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Energy balance of Litopenaeus vannamei postlarvae fed on animal or vegetable protein based compounded feeds

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Abstract:
L. vannamei postlarvae are normally raised with a protein dense diet (50% protein) rich in fishmeal. Part of the protein is utilized for energy purpose instead of protein synthesis. Based on a previous energy partitioning study, the effects of two isoenergetic compounded feed treatments – animal protein (AP) and vegetable protein and carbohydrates (VPC) – upon growth efficiency and energy budget of shrimp postlarvae and early juveniles were determined. Recovered energy (RE) or production (P) after 50 days trial was similar (2 J day− 1) in both treatments, from PL14 to PL19. However, early juveniles discriminated between animal protein (116 J day− 1) and vegetable protein and carbohydrates (88 J day− 1). The difference in respiration indicated a higher heat increment with AP compared to VPC. At maintenance level, energy used was lower with AP than VPC treatment. Postlarvae and early juveniles employed protein as a main energy substrate (O:N < 20). Differences in the efficiencies observed in the calculated energy budget were attributed to the presence of carbohydrates in diet and not to the protein source. The advantage of incorporating vegetable protein source in the diet of harvesting shrimp may eventually contribute towards a reduction of fishmeal costs and waste products as well as to achieve sustainable shrimp farming

Keywords: Postlarvae; Juvenile; Energy; Animal protein; Vegetable protein; Physiology; Shrimp

Introduction

Our current knowledge on the nutritional requirements of penaeid shrimp postlarval stages (PL's) is still limited. Few studies have been conducted on vitamins, lipids, proteins, and essential minerals needed for their development (Colvin and Brand, 1977; Rees et al., 1994; Camara et al., 1997; Sheen and Huang, 1998; Velasco et al., 1998; Immanuel et al., 2001). The digestive tract of postlarvae goes through several developmental stages (Lovett and Felder, 1989; Lovett and Felder, 1990a, 1990b, 1990c) before reaching its definitive structure with numerous hepatopancreas diverticula and full physiological functions. At PL stages also the feeding habits change in relation with the shift from planktonic to benthic habitats (Escobar-Briones, 1988; Garcia and Le Reste, 1981), as they immigrate from oceanic waters to the estuarine nursery grounds in coastal waters, particularly into mangrove systems (Dittel et al., 1997). In this environment, postlarvae adopt an opportunistic feeding strategy. However, there has been no strict determination of nutritional requirements in relation to their feeding activity under controlled conditions. Presumably, an opportunistic omnivorous invertebrate is capable of digesting most of the nutrients contained in its food, and shrimp postlarvae are not exception. Then, it would be highly relevant for the energy balance of PL's to establish possible differences in the digestion and absorption of food rich either in animal or plant protein and digestible carbohydrates (cbh).

There used to be a critical phase at M_{III}-PL_1 with a shift from live food sequence (algae-*Artemia*) to dry pellet (crumbles) (Aquacop, 1995). Since inert particles replace live food sequence during larval stages, the problem of weaning does not exist anymore. The obtainment of hardy postlarvae depends on feed quality and the feeding schedule applied at larvae stages (Gallardo et al., 2003). Whereas the adaptation to a category of protein sources and to a protein and carbohydrate balance at PL's stages will depend primarily on the composition of microcapsules provided at larvae stages.

Inert particles can replace live food sequence without significant difference in development duration. However, a more critical situation may arise when animal protein is replaced with vegetable protein sources (Argue et al., 2001).

Postlarval stages provide a good material to measure energy budget and to evaluate the respective growth promotion effects of feeds (whether under moist form or extruded with animal and/or vegetable protein sources). Several studies (Lemos and Phan, 2001; Lemos et al., 2001; Brito et al., 2004) provided methodology and results on energy expenditure; energy partitioning at PL's stages when replacing *Artemia* with inert particles or effect of salinities, proved feasible from a comparative point of view. Consequently, the PL's overall energy budget can be addressed in spite of an existing difficulty to assess feed intake with precision.

