Voluntary feed intake (VFI) followed the same trend with tissue growth. ► VFI or energy intake had a strong effect on molt frequency or intermolt cycle. Higher VFI level resulted in higher cumulative molts. ► Crabs fed on diet SPC-42 (40% crude protein content in which $\frac{3}{4}$ from soy protein concentrate-SPC) exhibited the best growth (tissue growth and molt frequency) then leading the highest feed efficiency (FCR, PRE, retention of protein and lipid). ► No effect of different SPC levels in the dietary diets on size increment after molt. ► Highest and lowest energy intake respectively allocated in RE (growth energy), HEm (maintenance energy) were observed for crabs fed on SPC-42.

Keywords : *Scylla serrata* ; Tissue growth ; Feed efficiency ; Protein requirement ; Soy protein concentrate ; Energy budget

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 production to triple in 5 years (from 10,000 tons in 2004 to 30,000 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).

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 fibre 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 (Christense 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 (Davis and Arnold, 2000; Amaya et al., 2007a,b). 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 (Heasmen, 1980). 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

A total of juvenile crabs, *S. serrata*, 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 x 20 x 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 hours using an automatic recording probe. During the experimental period, water environmental parameters were maintained with: $T^{\circ}\text{C} = 21.5 \pm 2.5$, $S \text{‰} = 34.0 \pm 1.5$, $\text{pH} = 7.97 \pm 0.09$ and $\text{O}_2 = 4.48 \pm 0.16 \text{ mg L}^{-1}$.

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, Germany) 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.

2.3. Experimental design

2.3.1. Growth trial

At the beginning of the experiment, the crabs were starved for 48 hours for weaning to the dietary diets and then weighed and measured. The initial average body weight and carapace size of the crabs was $20.43 \pm 9.81 \text{ g}$ and $4.92 \pm 0.81 \text{ cm}$, respectively. As each crab was held separately, the experimental unit in this study was the individual. Fifty four tanks with individual crabs were assigned to the five different dietary treatments. Thus, 11 tanks were randomly assigned to each treatment ($n = 11$), except for the treatment with diet SPC-12 ($n = 10$). The crabs were fed once each day *ad libitum*. The daily pre-weighed feed allocation for each tank was delivered at 6:00-7:00 am. Three hours after each meal, the uneaten feed was siphoned off, dried 24 hours at 60°C and weighed (Unnikrishnan and Paulraj, 2010).

The leaching rate (dry matter loss of the pellet in the water) was determined by using the method described in Johnston and Johnston (2007). A weighted quantity of each diet was placed in a 1-L glass beaker filled with 800 mL of seawater, gently aerated and then incubated in a water bath at 22°C for 3 h. The amount of voluntary feed intake (VFI) for each crab was obtained following this equation:

$$\text{VFI} = \text{dF} - \text{L} - \text{uF}$$

Where: VFI = voluntary feed intake; dF = distributed feed; L = leaching after 3 hours and uF = unconsumed feed.

During the experimental period, the date of ecdysis, carapace width and live body weight were recorded for each crab after molting, and then they were transferred back to the tanks. The exuviae were collected and weighed before and after drying at 80 °C for 24 h, then kept at -20 °C for energy analysis. The trial was maintained for 81 days. At the end of the experiment, all of the crabs were left unfed for 48 h and then weighed, measured, dried at 80 °C for 48 h and kept at -20 °C before biochemical and energy analysis.

2.3.2. Digestibility trial

All diets contained 1% chromic oxide (Cr₂O₃) as an inert maker to allow the calculation of digestibility coefficients (ADC) for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED). The chromium content of diets and fecal material used to calculate apparent digestibility values were determined using the method described by Furukawa and Tsukahara (1966). Apparent digestibilities of dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) were calculated following the equations:

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

$$\text{ACPD or AGED} = 100 - 100 [(\% \text{Cr}_2\text{O}_3 \text{ in feed} / \% \text{Cr}_2\text{O}_3 \text{ in feces}) \times (\% \text{protein or MJ kg}^{-1} \text{ energy in feces} / \% \text{protein or MJ kg}^{-1} \text{ energy in feed})].$$

2.3.3. Nitrogenous excretion trial

Three crabs from each treatment were individually fed *ad libitum* with their assigned experimental diet. Immediately after the meal, each crab was transferred into another individual aquarium containing a fixed volume of water (10 L). Ammonia-N excretion in each aquarium was measured twice: before and 24 h after transferring the crab, using the method described in Grasshoff (1983). The ammonia-N value was converted into energy by multiplying by 24.83 kJ g⁻¹ (Elliot, 1976). The potential loss of nitrogenous compounds through bacterial action or diffusion was quantified by setting controls without crabs. The values of ammonia-N concentration of this control treatment were used to adjust determined nitrogenous excretion. During the experimental period, this excretion trial was repeated every 25 days to get the average value for ammonia-N excretion.

2.4. Biochemical analysis

The proximate compositions of diets, feces, initial and final crabs were determined at the In vivo laboratory, Vietnam according to the methods of AOAC (1995). 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 content by an appropriate numerical factor, crude protein % = %N * 6.25. Crude lipid content was measured by solvent extraction with petroleum ether. Ash content was determined following combustion in 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. Definitions, calculations and statistics

2.5.1. Monitoring the molting

Cumulative molting (CM, %) can be calculated using the following formula:

$$CM = \left(\sum_{i=0}^n Mi/Cr \right) \times 100$$

Where i = the day i; Mi = number of molts on the day i; Cr = number of crabs in each treatment.

2.5.2. Growth measurements

Growth parameters of crabs in this study were determined as: tissue growth rate, tissue gain and nutrient gains (protein, lipid and ash). iBW and BWa.e. are initial body weight and body weight after ecdysis, respectively; fBW is final dry body weight. Dry BWa.e. was 15% of total body weight of 1 day-molted crab estimating from our extra sample assessment for 12 crabs molted after 1 day.

Tissue growth rate after ecdysis (% of dry BWa.e. d⁻¹) or during the course of experiment (% of dry iBW d⁻¹) and tissue gain (% of dry BWa.e.) were measured on the basis of the increase in dry weight (Passano, 1960):

Tissue growth rate after ecdysis = 100 [(fBW – dry BWa.e.) - ash gained] / (dry BWa.e. x days after ecdysis)

Tissue growth rate during the course of experiment = 100 [(fBW – dry iBW) - ash gained] / (dry iBW x days of experiment)

Tissue gain = 100 [(fBW - BWa.e.) - ash gained] / BWa.e.

Protein, lipid or ash gain = 100 (protein, lipid or ash retained) / BWa.e.,

2.5.3. Feed efficiency

Feed conversion ratio (FCR) was calculated as:

FCR = dry voluntary feed intake/dry weight gain

Protein efficiency ratio (PER) was calculated as:

PER = dry weight gain/total protein ingested

Protein, lipid or energy retention was calculated as:

Protein, lipid or energy retention (%) = 100 x (protein, lipid or energy retained/total protein, lipid or energy intake).

