Influence of dietary carbohydrate on the metabolism of juvenile *Litopenaeus stylirostris*

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**Abstract**

The effect of dietary carbohydrates (CBH) on glucose and glycogen, digestive enzymes, ammonia excretion and osmotic pressure and osmotic capacity of *Litopenaeus stylirostris* juveniles was studied. The increase of CBH, ranging between 1 and 33%, stimulates activities of \(\alpha\)-amylase and \(\alpha\)-glucosidase in the hepatopancreas. High levels of glucose in hemolymph and of glycogen in the hepatopancreas were reached at the highest level of dietary CBH; however, the kinetics of accumulation is different. Shrimps fed with low level of CBH needed 3 h to reached glucose peak, whereas only 1 h is necessary for high CBH levels. A saturation curve was observed in glycogen level and \(\alpha\)-amylase activity with maximum values in shrimp-fed diets containing 21% CBH. This level could be used to be included as a maximum shrimp dietary CBH level. Pre-prandial glycogen levels were observed in shrimp fed a diet containing 1% CBH, indicating an important gluconeogenesis, which affected the protein metabolism. The present results show that a diet containing 10% CBH may not be enough to cover the CBH requirement, which could be satisfied by dietary protein content. The low osmotic capacity observed in shrimp fed on a diet containing 10% CBH coincided with a relatively low post-prandial nitrogen excretion which reflects a low concentration of amino acids circulating in hemolymph, which affected the osmotic pressure and the osmotic capacity. These results reflect the high plasticity of shrimp species to use protein to obtain metabolic energy from food and its limited capacity for processing dietary CBH.

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**Keywords**: Carbohydrate metabolism; Penaeid shrimp; Enzymatic activity; Ammonia excretion; Osmotic capability; *Litopenaeus stylirostris* juveniles

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1. Introduction

A study on carbohydrates and the relation between this group of nutrients and protein is justified by a growing consideration of minimizing the negative impact of feeds on the environment at the pond level. If carbohydrates can represent up to 50% of the diet (in weight), and a part of it is loss in the water enhancing the pollution, a reduction of dietary carbohydrates is possible. Therefore, knowledge of the capacity of shrimp to utilize carbohydrates is important. Previous work (Andrews et al., 1972; Sick and Andrews, 1973; Deshimaru and Yone, 1978; Abdel-Rahman et al., 1979; Alava and Pascual, 1987) investigated carbohydrate utilization relative to the sparing of dietary protein which can be achieved and consequently lead to a decrease in the amount of nitrogen waste.

The blue shrimp *Litopenaeus stylirostris* is cultured in the South Pacific area and has a good potential to become an important shrimp species in the Americas (Cuzon and Aquacop, 1998). Numerous research projects have studied the nutrition of juveniles of *L. stylirostris*, mainly addressing protein requirements (Fenucci et al., 1982; De La Lanza et al., 1986; Kanazawa, 1993). Aquacop and Cuzon (1989) achieved average growth until they used multi-ingredient diets that satisfied the protein requirements through a native source of protein (from squid). The conclusions from many trials can be summarised as follows: (1) good quality protein is of paramount importance (for example, a shrimp meal product from one place can be definitely superior to a product from an other place) and (2) in practical diets having the same protein level, (30%) squid and shrimp meal gave better growth results than soybean concentrate or fish meal. However, results from a variety of culture systems indicate that, on average, about 25% of nitrogen added as feed or other nutrient input is recovered by the target organism, the remainder affecting the water quality of the waste water (Hargreaves, 1998).

