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## Effect of an herbivorous diet on energy balance of *Litopenaeus vannamei* at selected ontogenetic stages

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### Abstract:

Herbivorous (20% vegetable protein, 40% carbohydrate) and carnivorous diets (40% marine animal protein, 20% carbohydrate) were experimentally tested to assess their effect on the energy balance and energetic substrates utilized by postlarvae (PL's /15 days) and juvenile shrimp (3–6 g) of *Litopenaeus vannamei*. Postlarval stage 60 (PL<sub>60</sub>, early juveniles) shrimps fed HeD and CaD diets, then late juveniles (3–6 g) acclimated to the same diets were tested for their respective energy partitioning potential.

No significant differences ( $p > 0.05$ ) on growth were obtained in early juveniles (mean final wet weight of 0.19 g). However in late juvenile stages a significant difference ( $p < 0.05$ ) in growth rate was observed. In terms of energy partitioning, both early and late juveniles seem to spend more energy in respiratory metabolism than in the elimination of excretion products. A change in feed composition based on quality protein sources induced some modifications on shrimp's activity measured by heat increment. Shrimps fed with an herbivorous diet showed a higher heat increment. Interestingly, the early stages of *L. vannamei* display a remarkable capacity to assimilate a plant protein-based and a high carbohydrate level diet. Such capacity with a stimulation of genes would lead to a good adaptation of juveniles receiving all-plant diets to sustain growth performances up to a marketable size. The implications of these findings for the shrimp farming feeding costs are briefly discussed.

**Keywords:** Shrimp; Protein sources; Metabolism; Juveniles; Energy

## 1. Introduction

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In early 70's, postlarvae (PL's) were already fed formulated compounded feeds with *Penaeus californiensis* (Colvin and Brand, 1977). Different diet compositions were shown to change metabolism of *Litopenaeus setiferus* PL's (Brito et al., 2000), *Penaeus indicus* and *P. merguensis* early juveniles (Peñaflorida, 2002), and *L. stylirostris* juveniles (Rosas et al., 2000). Diet composition also affected energy budget in *L. setiferus* and *L. vannamei* PL's (Brito et al., 2004), *L. vannamei* PL's and early juveniles (Jiménez-Yan et al., 2006), and *Fenneropenaeus chinensis* juveniles (Huang et al., 2005).

One of the less studied aspects in crustacean bioenergetics was related with the calculation of daily digestible energy (DE) requirement. Generally, energy partitioning models gave ingestion rate and produced DE subtracting energy retained in faeces (non-digestible energy); from DE it was derived metabolizable (ME) and retained energy (RE) (Bureau et al., 2000).

The feed industry has recognized for many years that viable utilization of plant raw materials added in compound diets for the culture of aquatic organisms is a vital route for future development of aquaculture (Gatlin et al., 2007). Pond ecology would improved from use of feed ingredients, such as plant protein (Rowland et al., 2007) Moreover, Hari et al. (2006) shown that addition of carbohydrates to feed reduced total ammonia nitrogen in the water column and sediment in *Penaeus monodon* ponds, and that with a low (25%) protein and carbohydrates diet, it is possible to obtain similar yield than with a high (40%) protein without carbohydrates diet.

Energy partitioning obtained with *L. vannamei* PL's were not significantly affected by protein quality (animal versus plant protein, Jiménez-Yan et al., 2006). Then it was necessary to get a comprehensive approach on energy partitioning and substrate metabolism for *L. vannamei* PL's and juveniles with a drastic change in feed composition, as well as their physiological consequences.

In this paper, considering the high omnivorous capacity reported for *L. vannamei*, it was tested a carnivorous animal diet (CaD) based in marine animal protein (40%) and a herbivorous diet (HeD) with 17% plant protein and 40% carbohydrates (cbh) to evaluate *L. vannamei* performances. The objective was to analyze the effect of diet composition from 15 days old postlarvae until 3-6 g juveniles. Digestible energy (DE) estimation was obtained through oxygen consumption and nitrogen excretion of fasting and feeding shrimps. Energy channelled into growth was measured from carcass caloric content and daily growth rate. Metabolic substrates were determined according to O:N ratio variations. Additionally, it is emphasized on estimated values of DE requirement per day to define feeding rations and save resources in aquaculture.

## 2. Material and Methods

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### 2.1. Origin of the experimental animals

Shrimps used in this study were obtained from one spawn produced in the UMDI-Sisal laboratory. Larvae were reared until PL's of 15 days old using Galveston system, and feeding schedule previously in operation in the laboratory (Gallardo et al., 2003).