2.5.4. Energy budget assessment

The partitions of the energy budget suggested by the National Research Council (NRC, 2011) were adopted in this study with minor modification in order to comply with mud crab growth through ecdysis. The energy budget of growing crabs is expressed in our study as:

$$IE = FE + UE + SE + RE + HE_m \quad (1)$$

Where IE = the energy intake which was obtained for each crab by calculating the difference between the feed distributed and the feed leached and uneaten;

FE = energy lost from the animal through the feces for each crab, FE was calculated by the difference between the voluntary feed intake and the digested feed estimated from its apparent digestibility;

UE = energy lost from the animal through the total ammonia excretion which was assessed for each crab from the trial of nitrogenous excretion previously indicated;

SE = surface energy losses which is exuvia lost at ecdysis;

RE = recovered energy which is channeled into growth. It was calculated for each crab through the dry weight gain converted into energy;

HE_m = maintenance energy calculated for each crab from the equation (1):

$$HE_m = IE - (FE + UE + SE + RE) \quad (2)$$

2.5.5. Statistical analysis

Differences among the various treatments were considered significant when $P < 0.05$. Shapiro-Wilk and Levene's test were used for checking normality and homogeneity of variances, respectively. Percentage data were transformed to their arcsine values to get a normal distribution of the data. One-way ANOVA was applied to i) test the effect of dietary treatments on VFI, and ii) compare the digestibility coefficient of each diet. ANCOVA was used with energy intake as a covariate to assess the effect of the different diets on the tissue growth, feed efficiency parameters (FCR, PER, retention of protein, lipid, and energy), and the partitioning of the energy budget.

3. Results

3.1. Feed digestibility and voluntary intake

Apparent digestibility for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of the five experimental diets are given in Table 2. No significant differences between diets (ANOVA, $P > 0.05$) in ADMD, ACPD and AGED were found; apparent digestibilities were high with average values of $96.02 \pm 1.59\%$ for ADMD; $97.26 \pm 0.66\%$ for ACPD and $97.02 \pm 0.54\%$ for AGED.

The averages of voluntary feed intake (VFI), soy protein concentrate (SPC) and protein intakes for each diet are presented in Table 3. There were no significant differences in VFI (ANOVA, $P = 0.78$) among the treatments. However, the variability of the data was high whatever the diet (CV = 36.7%, $n = 50$) with a minimum VFI of 45.7 and maximum of 220.2 g kg^{-1} of fresh iBW week⁻¹. Soy and protein intakes significantly increased from the diet SPC-12 to the diet SPC-42 and then reached a plateau for the two following diets with higher inclusion levels of SPC.

As VFI was not affected by the diet composition, we pooled all the data to study the feed consumption during the course of the experiment (Fig. 1). During the first 14 days after ecdysis, crabs consumed about 200 g kg^{-1} of fresh iBW week⁻¹ and then the VFI decreased until Day 35. From Day 49 until the Day 77, VFI remained at a baseline value of around 100 g kg^{-1} fresh iBW week⁻¹. Over the last 5 days before ecdysis, the VFI dropped dramatically to the value of 50 g kg^{-1} of fresh iBW week⁻¹.

3.2. Molt cycle

The cumulative molts were clearly affected by the voluntary feed or energy intake levels, all diets combined (Fig. 2A). Two crab groups were identified; a low VFI group ($1.05 \pm 0.12\%$ of

iBW d⁻¹) and a high VFI group ($1.97 \pm 0.12\%$ iBW d⁻¹). Cumulative molts, during the course of experiment were 50% and 100%, respectively in the low and high VFI groups.

The cumulated molts were also affected by the SPC or protein contents in the diets (Fig. 2B). After 80 days, the molting rates was 60% for crabs fed on the diets SPC-12, and 90% for crabs fed on the diets SPC-32, SPC-52 and SPC-60. Whereas, only 50 days after starting the experiment, 90% of the crabs fed on diet SPC-42 had molted.

3.3. Growth

3.3.1. Body weight gain and carapace increment at molt

The fresh weight gain after ecdysis (molt stage B) averaged $89.5 \pm 4.1\%$. It was not affected by the diet, except significantly higher (ANOVA, $P < 0.05$) for crabs fed on diet SPC-42 compared with SPC-52 (Fig. 3A). The average carapace increment was $23 \pm 2.7\%$ and was not affected by the diet consumed (Fig. 3B).

3.3.2. Tissue growth during molt cycle

The water content of postmolt crabs was 85% one day after ecdysis (Fig. 4) and then decreased over the next 23 days to reach a baseline of $62.4 \pm 0.6\%$ of water which then remained relatively constant until the next molt.

The tissue gain, measured as the increase in dry organic matter as proportion of dry body weight after ecdysis (BWa.e.), increased for about 30 days after ecdysis and then it remained relatively constant until the next molt (Fig. 5A).

The tissue growth rate was highest two days after ecdysis (16.5%) and dropped dramatically during the first 10 days to 3.6%. Then the decrease was slower to the minimum value of 1.3% just before the next ecdysis (Fig. 5B).

Crab body protein and lipid change during the intermolt cycle and showed the same trend as the tissue growth (Fig. 6A, 6B, respectively) with two distinct phases: an initial increase followed by a plateau. The initial increase of body protein content started about one week earlier than the increase of the lipids. Ash gain did not follow the trends of protein and lipid. It increased rapidly during the first five days after ecdysis and then remained stable during most of the intermolt period (Fig. 6C).

3.3.3. Tissue growth and feed efficiency according to the dietary treatments

To compare the effect of the different diets on growth, feed efficiency and energy budget, only the crabs which had molted a minimum of 30 days before and had reached the plateau of

their tissue growth were retained for the experiment. In the treatment SPC-12, only 2 crabs satisfied this condition, thus we have not considered this treatment any further.

As voluntary feed intake (VFI) was highly variable, regardless of dietary treatment, we have to consider the effect of its level on crab tissue growth. Indeed, there is a significant linear relation between energy intake and growth rate for each dietary treatment, with the exception of diet SPC-32 which however has linear relationship (Fig. 7). Thus, ANCOVA was applied for all the diet treatments with energy intake as a covariate in order to reduce error variance within each treatment allowing us to more accurately assess the effect of dietary treatment on dependent parameters (tissue growth, feed efficiency).

Based on analysis of covariance (ANCOVA), dietary treatments did not significantly affect tissue growth rate ($P = 0.054$), nor the FCR ($P = 0.135$), PER ($P = 0.127$) and protein retention ($P = 0.112$), but they did affect lipid retention ($P < 0.001$), and energy retention ($P = 0.013$) (Table 4). For lipid retention, the highest values were found for the diet SPC-32 and SPC-42 and then it decreased with the diet SPC-52 and SPC-60. For energy retention there was no clear trend, with lowest value for diet SPC-32 and SPC-52 and highest values for diets SPC-42 and SPC-60.

3.3.4. Energy budget according the dietary treatments

To analyze the energy budgets for the different diets only crab juveniles which had molted a minimum of 30 days before were retained for the experiment.

ANCOVA analysis showed that the dietary treatments did not significantly affect the energy budget allocated in FE ($P = 0.15$) and SE ($P = 0.26$). Conversely, diet affected UE ($P < 0.001$), RE ($P = 0.005$), and HE_m ($P = 0.01$). UE increased in the similar way to SPC or protein content in the diets with significant rise for SPC-52 and SPC-60 (Table 5).

For RE and HE_m , there were no clear pattern but we noted significantly higher value for RE and lower for HE_m with crabs fed on diet SPC-42. The average energy budget accounted for RE was higher for crabs fed on diets SPC-32 and SPC-42 compared to other diets; however, this difference was not significant.

Under our experimental conditions, the main proportion of energy intake was allocated for maintenance (75%) and growth (21%). The remaining proportion of energy intake (4%) was shared among FE (2.5%), UE (0.6%) and SE (0.9%).

4. Discussions and conclusions

4.1. Feeds digestibility

In our study, digestibility of the experimental diets was not affected by the level of dietary soy protein concentrate (SPC). This result confirms the results of our previous study on crab *S. serrata* that showed high digestibility coefficients for dry matter, protein and energy of SPC, which were also similar to those of fish meal (unpublished). These results are also 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 to 95.7 %; 91.7 to 97.1% and 88.6 to 97.9, respectively (Catacutan et al., 2003; Tuan et al., 2006; Truong et al., 2008, 2009). Furthermore, Truong et al. (2008) showed that crude protein from soy bean meal was more completely digested by mud crabs than the other plant ingredients tested (canola, lupin and cotton seed meal). Although SPC is more expensive than SBM, it 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).