In recent years it has been demonstrated that the use of improper shrimp farming methods has damaged natural estuaries and bay systems (Lawrence, 1996). This condition has primarily been caused by both the intensification (increased production levels per ha of pond water) of shrimp farming and the increase of ponds and area of pond water in shrimp farm production. The shrimp farming industry is being constrained by the need to maintain adequate water quality and acceptable levels of potential pollutants in effluent discharges (Lawrence et al., 1998). Some researches have proposed different strategies to minimize the pollutants in the effluent discharge for the benefit of the estuarine and coastal ecosystems. Diets with low protein content (Molina-Poveda, 1998), the optimization of amino acid profile (Velasco et al., 1999), an optimum protein/energy (P/E) ratio of the food (Pedrazzoli et al., 1998), improvement of the fish meal quality (Ricque-Marie et al., 1998) an optimum feed management strategy (Cortés-Jacinto and Portillo, 1998; Velasco et al., 1999) and a deeper knowledge of nutritional physiology and biochemistry (Ceccaldi, 1998) have been proposed.

Knowledge of the optimum levels of the other components of the diet (lipids and carbohydrates) and their relation to the P/E ratio can be useful in reducing the production cost of the food and its effect as a pollutant. Although dietary carbohydrates are the most economical source of energy in food, little information about the utilization
of carbohydrate by shrimp is available (Pascual et al., 1983; Alava and Pascual, 1987; Shiau and Peng, 1992; Shiau, 1998). Some researchers have demonstrated that the type and level of carbohydrates affect growth rate of *M. japonicus* (Deshimaru and Yone, 1978; Abdel-Rahman et al., 1979), *F. aztecus* (Andrews et al., 1972) and *Litopenaeus vannamei* (Cousin, 1995). In *P. monodon* juveniles survival is affected by different carbohydrate levels and sucrose and glucose were better than trehalose in promoting growth of *P. monodon* (Pascual et al., 1983; Alava and Pascual, 1987).

The mechanisms responsible for the limited utilization of glucose by some species of penaeid shrimp are not yet fully understood. A negative physiological effect caused by glucose saturation due to a higher rate of absorption across the digestive tract is a possible explanation (Shiau, 1998). For this reason, many researchers have suggested the use of more complex carbohydrates such as starch which undergoes enzymatic hydrolysis before assimilation, in shrimp diets. Glucose from starch appears at gut absorption sites at a rate slower than free glucose (Pascual et al., 1983; Alava and Pascual, 1987; Shiau and Peng, 1992; Shiau, 1998). Although the activity of true α-amylases (EC 3.2.1.1, α-1,4-glucan 4-glucanhydrolases) from 40 crustacean species, including seven penaeid species, was recently reported (van Wormhoudt et al., 1995), no information about the carbohydrate-digesting enzymes of shrimp relative to different dietary carbohydrate levels is available.

Protein metabolism has been recognized as a key to the understanding of energy requirements of shrimp because growth depends strictly on protein (Deshimaru and Shigeno, 1972; Deshimaru and Yone, 1978; Lemos and Rodriguez, 1998). The high protein requirement and the limited capacity of shrimp to store reserve substances like lipids and carbohydrates (Dall and Smith, 1986) could be related to the capacity of shrimp to use proteins as a source of energy as well as for growth. In a recent study the maximum growth rate of *L. vannamei* juveniles was obtained when diets containing 50% protein and 1% carbohydrates were fed (Rosas et al., unpublished). The results of other studies showed that shrimp can change the metabolic substrates (measured as oxygen:nitrogen ratio) according to the physiological or nutritional requirements, passing from protein–lipid–carbohydrate metabolism to protein metabolism, indicating that shrimp are well adapted to use proteins as a source of energy (Rosas et al., 1995a,b, 1999; Taboada et al., 1998). The limited use of dietary carbohydrates by shrimp, therefore, may be the consequence of metabolic adaptation to use the proteins as a primary source of energy, because protein is the higher reserve substrate in shrimp, which can be converted to carbohydrates following the gluconeogenic pathway (Campbell, 1991).

