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Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific white shrimp (*Litopenaeus vannamei*)

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Abstract

Crustaceans are forced to fast during molting. Several physiological, metabolic and behavioral changes have been associated with starvation. Although some of these changes have been well studied, knowledge of the dynamics of fuel reserves during the molting process is limited. To understand the effects of short-term hunger stress on energy reserves, intermolt shrimp *Litopenaeus vannamei* were starved up to five days. This period corresponds to the normal time that juvenile shrimp starve during molting, since they can not eat. Glucose, glycogen, total soluble protein, total lipids, sterols, and acylglycerides were measured in plasma and hepatopancreas. The same metabolic substrates were measured in organisms that were fed after 96 h of starvation. It is widely accepted that protein is the main energy reserve used by shrimp to deal with starvation. However, under short-term starvation a rapid decrease of plasma and hepatopancreas glucose and an important decrease in hepatopancreatic glycogen were detected. Additionally, acylglycerides content in hepatopancreas decreased significantly at later times, while protein in plasma and hepatopancreas remained fairly constant during the experiment. This study may help understand some aspects of the nutrition physiology of the Pacific white shrimp related to its biology.

Keywords: Energy storage; Hepatopancreas; Plasma; Shrimp; Starvation

1. Introduction

Besides seasonal changes in food availability, crustaceans undergo periods of starvation due to molting or ecdysis, a natural continuous process, in which the animals shed their exoskeleton to grow. This process requires high amount of energy. Molt might last days or weeks, with morphological, physiological, hormonal and behavioral alterations occurring almost daily (Dall et al., 1990). The shrimp molt cycle is divided into five major stages: stage A (postmolt), when the exoskeleton is soft and limp and the animal is incapable of intaking food. Feeding begins until the animals are well into stage B, in which the exoskeleton is sufficiently rigid to support the weight and handle food. During stage C (intermolt), the exoskeleton becomes very rigid and the animal feeds actively. Prior to molting, feeding declines during stages D (premolt) just before ecdysis (E), when the old cuticle is shed and as a consequence, the organisms is unable to feed.

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Mammals and birds adapt to prolonged fasting by mobilizing fat stores and minimizing protein loss (Cherel et al., 1992). Certain fishes, such as the Atlantic cod (*Gadus morhua*) respond to starvation by mobilizing hepatic lipids first, then muscle and hepatic glycogen and finally muscle protein (Guderley et al., 2003). Lipids are the main source of energy for the flight muscle in long term-flying insects (Ryan and Van der Horst, 2000), while other insect species can use carbohydrate reserves or proline (Gäde and Auerswald, 2002). In contrast, it has been proposed that the primary source of energy for crustaceans is neither carbohydrates nor lipids, but protein (New, 1976).

However, several studies of crustacean metabolism have shown high variability of energy reserves mobilization, which makes it difficult to assume a standard metabolic profile. Even more, besides the class of reserves mobilized, the sequence of substrates used by crustaceans for energy during starvation varies considerably (Clifford and Brick, 1983). The numerous possibilities must be the result of the vast diversity of environments crustaceans inhabit and their long evolutionary history. Therefore, as more information is available, correlations and differences will become evident (Sánchez-Paz et al., 2006).

Protein utilization in crustaceans has been widely studied for several reasons: 1) it is generally accepted that protein is the main energy source in crustaceans (Cuzon et al., 1980; Barclay et al., 1983). Food proteins, rather than lipids and carbohydrates, are responsible for the quality of spermatophores and sperm in *L. vannamei* (Ceballos-Vazquez et al., 2003), an important issue for good quality shrimp seed production. 2) The feeds used for shrimp production, for example, in semi-intensive farms, feed may account for 28% of the total costs (Treece, 2000). Since the marine protein source is one of the most expensive components of the feed, lowering its proportion in the feed without decreasing the growth rate of the cultured species may result in lower production costs (Molina-Poveda and Morales, 2004). 3) The amount of proteases compared to other enzymes, on the crustacean midgut gland. For example, in the shrimp *Penaeus japonicus* the most abundant digestive proteinase is trypsin, contributing with 6% of the total soluble protein, while in the fiddler crab (*Uca pugilator*), trypsin is approximately 33% of the total hepatopancreatic protein (Eisen and Jeffrey, 1969; Galgani et al., 1985). Galgani et al. (1985, 1984) estimated that trypsin is responsible for the digestion of ~60% of the protein in the feeds. However, information related to the utilization of other energy reserves is scarce and confusing. Moreover, information related to the dynamics of energy reserves utilization during ecdysis is limited. Since marine crustaceans have limited capacity to store lipids and carbohydrates (Dall and Smith, 1986), understanding the mechanisms of energy mobilizations is a priority. Also, since simple sugars are poorly assimilated by shrimp (Dall and Smith, 1986), the metabolic processes of energy usage during molting must be an important topic.

