Journal of Shellfish Research
April 2004 ; Volume 23 (1) : Pages 243-249

The effect of starvation on refeeding, digestive enzyme activity, oxygen consumption, and ammonia excretion in juvenile white shrimp *Litopenaeus vannamei*

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Abstract:

Juveniles of the white shrimp *Litopenaeus vannamei* were kept Without food for between 0 to 15 clays to evaluate the impact of starvation oil physiologic state (oxygen consumption, poststarvation refeeding, index, nitrogen excretion, and O:N ratio) and digestive enzymes activity. Physiologic changes were found after 6 days of fasting, and refeeding ability declined as a result. Nevertheless. the shrimp were able to Survive 16 days Without food. Starvation Caused metabolism to drop progressively toward a basal level (21 J (.) h(-1 .) g(-1)) and a decrease in the rate of ammonia excretion, because of the catabolism of amino acids front soluble protein in the hepatopancreas. This decrease led to an increase in digestive enzymes specific activity (U/mg protein). But, expressed as total U. all digestive enzyme activities decreased in the absence of substrate from 0.016 to 0.007 U/hepatopancreas (HP) for alpha-amylase and 2.58 to 0.63 U/HP for total trypsin. *L. vannamei* juveniles showed a true physiologic adaptation mechanism to food depiivation: no changes in body weight but loss in hepatosomatic index, no exuviations, including the utilization of HP soluble proteins (a drop from 269 to 53 mg/mL). After 10 days. a neoglycogenic pathway and the corresponding tissue enzymes activities seemed enhanced, and the animals derived all energetic Substrates mainly from protein (O:N ratio of 17) to cover their metabolic costs. Estimates of basal metabolism (Hem) from the routine respiration rate per clay (from 361 to 725 J (.) g ww(-1) (.) day(-1) through the 15-day starvation period). and loss of nonfecal energy (HxE) from the nitrogen excretion rate (varying from 39 to 57 J (.) g ww(-1) (.) day(-1) during the same period) were used in a bioenergetic partition model of a fasting juvenile. Which indicated that the energetic requirement to Survive Without feeding was in the range of 418 and 771 J (.) g ww(-1) (.) day(-1) during the 15-day period of starvation.

Keywords: shrimp, *Litopenaeus vannamei*, starvation, metabolic rate, digestive enzymes.

1. Introduction

The Pacific white shrimp *Litopenaeus vannamei* is cultured in extensive, intensive, and semi-intensive systems and is, with *Litopenaeus stylirostris*, the most popular shrimp for aquaculture in Mexico and Central and South America. The state of Sinaloa on the Northwest coast of Mexico, considered the most important agricultural producer in the country, has observed in recent years an important increase of aquaculture farms, from approximately 100 ha of pounds in 1984, to 1850 ha in 1998, with a growth rate of about 150 ha/year. Nowadays, Sinaloa has more than 200 shrimp farms (about 75% of the national total) and produces around 10000 T yearly (65% of the national total) (Hernandez Cornejo & Ruiz Luna 2000).

The digestive gland is generally regarded as a major storage organ in decapods crustaceans (Huggins & Munday 1968, Allen 1971). The study of the digestive gland is of considerable interest because of its role in the accumulation and cyclic mobilization of reserves during the molting process, its contribution of nutrients to the ovary during vitellogenesis, the mobilization of its reserves during starvation, and its role in digestion and absorption. The level of the digestive enzymes in decapod crustaceans does not remain constant during the developmental cycles (Van Wormhoudt 1974) as a result of both external and internal factors. Among the external factors, the quantitative and qualitative variability of food is poignant.

Physiological and biochemical effects of starvation have been studied in several decapod species (Cuzon & Ceccaldi 1973, Cuzon et al. 1980, Anger 1986, Dall & Smith 1986, Wehrtmann 1991), but little is known about the effects of prolonged food deprivation. Changes in biochemical composition during starvation have been reported in *Marsupenaeus japonicus* where there was a progressive suppression of metabolism compared with normally fed shrimp (Cuzon et al. 1980). The study found that the shrimps utilized carbohydrates primarily, then lipids to meet their energy requirement; proteins were utilized significantly only during the fourth week of starvation (Cuzon et al. 1980). In *L. vannamei* postlarvae, triacylglycerol provided energy during short periods of starvation, whereas protein was utilized during prolonged starvation (Stuck et al. 1996).

Modifications in digestive enzyme activity have been found in several penaeid shrimp and related to the amount and quality of food (Le-Vay et al. 1993, Rodriguez et al. 1994, Le Moullac et al. 1996, Lemos & Rodriguez 1998). Rosas et al. (1985) found that the type and concentration of food influenced the ingestion rate of larval *L. setiferus*, which, in turn, affected metabolic rate. Changes in digestive enzyme activity were found under starvation conditions in juveniles of *Marsupenaeus japonicus* (Cuzon et al. 1980) and *Metapenaeus ensis* (Leung et al. 1990). Both studies pointed out a decrease in digestive enzyme activity compared with fed shrimps.