Ammonia accounts for 60–100% of the products of nitrogen metabolism and its formation may result directly from either deamination or transamination, both involved in amino acidic gluconeogenesis (Claybrook, 1983). The ammonia excretion has been used as a good indicator of the utilization of dietary protein for energy by shrimp (Dall and Smith, 1986; Lei et al., 1989; Taboada et al., 1998). The role of dietary proteins as a source of energy during post-absorptive processes that follow the ingestion of food has been evaluated through the measurement of post-prandial nitrogen excretion (PPNE) (Rosas et al., 1995a,b). According to Claybrook (1983), both routine ammonia excretion
and PPNE indicate the use of dietary protein as a source of energy and can be used as an indirect measure of gluconeogenesis.

Recently several authors showed that the osmotic capacity (Lignot et al., 1999) and metabolites, like hemolymph glucose (Racotta and Palacios, 1998) and digestive gland glycogen (Rosas et al., 1995b), are directly related to protein and carbohydrate metabolism. Coupled with ammonia excretion, these indices can be useful in developing an understanding of carbohydrate metabolism, relative to physiological adaptation. In this study the effects of dietary carbohydrate level on adaptation of digestive enzymes, as related to digestion, glucose hemolymph level, hepatopancreatic glycogen, ammonia excretion and osmotic pressure of *Litopenaeus stylirostris* juveniles are evaluated.

2. Material and methods

2.1. Animals and zootechnical measurements

Live animals (9.45±0.15 g wet weight) were obtained from the ‘Centre Océanologique du Pacifique’ (Ifremer) located in Tahiti. One hundred shrimp were reared for 15–18 days at a density of eight shrimp per 100-l tank, in a flow-through sea water system (35 ppt). The light–dark photoperiod was 12 h/12 h and water temperature was 28±1°C. The shrimp were fed ad libitum 3 times a day (08, 12 and 20 h). Uneaten food particles were removed regularly. Four tanks were randomly assigned to each carbohydrate level.

2.2. Diets

The juveniles were fed formulated semi-purified diets, prepared with different levels of carbohydrates: 1, 10, 21 and 33%. The ingredient composition of the experimental diets are presented in Table 1. The experimental diets were prepared by thoroughly mixing the dry ingredients with oil and then adding water until a stiff dough resulted. The dough was then passed through a mincer with a die, and the resulting spaghetti-like strings were air dried at 60°C. After drying, the diets were broken up, sieved to a convenient pellet size, and stored at −4°C.

2.3. Biochemical analysis

The specific enzymatic activities of α-amylase, α-glucosidase and total proteases of the midgut gland were evaluated after 15–18 days of acclimation to the different diets. Five digestive glands per dietary treatment were collected. These were individually homogenized (Ultraturrax) for 30 s with distilled water. The homogenates were centrifuged at 20 000 × g for 30 min at 4°C and the supernatants collected. Protein concentration was determined by the method of Bradford (1976) using reagents from Biorad (Hercules, CA) and bovine serum albumin (BSA) as the standard. α-Glucosidase
Table 1
Ingredient composition of experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Carbohydrate level (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>5</td>
</tr>
<tr>
<td>Fish meal</td>
<td>30</td>
</tr>
<tr>
<td>SPFC 90%</td>
<td>10</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>38.5</td>
</tr>
<tr>
<td>Gluten</td>
<td>5</td>
</tr>
<tr>
<td>Krill paste</td>
<td>2</td>
</tr>
<tr>
<td>Alginate Na</td>
<td>2.5</td>
</tr>
<tr>
<td>Fish oil</td>
<td>3</td>
</tr>
<tr>
<td>Lecitin</td>
<td>2</td>
</tr>
<tr>
<td>Rovimix¹</td>
<td>1</td>
</tr>
<tr>
<td>Minerals²</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>%CP</td>
<td>48</td>
</tr>
<tr>
<td>%CBH</td>
<td>1</td>
</tr>
<tr>
<td>% lipid</td>
<td>7</td>
</tr>
<tr>
<td>MJ/kg</td>
<td>13</td>
</tr>
</tbody>
</table>