In this study, we examined the effect of short-term starvation on plasma and hepatopancreas metabolite concentrations (glucose, glycogen, total protein, total lipids, acylglycerides and sterols) in the Pacific white shrimp (*L. vannamei*), to gain insights on the connection between episodes of food shortage, metabolic preferences and sequence of use of energy reserves. This study may contribute to the understanding of some aspects of nutrition and ecophysiology of this commercially important shrimp.

2. Materials and methods

2.1. Animals and experimental conditions

In laboratory indoor tanks, 135 organisms (average weight 21 g) from CIBNOR aquaculture facilities at Unidad Hermosillo were randomly distributed in three 70 L indoor tanks. Specimens were acclimatized 5 days at 28 °C, 34 psu and fed ad libitum twice daily with Camaronina 35®. Uneaten food particles and excretes were removed regularly. After acclimation, 50 shrimp were permanently fed (control group), and the remaining 60 were starved for up to 120 h. To test the effect of re-feeding after starvation, a group of 25 shrimp were starved 96 h and then fed. Three specimens of each group were weighed (control, C; starved, S; and re-feed, RF) at intermolt and sampled at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 120 h. Specimens were selected according to molt stage as described by Chan et al. (1988). Total weight and gender were recorded.

2.2. Tissues sampling

One volume (200 μL) of hemolymph was extracted from the base of the fifth pereiopod of each organism, with a 1 mL syringe containing two volumes (400 μL) of precooled (4 °C) shrimp anticoagulant solution (450 mM NaCl, 10 mM KCl, 10 mM Na_2–EDTA, 10 mM HEPES, pH 7.3) (Vargas-Albores et al., 1993). The hemolymph was centrifuged at 800 ×*g* for 5 min, and plasma transferred into a new tube and stored at −20 °C for further analysis. Shrimp were decapitated at 2, 4, 8, 12, 18, 24, 48, 72, 96 and 120 h, and the hepatopancreas was carefully dissected, weighed and cut into three portions and each portion was weighed. One portion was frozen at

−20 °C, another portion was submerged in TRIzol LS (GIBCO BRL) for RNA isolation and homogenized, and the remaining portion was used for biochemical composition analysis.

2.3. Biochemical analysis

Glucose, total protein, total lipids, acylglycerides and sterols concentrations in plasma were measured using commercial kits for medical diagnosis (RANDOX) according to the manufacturer's protocols and read in a Synergy microplate reader (Bio-Tek Instruments). Hepatopancreas were homogenized in one volume (w/v) of buffer A (100 mM potassium phosphate buffer, pH 7.2, 1.0 mM EDTA) containing 10 μM PMSF (stock solution 1 mM in ethanol) and one volume of a chloroform–methanol–water (2:2:1) mix was added. Samples were vigorously shaked, and centrifuged at 15,000 ×*g*. The upper aqueous phase was used to measure concentrations of glucose, glycogen and total soluble protein. The lower chloroform-containing phase was collected and air-dried in the dark to avoid photo-oxidation of lipids. The lipid extract was emulsified in a corresponding volume of water, and used to measure total lipids, acylglycerides and sterols levels. Glycogen measurement was based on the method described by Passonneau and Lauderdale (1974), and determined as glucose after acid hydrolysis. Briefly, one volume of aqueous extract of each sample was hydrolyzed with 2 M HCl and then heated for 2 h at 95 °C, followed by neutralization with 2 M NaOH. The extracts were analyzed in triplicate determining glucosyl units by using a commercial kit for glucose medical diagnosis (RANDOX).

2.4. Statistical analysis

To determine the effect of fasting on total body weight, hepatosomatic index and metabolites concentrations in plasma and hepatopancreas, statistical analyses were performed by one-way ANOVA, since the data were homogeneous. For post-hoc analysis, the Tukey honest significant difference (HSD) test was used. Statistical significance was considered when $p<0.05$. Analyses were performed using SigmaPlot for Windows v. 9.01 (Systat Software, Inc).

3. Results

3.1. Body mass and hepatosomatic index

No significant effect of fasting on the total weight of the specimens during the period of the experiment was found. Hepatosomatic index (HIS) was used to determine the effect on hepatopancreas. It was calculated individually as the ratio between hepatopancreas and total body wet weight. Even when the HSI slightly decreased in control organisms during the period of the study, no significant effect was observed when compared to starved organisms ($p<0.1488$). However, when the effect of starvation on the hepatosomatic index of starved specimens was analyzed, a significant decrease ($p<0.05$) was found (~35% decrease). An abrupt increase (~140%) of the HSI was noted by re-feeding following a 96 h starvation period (Fig. 1), reaching the same index as control specimens.