Nitrogen excretion and metabolic rates are influenced by many factors, such as moult stage, feeding conditions and level of activity. In this sense, O:N ratio has been used widely as an index of utilized substrate for oxidative metabolism (Chu and Ovsianico-Koulikowsky 1994, Regnault 1981, Dall & Smith 1986, Rosas et al. 1995). The catabolism of pure protein produced theoretical values of O:N of between 3 to 16, whereas the catabolism of equal quantities of proteins and lipids yield O:N values of between 50 and 60. Greater values of O:N correspond to an increase in lipid and carbohydrate catabolism (Mayzaud & Conover 1988).

The aim of this study was to evaluate the effect of starvation on digestive enzyme activity, oxygen consumption, ammonia excretion, and O:N ratio in *Litopenaeus vannamei* juveniles. Afterwards the effect of re-feeding of starved juveniles was analyzed to assess the capability of shrimp to recover after prolonged starvation.

This information may contribute to a better understanding on the physiology of this commercially important shrimp species and will have an application in assisting the management in farms.

2. Materials and methods

Experimental conditions and design.

Juveniles of *Litopenaeus vannamei* were provided by a commercial farm, Maricultura del Pacífico, located in Mazatlán, Sinaloa, Mexico, during March 2001. The organisms were transported to the laboratory culture system of the Centro de Investigación en Alimentación y Desarrollo (CIAD) and fed with a commercial food during the acclimatizing period in 1000-L tanks. They were then transported to the laboratory, and groups of 20 organisms (wet weight = 0.998±0.213 g) were maintained for different starvation periods (0-control group-, 3, 6, 9, 12, and 15 days) in 10-L glass aquaria. Experimental conditions were: 34o/oo salinity, 24°C water temperature and 12D:12L. Water was completely renewed daily and molts and dead organisms were removed.

Oxygen consumption, ammonia excretion, and atomic ratio (O:N).

After each starvation period groups of 5 individuals were placed in individual 500-mL respirometer chambers in a flow-through system using a continuous pump flow (ISMATEC®, 12 mL/min flow). All measured were done at the same day time in order to obtain comparable data. Organisms were acclimated for 2 h in the beakers and a sample of water from each chamber was taken to determine the initial concentration of oxygen and ammonia; flasks were sealed for 30 min, after which new samples were taken to measure final concentrations. The flow-through and the sealed periods were adjusted to avoid a depletion of oxygen concentration by more than 0.5 mg/L. Samples for ammonia excretion were filtered and fixed with H2SO4 (pH 2) and then frozen until processing.
The concentration of oxygen was measured using a polarographic oxygen electrode (YSI® 59) and ammonia was determined by the indophenol technique (Parsons et al. 1984). Consumed oxygen and excreted ammonia were taken as being the net difference between the start and end of the sealed period. One out of every six chambers was left without a shrimp and measured as a control. The atomic O:N ratio was estimated according Taboada et al. (1998) using the individual values of oxygen consumption and ammonia excretion transformed to µg At / g/ h as follows: oxygen values were multiplied by 62.5 (1000 to convert the milligrams into micrograms divided by the atomic weight of oxygen, 16), ammonia values were multiplied by 58.9 (obtained through dividing 1000 by the product of the atomic weight of nitrogen (14) and the fraction of nitrogen in NH3 (0.824)).

Digestive enzyme activities

After physiological measurements, shrimps were dissected and the hepatopancreas stored at –70°C in individual 1-mL microtubes until enzyme assays were done.
Frozen samples were homogenized in 1 mL ice-cold pure water. Homogenates were centrifuged (at 14000 x g for 6 min at 4°C) and the aqueous supernatant, crude or diluted (1:10 v/v), was immediately used for enzyme analysis. The soluble-protein content was measured by the method of Bradford (1976), using a micro-plate reader at 495 nm.

Duplicate assays for each sample were made. Trypsin activity was measured by the method of Erlanger et al. (1961) with Nα-benzoyl-DL-Arg-p-nitroanilide (BAPNA) as substrate. Chymotrypsin activity was assayed by the method of Delmer et al. (1979) using Nα-succinyl-L-alanyl-L-prolyl-L-phenyl-alanine-4-nitroanilide (SAPNA) as substrate. Hydrolysis for both enzyme activities was made in 0.1 M Tris-buffer, pH 8 at 25°C and the absorbance measured at 405 nm. One unit of enzyme activity was defined as 1 µmole of p-nitroanilide liberated in 1 min at 25°C.
α-amylase activity was assayed according to Bernfeld (1955) with 1,5% oyster glycogen as substrate in 10 mM phosphate buffer, pH 7. Absorbance measurements were made at 520 nm. For this method, one unit of enzymatic activity was defined as 1 mg of maltose liberated in 1 min at 37°C.
General protease activity was estimated in homogenates using azocoll as substrate in phosphate buffer, pH 7.5 (Todd, 1949). Absorbance was measured in a spectrophotometer at 520 nm. For this method, one unit was defined as the amount of enzyme that catalyzes the release of azo dye causing a $\Delta A/\Delta t=0.001$min (Walter, 1988).
α-glycosidase activity was estimated using p-Nitrophenyl- α-D-glycopyranoside as substrate in 50 mM phosphate buffer, pH 6. Absorbance was measured at 410 nm. One unit of enzymatic activity was defined as the amount of enzyme that hydrolyzes 1 µm of substrate per minute.