## 2.2. Feeding trials

Postlarvae of 15 days old (PI<sub>15</sub>) were transferred to 30 L plastic tanks, at a stocking density of 30 animals per tank. Temperature was maintained at 28±0.1°C, salinity at 32.4±0.1‰, photoperiod 12h light: 12h dark. Tanks were supplied with constant aeration, which kept oxygen near saturation level (6.3±0.04mg O<sub>2</sub> L<sup>-1</sup>). Natural seawater was filtered through sand layer and then cartridges (5 and m) plus UV sterilization. A 50% daily water exchange was applied. Shrimps were fed two artificial feeds, an herbivorous diet (HeD) based on plant protein and carbohydrates, and carnivorous animal diet (CaD) based on marine animal protein (Table 1). Proximal analysis of the diets appeared in Table 2.

Animals were fed three times daily (0800, 1400, and 2000), and feed ratio adjusted every fortnight at 300% biomass from day 0 to 15, followed by 200% biomass from day 15 to 30, and 80% biomass from day 30 to 45. Crumbles of 250-350µm were used at the beginning of the experiment, increasing size until 800-1000µm at end. After 45 days PI<sub>60</sub> (thereafter referred as early juveniles) were sampled. Other group of shrimps was transferred to 400 L tanks and fed with the same diets (at 10% of biomass as daily ration) during six weeks, then sampled for weight, and after 45 days, new samples were obtained for physiological measurements (thereafter referred as late juveniles).

The experimental feeds were prepared in the laboratory by thoroughly mixing dry ingredients with oil and then adding water until firm dough was obtained. The dough was passed through a meat-mincer equipped with a 2mm die, and resulting strands were air-dried at 60°C overnight. After drying, pellets were broken, sieved to a convenient size and stored at -40°C until use.

## 2.3. Energy partitioning

The amount of energy channeled into growth (RE), maintenance (H<sub>e</sub>E), excretion (UE+ZE) for each treatment was obtained during the experiment through several measurements. Individual wet and dry weight was measured using a Cahn model C-33 microbalance with 0.001mg accuracy. Initial wet and dry weight of shrimp was determined from samples of 50 individuals. Shrimps were washed with distilled water and oven-dried at 60°C for 24h. The same procedure was used to obtain final weight of animals in each treatment. Initial and final energy content of shrimps from each treatment were obtained by means of an adiabatic bomb calorimeter (Parr), previously calibrated with benzoic acid. Data on individual dry weight gains in mg<sup>-1</sup> day<sup>-1</sup> were transformed into energy with corresponding units to express values of RE in J d<sup>-1</sup> shrimp<sup>-1</sup>.

Oxygen consumption and ammonia excretion were measured individually in 10 early juvenile for each diet treatment using 100 mL respirometric chambers. For late juveniles (10 animals for treatment) chambers of 250 mL were set up. A flow through respirometer system in a closed system was used according to Rosas et al. (1998). Oxygen tension in water at the entrance and exit of respirometric chambers was measured using an oxygen electrode (YSI 50B). Oxygen consumption was first determined (0800) in 24 hours fasting shrimps, which were previously acclimated during 14 hours to the respirometric chambers. Then the animals were fed the corresponding diet for each treatment and oxygen consumption was measured every one hour from 0900 until 1400 in early juveniles and from 0900 until 1500 in late juveniles. At the same time water samples of the entrance and exit of the chambers were stored in Ependorff tubes with sublimated iodine and preserved frozen until analysis for ammonia. Ammonia concentration in samples was always determined less than 12 h after sampling, following the method proposed by Strickland and Parsons

(1972), adapted by Hernández-López and Vargas-Albores (2003) for small water samples. Only shrimps fed in the chamber were used for the analyses.

Respiration ( $\text{mgO}_2 \text{ h}^{-1} \text{ shrimp}^{-1}$ ) was estimated as basal ( $H_eE$ ) + heat increment of feeding ( $H_iE$ ), where  $H_eE$  (routine metabolism) was the oxygen consumption of unfed animal and  $H_iE$  was the value of the peak of heat increment after feeding. These values were converted to energy by the coefficient  $14.3 \text{ J mg}^{-1} \text{O}_2$  (Lucas, 1996).  $H_eE$  ( $\text{J d}^{-1} \text{ shrimp}^{-1}$ ) was estimated considering day time where shrimps were not fed,  $H_iE$  ( $\text{J d}^{-1} \text{ shrimp}^{-1}$ ) was measured by the peak of oxygen consumption after feeding and number of rations ( $n=3$ ) fed to shrimps per day. Similarly, excretion was estimated as routine nitrogen excretion ( $UE+ZE$ ) plus post-alimentary nitrogen excretion ( $PANE$ ) and these values were converted to energy by the coefficient  $20.5 \text{ J mg}^{-1} \text{ N-NH}_3$  (Lucas, 1996). Excretion ( $\text{J d}^{-1} \text{ shrimp}^{-1}$ ) was estimated taken into consideration the same factors as in respiration.

Digestible energy (DE) for shrimps was calculated as:

$$DE = RE + (H_eE + H_iE) + (UE + ZE)$$

where RE is the energy channelled into growth, and came from the conversion of daily growth rate in energy values using the carcass caloric content; ( $H_eE + H_iE$ ) is the energy lost in respiration, ( $UE + ZE$ ) the energy lost in excretion.