3.2. Plasma biochemical analysis

3.2.1. Glucose

Mean plasma glucose concentrations for each group are shown in Fig. 2. Significant differences in plasmatic glucose were observed among treatments ($p<0.05$). The starved group had significantly lower glucose concentration than control specimens, showing a constant reduction of plasmatic glucose from the beginning of the study, remaining at low levels from 18 h of starvation until the end of the experiment. An abrupt increase in plasmatic glucose levels was observed immediately after organisms were re-fed, reaching values similar to those of the constantly fed group.

3.2.2. Total soluble protein

Total soluble protein content (Fig. 3A) in plasma from control specimens decreased from 137.53 mg/mL to 83.51 mg/mL, (approximately 40% less). After 48 h

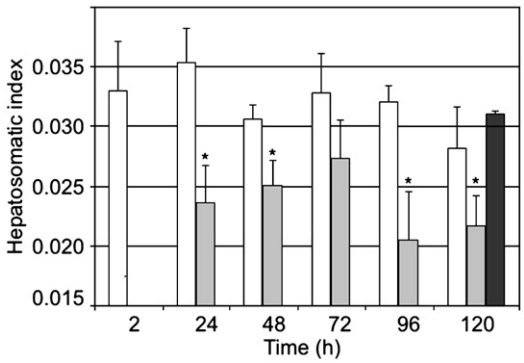

Fig. 1. Changes in hepatosomatic index during starvation in *Litopenaeus vannamei*. Significant differences were observed among groups ($p<0.05$). Values represent the mean ± SD of a triplicate analysis for $N=36$ animals. Empty bars: control group; gray filled bars: starved group; black filled bars: re-fed group. *$p<0.05$ versus fed (control) group.

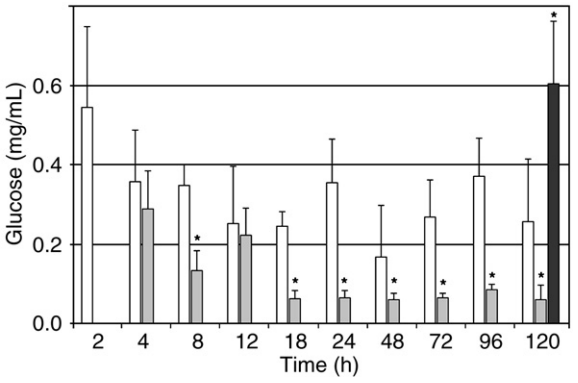

Fig. 2. Glucose in plasma after a short-term starvation period (up to 120 h) and re-feeding (96 h) for *Litopenaeus vannamei*. Significant differences were detected among fed, starved and re-feeding groups ($p<0.05$). Values are means ± SD of a triplicate analysis for $N=60$ animals. Empty bars: control group; filled bars: starved group; black filled bars: re-fed group. *$p<0.05$ versus fed (control) group.

of food deprivation, shrimp plasma protein concentration presented the lowest concentration (76.65 mg/mL, approximately 45% less), although no statistically significant differences were observed when compared to the constantly fed group ($p<0.05$). Protein concentration slightly increased (~27%) at 72 h in the control and starved groups. Re-fed shrimp did not reach the initial protein content values, but its concentration was similar to the control group at 120 h.

3.2.3. Total lipids, sterols and acylglycerides

During experimentally-induced starvation, total lipid content in plasma from control, starved and re-fed organisms showed minor fluctuations during the course of the study (Fig. 3B). No significant differences were detected among groups ($p<0.4610$). However, a major decline of lipids in starved organisms was observed after 120 h of food deprivation (43.8% loss), probably due to the strong utilization of lipids. Re-fed specimens reached similar levels as controls at the end of the experiment.

Sterols levels were not significantly affected (Fig. 3C) by starvation ($p<0.05$). A decrease (8%) at 48 h was detected in starved shrimp, recovering its original concentration 24 h later. No significant differences were observed between control, starved and re-fed groups ($p<0.4932$). The control group slightly increased its