This paper addresses the comparative effects on growth efficiency and energy budget of *L.vannamei* postlarvae and early juveniles -previously weaned on inert particles- while being exposed to two types of diets: one composed of vegetable protein and carbohydrates (soybean soluble protein concentrate, *Spirulina* powder, wheat gluten, and wheat starch) and another containing animal protein sources (fish, squid, and shrimp meals, and fish soluble protein concentrate).

Material and Methods

Experimental shrimp

L. vannamei postlarvae from 8th generation farmed population obtained from a commercial hatchery (Grupo Pecis, Yucatan, Mexico) were transported to the laboratory as postlarvae 14 (PL_{14}, fourteen days after metamorphosis). Postlarvae (1.08 mg wet weight) were transferred to 25-L plastic tanks, stocked at a density of 30 PL per tank and acclimatized at 28±0.05ºC and 32.4±0.1‰ salinity, photoperiod (13-h:11-h, light: dark). Tanks were supplied with constant aeration maintaining oxygen near saturation levels (6.3±0.04 mg O_2 L^{-1}). Sand filtered seawater was filtered through 5 and 1μm cartridges and UV sterilized before a 50% daily exchange.

Diet preparation and feeding schedule

Postlarvae were fed two artificial diets. One group was fed an animal protein based diet (AP), and the other was fed a vegetable protein concentrate plus carbohydrate (VPC). Proximate composition of both experimental feeds (Table 1) was similar. The experimental feeds were prepared in the laboratory by thoroughly mixing dry ingredients with oil and then adding water until a firm mass was obtained. The mass was passed through a meat-grinder equipped with a 2mm die, and resulting strands were air dried at 60^0C. After drying, pellets were broken, sieved to a convenient size and stored at -4^0C until use. At the beginning of the experiment 250-350μm crumbles were used, increasing its size up to 800 -1000μm at the end of the experiment. Animals were fed three times daily (0800, 1400, and 2000), and food ratio adjusted every fortnight as 300% of the biomass from day 0 to 15, then 200% of the biomass from day 15 to 30, and in final 80% of the biomass from day 30 to 45.

Energy balance

The amount of energy channeled into growth (P), maintenance (R), excretion (U), and exuviae (E_v) in each treatment during the experiment was obtained through several measurements and estimations. Initial dry weight of shrimp was determined from samples of 50 individuals. Shrimp were washed with distilled water and oven-dried at 60°C for 24 h. Individual dry weights were measured using a CAHN model C-33 microbalance with 0.001 mg precision. The same procedure was used to obtain the final weight of the animals in each treatment.

Initial and final energy content of shrimp from each treatment was obtained by means of a calorimeter (Parr), previously calibrated with benzoic acid. The individuals dry weight were transformed into energy units (J mg dw^{-1}), and the energy channeled into growth (P) calculated.

Oxygen consumption and ammonia excretion of PL_{19} were measured in a closed micro-respirometer (RC-300 respiration glass cell, Strathkelvin Instruments, Glasgow, UK). Twenty-four micro-respirometer chambers were connected in series with a constant temperature bath (28 ± 0.1°C) using a thermocirculator (Fisher Scientific Isotemp Refrigerated Circulator, model 900) (Brito et al., 2000). Twelve hours fasting postlarvae were individually placed in the respirometric cells with 1.5-mL clean seawater at 10 minutes interval. Before the oxygen consumption measurements, postlarvae were acclimated during 30 minutes in the respirometric chambers. Thereafter, oxygen tension in the chambers' water was measured for a

10-min period using an oxygen microelectrode (model 781, Strathkelvin Instrument, Glasgow, U.K.). Oxygen meter was calibrated to zero with sodium sulfite solution and to 100% saturation with full aerated seawater. Oxygen tension in the chambers' water never fell below 80% saturation level.