Post-starvation re-feeding index (PSRFI)

Other subgroups of organisms for each treatment (n=6) were kept in individual chambers and exposed to a known amount of food previously lyophilized. The proximate composition of the food was 35% protein, 3.5% oil, 30% fiber, 16% ash, 12% humidity; average weight of each lyophilized pellet was 15.14±2.28 mg; calorific content, 5.5 cal/mg of food. After 2 h 30 min the non consumed food was removed and lyophilized again. The consumed food was calculated to measure the post-starvation re-feeding index, defined as PSRFI (Food consumed W/Body W).

Bioenergetics model

From a bioenergetic point of view, in order to integrate a model of energy partition for starved shrimp, we followed an equation (Bureau et al. 2000):

DE requirement = (RE+Hem+HiE+HxE (UE+ZE)+SE),

This model originally included parameters where DE is digestible energy requirement; RE is energy gain; HEm is maintenance of energy requirement; HiE is heat increment of feeding; HxE is non-fecal energy losses; and SE surface loss or exuviae. With this model a general energy balance model for starvation conditions in shrimp can be calculated.
However, in starved shrimps, the following parameters can be considered as 0:

DE= 0 (no feeding)

HiE= 0 (no heat increment of feeding)

SE = 0 (no exuvia),

Hence, a model for starving shrimp could be:

$$RE = -(Hem+HxE\ (UE+ZE)$$

in which RE can be understood as endogenous energy needed to survive (Rosas, pers. comm.) and it is negative because in absence of energy input it is not possible to have energy gain *sensu stricto*. It is called also the scope for growth (SFG). Using this model, we calculated the endogenous energy (RE) for starving shrimp.

Statistical analysis

To determine significant differences among starvation periods one way ANOVA and Tuckey range test were used, when the data were normal. Those results with no normality were evaluated through the Kruskal Wallis test and Dunn's multiple comparisons (Daniels 1978). For both analyses, *p* was set at 0.05.

3. Results

Weight and Survival

No significant differences were observed ($p>0.05$) in relation to individual wet weight among treatments. However, survival decreased the longer the period of starvation (Table 1). The percentage of molts were approximately 10% for control, 3 and 6 day treatments, after that no molt were recorded.

Oxygen consumption, ammonia excretion, and O:N ratio

Oxygen consumption increased significantly at 3, 6, and 9 days in starved animals (mean value 1.75 mg O_2 /h /g) compared with the control. At 12 and 15 days values were not significantly different from the control group (mean value 1.13 mg O_2 /h /g). (Kruskal-Wallis, H=14.1; p=0.015) (Figure 1).

There were no significant differences in the amount of ammonia excreted between starved and control animals (mean value 0.08 mg $N\text{-}NH_3$ /h /g, Kruskal-Wallis, H=1.74, p=0.88) (Figure 2). This may be because high standard deviations were observed, especially in the 6 and 12 days starvation groups (0.03 and 0.067 mg $N\text{-}NH_3$ /h /g, respectively).

No significant differences between treatments were observed with regards O:N atomic ratios (Kruskal-Wallis, H=10.14, p=0.071) (Figure 3). However, there was a trend towards increased ratios between days 3 and 9, suggesting that lipid was being catabolised. By day 12 the drop in the ratio to 10-20 suggests that protein was once again the main source of energetic fuel.

Enzyme activity

Significant differences were found in the hepatosomatic index after different periods of starvation (Kruskal Wallis, H=25.6, p=0.0001). The index was significantly lower after animals were starved beyond 9 days. Beyond the 9th day, index decreased a further 50% until reaching the end of the experiment (Table 1). Hepatopancreatic total soluble protein was significantly affected by the period of starvation (ANOVA, $p<0.05$). The highest value

(268.7 ± 52.13 mg/mL) was obtained in the control, followed by the 3 and 6 day starvation experiments (mean value 159.8 mg/mL). The lowest significant values were found in the 9 to 15 day experiments (53.23 mg/mL) (Figure 4).
Digestive enzyme activity was, in general, significantly affected by different periods of starvation.