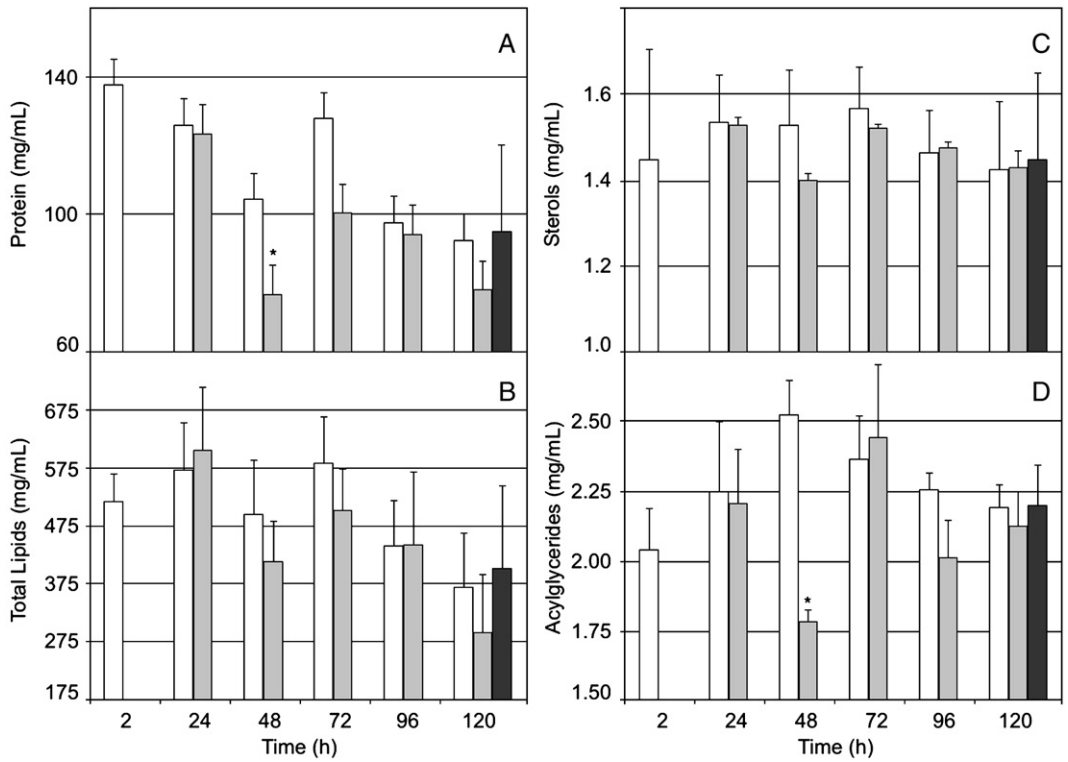

Fig. 3. Plasma protein, total lipids, sterols, and acylglycerides concentration during a short-term starvation period (up to 120 h) and re-feeding (96 h) for *Litopenaeus vannamei*. Values are means ± SD of a triplicate analysis for $N=36$ animals. Empty bars: control group; gray filled bars: starved group; black filled bars: re-fed group. *Denotes significant differences from fed controls.

sterols content from the beginning of the experiment until 72 h, returning to initial levels at 120 h.

Analysis of the effect of starvation on plasma acylglycerides (Fig. 3D) showed that their concentrations were similar in control, fasting, and re-fed organisms ($p<0.2320$), remaining fairly constant during the experiment. However, a sharp decreased (70%) at 48 h in starved shrimp was observed (~13%). At the end of the experiment acylglycerides levels between controls, starved and re-fed organisms showed no significant differences ($p>0.05$).

3.3. Hepatopancreas biochemical analysis

3.3.1. Glucose and glycogen

Glucose concentration in control shrimp showed some variations during the course of the experiment (Fig. 4A). In control specimens hepatopancreatic glucose content showed some variation. However, no significant changes were observed during the study in the control group ($p<0.5708$). Significant differences in hepatopancreatic glucose were observed among control and starved organisms ($p<0.05$). Glucose concentration was significantly reduced after 24 h of starvation (~93% less), reaching at 120 h of starvation 0.832 mg/g. After re-feeding hepatopancreatic glucose rose, returning to the levels of constantly fed organisms. These values were significantly different from those of starved shrimp.

Hepatopancreatic glycogen content in fed shrimp showed slight variations (Fig. 5). In contrast, hepatopancreatic glycogen content gradually decreased as starvation progressed ($p<0.05$). Glycogen content after 24 h dropped about 50%, reaching its lowest concentration at 96 h of starvation (~80% less). Interestingly, after re-feeding glycogen content increased abruptly and surpassed the levels of control specimens.

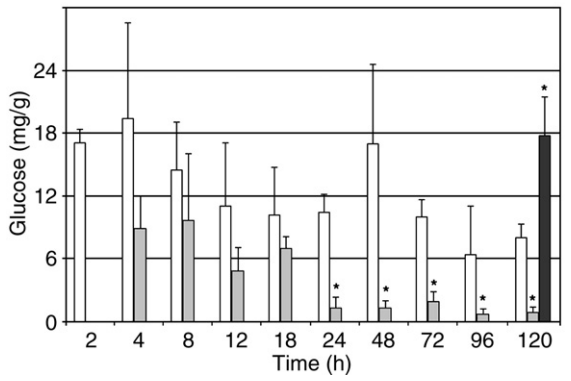

Fig. 4. Hepatopancreatic glucose during a short-term starvation period (up to 120 h) and re-feeding (96 h) for *Litopenaeus vannamei*. Significant differences were observed among fed, starved and re-feeding groups ($p<0.05$). Values are means±SD of a triplicate analysis for $N=60$ organisms. Empty bars: control group; gray filled bars: starved group; black filled bars: re-fed group. *Denotes significant differences from fed controls.