Ammonia excretion was evaluated in the same postlarvae used for oxygen consumption measurements. For this purpose, postlarvae were maintained in the respirometric cells for 1.5 hours. Ammonia excretion corresponded to the difference between the ammonia concentration in the water after and before this period. Samples for ammonia determination were stored in Ependorff tubes with sublimated iodine and kept frozen until analysis. After these measurements, postlarvae in the chambers were fed with the corresponding diet for each treatment; one hour later, the procedure was repeated to determine oxygen consumption and ammonia excretion of feeding animals. One control chamber without animals was used for each five experimental chambers. Twenty measurements were recorded per treatment.

Oxygen consumption and ammonia excretion were measured individually in 10 early juvenile (PL_{59}) from each diet by a continuous flow respirometer in a closed system (Rosas et al., 1998). Oxygen tension in water at the entrance and exit of respirometric chambers was measured using an oxygen microelectrode (model 781, Strathkelvin Instrument, Glasgow, U.K.). Oxygen consumption was first determined in 24 hours fasting shrimp which were acclimated during 14 hours in the respirometric chambers. Then, the animals were fed the corresponding diet for each treatment and oxygen consumption measured every one hour from 0900 until 1700. At the same time samples for ammonia determination were stored in Ependorff tubes with sublimated iodine and preserved frozen until analysis. Moulting stage was determined according to the method of Drach and Tchernigovtzeff (1967) adapted for penaeids (Aquacop et al., 1975); only data from shrimps in intermoult stage were retained.

Ammonia concentration in samples was always determined less than 12 hours after sampling, following the method proposed by Strickland and Parsons (1972), adapted by Hernández-López and Vargas-Albores (2003) for small water samples. Water samples (250 μL) were mixed in a microplate with 20 μL of 10% phenol solution (in 95% ethanol), 20 μL of 0.5% sodium nitroprussiate, and 30 μL oxidant reactive (20% sodium citrate, 1% sodium hydroxide, and 2.5-mL sodium hypochlorite). The mixture was incubated for 60min at room temperature and optical density was recorded at 655 nm in a microplate reader (model 550, BioRad, Hercules, CA, USA). Ammonium sulphate was used as a standard (1.5mM).

R was estimated as $R_{rout}+R_{AHI}$, where R_{rout} (routine metabolism $mgO_2\ h^{-1}\ mg\ dw^{-1}$) was the oxygen consumption of unfed animal and R_{AHI} (apparent heat increment, $mgO_2\ h^{-1}\ mg\ dw^{-1}$) was the difference between the oxygen consumption of unfed animal and the maximum value obtained after feeding. These values were converted to energy by the coefficient 14.3 J $mg^{-1}O_2$ (Lucas, 1996). R_{rout} ($J\ d^{-1}\ mg\ dw^{-1}$) was estimated considering day time where shrimp were not fed. R_{AHI} ($J\ d^{-1}\ mg\ dw^{-1}$) was estimated by the peak of oxygen consumption after feeding and number of rations (n=3) fed to shrimp per day. In the case of young juveniles also the extent (2h) of the peak was taken into account in the estimations.

Similarly, U was estimated as $U_{rou}+U_{PPNE}$, where U_{rout} (routine excretion, mg N-NH_3 h^{-1} mg dw^{-1}) was the excretion of unfed animals and U_{PPNE} (post prandial excretion) was the difference between ammonia excretion of unfed animals and the maximum value obtained after feeding. These values were converted to energy by the following coefficient 20.5 J mg^{-1} N-NH_3 (Lucas, 1996). U_{rout} (J d^{-1} mg dw^{-1}) was estimated considering day time period where animals were not fed, U_{PPNE} (J d^{-1} mg dw^{-1}) was obtained with the ammonia excretion peak after feeding and number of rations (n=3) fed to shrimp per day. In the case of young juveniles also the extent (2h) of the peak was taken into account for such estimations. The energy content of exuviae (Ev) was considered as 5% of the total individual energy content (Kurmaly et al., 1989). Energy absorbed (Ab) from feed was calculated as $Ab=P+R+U+E_v$, where P is the energy channeled into growth, R is the energy lost in respiration, U the energy lost in excretion, and E_v is energy content in exuviae. Assimilated energy was calculated as the energy used for biomass production (P), respiration (R), and energy content in exuviae: $As=P+R+E_v$. Net growth efficiency (K_2) that represents the proportion of assimilated energy (or metabolizable energy) channeled into growth was calculated as $K_2=(P+E_v)/(P+R+E_v)$ and expressed in percentage (Lucas, 1996). The proportion of assimilated energy invested in production or respiration (P/As, R/As) and a ratio between respiration and production (R/P) were also calculated.