4.2. Voluntary feed intake (VFI)

In the present study, VFI varies greatly between individuals (global CV = 36.7%) with a general average of 105.5 g kg⁻¹ of iBW week⁻¹. As the experimental diets were formulated to be isoenergetic, the variability of energy intake was similar to the VFI variability. Interestingly, the average VFI did not differ among diets tested, even the corresponding SPC intake increased significantly as its concentration in the diet increased. Jiang et al. (2013) obtained a similar result with the crab *Eriocheir sinensis* and Suárez et al. (2009) showed no difference in feed intake for the shrimp *Litopenaeus vannamei* when the replacement of fish meal by soybean-canola meal reached 100%. Nevertheless, Luo et al. (2011) reported that feed intake of Chinese mitten crab (*Eriocheir sinensis*) was significantly reduced when a mixed source of dietary soybean meal and rapeseed meal exceeded 30%. As the VFI were very variable in our study, whatever the diet tested, the amount of protein or SPC ingested relied not only on the diet composition (dietary level of SPC) but also on the VFI. Thus, several crabs fed on the diets with higher protein contents, did in fact ingest less protein than some animals fed on diets with lower protein contents. However, the general trend showed that the protein intake increased significantly from the diet SPC-12 to the diet SPC-42 and then remained stable. Consequently, the surplus of SPC in the diets SPC-52 and SPC-60 did

not result in higher protein consumption. We can then assume that the diet SPC-42 is the best compromise between dietary levels of SPC and availability of proteins for crabs.

4.3. Molt frequency

The duration of molt cycle stages in *S. serrata* (and all decapod crustaceans) are the net result of complex interactions between many endogenous (genetic) and exogenous factors (Heasman, 1980). Amongst these factors, temperature, nutrition, autotomy and regeneration, size and sex and sexual maturity are important (Heasman, 1980). Few studies have been devoted to the quantitative and still less to the qualitative influence of nutrition on the molting frequency of crustaceans. However, the general trend is that if food supply is reduced below the optimum level, there is invariably a reduction in growth rate (Hartnoll, 1982). This reduction is due to the combination of an extended intermolt period (lower molt frequency) and a reduced molt increment (carapace and body weight growth at molt). Our study confirmed this trend as the level of voluntary food intake (VFI) greatly influenced the molt frequency. One hypothesis on the mechanisms involved in triggering molting when threshold of energy become sufficient is the implication of the hormonal control involving the molt inhibiting hormone (MIH) and the hyperglycaemic hormone (CHH) (Chang and Mykles, 2011). It is accepted that ecdysis is initiated when blood levels of the MIH fall, which could be triggered by accumulated nutritional energy level. The CHH, which has also a molt inhibiting activity, is produced under various stresses, including food reduction or starvation (Keller and Orth, 1990). Raised CHH levels have been suggested as a possible factor in extending the intermolt period beyond its normal length with reduction in food supply (Oh and Hartnoll, 2000). We showed also that, for an equivalent VFI or energy intake, the dietary level of SPC or protein influenced the molt frequency which was highest for the diet SPC-42. The molt frequency increased from diet SPC-12 to diet SPC-42 and then decrease for the diets SPC-52 and SPC-60. These results are novel and we could hardly find an equivalent study showing the influence of dietary protein on the molts frequency of crustaceans (Regnault and Luquet, 1974). The lower molt frequency of the crabs fed on diet SPC-12 could be explained by a deficiency in protein and essential amino acids required for tissue growth especially during postmolt period. This can also be explained by the high carbohydrate content of the diet SPC-12 which can lead to glucose load resulting in persistent hyperglycemia and growth impairment. However, this second explanation is unlikely, because base on the reported findings for different fish and shrimp species, the maximum recommended levels of digestible

starch inclusion in feed fall within 20-40% in shrimp and up to 50% in omnivorous species among which is the mud crab (La Sara et al., 2007; NRC, 2011).

To explain the decline in molts frequency for the diets SPC-52 and SPC-60, two hypotheses can be put forward: the first relates to the toxicity of ammonia and the second to the antinutritional factors encountered in soy bean.

Regarding the first hypothesis, after feeding the majority of amino acids in excess of what is required for protein synthesis, are catabolized in the liver (Campbell, 1991), releasing ammonia and resulting in transient increases in plasma ammonia level and ammonia excretion rate in fish (Wicks and Randall, 2002). In crustaceans, ammonia, excreted by the gills as the forms of non-ionic ammonia (NH_3) and ammonium ions (NH_4^+) existing in a pH-dependent equilibrium, is the major form of nitrogen excreted, accounting for more than 80% of the total nitrogen excretion (Needham, 1957; Krishnamoorthy and Srihari, 1973; Weihrauch et al., 2009). During the molt cycle an increase in nitrogen metabolism is correlated with a simultaneous increase in general metabolism (Regnault, 1979). Thus a good correlation exists between ammonia excreted and oxygen consumed. Both were highest immediately after ecdysis or after a meal (Regnault, 1979). For *S. serrata*, there is also a positive correlation between ammonia excreted and meal size as well as dietary proteins of the diets found in our previous study (unpublished). Thus, a transient postprandial surge in blood ammonia concentration could occur in crabs as it has been described for fishes (Kaushik and Teles, 1985; Wicks and Randall, 2002) and lead to postprandial ammonia toxicity. In our study, crabs fed on diets SPC-52 and SPC-60, with the highest dietary protein content, could be confronted with ammonia toxicity after feeding, leading to lower molt frequency compared to animals fed SPC-42 diet.

The second hypothesis could explain the negative effect of high SPC inclusions on molt frequency of juvenile crabs *S. serrata*. Anti-nutritional factors (ANFs) are present in soy bean meal, such as protease inhibitors, tannins, lectins and non-starch polysaccharides (Francis et al., 2001). However, in our study SPC has been used and produced by aqueous alcohol extraction from defatted soybeans, which removes a majority of the phytate, lectins, saponins and oligosaccharides (Anderson and Wolf, 1995; Bureau et al., 1998; Deng et al., 2006; Refstie et al., 2001). Although SPC has a low content of ANFs, they could still be a significant problem when SPC is included at high levels in the diet. *S. serrata* may be particularly susceptible to these anti-nutritional factors.

4.4. Growth

Most growth studies on crabs only consider the increase in size and fresh weight with time. The highest increase in fresh weight (almost 90%) of the crab *S. serrata* occurs in the very brief period of rapid water uptake (Heasman, 1980). Fresh weight changes follow a basic pattern through the molt cycle in all species of crustaceans, i.e. large and abrupt increases associated with rapid water uptake at ecdysis. Further moderate gains are associated with mineralization of the integument and the accumulation of organic matter during late molt stages and a relative stabilization of fresh weight during intermolt until the onset of the successive ecdysis (Heasman, 1980). The water content (% of fresh body weight) of juvenile *S. serrata* had a clear and progressive decline from a maximum of 88% following ecdysis to an average baseline of $62.4 \pm 0.6\%$. Our results are in close to those of Heasman (1980). The high percentage of free water levels after ecdysis largely reflect the relative amount of water absorbed during and immediately after ecdysis and hence the relative increase in volume of the animal. Although the weight growth in crabs in connection with the molt has an interrupted character, the somatic growth and the accumulation of energy reserves in tissues are continuous processes. Consequently, such growth is accompanied by definite changes in the ratio of dry and fresh weight during the molt cycle. Thus the period of rapid water uptake associated with ecdysis in *S. serrata* is followed by an increase in dry matter content in the relative proportion of initial body dry weight, reaching a plateau 30 days after ecdysis. The most rapid growth rate occurred during the first days after molt and then decreased until the next molt. Our results are in similar to those of Heasman (1980) who showed that increases in organic matter, as a proportion of fresh weight, were higher from molt stages B to C3 (postmolt stages) than from molt stage C4 to D2 (intermolt-premolt stages) for *S. serrata*. According Passano (1960) and Skinner (1966), the growth of the muscle is thought to occur during the postmolt period when the carapace is already hardened, whereas immediately after the molt when the carapace is still soft the muscles appear to be watery and atrophied.