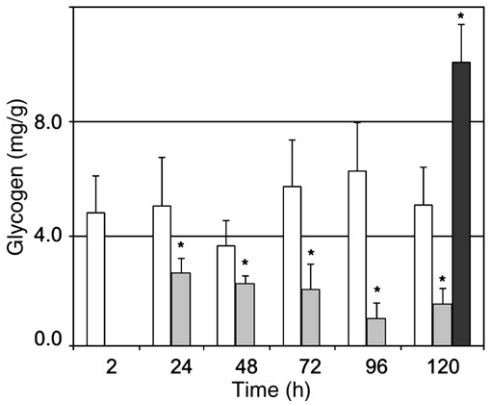

Fig. 5. Hepatopancreatic glycogen during a short-term starvation period (up to 120 h) and re-feeding (96 h) for *Litopenaeus vannamei*. Significant differences were observed among fed, starved and re-feeding groups ($p<0.05$). Values are means±SD of a triplicate analysis for $N=36$ animals. Empty bars: control group; gray filled bars: starved group; black filled bars: re-fed group. *Denotes significant differences from fed controls.

3.3.2. Total soluble protein

The concentration of total soluble protein in hepatopancreas (Fig. 6A) displayed slight fluctuations in control, starved and re-fed organisms, however no significant differences were observed between groups ($p<0.4713$), except in the last value of fed organisms. In general, soluble protein concentration remained relatively stable during the experiment.

3.3.3. Total lipids, sterols and acylglycerides

Total lipids in hepatopancreas (Fig. 6B) showed slight fluctuations in control shrimp. However, significant differences were observed between groups ($p<0.05$). In starved shrimp, hepatopancreatic lipids decreased noticeably, reaching its lowest concentration after 96 h of starvation (137.7 mg/g, ~84% loss). Re-fed organisms at the end of the experiment had slightly increased hepatopancreatic lipids, but did not recover from starvation to initial values.

Fluctuations in hepatopancreatic sterols (Fig. 6C) occurred in control and starved groups, but no significant changes were noticed ($p<0.2485$). A slight decrease (50% loss) was observed in starved specimens at 96 h. A marked reduction was detected at 24 h in control organisms (nearly 50%). After 96 h of starvation, re-fed organisms increased sterol levels in the hepatopancreas but did not recover to initial levels.