Hepatopancreatic digestive carbohydrases results are shown in figure 5. Total amylase activity was significantly decreased by starvation (ANOVA, $p<0.05$), from the highest value in fed juveniles (control) (0.016 ± 0.002 total U), followed by the 3 and 6 day experiments (mean value 0.011 total U), and the lowest value in the 9, 12, and15 days starved groups (mean value 0.007 total U) (Figure 5a). However, the specific activity increased as the soluble protein content decreased (Figure 5b).
Glycosidase activity was significantly decreased (mean value 1.47 total U) by all starvation periods as compared to control fed animals (3.6 ± 1.5 total U) (Figure 5c). For this enzyme, the specific activity also increased as the soluble protein content in the hepatopancreas decreased (Figure 5d).
Proteinase activity was significantly affected by the starvation periods (ANOVA, $p<0.05$) (Figure 6). The highest value (5.3 ± 2.4 total U) was observed in the control, which was not significantly different from 3 and 6 day experiments, whereas the lowest value of total proteinase activity (1 ± 0.12 total U) was observed in the shrimps starved for 15 days (Figure 6a). As with the carbohydrases, the specific activity of total proteinases was highest in shrimps starved for 12 days (Figure 6b).
Total trypsin activity was significantly lower from the 9th day to the end of starvation (mean value 0.63 total U). Fed shrimp and those deprived for 3 and 6 days yielded a mean trypsin activity value of 2.58 total U (Figure 6c).
Total chymotrypsin activity diminished along the starvation days. Significant differences were found among the fed shrimps (45.41 ± 11.89 total U), the 3 and 6 days starvation groups (mean value 25.02 total U) and the 9, 12 and 15 days starvation groups (mean value 7.87 total U) (Figure 6e). The specific activities of these latter two endoproteinases were not significantly affected by starvation (Figure 6d and 6f).

Post-starvation re-feeding index (PSRFI)
The PSRFI was no significantly different among control, 3 and 6 day starvation experiments, conforming an homogenous group (5.09%; 4.71% and 4.75%, respectively), whereas the amount of food consumed decreased for the 9, 12 and 15 day starvation groups being another homogenous group (3.72%, 3.3%, and 2.97% respectively, Figure 7) (Kruskal-Wallis, $H= 21.5$; $p=0.006$).

Bioenergetics model
Estimation of RE values indicates that at the beginning as well as at the end of the starvation period, shrimp needed the same endogenous energy to survive, while between 3 and 9 days they needed more energy, used essentially in respiration to maintain basal metabolism (Table 2). However, the routine respiration rate measured was 61% higher at day 3 of starvation than in fed shrimp, and remained in the same high trend until day 9 of starvation. After then, values diminished to the same value as in controls.

4. Discussion

In the present study the wet weight did not change with starvation, but survival rate decreased. Under starvation conditions, a first event commonly observed is a weight loss in relation with energy expenditure for basal metabolism; after a few days, shrimp in premolt stages will not evolve further and refrain exuviation, saving around 1.4 kJ (Read & Caulton 1980), which is the energy expenditure at molt. This means that shrimp have the adaptation to tolerate starvation saving energy from exuvia, including the energy challenged to mobilize reserves, chitin digestion, and exuviation. In this way shrimp could compensate the weight loss, maintaining body weight without significant changes as observed in the present study.

Homeostasis in shrimp can change to help animals sustain severe food deprivation and survive; it is not instantaneous as the post-starvation and re-feeding showed; however not all starved shrimps stayed alive (Table 1). It means that under the experimental conditions tested, there was a kind of discrimination among shrimp capable or not to adapt and survive. Part of the explanation could come from previous life history before animals were sampled in the farm and placed under laboratory conditions. Also, individuals will resist food deprivation for several days without significant morbidity and a change in some metabolic routes as an adaptation to the severe trophic conditions is hypothesized: robust and fastest growing individuals would have been selected through farming.

L. vannamei juveniles as many species of crustaceans showed a biochemical adaptation response to an absence of food (decrease of digestive enzyme activities) using their own reserves (hepatopancreatic glycogen, protein and probably lipids, estimated through O:N ratio variations) for homeostasis and to channel enough energy for basal metabolism in that period.

According to these results, a high rate of mobilization of reserves was observed between day 3 and 9 of starvation, when the recovered energy (RE) was maximal. Shrimp reserves are mainly limited to lipids stored in the digestive gland. When shrimp are starving, they will use those reserves, increasing the energy debt, reducing the digestive gland weight and its components. Reduction of enzyme activity and soluble protein during starvation underlined the change. It means the shrimp are biochemically and energetically well adapted to fasting because they could mobilize their reserves to be used as energetic sources, at the same time, the enzyme activity in the digestive gland was maintained. This type of strategy has been observed in other shrimp species. Cuzon et al. (1980) showed that *M. japonicus* used protein to obtain energy though the catabolism of amino acids present in digestive gland cells during prolonged starvation period. Similar response was observed in *Penaeus esculentus* by Smith and Dall (1991), evidencing that shrimp can mobilize their own energetic reserves through catabolizing lipids (after 3 to 6 days of starvation) or protein to sustain at food deprivation.

Otherwise a negative correlation was observed in starvation condition between total hepatopancreatic soluble protein and total activity for all measured digestive enzymes. This correlates well with the lower values of post-starvation re-feeding rate.