Protein gain expressed as the relative proportion of initial body dry weight followed the tissue growth rate pattern. The proportion increased during the first 35 days after ecdysis. This increase reflected a high protein accretion during the postmolt period. El Haj and Houlihan (1987) also showed an increase of the protein synthesis rate in the extensor muscle during the post-ecdysial period of the crab *C. maenas*.

The changes in the lipid gain were similar to those of tissue and protein. Lipids were accumulated by the crab during the first 40 days following ecdysis reaching a plateau at $8.49 \pm 0.67\%$ of the body dry weight. In our previous study we showed that lipid level was higher

in premolt crabs than in postmolt crabs (unpublished). Furthermore, Copeman et al. (2012) found that triacylglycerol levels in “red king crab” (*Paralithodes camtschaticus*) rapidly accumulated during seven days following a molt. In *Eriocheir sinensis*, the accumulation of lipids prior to molting appeared to result from the increase in neutral lipids because the polar lipid content remained constant during the molt cycle. The neutral lipids, particularly triacylglycerides, are the predominant molecules of energy storage and, consequently, the major source of energy (Stoner et al., 2010). In our previous study (unpublished), lipid concentration of dry body in premolt *S. serrata* (2.8%) was lower than those reported for the crab *Rhithropanopeus harrisi* (5%) (Nates and McKenney, 2000), the rock lobster *Panulirus cygnus* (7%) (Liddy et al., 2005) and red king crab, *Paralithodes camtschaticus* (2 to 3.6%) (Stoner et al., 2013).

Conversely to tissue, protein and lipid gains, the proportion of mineral salts (in dry weight) increased rapidly during the 5 days following ecdysis and then reach a plateau until the next molt. Heasman (1980) already noted the rapid mineralization of the cuticle for newly molted *S. serrata*. Similarly, the crab *Eriocheir sinensis* actively incorporated minerals after ecdysis (Tian et al., 2012).

In our study we found a linear relationship between VFI (or energy intake) and growth and based on this correlation we calculated the adjusted means of growth for a same amount of energy intake. Tissue growth for a same amount of VFI was not significantly affected by the feed composition, although a higher value was observed with treatment SPC-42. This diet resulted in a better FCR, PER, and retention of protein, lipid and energy. The higher lipid and energy retention of crabs fed on SPC-42 diet must be related to the increase in their molt frequency. This result is consistent with Adelung (1971) who found that the principal determinant of molt frequency in crab *C. maenas* was its rate of tissue growth. This author also stated that reserve of energy must be accumulated before a molt can occur. In our study, tissue growth and energy storage were faster with diet SPC-42 and the next molt occurred earlier compared with crabs fed on other diets. This would explain the higher molt frequency observed for the crabs fed on SPC-42.

This study has also shown that the quantity or composition of the diet greatly influenced somatic growth and molting frequency, but that they have no influence on the increase in size at ecdysis. According to Hartnoll (1982), the overall growth rate of crustaceans is a function of the molt cycle duration and the molt increment. The relative importance of these two processes varies between species, and within species between different experimental

protocols. However, in most cases the most important factor is molt cycle duration. Our results on *S. serrata* are consistent with those in the literature (Hartnoll, 1982).

The best growth (tissue growth and molt frequency) and the best feed efficiency (FCR, PER, retention of energy, proteins and lipids) obtained with SPC-42 diet was confirmed by the bioenergetic study. A decreased of retained energy (RE) was observed when dietary protein content increased. The best RE was observed for the diet SPC-42. Increased energy retention in lower dietary protein diets (SPC-42 and SPC-32) may be related to the efficiency of nutrient utilization. Excess dietary protein is diverted for catabolism creating an imbalance in the nutrient profile of the diets, reducing protein retention (Guzman et al., 2001). Furthermore, it has been demonstrated in young pigs that dietary protein is used less efficiently than carbohydrate or lipid for energy retention (Van Milgen et al., 2001). If a similar process is occurring for crabs in our study, it should result in increased heat production leading to higher energy allocated for maintenance (HEm) and increased urinary energy losses in diets SPC-52 and SPC-60.

The best growth (somatic growth and molt frequency) and the best feed efficiency were obtained, under our experimental conditions, when 1 kg crabs consumed daily 6.5 ± 1.1 g of protein ($P/E = 22.13 \text{ g MJ}^{-1}$). This ingestion rate was obtained with the diet SPC-42 that contained 40% protein of which almost three quarters was derived from SPC. Intake of protein with diets SPC-12 and SPC-32 was obviously insufficient for optimal growth, while the excess intake of protein (or SPC) with diets SPC-52 and SPC-60 adversely affected the growth of juvenile crabs. Our results are similar to those of Catacutan (2002) who has shown that the optimal dietary protein in diet was 40% for juvenile crabs. However, at a younger stage, crabs had much higher voluntary feed intake (21.8 g kg^{-1} of BW d^{-1}) and the optimum level of crude protein in the diet was 45% (Unnikrishnan and Paulraj, 2010). Finally our work demonstrated the advantage of following somatic growth and molt frequency when investigating nutritional requirements of the crab which is subject to very significant changes in water content during the molting cycle.

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Captions of tables and figures

Table 1. Ingredients and proximal composition of five experimental diets.

Table 2. Apparent digestibilities for dry matter (ADMD), crude protein (ACPD) and gross energy (AGED) of five experimental diets.

Table 3. Voluntary feed intake, SPC (soy protein concentrate) intake and protein intake for crabs fed on five different experimental diets.

Table 4. Feed efficiency in crab juveniles fed on different experimental diets.

Table 5. Energy budget of crabs fed on different experimental diets with the same energy intake.

Figure 1. Voluntary feed intake of crabs fed on whatever the diet, during a molt cycle. Homogenous groups determined from Tukey test are presented above the curve according time.

Figure 2. Cumulative molts of crabs (% of initial crab numbers) according to the voluntary feed intake (VFI) levels (A) and different diets (B). iBW = fresh initial body weight.

Figure 3. Fresh body weight gain after one day of ecdysis (A) and carapace increment after

one day of ecdysis (B) for crabs fed on different experimental diets.

Figure 4. Crab water content during a molt cycle. Mean value of the data calculated for crabs after 25 days of ecdysis. The fast decline trend of water content during the first 25 days after ecdysis “○” while stable trend for crabs after 25 days of ecdysis “●”.

Figure 5. Growth of crabs during a molt cycle: (A) is tissue gain expressed in % of dry body weight after ecdysis - % of dry BWa.e.; mean value was determined for crabs after 25 days of ecdysis and (B) is tissue growth rate expressed in % of dry body weight after ecdysis per day - % of dry BWa.e. day⁻¹.

Figure 6. Nutrient gains of crabs expressed as % of dry body weight after ecdysis during a molt cycle: (A) protein gain; (B) lipid gain and (C) ash gain.

Figure 7. Linear relationships between growth rates (% of dry iBW.day⁻¹) and energy intakes of crabs fed on different experimental diets: SPC-32; SPC-42; SPC-52 and SPC-60. Significant r^2 , $P = 0.10; 0.02; 0.04; 0.03$ for SPC-32; SPC-42; SPC-52; SPC-60, respectively.