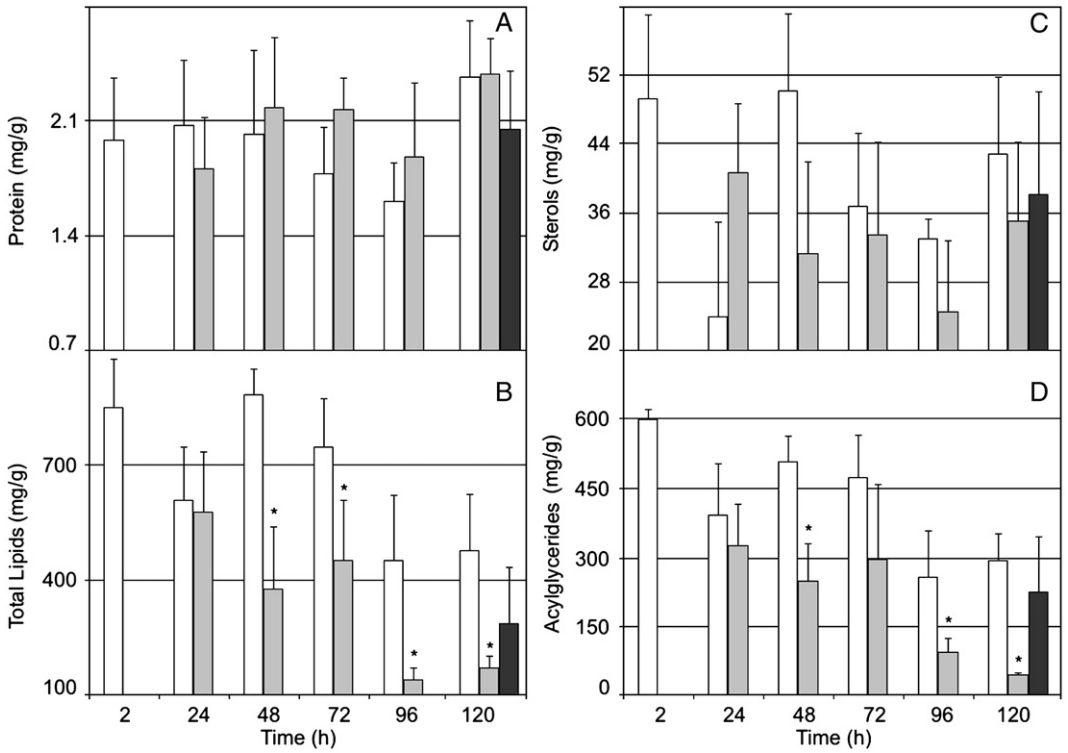

Fig. 6. Hepatopancreatic protein, total lipids, sterols, and acylglycerides concentration during a short-term starvation period (up to 120 h) and re-feeding (96 h) for *Litopenaeus vannamei*. Values are means±SD of a triplicate analysis for $N=36$ animals. Empty bars: control group; gray filled bars: starved group; black filled bars: re-fed group. *Refers to significant differences against fed controls.

Hepatopancreatic acylglycerides showed slight fluctuations in controls. Significant differences exist among groups ($p<0.05$). In starved shrimp, hepatopancreatic acylglycerides decreased noticeably (94% below original levels), reaching its lowest amount after 120 h of starvation (47.25 mg/g) (Fig. 6D). In re-fed organisms acyglycerides increased significantly (80% over 120 h starved organisms), however, final values did not return to the initial concentration.

4. Discussion

Organisms constantly subjected to a stressful environment must possess mechanisms to deal with factors that may have adverse fitness consequences. In cases where organisms must undergo temporary food-depriving conditions, strategies to prevent damage or death had been selectively favored, such as encystment, hibernation, or storage and utilization of energy reserves. New (1976), proposed that the main energy source for crustaceans is protein, contrasting with mammals and birds, which utilize carbohydrates and lipids for energy supply, in order to minimize protein loss (Cherel et al., 1992). We report that the Pacific white shrimp, under short-term starvation conditions, for periods similar to those experienced during molting stage, uses non-protein sources of energy, mainly, and immediately, glucose followed by acylglycerides.

4.1. Impact of starvation and re-feeding on hepatosomatic index

The crustacean hepatopancreas is a multilobate diverticulum of the midgut, which consists of a multitude of blind tubules lined with a single layer of epithelial cells (Ahearn et al., 1985). It is a vital organ involved in excretion, molting, lipid and carbohydrate metabolism, and diverse metabolic activities, including synthesis and secretion of digestive enzymes, absorption of nutrients, synthesis of plasma proteins such as hemocyanin and lipoproteins (oxygen and lipid transporters and components of the defense system) (Yepiz-Plascencia et al., 2000), and storage of energy reserves (Gibson and Barker, 1979). Thus, reduction of ~35% of the hepatosomatic index in starved organisms may be a consequence of the use and mobilization of energy reserves. In the shrimp *P. japonicus*, a ~50% decline in the hepatosomatic index was observed in specimens starved for

28 days (Cuzon et al., 1980). Similarly, in the shrimp *Metapenaeus ensis* (Cuzon et al., 1980), the hepatosomatic index after 4 days of starvation was less than 50% compared to the value in daily fed shrimp. Pascual et al. (2006) reported that juveniles of *L. vannamei* fed with different feeds (5 or 40% protein diet) during 21 days, and then fasted for 21 days, showed a decline on the hepatopancreas weight of about 50%. However, the immediate increase observed on hepatosomatic index after re-feeding was unexpected. After 96 h of starvation, the hepatosomatic index dramatically increased (140%) after re-feeding, reaching the same value at 120 h as the constantly fed shrimp.

4.2. Effect of starvation and re-feeding on plasmatic metabolites

Plasma metabolites underwent striking variations during starvation. Glucose is the major component of circulating carbohydrates in crustaceans, but its concentration varies markedly between species (Chang and O'Connor, 1983). From the entire set of metabolites studied, glucose was the most drastically affected by starvation, dropping constantly from the beginning of the study and stabilizing to ~11% of its initial value after 18 h of food deprivation. Interestingly, glucose content in plasma increased strikingly after re-feeding, slightly above the initial values. How fast glucose is absorbed and present in blood in humans, is a measure of the tolerance to glucose. In fact, glucose intolerance is the inability to rapidly deal with a glucose load. Humans are diagnosed as glucose intolerant by means of a glucose tolerance test (GTT), if after an oral or intravenous administration of a bolus of glucose, plasmatic glucose values do not return to baseline within 1–2 h (Moon, 2001). In this way, shrimp have been compared to carnivorous fishes due to its low capacity to cope with a glucose load, maintaining hyperglycemic levels (Cuzon et al., 2000). Our results showed that after re-feeding glucose is absorbed and peaks in hemolymph very fast, which is in agreement with the report by Santos and Keller (1993).