The proportion of digestible energy (DE) invested into growth ( $RE/DE$ ) was calculated.

#### 2.4. Metabolic substrate

O:N atomic ratio was calculated transforming oxygen consumption and ammonia excretion of fast and fed shrimps data to gAt. Feeding O:N ratio were estimated with maximum oxygen consumption and ammonia excretion after shrimps feeding. O:N values from 3-16 correspond to pure protein substrate metabolism; between 50-60 for a mix of protein plus lipids; above this value represents the mix of lipids plus carbohydrates (Mayzaud and Conover, 1988).

#### 2.5. Statistical analysis

To test differences between initial weight of experimental shrimps and the effect of diets on survival, final weight, growth rate, digestible energy, as well as on the energy channelled into growth, respiration and excretion, data were contrasted by Student's *t* test, February 3, 2010. Survival data were transformed to arcsin prior to apply the test.

### 3. Results

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#### 3.1. Zootechnical parameters

Survival of early juveniles were significantly affected by diets, shrimps fed CaD presented a lower value (73.3%) than those fed HeD with 91.7% ( $p < 0.05$ ). In late juveniles diets had not effect on survival (mean value 95.8%,  $p > 0.05$ ). Final wet weights were similar in early juveniles fed both diets ( $p > 0.05$ ), but in late juveniles HeD affected negatively shrimp growth ( $p < 0.05$ ) with lower final weight (3.3 g), than those fed CaD that reached 6.09 g (Table 3).

### 3.2. Oxygen consumption

During fasting, early juveniles from CaD treatment respired at a rate of  $1.35 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$ , similar value was obtained in those fed HeD ( $1.14 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$ ) (Fig. 1a and b). Feeding increased oxygen consumption in both treatments, in early juveniles from CaD, heat increment reach a peak one hour after feeding ( $2.91 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$ ), and returned to fasting levels in the next hour, whereas in those fed HeD oxygen consumption paullatinaly rose until three hours after feeding with a peak in  $3.28 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$ .

Late juveniles from CaD treatment consumed  $3.9 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$  during fasting, while in fasting animals from HeD treatment the oxygen consumption was  $1.6 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$  (Fig. 1c and d). After feeding, oxygen consumption rose until  $5.4 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$  in CaD fed shrimps and up to  $3.5 \text{ mgO}_2\text{h}^{-1}\text{shrimp}^{-1}$  in HeD fed shrimps. Both treatments shown a similar pattern with a peak in heat increment two hours after feeding, but in CaD, oxygen consumption returned to basal level four hours after feeding while HeD fed shrimps required seven hours to reach basal level.

### 3.3. Nitrogen excretion

Ammonia excretion in early juveniles from CaD treatment (Fig. 2a) was  $0.006 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$  in fasting shrimps, with a peak ( $0.02 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$ ) four hours after feeding. In HeD treatment early juveniles excreted  $0.011 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$  during fasting period reaching a peak ( $0.022 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$ ) in the first hour after feeding (Fig. 2b).

Late juveniles from both treatments (Fig. 2c and d) shown similar excretion values in fasting condition ( $0.036$  and  $0.029 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$  in CaD and HeD respectively), shrimps from CaD reach a peak in ammonia excretion four hours after feeding ( $0.161 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$ ), while HeD shrimps had a peak in ammonia excretion only two hours after the meal ( $0.105 \text{ mg N-NH}_3 \text{ h}^{-1}\text{shrimp}^{-1}$ ). Post-alimentary peak in ammonia excretion represent an increment of 440% respect to basal leves in CaD animals, whereas in HeD shrimps the highest value in excretion represent 350% of basal level.

### 3.4. Energy partitioning

In early juveniles retained energy showed similar values ( $p < 0.05$ ) in both treatments with  $59.4$  and  $76.9 \text{ J shrimp}^{-1} \text{ day}^{-1}$  for CaD and HeD respectively (Table 4). Energy partitioning showed significant differences associated with digestion process of the different diets, energy spent in post-alimentary excretion (PANE) was significantly higher when animals fed CaD ( $2.25$  versus  $0.61 \text{ J shrimp}^{-1} \text{ day}^{-1}$  in HeD), meanwhile energy corresponding to heat increment (H<sub>i</sub>E) was significantly higher in HeD ( $164.4 \text{ J shrimp}^{-1} \text{ day}^{-1}$ ) than in CaD shrimps ( $67.1 \text{ J shrimp}^{-1} \text{ day}^{-1}$ ).