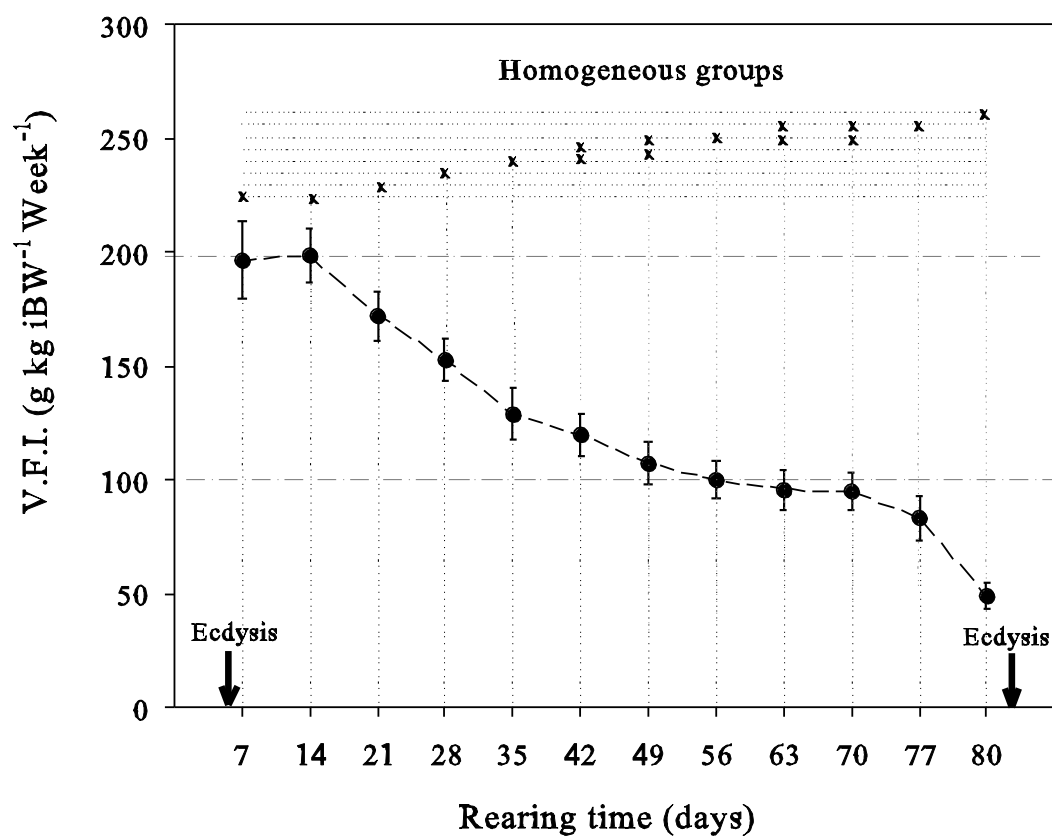


Figure 1

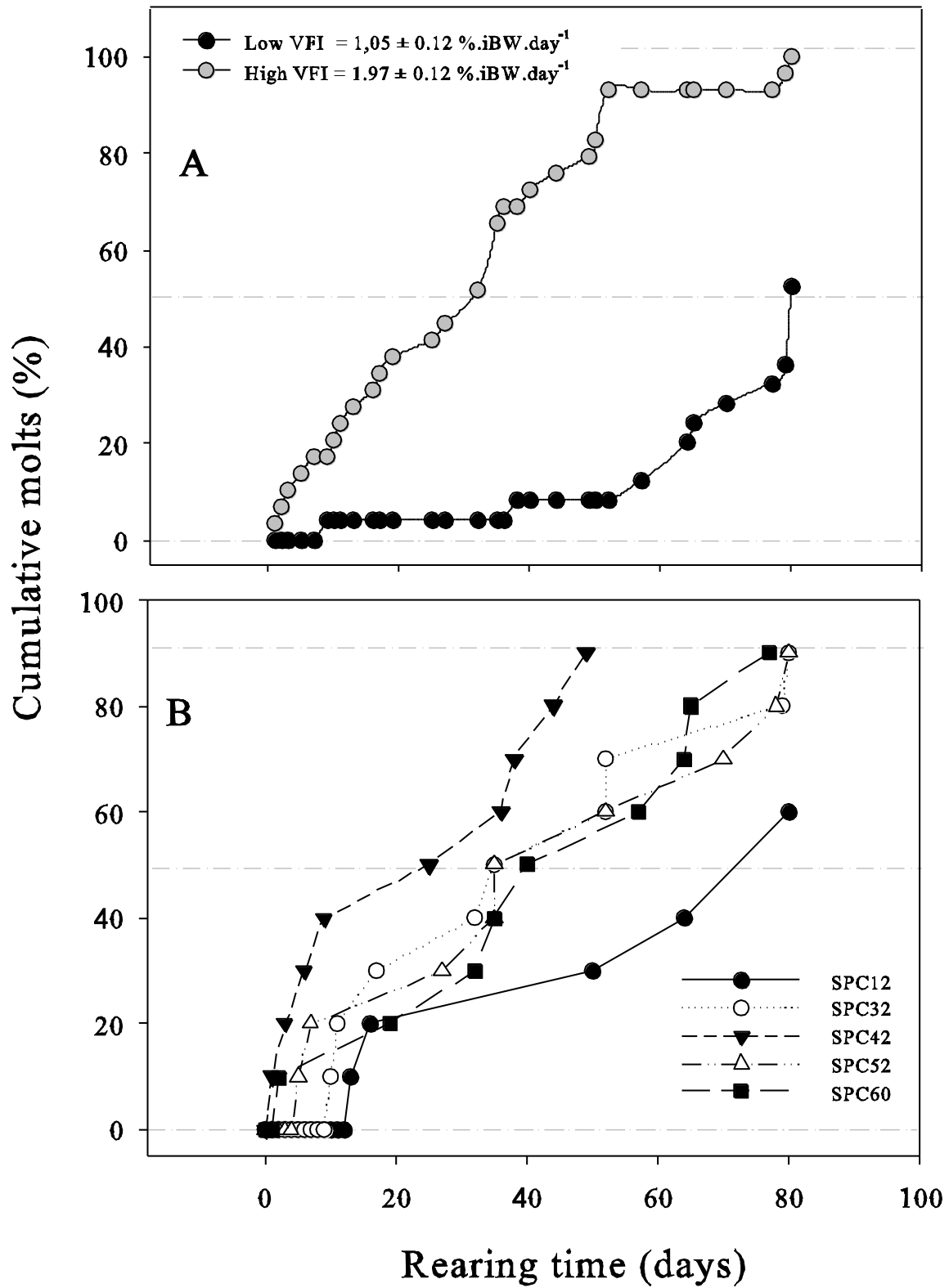


Figure 2

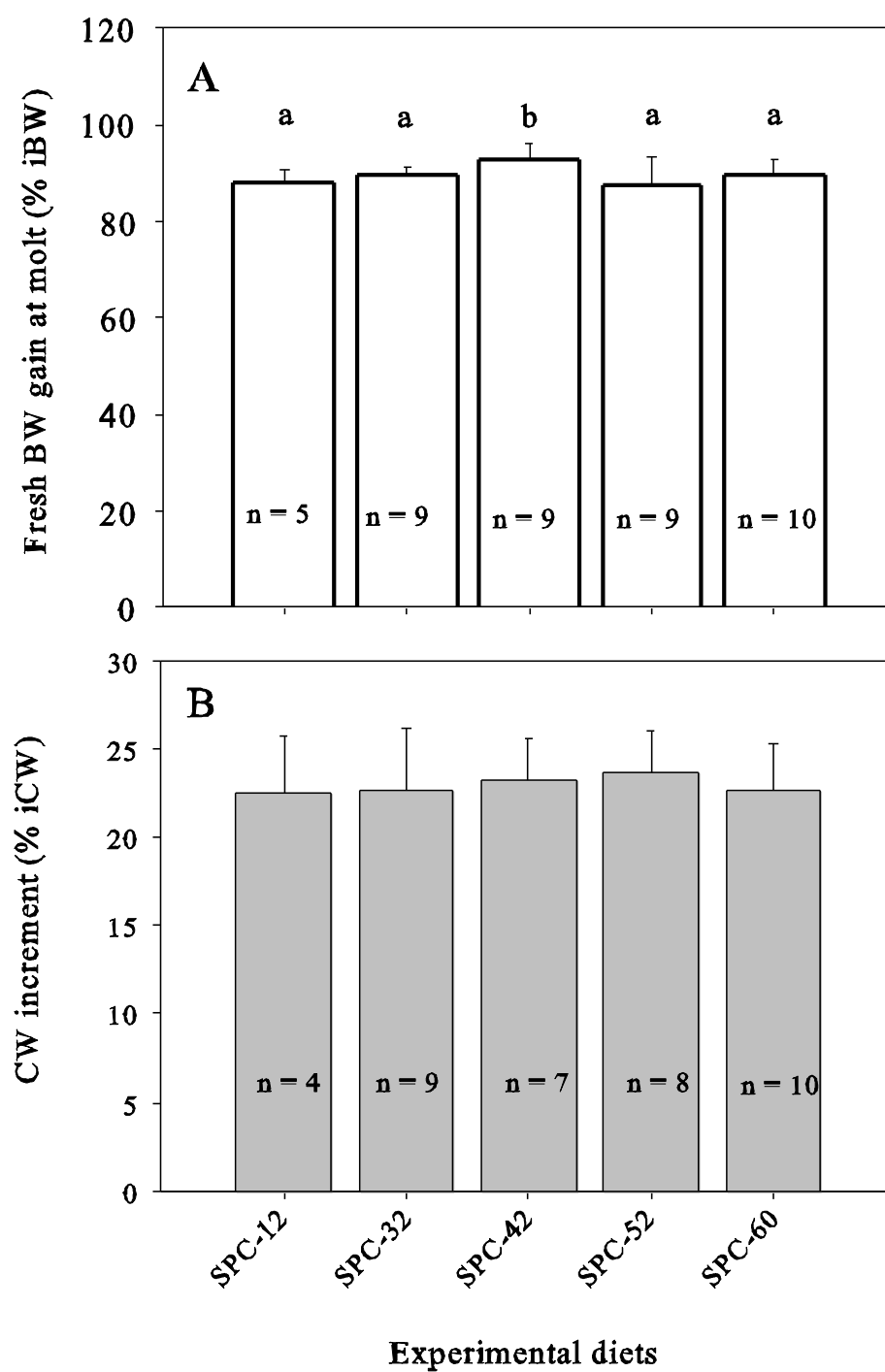