During starvation, protein concentration in plasma dropped 32% from initial values. However, protein mobilization was slow. A decrease of 26% occurred after 48 h starvation, although plasma protein hardly recovered without reaching initial levels. After re-feeding, protein in plasma reached the same levels as the control group. These results are important because, 1) it has been widely accepted that protein acts as the main fuel reserve used by shrimp to deal with food shortage (Cuzon et al., 1980; Barclay et al., 1983). Serum proteins have been proposed as the most likely source of catabolites during the initial stages of starvation (Clifford and Brick, 1983), and 2) recognition of the limited capacity of shrimp to store carbohydrates (Dall and Smith, 1986; Rosas et al., 2001); and reduced assimilation of simple sugars (Shiau, 1998). Therefore, it was not expected that shrimp would exploit carbohydrates, as an immediate source of energy during starvation. Our results indicate that glucose is the first source used by shrimp for dealing with lack of food. While glucose was rapidly consumed, protein concentration decreased slightly. Although it can not be ruled out that proteins are used as an energy source, the rapid decrease of glucose indicates that it is the first fuel utilized. This response may be an adaptative strategy to avoid usage of high cost energy macromolecules, at the beginning of a food shortage episode. Prudent utilization of protein in very short starvation periods could represent energy protection in case of prolonged food scarcity intervals.

The amount of acylglycerides and sterols in plasma remained fairly stable during the course of the experiment. However, a conspicuous decline occurred 48 h after starvation began, just after glucose levels dropped to its minimum. Low concentration of sterols and acylglycerides could reflect the limited ability of shrimp for de novo synthesis of polyunsaturated and highly unsaturated fatty acids (Chang and O'Connor, 1983; Mourente, 1996; D'Abramo, 1997; González-Felix et al., 2003a,b).

4.3. Effect of starvation and re-feeding on hepatopancreatic metabolites

The shrimp hepatopancreas is considered the main storage organ, mainly accumulating lipids (Adamczewska and Morris, 1994; Yepiz-Plascencia et al., 2000; Luvizotto-Santos et al., 2003), and to a lesser degree, glycogen (Verri et al., 2001). Glycogen in the hepatopancreas is a glucose supply, and an important precursor for chitin synthesis (Cuzon et al., 2000), consequently, it is closely related with the molt cycle. Therefore, factors affecting hepatopancreas homeostasis could result in changes of concentration of its macromolecules constituents. Our results indicate that glucose is the primary source of energy used by the hepatopancreas during food scarcity of juvenile *L. vannamei*. Moreover, glucose concentration in re-fed organisms rapidly returned to the level found in the control group. Similar results were observed by Hervant et al. (1999) for the surface-dwelling amphipod *Gammarus fossarum* subjected to a 28-day starvation period.

Glycogen is the most widely distributed animal polysaccharide (Awapara and Simpson, 1967), and a readily energy source. In our study, a decline of nearly 80% of hepatopancreatic glycogen occurred after 120 h of

food deprivation. In the estuarine grapsid crab *Chasmagnathus granulata* maintained on a high protein (HP) feed (21.59%) for two weeks, and then to 21 days of food deprivation, hepatopancreatic glycogen diminished 51%, while in crabs maintained during the same period on a high carbohydrate (HC) feed (34.56%), hepatopancreatic glycogen was reduced to approximately 64%. Hemolymph glucose levels in both treatments (HC and HP) were reduced after 3 days starvation (76 and 39%, respectively), yet, after 6 days hemolymph glucose concentration tended to increase and stabilize, and the effect was lower in HC treated organisms than in fed specimens. In HP fed organisms, glucose concentration did not differ from non-fasted crabs (Oliveira et al., 2004). In our study, once glucose or glycogen concentration dropped to a minimum level, there was no recovery until the shrimp were re-fed. Moreover, a fast decrease on plasmatic glucose concentration on *L. vannamei* and a constant, but slow decrease on hepatopancreatic glycogen may be a response of the enzymatic machinery for cautious utilization of glycogen hepatopancreatic reserves to preserve the valuable reserves in case fasting is prolonged. In *Marsupenaeus japonicus* starved for 28 days, the hepatosomatic index decreased (3.3 to 1.8) during the first week, indicating degradation of glycogen, but HSI remained constant over the next three weeks. In crabs, hepatopancreas glycogen appears as the major source of energy during the early stage of starvation (Cuzon et al., 2000).