Contrary to early juveniles, in late juveniles recovered energy was significantly higher in CaD treatment ( $329.4 \text{ J shrimp}^{-1} \text{ day}^{-1}$  versus only  $131.8 \text{ J shrimp}^{-1} \text{ day}^{-1}$  in HeD shrimps). Here again, energy channeled to H<sub>i</sub>E was significantly higher in HeD shrimps ( $284.8 \text{ J shrimp}^{-1} \text{ day}^{-1}$ ) than in animals from CaD treatment ( $128.4 \text{ J shrimp}^{-1} \text{ day}^{-1}$ ), but energy associated with PANE was similar in both treatments (Table 4). The proportion of digestible energy (DE) invested into growth (RE/DE) was similar in early juveniles from both diets, however in late juveniles this proportion was higher in CaD than in HeD fed animals.

### 3.5. Metabolic substrate

The metabolic substrate was estimated from the O:N ratio in early and late juveniles during fast and fed status (Table 5). Substrate metabolism showed differences during starvation in early juveniles, with a mixture of lipid plus cbh in CaD animals and a mixture of protein plus lipids in HeD shrimps. In both treatments a mixture of lipids and cbh is utilized as metabolic substrate in fed animals. Late juveniles from CaD treatment used lipids plus cbh as substrate during fasting period, same as in early juveniles, but in HeD protein had greater importance as substrate during starvation. In both treatments later juveniles changed their substrate metabolism to protein and lipids when feeding. In general, later juveniles showed a higher use of protein as metabolic substrate than early juveniles.

## 4. Discussion

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Shrimp during its life cycle in nature from larvae to PL's migrate from oceanic to brackish waters in mangrove areas. There is a drastic change in feeding habits with presence of plant debris, benthic organisms, bacteria and also due to its herbivorous tendency and a trypsin activity that is high compared to carnivorous species (Lemos and Rodriguez, 1998). Moreover, PL's will probably show a remarkable flexibility to utilize protein (Brito et al., 2001) as well as cbh sources to prepare for juvenile stages (Jiménez-Yan et al., 2006); its demand in energy from all food sources to sustain a rapid growth rate is obviously high. In *L. vannamei* juveniles, reared in commercial ponds under semi-intensive conditions, Gamboa-Delgado et al. (2003) observed that plant matter contributed above 30% of stomach content and detritus represented up to 58%, meanwhile artificial feed contributed in less than 20%, showing the capability of this species to use vegetal food, and suggested an ontogenetic adaptation of the enzymatic activity with a decrease in total protease and an increase in lipase and amylase activities as shrimp growth.

Variations on oxygen consumption and ammonia excretion according to the diet composition were observed in this study. The main difference between treatments was related to higher lapse needed for HeD fed animals for return oxygen consumption to basal levels after feeding. Apparent heat increment has been related with dietary protein levels in different species of shrimps PL's (Rosas et al., 1996), as well as in *L. vannamei* early juveniles (Jiménez-Yan et al., 2006), but these authors used diets with protein levels higher than 40%CP. On line with this study, Rosas et al. (2001) found that in *L. vannamei* juveniles a diet with high cbh level produced a heat increment 244% higher than the obtained when shrimps fed with a diet containing low cbh, therefore, in this case the pattern in heat increment seems not to be related with protein content but with the cbh level in the diet.  $H_iE$  varied according to the nature of protein source as well. Already with *Marsupenaeus japonicus* juveniles, soy-protein concentrate produced a larger variation in  $H_iE$  than on crab protein (Teshima, 1998) and in a first approximation it could be higher with vegetable protein than with animal protein diet.  $H_eE$  did not vary significantly either in early or in late juveniles fed both diets. By and large, RE ( $J \text{ shrimp}^{-1} \text{ day}^{-1}$ ) did not vary significantly under dietary alteration in early juveniles. It meant that the partition of energy did not differ from one diet to another at this stage.

Jiang et al. (2000) indicated that *L. vannamei* is ammonotelic, with ammonia accounting for up to 84% of total nitrogen excretion. In this sense Burford and Williams (2001) stated that in *P. monodom* the main source of dissolved nitrogen is ammonia excreted from the gills, they also found that excretion rate was three times higher after feeding than that for starved animals. In this study, ammonia excretion after feeding

presented higher values in CaD (440% respect to basal levels) than in HeD (350% respect to basal levels), which could be related to higher protein contents in CaD (40%CP) than in HeD (20%), but also with cbh levels in diet. When analyzing the effect of cbh upon excretion, Rosas et al. (2001) found that ammonia excretion as a percentage of fasting rates was higher in shrimps fed low cbh diet than the obtained in animals fed a diet with high cbh level. In this sense Hari et al. (2006) showed that addition of cbh to the diet reduced total ammonia nitrogen in *P. monodon*.

From energy point of view, the only difference in excretion found in this study was related to higher energy expended in PANE when early juveniles fed CaD. Nevertheless excretion represent a minor item in the shrimp budget, here the proportion of excretion to DE was less than 2% in all cases, so the contribution of excretion on energy balance was negligible.