Figure 3

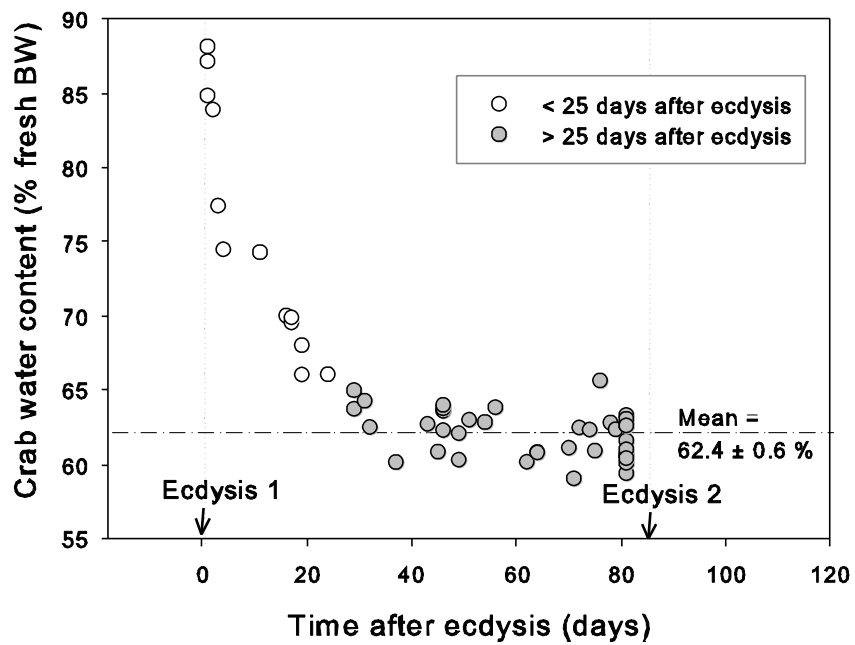


Figure 4

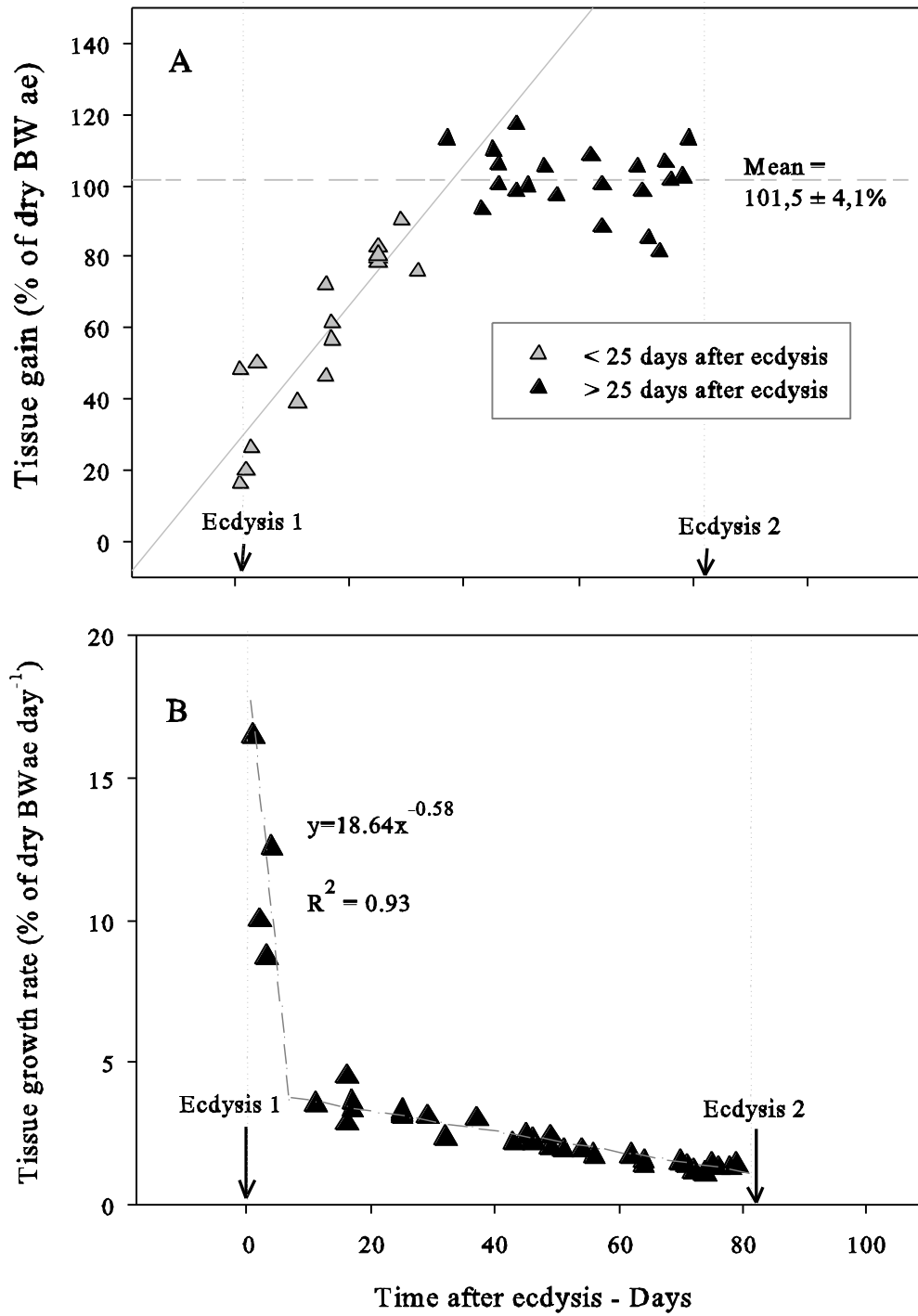


Figure 5

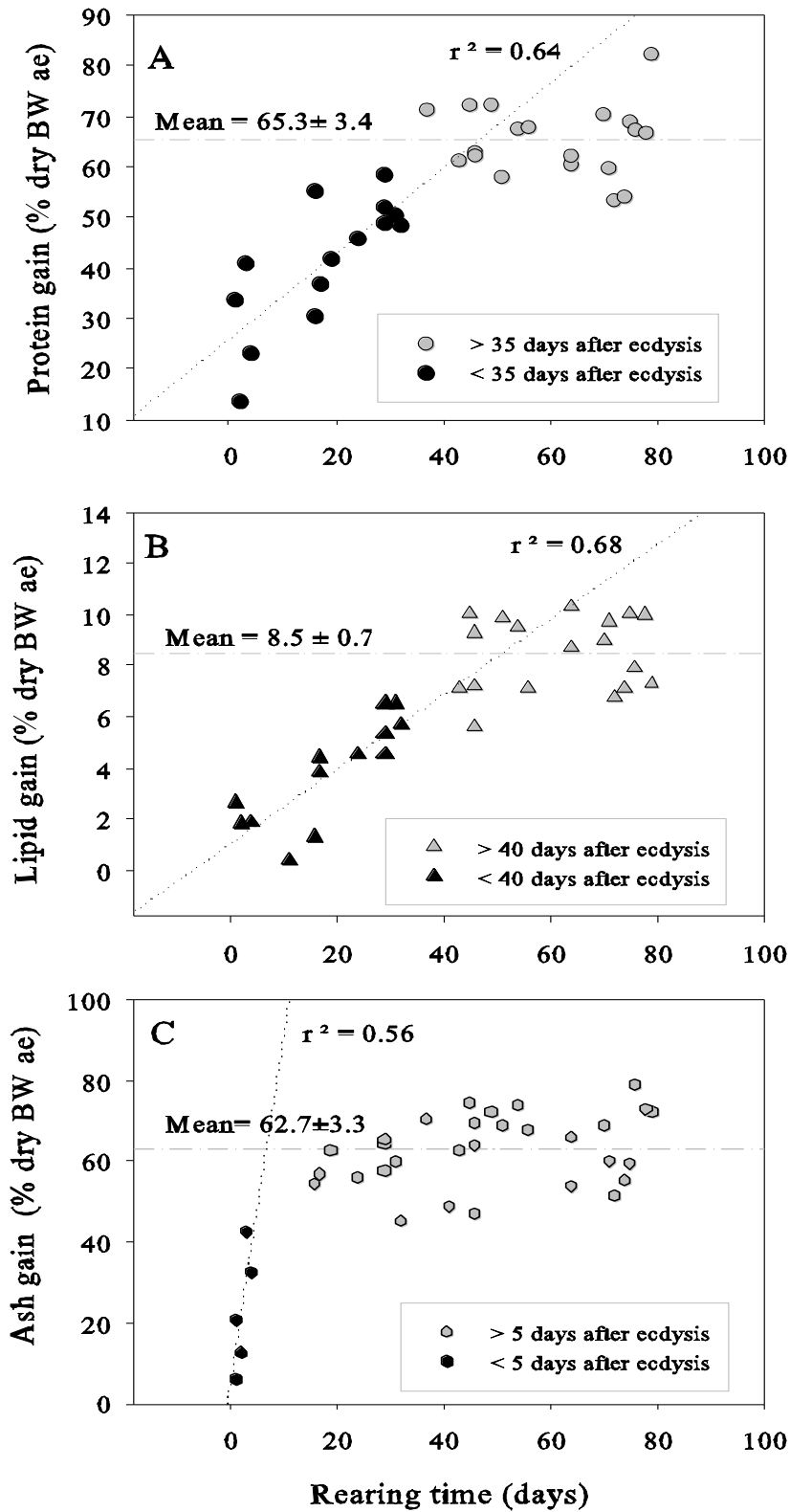