Using oxygen consumption and ammonia excretion data, O:N ratio was calculated transforming data to μg At h^{-1} mg dw^{-1}. O:N values of fed shrimp were estimated with maximum oxygen consumption and ammonia excretion after shrimp feeding.

Statistical analysis

The Student's t analysis was applied to test the effect of feeds on the energy channeled into production, respiration, excretion, exuviae, as well as on the amount of energy absorbed and assimilated. The same procedure was used to test the effect of feeds on O:N ratios after fasting or feeding periods.

Results

As result of calorimetric analysis, the initial shrimp (PL_{14}) energy content (mean value±standard error) was 21.11±0.81J mg dw^{-1}. Final energy content of shrimp fed animal protein diet (AP) was estimated as 21.11±0.82J mg dw^{-1}, and final energy content of shrimp fed vegetable protein plus carbohydrate diet (VPC) was 21.10±0.92J mg dw^{-1} ($P>0.05$). Daily energy channeled into respiration and nitrogen excretion values were separated (Table 2) according to routine (fasting period) and active metabolism (feeding period). PL's utilized energy in respiration during routine metabolism (R_{ROUT}) significantly higher ($P<0.05$) in animals fed VPC than AP. Energy expenditure during post-absorptive process (R_{AHI}) increased significantly ($P<0.05$) in case of animals fed AP. Early juvenile shrimp follow a similar pattern, although only R_{AHI} was significantly different ($P<0.05$). Nitrogen excretion showed a significant increment in energy lost in fasting postlarvae (PL_{19} /U_{ROUT}) from AP treatment, while the postprandial excretion increased in shrimp fed VPC. In early juveniles (PL_{59}), there was no difference among energy loss in U_{ROUT} or U_{PPNE} between treatments. However, slightly high values in routine excretion were obtained in shrimp fed AP. Energy balance showed less difference in shrimp fed the two types of feeds on short (PL_{14} to PL_{19}) rather than on long term (PL_{14} to

PL_{59}) periods (Table 3). The amount of energy recovery into production was similar for both diets in a short period, but increased significantly ($P<0.05$) in shrimp fed AP during a long trial. Postlarvae fed VPC consumed significant higher amount of energy in maintenance ($P<0.05$). During long periods, energy invested in respiration was the same in both diets. Energy lost in nitrogen wastes was significantly ($P<0.05$) affected by diets from PL_{14} to PL_{19} with higher values in AP, but was similar ($P>0.05$) from PL_{14} to PL_{59}. Energy lost in exuviae was the same in both treatments during short period and increased significantly when the long period was analyzed ($P<0.05$). In relation to absorbed and assimilated energy there was no difference between feeds during short or long periods. Assimilation efficiency, calculated as the proportion of absorbed energy, was high (99 and 90% during short or long periods respectively) in animals fed VPC. In shrimp fed AP, assimilation efficiency was 89% during the short period and 86% for the long term period. Between 41 and 52% of assimilated energy (As) went to production and it changed according to feed quality and period analyzed (Fig. 1) with a high range of values in shrimp fed AP for both periods. The proportion of assimilated energy invested in maintenance (R/As) fluctuated between 47 and 57%, with a higher value in the VPC treatment for both periods (Fig. 2). The ratio between respiration and production showed that the highest proportion of energy allocated into growth fitted with animals fed AP in both periods. Those animals derived equal quantities of energy for maintenance and production (Fig. 3). Shrimp on VPC treatment during the long period invested for maintenance 50% energy going to production. Net growth efficiency (K_2) or the proportion of metabolizable energy channeled into growth was similar during both periods in shrimp fed VPC, though its values were slightly lower than K_2 value obtained for shrimp fed AP. The highest value was found in shrimp fed AP during long term period (Fig. 4).