On line with this study, *L. vannamei* that fed animal or plant protein sources during the PL's phase provided consistent results in terms of energy partitioning with equal amount for RE (Jiménez-Yan et al., 2006). RE expressed in %DE was similar in early juveniles fed respectively on CaD or HeD, but in late juveniles a greater amount of DE was channelled into RE when fed on CaD. H<sub>i</sub>E presented some variations and protein source tended to decrease H<sub>i</sub>E with CaD compared to HeD. By and large, animal or plant protein sources had low incidence on PL's RE in a range of 26-27% respectively even though level of variability was important (Jiménez-Yan et al., 2006).

O:N ratio brought additional information on metabolic substrate related to feed composition. O:N was quite high at 160-164 in fed early juveniles from CaD or HeD; it indicated lipid and cbh as energy substrates (Mayzaud and Conover, 1988). In the same way in fed late juveniles from either CaD or HeD, the O:N ratio at 34 was for an utilization of a mix protein plus lipid. It emphasized the role of protein as main energy substrate. In *L. stylirostris* juveniles, increased levels of dietary protein produced a decrease in O:N ratio (Gauquelin et al., 2007). In the present study, due to feeding background of juveniles, metabolism was driven on same substrate indifferently of actual diet composition. This trend signed a nutrition status that was more omnivory than herbivory as described previously with zooplankton (Bougis, 1974; McTigue and Zimmerman, 1991). The use of protein as source of energy is well documented in shrimp (Dall and Smith, 1986). The effect of dietary protein levels and protein/energy ratio on O:N has also been reported. Rosas et al. (2001) found that when shrimps were fed a low cbh diet used proteins as source of energy at fasting or feeding status, but when animals fed a high cbh diet, metabolic substrate during fasting are mainly protein and changed to a mixed substrate when feeding. In *L. setiferus* juveniles O:N changed in relation to dietary protein level, with a mixture of protein and lipids when shrimp fed a 30% CP diet (Taboada et al., 1998). Also in *L. vannamei* juveniles fed animal or plant protein diets, O:N indicated an energy derived mainly from protein and lipid (Jiménez-Yan et al., 2006).

A replacement of protein with cbh reduced weight gain in late juveniles, however in early juveniles produced similar values. Addition of cbh to a low protein diet (25% CP) give similar yield and improved protein efficiency ratio when compared with a high protein diet (40% CP) without cbh in PI<sub>20</sub> *P. monodon* (Hari et al., 2006). Eventually, it was shown a sparing-effect of cbh as energy substrate preserving amino acid (aa) for protein synthesis but all aspects of feed utilization should be considered such as intake, DE, (UE+ZE), H<sub>i</sub>E, H<sub>e</sub>E and RE. However, some elements of the budget were difficult to address such as feed intake. H<sub>i</sub>E varied according to the nature of protein source as well. Already with *M. japonicus* juveniles, soy-protein concentrate produced a larger variation in H<sub>i</sub>E than on crab protein (Teshima, 1995) and in a first approximation; it could be higher with plant protein than animal protein diet. H<sub>e</sub>E did not vary significantly. RE (J shrimp<sup>-1</sup> day<sup>-1</sup>) did not vary significantly under dietary alteration in early juveniles, but was increased in late juveniles fed CaD.

The category of plant protein sources such as *Spirulina*, wheat gluten or soy-concentrate seemed to bring a reasonable potential for partial or total replacement of fish meal in diets. Replacement of 50% fish meal with soybean meal enhanced retention of protein due to a decline in protein degradation in *L. vannamei* juveniles (Mente et al., 2002). Moreover, evidence appeared of a cbh sparing-effect on dietary protein with wild *L. vannamei* juveniles raised under laboratory conditions (Arena et al., 2003). *L. vannamei* early juveniles helped to verify this concept.

There was a conflicting situation between a protein-rich feed provided more RE than low protein diet. In a protein-rich diet such as CaD, aa that were not used for protein synthesis were more efficiently utilized as energy source than glucose. On the other hand, a high-cbh based diet (mainly starch) such as HeD provided glucose that could limit deamination and divert more aa to protein synthesis. Up to now, the most efficient pathway has been described in the first option, i.e. more protein, more weight gain. But this case was described mainly with carnivorous species such as *L. stylirostris* late juveniles (Gauquelin, et al, 2007). The second option, a pathway with cbh-sparing effect on dietary protein could be more suited for a species such as *L. vannamei* for which a low muscle protein turnover was measured (Mente et al., 1997). Prerequisites could be (i) enough amylase isoforms, i.e. enough polymorphism given by amylase genes allelic frequency on the population, (ii) a stimulation of amylase genes in early stages of development by feeding with plant sources, and (iii) a well-balanced aa profile in order to contribute to make available simultaneously all essential aa in free pool for efficient on-site muscle protein synthesis.

A mixture of marine protein sources benefited shrimps because of an excellent aa profile and a limiting factor bearing on tryptophan. Are there some essential aa missing in plant protein? The improbability of lysine and methionine being limiting aa in practical rations was discussed previously. The use of uncoated crystalline aa was proved inefficient in fish (Cowey, 1994) or shrimp as well (Deshimaru and Kuroki, 1975) and then it was preferred to use the formulated diets as such taking into account a frequent supply of minute amount of feed that probably reduced the impact of a limiting factor. Coated aa would help solve the problem as well.