¹ Soluble fish protein concentrate, 90% protein (Sopropeche, Boulogne s/mer, France).
² Retinyl palmitate (vitamin A), 8 000 000 IU; cholecalciferol (vitamin D₃), 196 000 IU; α-tocopherol acetate (vitamin E), 10 000 mg/kg; vitamin K₃, 800 mg/kg; ascorbyl phosphate (vitamin C), 15 000 mg/kg; thiamine (vitamin B₁), 700 mg/kg; riboflavin (vitamin B₂), 2000 mg/kg; pyridoxin (vitamin B₆), 1000 mg/kg; niacin (vitamin PP), 10 000 mg/kg; calcium pantothenate, 5000 mg/kg; cyanocobalamin (vitamin B₁₂), 50 mg/kg; folic acid, 250 mg/kg; biotin, 30 mg/kg; inositol, 30 000 mg/kg (Hofmann La Roche, Bâle, Switzerland).
³ Disodium phosphate and monopotassium phosphate in equal amounts.

(EC 3.2.1.20) was assayed spectrophotometrically using p-nitrophenyl-α-β-glucopyranoside (Sigma, St. Louis, MO) as the substrate. The standard reaction mixture (1 mM substrate in 50 mM sodium phosphate buffer, pH 6, containing an aliquot of enzyme solution) was incubated at 37°C for 30 min. The reaction was stopped with Na₂CO₃ (1 M) (Thirunavukkarasu and Pries, 1983) and the absorbance was read at 410 nm. One unit (U) of enzyme activity is defined as the amount of enzyme capable of hydrolysing 1 μmol of substrate per min (ε = 18 000 l mol⁻¹ cm⁻¹). Amylase activity was measured according to a modified Bernfield’s method (1955) using 1% glycogen as substrate diluted in a 2.5 mM MnCl₂, 10 mM NaCl, 10 mM, pH 7, phosphate buffer. Enzymatic activity was expressed as mg of maltose liberated during 10 min at 37°C.

Proteolytic activity was measured using 1% azo-casein as substrate diluted in a sodium phosphate 10 mM, pH 7, buffer at 37°C for 30 min. Total proteases is defined by mg hydrolysed azo-casein min⁻¹ mg⁻¹ of protein.

Glycogen content of the midgut gland was determined from a sample of five shrimp from each treatment, at time 0 (after 12 h fasting) and 1, 2, 3, 6 and 7 h after feeding. Glycogen was extracted following the method reported by Dubois et al. (1956). Each midgut gland was first homogenized in trichloroacetic acid (TCA, 5%) for 2 min at 16 000 rpm, and centrifuged (3000 rpm for 5 min). This procedure was performed two
times. One ml of TCA was pipetted into tubes and mixed with 5 vol of 95% ethanol. The tubes were then placed in an oven at 37–40°C for 3 h. After precipitation occurred, the tubes were centrifuged at 3000 rpm for 15 min. The precipitate containing the glycogen was dissolved by addition of 0.5 ml of boiling water and 5 ml of concentrated sulfuric acid and phenol (5%). The contents of the tubes were transferred to a colorimeter tube and read at 490 nm in a microplate spectrophotometer.

The level of glucose in the hemolymph of the same shrimp used for the glycogen determination was measured. Before the midgut gland was excised, 100–300 µl of hemolymph were extracted with a syringe inserted at the base of the fifth pereiopod after the shrimp had been blot dried. A sub-sample was obtained with another syringe containing a 12.5% solution of sodium citrate, which was used to prevent clotting (Rosas et al., 1995b) The glucose concentration in the hemolymph was measured with a commercial kit for medical diagnosis (Merckotest 3306). Glucose content of the extract of the midgut gland was measured with a commercial kit (Biotrol Diagnostic), using as reactive a phosphate 100 mM buffer at pH 7.3 which contained phenol 16 mM, 4-aminoantipyrine 0.25 mM, glucose oxidase at 20 000 U/l and peroxidase 1000 U/l. The absorbance was measured at 500 nm.