Postlarvae under fasting or feeding conditions showed variations in O:N ratio according to the type of feed (Figure 5). Postlarvae fed AP used protein as metabolic substrate during the fasting period. However, the O:N ratio increased significantly ($P<0.05$) after feeding showing a change towards a mixture of metabolic substrates. On the contrary, postlarvae fed VPC utilized lipid and protein as metabolic substrate during fasting with a significant ($P<0.05$) decrease in O:N ratio after feeding. This reveals the importance of protein as metabolic substrate. In juveniles, O:N ratio variations showed that protein was the main metabolic substrate independently of diet composition or physiological condition (fasting or feeding).

Discussion

From an ontogenetic point of view, daily energy intake rate of shrimp larvae fed on rotifers was found as high as 5J $larvae^{-1}$ day^{-1} at $Protozoea_{III}$ before decreasing to 2.6J $larvae^{-1}$ day^{-1} at PL_1 (Emmerson, 1984). Larvae fed on rotifers or *Artemia*, ingest an amount of live preys estimated in a similar range as it is on unicellular algae (Gaxiola et al., 2005). At PL's stage, while feeding on microdiets, the precise assessment of feed intake becomes quite complex. That is the reason why energy partitioning has been expressed from energy density of larvae or PL's (Brito et al., 2004). Feed intake is regulated by energy density (Cuzon and Guillaume, 1997) and it is equivalent in terms of digestible energy (16kJ g^{-1}) between the two feeds. Hence, it was assumed that both crumbled feeds were consumed in a similar

manner. On the contrary, live food can be consumed differently whether rotifers (2.5J larvae^{-1} day^{-1}) or ***Artemia*** (8J larvae^{-1} day^{-1}) were ingested probably due to a raptorial effect acting differently (Emmerson, 1984) or a difference in energy content or both. Excretion and respiration rates were measured in order to obtain an indication of digestible energy (DE). This avoided the difficult procedure of collecting feces to obtain a value of apparent digestibility coefficient (ADC) attributed to each category of feed. Protein or energy ADC can be measured on early juveniles even though most studies have been conducted on large size shrimp (Cousin, 1995; Merican and Shim, 1997). Herein, energy partitioning was determined using the method applied to bivalve microphages (Lucas, 1996) and shrimp (Rosas et al., 2001). Coefficients derived from the energy budget expressed differences observed between the two treatments. Recovered energy (P or RE) did not change with diets on a short period of time (PL_{14}-PL_{19}). However, on long term (PL_{14}-PL_{59}) RE from animal fed AP was higher than that from shrimp feed VPC. Two explanations are put forward: (i) VPC diet reduces feed intake (ii) expenses from metabolism (respiration, excretion) are much higher on PL's fed VPC than with AP. In joules per day, from both treatments PL's respired 2.5J and 1.9J ($P<0.05$) and young juveniles 134J and 118J ($P>0.05$) when receiving VPC and AP, respectively. In this sense, the second assertion may be correct at least during the stage PL_{14}-PL_{19}. Ammonia excretion rate was slightly higher with AP but due to the relatively low incidence of excretion on energy budget (U/Abs: 3-10%), it appeared negligible. Higher apparent heat increment (AHI) with AP than with VPC might be related with a relative higher ammonia excretion rate, but when PPNE/AHI ratio is analyzed, lower values were obtained with AP (4 and 34% for PL_{19} and juveniles respectively) than with VPC (12 and 49% for PL_{19} and juveniles respectively). Therefore, higher AHI with AP could be associated with higher protein content in this diet as AHI could be related with dietary protein level (Rosas et al., 1996).