In general terms, digestive enzymes follow the presence or absence of food. Samain et al. (1983) found that amylase increases in case of food deprivation with a peak and then the enzyme production decreases as an adaptation to low nutrition status and to save energy. Digestive enzymes of *M. japonicus* showed a similar trend that supports previous results (Cuzon et al. 1980). However, results obtained after re-feeding indicate that the necessary time to recover the digestive gland integrity depends on the fasting period. These results show that although *L.*

vannamei have an adaptation mechanism to tolerate fasting conditions, recovery cannot be achieved if starvation is long enough to produce physical damage in the digestive gland and loss of enzyme synthesis. A pattern of variation for digestive enzymes presented by *L. vannamei* during a shorter experimental period differed from the one of *M. japonicus*. At day 15, for example, specific enzymes activity increased as a sort of adaptation to absorb the minute amount of food, but as energy expenditure increased, the peak disappeared shortly. Both amylases and proteases exhibited the same trend.

Mayzaud and Conover (1988) described starvation condition regarding the use of energetic substrates estimated through O N: ratio. It can be assumed that acetyl CoA, which is the final product of β-oxidation of fatty acids. Such oxidation required 46-52 oxygen atoms whereas, in normal condition, β-oxidation of neutral lipids required 14-16 oxygen atoms to produce acetyl CoA. Increase in O_2 consumption of shrimps between days 3 and 9 of starvation with no increase of nitrogen excretion, brought a high O:N ratio. Then, β-oxidation of hepatopancreatic lipid reserves could be occurring in this period. An explanation for a change can be found in the O:N ratio in which the use of lipid as energetic substrate was clear followed by protein as the main energy source. Ammonia excretion values provided indication on substrate oxidation; although nitrogen excretion was not significantly different from the control along the starvation period, O:N ratio values showed a trend to use lipids as energetic substrate between days 3 and 9 of starvation. Such a trend has also been evidenced in *M. rosenbergii* (Clifford & Brick 1983). After 12 starvation days, shrimp returned to the use of protein to derive energy. Likewise, as Mayzaud and Conover (1988) reported for planktonic crustaceans, a decrease in O:N ratio with time of starvation seemed to be common to all species with a predominantly protein-based metabolism. O:N ratio variation can be related to glucose homeostasis, through the regulation of glyconeogenesis (Cuzon et al. 2001) and glycolysis (Hochachka et al. 1988). These results are not in contradiction to those reported by Dall and Smith (1986) for *Penaeus esculentus*. These authors pointed out a reduction in metabolic rate with starvation providing a mechanism for prolonged survival. O:N ratios gave an explanation for successive fuel substrates as starvation increased in intensity (Table 3). Glycogen in the hepatopancreas is affected first, as in *Crangon crangon* (Regnault 1972), then neutral lipids are hydrolyzed, and towards the end of the starvation period, protein are utilized similarly as in *M. japonicus* juveniles (Cuzon et al.1980).

By and large, it appears that tropical species *L. vannamei* living at 24 up to 27°C seawater would hardly sustain food deprivation over 15 days; on the contrary, species such as *M. japonicus* under temperate conditions (20°C) can stand on a 4-week starvation period. They possess a similar level of digestive enzyme activities; then, there are good reasons, according to values of metabolism rates obtained in this study to think that tropical species present a higher basal metabolism and activity that leaves them more dependent on regular food supply for their development.

Acknowledgments

This study was funded by CONACYT- project N°34952-b (A. Roque), UNAM-DGAPA-IN-231599 (G. Gaxiola), CONICET-Argentina (L.Comoglio and O. Amin) and IOC (O. Amin). We thank very especially Dr. Carlos Rosas for critically reviewing the manuscript and Mrs. Ingrid Mascher for editorial assistance.

Tables

Table 1. Wet weight (g), survival (%) and hepatosomatic index (%) of the food deprived shrimps *L. vannamei*. Mean ± SD.

	STARVATION DAYS					
	0	3	6	9	12	15
wet weight (g)	1.07±0.1	0.86±0.2	0.97±0.23	0.99±0.3	0.85±0.1	1.04±0.12
survival (%)	100	90	90	65	65	55
HSI (%)	4.1±0.7	3.3±0.4	2.90±0.45	2.5±0.4	2.1±0.3	1.98±0.9

Table 2. Energetic balance of starved juveniles of *L. vannamei* at different starvation days. HEm (maintenance of energy requirement); HxE is non-fecal energy losses; RE (the endogenous energy). Mean ± SD. Different letters indicate significant differences ($p<0.05$).

Starvation days	HEm (joules/d/g ww)	HxE (joules/d/g ww)	RE (joules/d/g ww)
0	449 ± 94^{b}	53 ± 5^{a}	-473 ± 77^{b}
3	725 ± 68^{a}	46 ± 12^{a}	-771.7 ± 65^{a}
6	664 ± 187^{ab}	39 ± 22^{a}	-696 ± 178^{ab}
9	598 ± 233^{ab}	43 ± 15^{a}	-655 ± 262^{ab}
12	361 ± 167^{bc}	57 ± 39^{a}	-418 ± 166^{b}
15	428 ± 138^{b}	45 ± 16^{a}	-473 ± 152^{b}

Table 3. Theoretical limits of the O:N ration according to Charmantier (com pers).