A mixture of digestible ingredients (*Spirulina*, wheat gluten, and soy-concentrate) gave such aa profile in spite of a lysine deficit in relation to the presence of plant protein sources. Complementation with fish meal and CPSP<sup>80</sup> helped make adjustment on aa profile. On top of that, feed distribution was fractionated during the day rendering a possible limiting factor less critical regarding any imbalance of indispensable amino acids for protein synthesis. All the prerequisites described above were found achievable through this study even though confirmation and adjustments in formulations still remain to be done. According to Mente et al. (2002), it appears that in shrimps there is a little scope for recycling of essential amino acids if these become limiting in the diet. These authors suggested that high growth rates seem to involve a reduction in the turnover of proteins, a lower protein turnover may conserve energy for growth (or other processes), as protein turnover is responsible for a large fraction of the energy budget (Conceição, 1997).

In this study early juveniles utilized indifferently and efficiently a mix of animal protein or another diet with plant protein. This flexibility is shown not so in terms of protein balance because of similar aa profile rather than on energy partitioning, animals derived equal DE amount to RE in spite of difference in excretion. Those aspects evidenced an omnivory-herbivory trend found previously with this species. The ability of *L. vannamei* to spare protein with an upper level of digestible starch appeared already in a previous work on juveniles captured from the wild (Arena et al., 2003).

Replacing fish meal by plant protein, maintaining low CP percentage increasing cbh levels, multiple meals, and a good attractiveness of the diet, should guide for feeds in perspective of sustainable farming nursery production. Level of animal protein



replacement by plant protein from a culture viewpoint is verified with an incidence on metabolism and energy partitioning. Similar performances on early juveniles with CaD or HeD showed a certain potential for adaptation to plant protein with a lot of starch. The course of the project aimed at demonstrate a better adaptation to a cbh-rich diet based on plant protein sources given as early as possible in the life cycle. Such preparation of animals with a stimulation of genes would lead to a good adaptation of juveniles receiving all-plant diets to sustain growth performances up to a marketable size. Such premises examined not only performances, but physiological aspects with energy partitioning, body reserves and tissue metabolism to give evidence of proper activation of key enzymes under diet variations, set the ground to further selection program. *L. vannamei* represent a good model to study such potentialities while using these and previous data accumulated at all stages of its life cycle.

## Acknowledgements

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We thanks for the financial support to UNAM IN 216406 and CONACyT 49406/24750 and the technical support of Biol. Gabriela Palomino in live food for larvae production; Ing Miguel Arévalo for nauplii production; Ing. Adriana Paredes for larval rearing; Biol Manuel Valenzuela, for broodstock management and Biol. Gabriel Taboada for artificial feed elaboration.

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## Tables

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Table 1. Diets composition (g kg<sup>-1</sup>) for early and late juveniles.

	CaD	HeD
<sup>(1)</sup> squid meal <sup>72</sup>	200	-
<sup>(1)</sup> fishmeal <sup>65</sup>	190	-
<sup>(2)</sup> CPSP <sup>70</sup>	40	45
dry whey <sup>80</sup>	120	-
<sup>(3)</sup> wheat gluten <sup>80</sup>	-	70
<sup>(2)</sup> soybean concentrate <sup>60</sup>	-	150
<i>Spirulina</i> <sup>72</sup>	-	45
<sup>(3)</sup> wheat starch	200	400
cod liver oil	37	60
soy lecithin	20	20
cholesterol	5	5
<sup>(4)</sup> vitamin and mineral premix	10	10
<sup>(4)</sup> vitamin Stay-C-35%	4.5	4.5
<sup>(4)</sup> carophyl	0.15	0.15
Na-alginate	10	10
filler	163.3	180.3

CaD, carnivorous animal diet; HeD, herbivorous diet; upper digits for protein content of ingredients (%). <sup>(1)</sup>Local products; <sup>(2)</sup>Profine, Argentina; <sup>(3)</sup>Gluten y Almidones SA de CV, Mexico; <sup>(4)</sup>DSM, Nutritional Products, México.

Table 2. Proximal analysis of the diets.

	CaD	HeD
Moisture	4.3	7.4
Crude protein	39.5	17.5
Ether extract	10.0	8.7
Nitrogen free extract	23.0	44.5
Ash	22.8	21.9
Fiber	0	0
Gross energy (kJ/g)	14.2	13.5
Lysine	2.7	1.5
Arginine	2.5	1.6
Histidine	0.8	0.6
Theronine	1.5	1.0
Methionine	1.0	0.5

CaD, carnivorous animal diet; HeD, herbivorous diet. Values of amino acids from analyses made by CENCOM SA de CV, Mexico.