2.4. Physiological analysis

Osmotic pressure of the hemolymph of the same shrimp used for the glycogen and glucose determinations, was measured with a Wescor 5500 vapor pressure osmometer that required 8 µl of sample per titration.

Osmotic capability (OC) of shrimp was measured for 15 intermolt shrimp selected from each treatment and exposed to a rapid change in salinity from 35 to 5 ppt. These shrimp were held in 75-l tanks containing water at a salinity of 35 ppt for 12 h prior to the experiment. During this time the shrimp were fed with the experimental diets. Then these shrimp were transferred to a similar tank containing water at 5 ppt and held there for 2 h. Fifty shrimp from each treatment were maintained in 35 ppt and served as a control group. After a 2-h period, hemolymph samples were collected similar to the procedure followed to collect samples for pre- and post-prandial glucose and osmotic pressure of the hemolymphs. OC is defined as the difference between the osmotic pressure of hemolymph and of the external medium, at a given salinity. The osmotic pressure of the water was measured in a Wescor 5500 vapor pressure osmometer requiring 8 µl of sample per titration.

Ammonia excretion was measured using the indophenol blue technique. Fifteen shrimp from each dietary treatment were starved for 12 h. Groups of five shrimp each were placed in 30-l chambers which were connected to a water recirculating system (Charmantier et al., 1994). To prevent handling stress, the shrimp were conditioned in the chambers for 12 h before any reading was recorded. Ammonia excretion was calculated as the product of the difference in the water ammonia concentration at the input and output of each chamber, timing the water flux (1 l min⁻¹). The readings were corrected relative to a control chamber that contained no shrimp. The first reading was considered to be the rate of ammonia excretion under fasting conditions. Thereafter, 2 g of food per five animals were added to each chamber, and the amount of of ammonia
excretion after 1, 2, 3, 4, 6 and 7 h was determined. The shrimp were then sacrificed, dried (60°C) and weighed until a constant weight was attained.

The ingestion rate was measured in the chambers used to determine ammonia excretion. At the end of the experiments, all remaining food was recovered, carefully separated and dried (60°C) until a constant weight was attained. At the same time, a control experiment was conducted to evaluate loss of weight due to the natural leaching of the food in the chambers. Two grams of dry food were placed in a similar chamber without animals. After 7 h, the remaining food was recovered, dried, and weighed. The amount of ingested food was determined as the original amount minus the remaining amount plus the amount lost due the leaching. The ingested food was expressed as J day⁻¹ g wet weight⁻¹.

2.5. Statistical analysis

The effect of different dietary levels of carbohydrate on the digestive enzymes, glucose content of midgut gland extract, glucose hemolymph level, digestive gland glycogen, ammonia excretion rates, osmotic pressure, ingestion rate and osmotic capability was analyzed separately using ANOVA. Homogeneity of variances was verified with the Cochran’s test. Means for each treatment were compared using Duncan’s multiple range test, only after ANOVA indicated significant differences among all the dietary treatments (Zar, 1974)

3. Results

3.1. Ingestion rate and survival

A significant reduction in ingestion rate was recorded as dietary CBH increased with comparatively high values for animals fed diets containing 1% CBH (1.4 J/day per g wet weight (ww)) and low values in shrimp fed diets containing 21 and 33% CBH (0.5 and 0.45 J/day per g ww, respectively) (P < 0.05) (Fig. 1). Survival ranged from 96 to 100% and was unaffected by CBH level (P > 0.05).