Figure 6

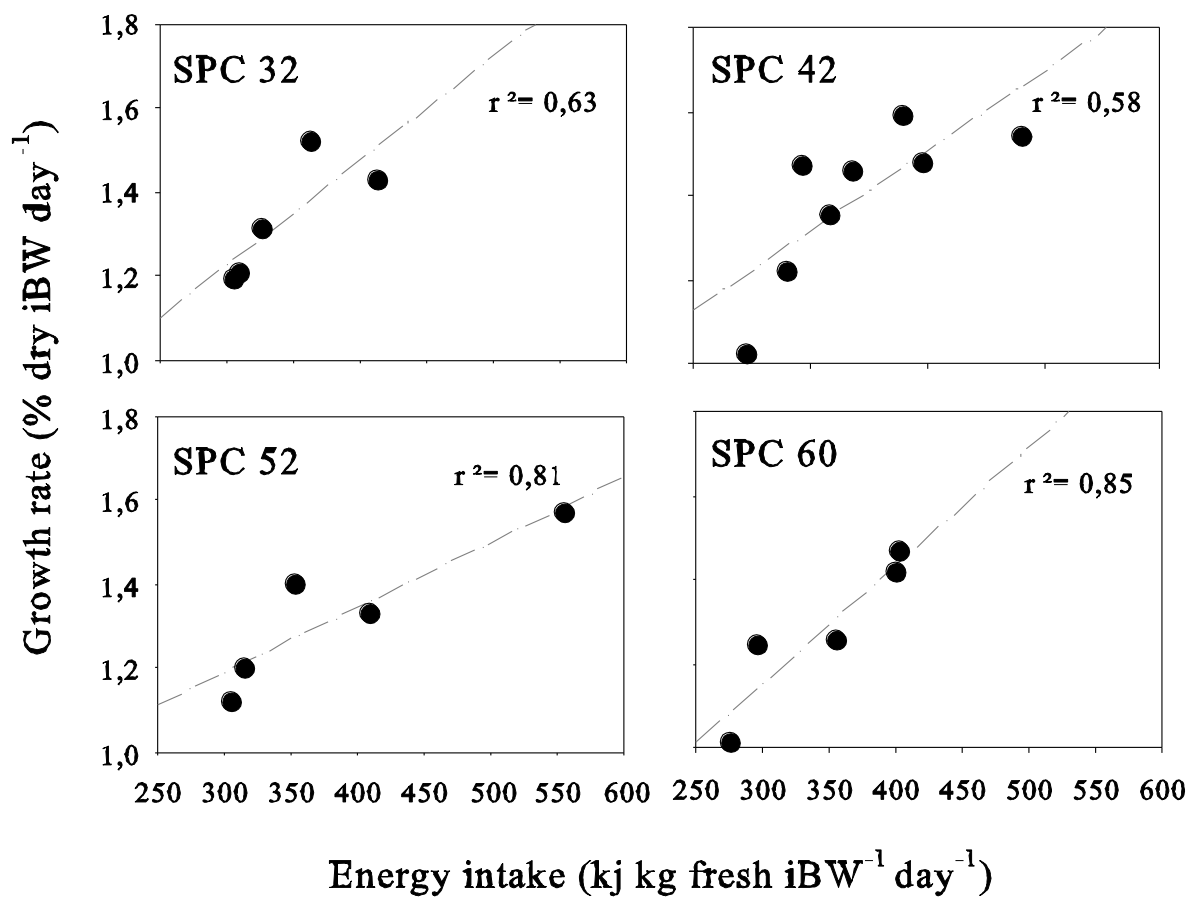


Figure 7

Table 1. Ingredients and proximal compositions of five experimental diets.