AHI and production in farmed juveniles of *L. vannamei* (25th generation), fed a low cbh-diet were higher than when fed on a high cbh-diet (Arena et al., 2003). Low PPNE/AHI ratio associated with high weight gain was also found in young juveniles *L. vannamei* (Rosas et al., 1996; 2001) and *L. setiferus* (Taboada et al., 1998). Ratios such as P/As, R/As, R/P compared for the two diets showed an overall efficiency higher on AP than on VPC. It revealed a greater proportion of energy channeled to production and a lower one to maintenance in animals fed on AP. Metabolic routes for cbh in shrimp coming from massal selection and fed different cbh levels were modified and seemed the result of a drastic reduction in amylase genes allelic frequency (Arena et al., 2003). Then, adaptation of shrimp to use dietary cbh as energy source is not obvious. Farmed population seemed protein dependent and cbh could decrease production efficiency. Net growth efficiency (K_2) that represents a proportion of assimilated energy (or metabolizable energy) channeled into growth is a good condition index (Lucas, 1996). Assimilation efficiencies of animals fed different diets result in different energy yield for metabolic process and weight gain (Capuzzo, 1981). Estimates of K_2 for both short and long term period ($PL_{14\text{-}19}$ and $PL_{14\text{-}59}$) confirmed superior efficiency of animals fed AP diet. Values of K_2~45-50 corresponding to AP and ~40 to VPC remained in the same order of magnitude as ***L. vannamei*** early post larvae (K_2~45) fed on inert

diet (Brito et al., 2004), and with those for *Farfantepenaeus paulensis* early post larvae (K_2~44-56) fed artificial diet at different salinities (Lemos et al., 2001). In essence, maintenance could be at a higher level with PL's fed on VPC than on AP. It is possible that shrimp fed VPC display more mobility while searching for food in experimental tanks. Such an assumption was already addressed by Teshima (1996; pers. com.) with *Marsupenaeus japonicus* juveniles receiving squid or a soybean concentrate based feed which invested in respiration 20 vs more than 50%DE respectively. However, considerations taking into account behavioral observations remain uneasy to apply. A similar hypothesis was done in experimental tanks with *L. stylirostris* and *L. vannamei* juveniles under a post prandial status with glycaemia (3mg ml^{-1}) on a rich-starch feed; unfortunately, no precise observation was done to substantiate such hypothesis (Cousin, 1995).

O:N ratio (Mayzaud and Conover,1988) varied between PL_{19} and PL_{59}. After 5 days adaptation to diets, PL_{19} shrimp had an O:N~10 signaling protein as main metabolic substrate in shrimp from AP during fasting, the ratio increased to 60 (use of mixture of lipid and protein) in fed animals. The O:N ratio in the VPC treatment was ~25 (indicating lipid and protein as metabolic substrates) in fasted shrimp and in post prandial stage this ratio decreased showing an increase in protein use. Interestingly, after 45 days acclimated to diets, PL_{59} shrimp presented no change in O:N. The ratio values obtained indicate a use of protein as the main metabolic substrate. These differences might be related on the one hand, to an adaptation to different type of feed that is on line with the omnivorous-opportunist character of *L. vannamei* postlarvae, and to digestive tract development, on the other (Lovett and Felder, 1990b). O:N values with animals fed either AP or VPC indicated an energy derived mainly from protein and lipid. In VPC fed shrimp, difference in energy amount going to production was not related to dietary cbh used as metabolic substrate. The use of cbh as energy source resulting in poor weight gain in *L. vannamei* young juveniles (Rosas et al., 2001) may be attributed to cbh inhibiting absorption of amino acids. Energy amount channeled into growth (P) showed differences between shrimp fed AP or VPC diets that do not seem to be related with a use of different metabolic substrate (lipid-protein or cbh-lipid-protein) but rather to energy expended in maintenance. Changes in behavior (mobility in tanks, time spent in searching for food) would explain differences in routing metabolism. This is difficult to quantify and it requires specific designed experiments aimed to that purpose.