3.2. Biochemical analysis

The specific activities of the α-amylase and α-glucosidase were significantly affected by the level of dietary carbohydrates (P < 0.05) (Table 2). For both carbohydrases, as the level carbohydrate increased, the specific activities increased. A saturation curve of α-amylase activity was observed, with the maximum activity in shrimp fed diets containing 21 and 33% CBH (P > 0.05). The maximum values of α-glucosidase were obtained at dietary levels of 21 and 33% (Table 2). Total proteases did not significantly differ among diets (P > 0.05). Glucose content in the midgut gland extract for the 1% dietary carbohydrate treatment was significantly lower than that of each of the other three treatments (P < 0.05) (Table 2)

No significant differences in pre-prandial glucose level were observed among the
shrimp fed diets with the different levels of CBH, with values ranging from 13.7 mg/100 ml (10% CBH) to 21.9 mg/100 ml (21% CBH). The pre-prandial level of glycogen in the digestive gland ranged between 4.3 and 6.9 mg/g of tissue, with low values in animals fed the diet containing 1% CBH and higher values in animals fed diets containing 21 and 33% CBH (Table 2) (P < 0.05). Increases in mean post-prandial glucose and glycogen concentration (Table 3) were observed in response to different dietary CBH levels. In all cases, glucose and glycogen levels notably increased at 1–3 h after feeding, and returned to the pre-feeding rate 6–7 h later (Fig. 2). The maximum post-prandial glucose level was found in shrimp fed the diet containing 10% CBH (73

Table 2

<table>
<thead>
<tr>
<th>Diet (%)</th>
<th>Low carbohydrate</th>
<th>High carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP^d</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL^c</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>CBH^d</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>P/E ratio (mg/kJ)</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Total proteases</td>
<td>2.07±0.29*</td>
<td>1.99±0.22*</td>
</tr>
<tr>
<td>Glucose (mg ml^-1) homogenized in hepatopancreas</td>
<td>52±13*</td>
<td>110±17**</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>0.44±0.09*</td>
<td>0.70±0.06*,**</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>264.3±7.46*</td>
<td>256.35±16.92*</td>
</tr>
<tr>
<td>Total protein</td>
<td>23.7±5.33*</td>
<td>41.03±4.96**</td>
</tr>
</tbody>
</table>

^ Values mean±S.E.
^ Protein concentration (%).
^ Lipid concentration (%).
^ Carbohydrate concentration (%).

Entries with differing numbers of asterisks are significantly different (P < 0.05).
Table 3
Effect of carbohydrate levels (%) on growth rate (mg/day), survival, glucose in hemolymph, and glycogen in digestive gland of *Litopenaeus stylirostris*.

<table>
<thead>
<tr>
<th>Diet (%)</th>
<th>Low carbohydrate</th>
<th>High carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>CP (b)</td>
<td>48</td>
<td>43</td>
</tr>
<tr>
<td>CL (c)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>CBH (d)</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>P/E ratio (mg/kJ)</td>
<td>40</td>
<td>33</td>
</tr>
</tbody>
</table>

**Pre-prandial**
- Plasma glucose level (mg/100 ml): 18.4±0.4* 13.7±0.4* 21.9±0.8** 17.7±0.5*
- Digestive gland glycogen (mg/g of tissue): 4.3±0.1* 6.0±0.2** 7.1±0.2** 6.9±0.2**
- Number of animals: 6 6 5 6

**Post-prandial at the peak**
- Plasma glucose level (mg/100 ml): 38.1±2.7* 73.0±4.0** 55.1±2.2*** 37.7±2.3*
- Time to reach the peak (h): 3 3 2 1
- Digestive gland glycogen (mg/g of tissue): 6.4±0.1* 9.3±0.5** 13.3±0.7*** 11.7±0.4***
- Time to reach the peak (h): 1 3 2 2
- Number of animals: 6 5 5 5

**Glucose level 7 h after feeding**
- Plasma (mg/100 ml): 19.1±0.6* 20.1±1.2* 20.0±0.4* 32.0±1.3***
- Glycogen level 7 h after feeding (mg/g of tissue): 4.5±0.1* 5.0±0.2* 2.7±0.1** 5.5±0.1***
- Number of animals: 5 5 6 6