4.1. days of fasting	J_1	J_3	J_6	J_{10}	J_{15}
O:N	70-100		50-60		2-16
substrate	glycogen		triglycerids		amino acids

Figures

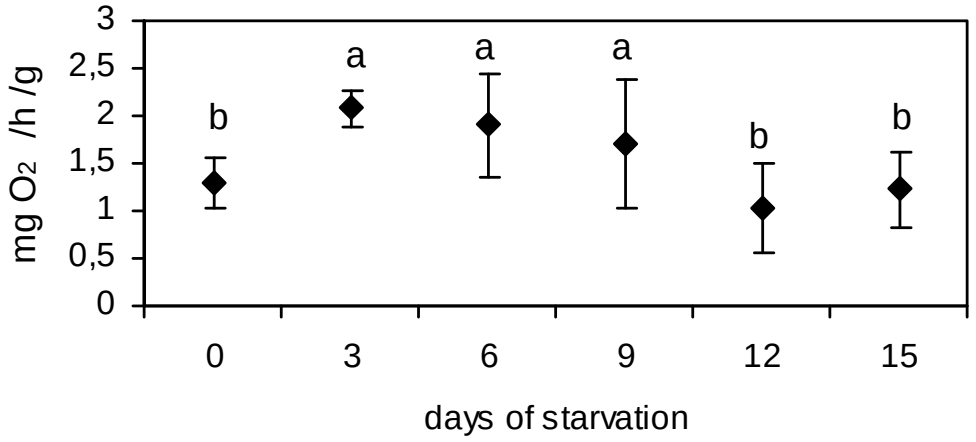

Figure 1. Routine Oxygen consumption of juveniles of *Litopenaeus vannamei* after each starvation period. Mean± SD. Different letters indicate significant differences ($p<0.05$).

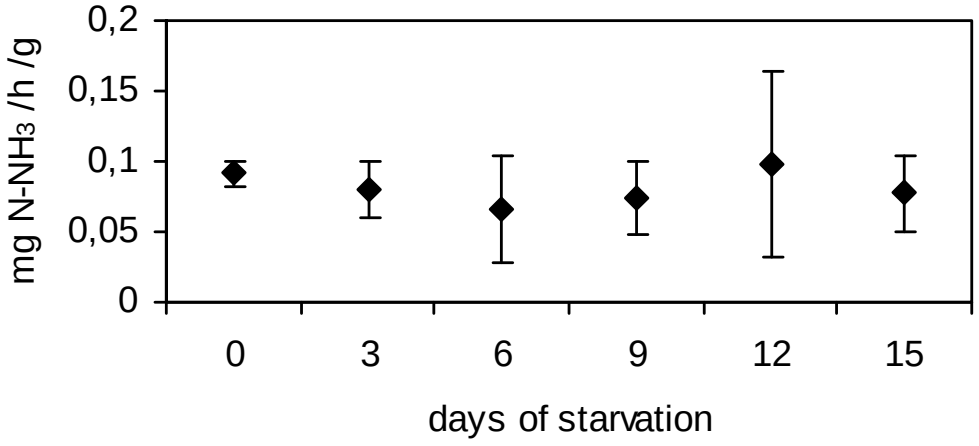

Figure 2. Routine Nitrogen excretion of juveniles of *Litopenaeus vannamei* after different starvation periods. Mean ± SD.

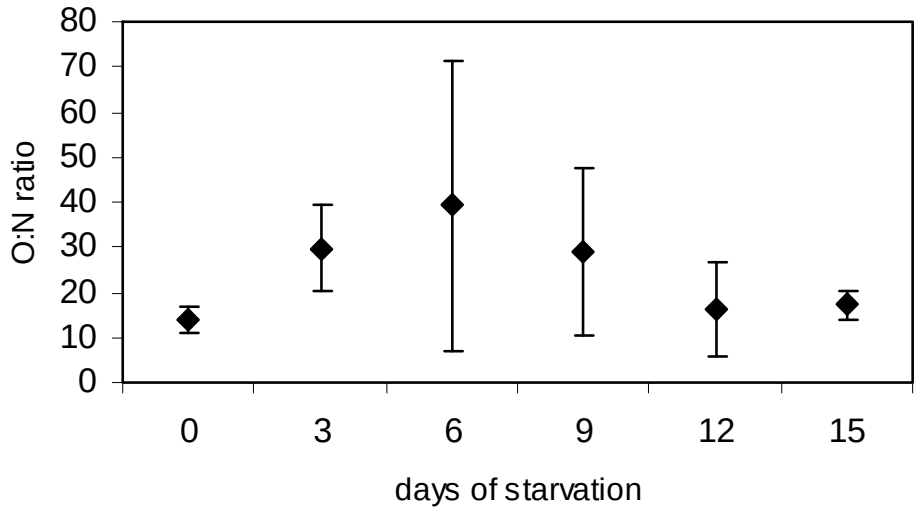

Figure 3. O:N ratio of food deprived juveniles of *Litopenaeus vannamei*.