Sparing-protein effect with dietary cbh that has previously been documented on wild juveniles of *L. vannamei* (Arena et al., 2003), is lost through massal selection. This could cause an increase of maintenance cost when cbh are including into the diet. Nevertheless, early juveniles maintained on a grower feed based on vegetable protein instead of animal protein remains a feasible option. Differences in efficiency found through energy budget evaluation seem to be related to the presence of cbh in diet and not to the origin of protein sources. This knowledge may eventually lead to reduce both the fishmeal content in feeds and the amount of waste products, and to contribute to achieve sustainable farming.

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Table 1. Composition (% dry weight) and analysis of experimental feeds.

Ingredients	AP	VPC
fish meal	19	-
CPSP-70*	9	5
shrimp meal	5	-
squid meal	30	-
wheat starch	-	14
soybean SPC**	-	20
wheat gluten	-	10
Spirulina	-	21
cholesterol	0.56	0.56
super Selco^{R}	3.7	4
soybean lecithin	2	2
carophyll-red	0.02	0.02
vitamin mix***	1	1.5
sodium alginate	1	1
filler	19	21
protein %	51	40
lipid %	11	11
carbohydrate %	3	20
digestible energy (J/mg)	16	16

Animal protein diet (AP) and vegetable protein and carbohydrate diet (VPC).

* Soluble fish protein concentrate (Sopropêche, Boulogne s/mer, France)

** Soy soluble protein concentrate

*** Rovimix # 1720, Roche, Bâle, Suisse

Table 2. Respiration and excretion in fasting and fed postlarvae (PL_{19}) and early juveniles (PL_{59}) of *L. vannamei* fed different diets (mean values±standard error). All values expressed in J day^{-1}.

Stage/ Diet	R_{ROUT}	*p*	R_{AHI}	*p*	U_{ROUT}	*p*	U_{PPNE}	*p*
(PL_{19})								
AP	1.19 ± 0.13		0.68 ± 0.07		0.195 ± 0.021		0.025 ± 0.003	
		0.023		0.000		0.002		0.015
VPC	1.77 ± 0.25		0.33 ± 0.04		0.114 ± 0.014		0.039 ± 0.005	
(PL_{59})								
AP	84.36 ± 13.97		35.05 ± 3.02		18.97 ± 4.46		11.95 ± 2.89	
		0.133		0.006		0.154		0.435
VPC	110.76 ± 17.54		23.18 ± 2.57		13.91 ± 1.33		11.34 ± 2.18	

(AP) animal protein diet, (VPC) vegetable protein and carbohydrate diet. *p* is the probability from Student's *t* analysis at 0.05.

Table 3. Energy channeled into production (P), respiration (R), excretion (U), and exuviae (E_v) during postlarvae (PL_{14}-PL_{19}) and early juvenile development (PL_{14}-PL_{59}) of *L. vannamei* fed different diets (mean values±standard error). Ab, absorbed energy (Ab=P+R+U+E_v), As, assimilated energy (As=P+R+E_v).
All values expressed in J day^{-1}.