The effect of re-feeding on glycogen reserves is notable, because of the recognized limited capacity of shrimp to store carbohydrates (Dall and Smith, 1986; Rosas et al., 2001). Similar results were observed by Hervant and Renault (2002) in the hypogean aquatic isopod *Stenasellus virei* and in surface-dwelling isopod *Asellus aquaticus* after 180 days of starvation. In both animals, glycogen increased significantly within the first week of re-feeding. *S. virei* presented an abrupt increase (reaching 121% of the level of fed specimens), before returning to the pre-fasting level, while *A. aquaticus* had a smaller increase in glycogen levels (60% of the initial content). This was proposed as an adaptation response that results in storing of food energy that can be mobilized later for the synthesis of body materials, such as triglycerides and proteins (Hervant and Renault, 2002). The ability to maintain and rapidly restore high levels of metabolic energy stores for use during periods of food deficiency allows the animal to fuel successfully in unpredictable fasting periods and therefore, increase their competitive abilities.

In fed and starved shrimp, total soluble protein in hemolymph and hepatopancreas remained constant during the experiment. These results disagree with many reports that maintain that protein is the main energy source for most crustaceans but may explain previous findings. Muhlia-Almazán and García-Carreño (2002) showed that in *L. vannamei*, hepatopancreatic trypsin activity was significantly affected by food shortage (differences of 35% between 2 and 120 h of starvation), while chymotrypsin activity declined 40% on the same starvation interval. A similar effect was reported in trypsin activity in *M. japonicus* (Cuzon et al., 1980). Also, Sánchez-Paz et al. (2003) showed that hepatopancreatic trypsin mRNA levels in juvenile *L. vannamei* dropped sharply after 24 h of starvation and it was proposed that such reduction of transcript concentration might be a strategy to reduce enzyme synthesis, which would, in turn, reduce the energy required for protein synthesis, and thereby prevent autolysis. We propose now, that a decrease on trypsin activity and the abundance of transcripts, could be a strategy to avoid protein utilization as the main energy source in the beginning of a food-deprivation period. This type of down-regulation in enzyme synthesis levels would be very important to carefully save an expensive energy supply.

Total lipids decreased sensibly (84%), reaching the lowest values after 120 h of starvation. Both, sterols and acylglycerides may be the main cause of this effect, since their concentration in the hepatopancreas dropped sharply, especially acylglycerides. It has been proposed that in crustaceans, neutral lipids (mainly triglycerides) are preferentially catabolized during starvation, while polar lipids (phospholipids and cholesterol) are conserved due to their role as structural components of cell membranes (Heath and Barnes, 1970; Bourdier and Amblard, 1989; Stuck et al., 1996). A large reduction in total lipids (particularly a total depletion of tryacylglycerides stores) as a response to starvation for the lake-dwelling copepod *Acanthodiaptomus denticorni* was reported (Bourdier and Amblard, 1989). Similar results were found for larvae, adult and sub-adult lobsters (Stuck et al., 1996). Ritar et al. (2003) reported that lipid dry weight in lobster larval stages II, IV and VI, declined during starvation to 81, 41, and 73%, respectively, compared to fed larvae. Additionally, polar lipids were the only lipid class significantly reduced during starvation (45, 38, and 70%) for stages II, IV and VI, respectively. The next most abundant lipid class in phyllosoma was sterol, and was the only lipid class conserved during starvation at all stages.

An improved knowledge of the physiology of nutrition in shrimp is important for aquaculture strategies. The type of energy reserves used by starved shrimp depends on the dietary protein level previously

used for shrimp food (Pascual et al., 2006). It was also proposed that protein levels modulate the use of energy reserves in shrimp because proteins are the main nutritional store (Rosas et al., 2002). An appropriate nutrient level is, obviously, an important requirement for all living organisms. However, nutrient quality should be also of paramount importance also. Protein content in aquafeeds needs to be evaluated in terms of chemical composition, biological value, digestibility (Molina-Poveda and Morales, 2004), and amino acid composition. We propose that future studies on the effect of starvation on energy stores usage, should consider a detailed analysis of the food quality provided days before the experimental procedure is initiated. This is particularly true after reviewing the results obtained by Pascual et al. (2006) that concluded that shrimp are well adapted to tolerate food deprivation depending on its previous nutritional condition. In this context, Gaxiola et al. (2005) mentioned that, after reviewing information related to shrimp nutrition, it appears that carbohydrates could be one of the more interesting nutrients in shrimp diet. Increasing carbohydrates levels in shrimp feed could alleviate the natural effects of starvation on the organism, improve farm productivity and reduce deleterious impact of nitrogen pollution generated by rich-protein feeds currently used on shrimp farming.

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