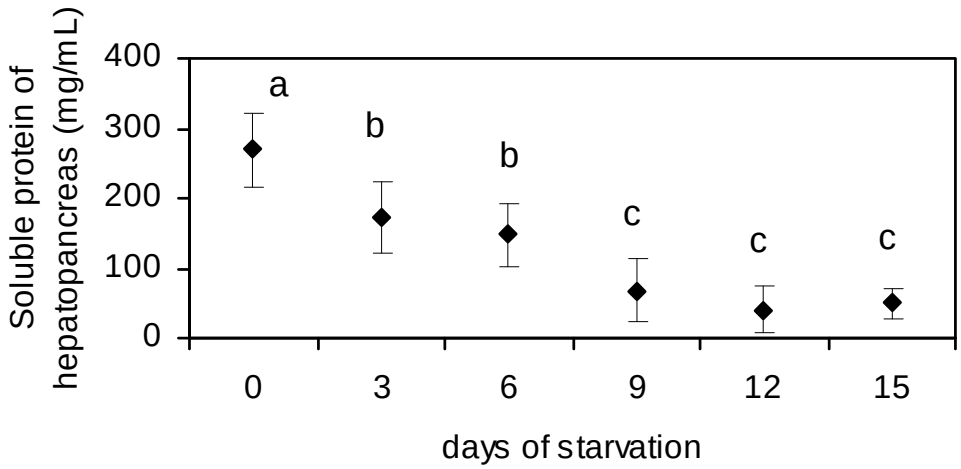

Figure 4. Hepatopancreatic total protein of the juveniles of *Litopenaeus vannamei* food deprived. Mean ± SD. Different letters indicate significant differences ($p<0.05$)

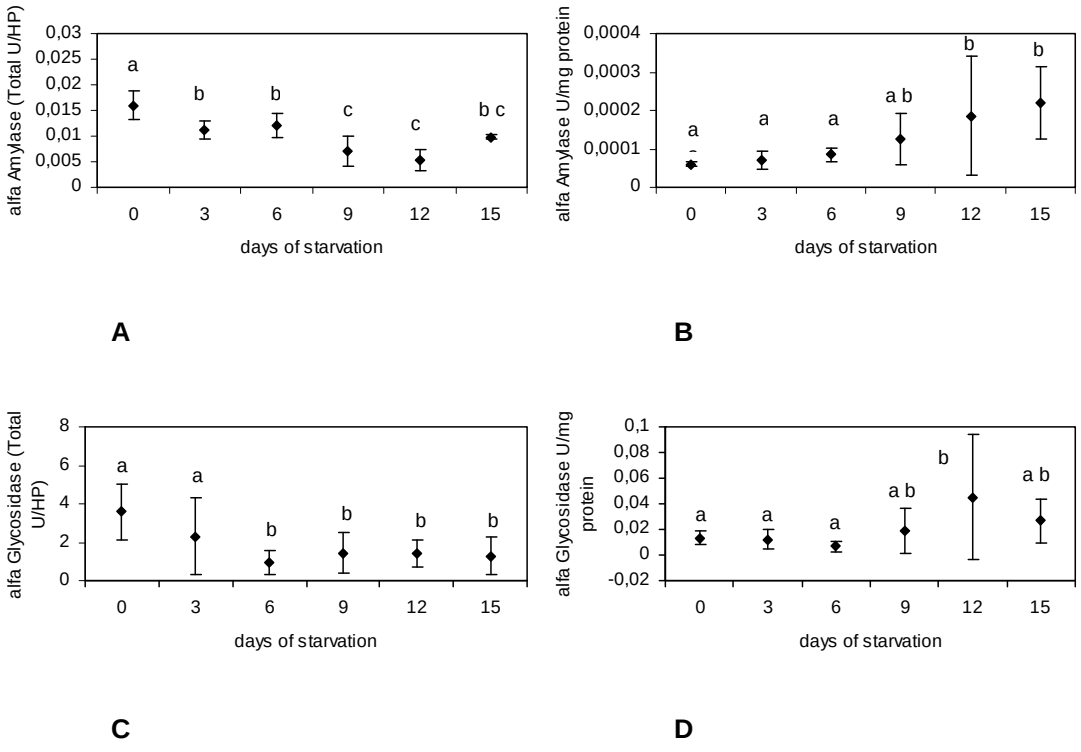

Figure 5. Total and specific acitivities of α-amylase (A and B) and α-glycosidase (C and D) of food deprived juveniles of *Litopenaeus vannamei*. Mean ± SD. Different letters indicate significant differences ($p<0.05$)