Table 3. Zootechnical parameters of the experiments. Each experiment lasting 45 days. Mean values  $\pm$  SD.

	Early juveniles		Late juveniles	
	CaD	HeD	CaD	HeD
Survival (%)	73.3 $\pm$ 3.6 <sup>b</sup>	91.7 $\pm$ 3.2 <sup>a</sup>	97.7 $\pm$ 5.4 <sup>a</sup>	94.0 $\pm$ 0.3 <sup>a</sup>
Initial wet weight (g)	0.004 $\pm$ 0.001	0.004 $\pm$ 0.001	3.08 $\pm$ 0.7	2.07 $\pm$ 0.6
Final wet weight (g)	0.21 $\pm$ 0.01 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	6.09 $\pm$ 0.09 <sup>a</sup>	3.3 $\pm$ 0.02 <sup>b</sup>

CaD, carnivorous animal diet; HeD, herbivorous diet. Different letters in the superscript indicate significant difference between treatments in each experiment ( $p < 0.05$ ).

Table 4. Energy partitioning ( $\text{J animal}^{-1}\text{day}^{-1}$ ) measured for early and late juveniles. Mean values  $\pm$  SD ( $n = 9$ ).

	Early juveniles		Late juveniles	
	CaD	HeD	CaD	HeD
DE	594.66 $\pm$ 75.9 <sup>a</sup>	639.3 $\pm$ 172.5 <sup>a</sup>	1291.5 $\pm$ 109 <sup>a</sup>	1002.5 $\pm$ 94.2 <sup>a</sup>
UE+ZE	2.9 $\pm$ 1.2 <sup>a</sup>	5.4 $\pm$ 2 <sup>a</sup>	6.50 $\pm$ 0.8 <sup>a</sup>	4.64 $\pm$ 0.6 <sup>a</sup>
PANE	2.25 $\pm$ 0.7 <sup>a</sup>	0.61 $\pm$ 0.04 <sup>b</sup>	9.7 $\pm$ 1.3 <sup>a</sup>	10.3 $\pm$ 3 <sup>a</sup>
H <sub>e</sub> E	463 $\pm$ 97.8 <sup>a</sup>	392 $\pm$ 114 <sup>a</sup>	817.5 $\pm$ 32.7 <sup>a</sup>	571. $\pm$ 12.2 <sup>a</sup>
H <sub>i</sub> E	67.1 $\pm$ 14.3 <sup>a</sup>	164.4 $\pm$ 74 <sup>b</sup>	128.4 $\pm$ 12.1 <sup>a</sup>	284.8 $\pm$ 7.1 <sup>b</sup>
RE	59.4 $\pm$ 6.6 <sup>a</sup>	76.9 $\pm$ 19 <sup>a</sup>	329.4 $\pm$ 9.0 <sup>a</sup>	131.8 $\pm$ 8 <sup>b</sup>
RE/DE (%)	10.0	12.0	25.5	13.1

CaD, carnivorous animal diet; HeD, herbivorous diet; DE, digestible energy; UE+ZE, routine nitrogen excretion; PANE, post-alimentary nitrogen excretion; H<sub>e</sub>E routine respiration; H<sub>i</sub>E heat increment due feeding; RE, recovered energy obtained from energy content of the carcass multiplied by weight gain per day. Different letters in the superscript indicate significant difference between treatments in each experiment ( $p < 0.05$ ).

Table 5. Values of O:N ratio for early and late starved and fed juveniles.

	Early juveniles				Late juveniles			
	starved		fed		starved		fed	
	CaD	HeD	CaD	HeD	CaD	HeD	CaD	HeD
O:N	177	69	160	164	98	20	34	34
Metabolic substrate*	cbh + lipid	prot + lipid	cbh + lipid	cbh + lipid	cbh + lipid	prot	prot + lipid	prot + lipid

CaD, carnivorous animal diet; HeD, herbivorous diet; cbh, carbohydrates; prot, protein; (\*) Range according to Mayzaud and Conover (1988): from 3- 6 protein, between 50-60 a mix of protein and lipids, above this a mix of lipids and carbohydrates.