	Experimental diets				
	SPC-12	SPC-32	SPC-42	SPC-52	SPC-60
Composition (%)					
Soy protein concentrate (SPC) ¹	12	32	42	52	60
Crab meal ²	20	20	20	20	20
Wheat flour ³	53.5	35.0	25.5	16.0	8.0
Fish oil	4	4	4	4	4
Soy oil	2.5	1.0	0.5	0.0	0.0
Gluten	5	5	5	5	5
Dicalcium phosphate	3	3	3	3	3
vitamin C ³	0.25	0.25	0.25	0.25	0.25
Vitamin premix ⁴	0.5	0.5	0.5	0.5	0.5
Trace elements ⁵	1	1	1	1	1
Analysis (dry matter basis)					
Crude protein (%)	26.87	35.52	39.69	44.15	49.17
Lipid (%)	6.30	4.80	4.62	4.36	4.43
Ash (%)	13.95	14.54	22.36	21.80	27.43
Gross energy (MJ kg ⁻¹)	17.96	17.26	17.87	18.61	18.44
Protein/Energy (g MJ ⁻¹)	16.79	20.58	22.13	23.72	25.56

¹SPC purchased from "Sementes Selecta SA", a Cie in Brazil.

²Crab meal purchased from "Comercial Bahia SPA", a Cie based in Chile.

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

⁴Vitamin 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, vitamine B6 98% Pyridoxine, vitamin B9 97% Folic acid, vitamin D3 500, Vitamin E 50 ADD, vitamin K3 43.7%.

⁵Trace elements from SICA Cie (Goodman Fielder[®]). SICA is the main animal feed producer Cie in New Caledonia.

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Table 2. Apparent digestibilities for dry matter (ADMD), crude protein (ACPD), and gross energy (AGED) of five experimental diets.

Diets	Apparent digestibilities (%)		
	ADMD	ACPD	AGED
CP25	95.96 ± 1.44 ^a	96.85 ± 0.82 ^a	96.84 ± 0.70 ^a
CP35	95.80 ± 2.02 ^a	96.99 ± 0.92 ^a	96.47 ± 0.55 ^a
CP40	96.29 ± 1.82 ^a	97.65 ± 0.57 ^a	97.09 ± 0.42 ^a
CP45	96.03 ± 1.04 ^a	97.12 ± 0.63 ^a	97.04 ± 0.55 ^a
CP50	96.00 ± 1.62 ^a	97.68 ± 0.34 ^a	97.67 ± 0.47 ^a

Values are means ± SD (n = 3 replicates per treatment). Within the same column, means with the same letters are not significantly different ($P > 0.05$).

Table 3. Voluntary feed intake, SPC (soy protein concentrate) intake and protein intake for crabs fed on five different experimental diets.

	Experimental diets				
	SPC-12 (n = 10)	SPC-32 (n = 11)	SPC-42 (n = 11)	SPC-52 (n = 11)	SPC-60 (n = 11)
Voluntary feed intake (g kg ⁻¹ of iBW week ⁻¹)	96.7 ± 25.3 ^a	106.7 ± 18.6 ^a	114.0 ± 18.7 ^a	107.4 ± 31.0 ^a	102.7 ± 21.5 ^a
SPC intake (g kg ⁻¹ of iBW week ⁻¹)	11.6 ± 3.0 ^a	34.1 ± 5.8 ^b	47.9 ± 7.9 ^{bc}	55.9 ± 16.1 ^{bc}	61.6 ± 12.9 ^c
Protein intake (g kg ⁻¹ of iBW week ⁻¹)	26.0 ± 6.8 ^a	37.9 ± 6.5 ^a	45.3 ± 7.4 ^b	47.4 ± 13.7 ^b	50.5 ± 10.6 ^b

Values are means ± SD. Within the same row, means with different letters are significantly different ($P < 0.05$); iBW = fresh initial body weight.

Table 4. Feed efficiency in crab juveniles fed on different experimental diets.

Parameters	Experimental diets				
	SPC-12	SPC-32 (n = 5)	SPC-42 (n = 7)	SPC-52 (n = 5)	SPC-60 (n = 5)

Tissue growth rate (% of dry iBW d ⁻¹)	ND	0.81 ± 0.05 ^a	0.86 ± 0.06 ^a	0.76 ± 0.04 ^a	0.78 ± 0.05 ^a
FCR	ND	3.88 ± 0.22 ^a	3.68 ± 0.26 ^a	4.07 ± 0.26 ^a	4.05 ± 0.28 ^a
PER	ND	0.62 ± 0.04 ^a	0.65 ± 0.04 ^a	0.59 ± 0.04 ^a	0.59 ± 0.03 ^a
Protein retention (%)	ND	24.95 ± 1.53 ^a	26.45 ± 1.75 ^a	23.80 ± 1.55 ^a	23.98 ± 1.56 ^a
Lipid retention (%)	ND	32.65 ± 2.32 ^a	35.27 ± 2.33 ^a	24.57 ± 1.21 ^b	25.20 ± 1.64 ^b
Energy retention (%)	ND	18.48 ± 1.13 ^{abc}	18.92 ± 1.25 ^b	16.35 ± 1.07 ^c	19.62 ± 1.08 ^b

Values are adjusted means ± confident intervals, with energy intake (EI = 0.93 ± 0.06 kJ g⁻¹ of dry iBW d⁻¹) as covariate. Within the same row, different letters indicate significant differences ($P < 0.05$) between the diets; ND is not determined.

Table 5. Energy budget of crabs fed on different experimental diets with the same energy intake.

Diets	Energy intake (IE) (kJ g ⁻¹ of dry iBW d ⁻¹)	Partitions of energy (kJ g ⁻¹ of dry iBW d ⁻¹)				
		Faeces (FE)	Excretion (UE)	Ecdycis (SE)	Growth (RE)	Maintenance (HE _m)
SPC-12	ND	ND	ND	ND	ND	ND
SPC-32 (n = 5)	0.93 ± 0.06	0.025 ± 0.002 ^a	0.004 ± < 0.001 ^a	0.008 ± 0.001 ^a	0.195 ± 0.011 ^{abc}	0.690 ± 0.012 ^{abc}
SPC-42 (n = 7)	0.93 ± 0.06	0.023 ± 0.001 ^a	0.004 ± < 0.001 ^a	0.009 ± < 0.001 ^a	0.211 ± 0.014 ^b	0.682 ± 0.034 ^b
SPC-52 (n = 5)	0.93 ± 0.06	0.023 ± 0.002 ^a	0.006 ± < 0.001 ^b	0.008 ± 0.001 ^a	0.178 ± 0.008 ^c	0.713 ± 0.008 ^c
SPC-60 (n = 5)	0.93 ± 0.06	0.024 ± 0.002 ^a	0.009 ± < 0.001 ^c	0.009 ± < 0.001 ^a	0.184 ± 0.016 ^c	0.703 ± 0.018 ^c

Values are adjusted means ± confident interval, with energy intake (EI = 0.93 ± 0.06 kJ g⁻¹ of dry iBW d⁻¹) as covariate. Within the same column, different letters indicate significant differences ($P < 0.05$) between the diets; ND is not determined.