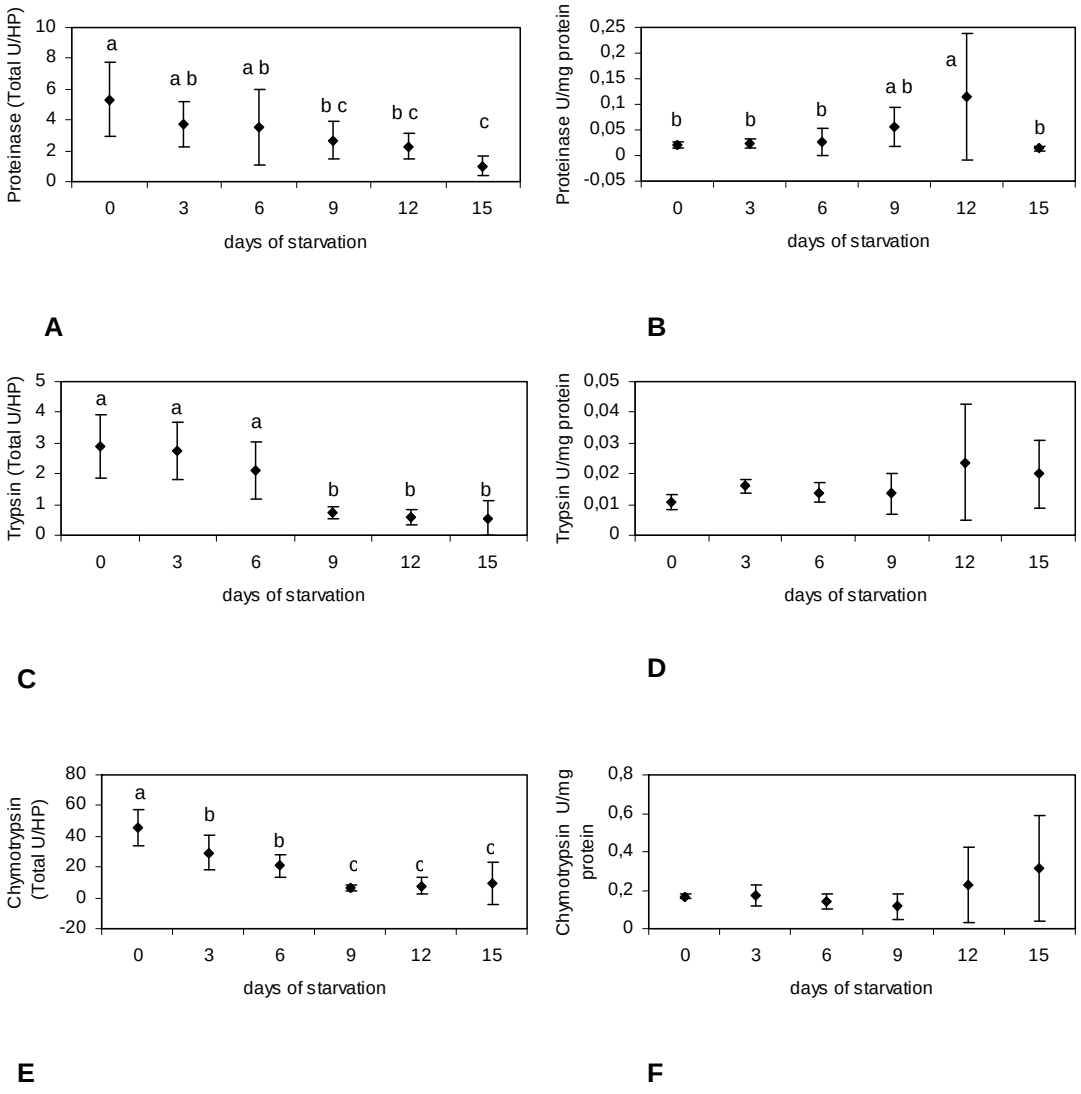

Figure 6. Total and specific digestive proteinases activities of juveniles of *Litopenaeus vannamei* after different starvation periods. Mean ± SD. Different letters indicate significant differences ($p<0.05$).

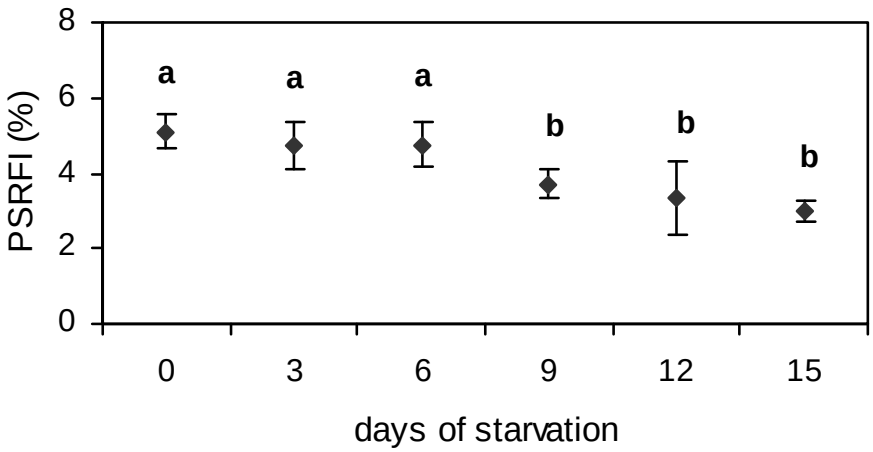

Figure 7. Ingestion rate of juveniles of *Litopenaeus vannamei* after the different starvation periods (PSRFI %). Mean ± SD. Different letters indicate significant differences ($p<0.05$).

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