## Figures

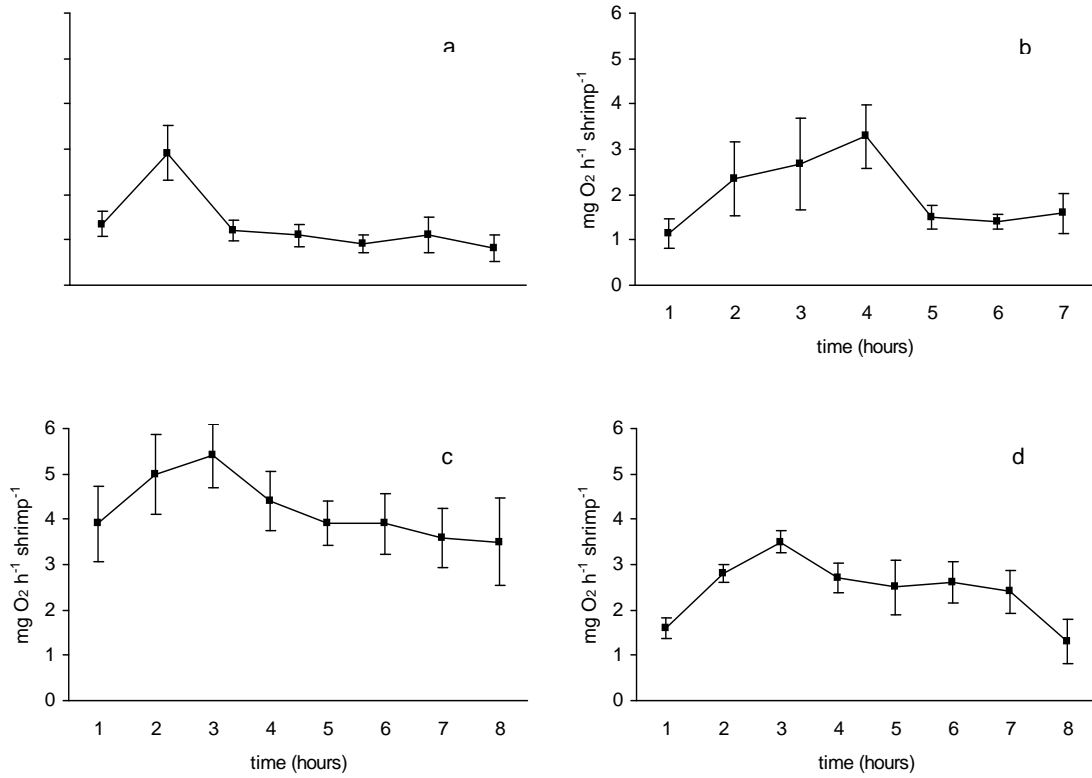


Figure 1. Oxygen consumption (mean  $\pm$  SE) of *L. vannamei* early juveniles fed control diet (a) or extreme diet (b), and late juveniles fed control diet (c) or extreme diet (d).



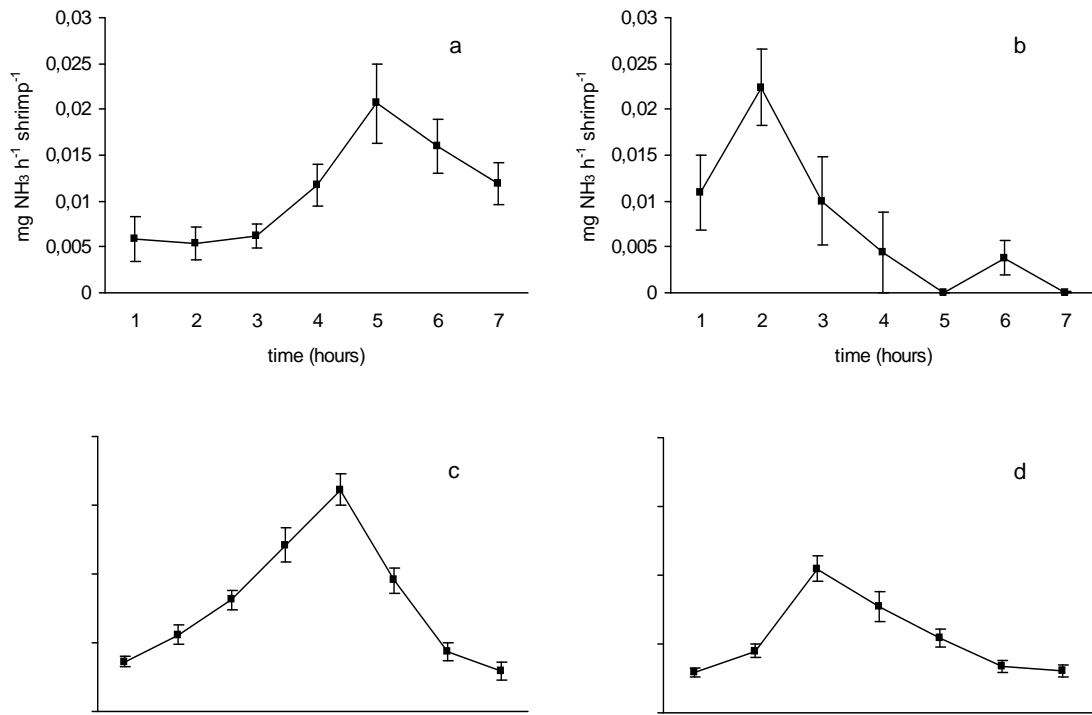


Figure 2. Ammonia excretion (mean  $\pm$  SE) of *L. vannamei* early juveniles fed control diet (a) or extreme diet (b), and late juveniles fed control diet (c) or extreme diet (d).