Stage/Diet	P	R	U	E_v	Ab	As
(PL_{14}-PL_{19})						
AP	2.04± 0.45	1.87[b]± 0.16	0.23[a]± 0.03	0.11± 0.02	4.46± 0.77	3.95±0.67
VPC	2.05± 0.44	2.50[a]± 0.29	0.15[b]± 0.01	0.10± 0.02	4.79± 0.74	4.76± 0.74
(PL_{14}-PL_{59})						
AP	115.9[a]±10.6	118.5± 16.0	31.0± 5.4	5.8[a]± 0.5	283.3± 29.8	243.8± 23.8
VPC	88.4[b]± 10.1	133.9± 20.0	20.2± 5.8	4.4[b]± 0.5	214.0± 43.6	192.5± 46.9

Different letters means statistical differences (P<0.05). (AP) animal protein diet, (VPC) vegetable protein and carbohydrate diet.

Fig. 1. Proportion of assimilated energy channeled into production (P/As) for PL_{14-19} and PL_{14-59} *L. vannamei* fed AP animal protein feed or VPC vegetable protein and carbohydrate feed.

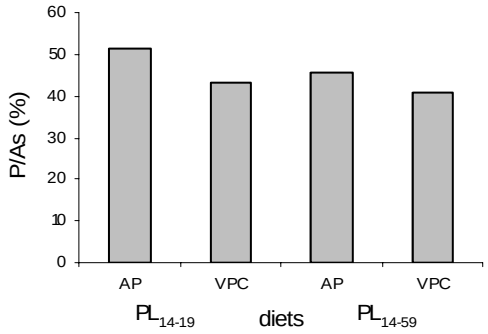

Fig. 2. Proportion of assimilated energy used in respiration (R/As) for PL_{14-19} and PL_{14-59} *L. vannamei* fed AP animal protein feed or VPC vegetable protein and carbohydrate feed.

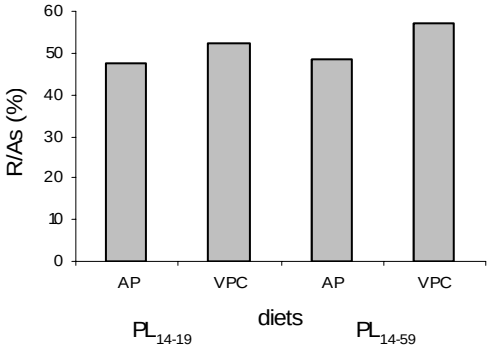

Fig. 3. Ratio between energy channeled into respiration and growth (R/P) for PL_{14-19} and PL_{14-59} *L. vannamei* fed AP animal protein feed diet and VPC vegetable protein and carbohydrate feed.

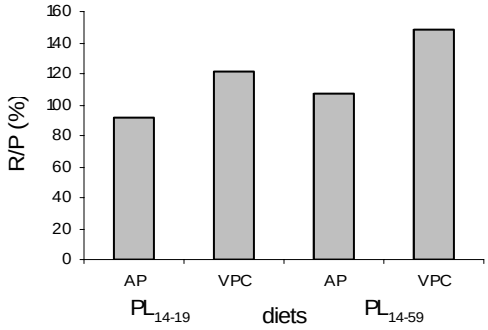

Fig. 4. Net growth efficiency (K_2) for PL_{14-19} and PL_{14-59} *L. vannamei* fed AP animal protein feed or VPC vegetable protein and carbohydrate feed. (*) $p<0.05$), (ns) $p>0.05$. Lines represent the standard error.

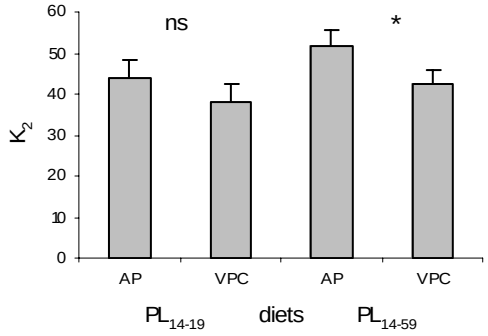

Fig. 5. O:N ratio for PL_{19} and juveniles (PL_{59}) L. vannamei fed AP animal protein feed or VPC vegetable protein and carbohydrate feed. (*) $P<0.05$, (ns) $P>0.05$. Lines represent the standard error.