\(a\) Values mean±S.E.
\(b\) Protein concentration (%).
\(c\) Lipid concentration (%).
\(d\) Carbohydrate concentration (%).
Entries with differing numbers of asterisks are significantly different: \(P < 0.05\).

mg/100 ml), followed by shrimp fed diets containing 21% CBH (55.1 mg/100 ml) and 1 and 33% CBH (mean value of 37.9 mg/100 ml) \(P < 0.05\) (Table 3). The maximum post-prandial level of glycogen increased as levels of dietary carbohydrate increased with low values in animals fed the diet containing 1% CBH (6.4 mg/g of tissue) and significantly higher levels in shrimp fed diets containing 21% CBH (13.3 mg/g of tissue) and 33% CBH (11.7 mg/g of tissue) \(P < 0.05\) (Table 3).

The time to reach the peak level of glucose in the hemolymph and glycogen in the digestive gland varied according to the dietary CBH levels (Table 3, Fig. 2). The time to reach the peak of glucose hemolymph level was inversely related to dietary carbohydrate levels, 3 h in animals fed low CBH levels (1 and 10%), 2 h in animals fed diets containing 21% CBH and 1 h in animals fed diets containing 33% CBH (Table 3). The time to reach the peak of digestive gland glycogen was 1 h in animals fed diets...
Fig. 2. Effect of feeding on midgut gland glycogen concentration (mg/g tissue) and hemolymph glucose levels (mg/100 ml) in *L. stylirostris* juveniles fed with different carbohydrate levels (%). Mean±S.E.

Fig. 3. Post-prandial glycogen concentration (mg/g tissue) and α-amylase activity (IU/mg protein) of *L. stylirostris* juveniles fed diets containing different levels of carbohydrates (%). Mean±S.E.
containing 1% CBH, 3 h in animals fed diets containing 10% CBH and 2 h in animals fed diets containing 21 and 33% CBH (Table 3).

A saturation curve was observed in post-prandial glycogen concentration and α-amylase activity in relation to dietary CBH levels (Fig. 3). Significantly greater glycogen accumulation and enzymatic activity was observed in animals fed diets containing 21 and 33% CBH ($P < 0.05$).

3.3. Physiological analysis

Ammonia excretion was affected by dietary carbohydrate levels (Fig. 4). For all dietary treatments a higher rate of ammonia excretion was observed for animals after feeding ($P < 0.05$). Post-prandial nitrogen excretion (PPNE) resulted in an increase of 92% (1% CBH), 46% (10% CBH), 33% (21% CBH) and 84% (33% CBH) relative to the rate obtained in fasting animals (Fig. 4) ($P < 0.05$). The maximum pre- and post-prandial ammonia excretion was observed in shrimp fed diets containing 1% CBH followed by that observed for shrimp fed diets containing 10, 21 and 33% CBH ($P < 0.05$) (Fig. 4).

Osmotic pressure was affected by dietary carbohydrate levels and nutritional condition of the shrimp (Fig. 5a). In shrimp fed diets containing 10 and 33% CBH, the pre-prandial osmotic pressures were significantly higher than those observed in feeding animals ($P < 0.05$). In shrimp fed diets containing 1% CBH post-prandial osmotic pressure was higher than that observed in fasting animals ($P < 0.05$). In animals fed diets containing 21% CBH no differences were observed in pre- and post-prandial osmotic pressure ($P < 0.05$). The lowest osmotic pressure was observed in feeding animals fed diets containing 10% CBH and the highest in feeding animals fed diets containing 1% CBH (Fig. 5a) ($P < 0.05$).

No significant differences were observed in the osmotic capability (OC) obtained between shrimp fed diets containing 1, 21 and 33% CBH ($P > 0.05$). The OC determined for animals fed diets containing 10% CBH was significantly lower than that

![Fig. 4. Pre and post-prandial nitrogen ammonia excretion of *L. stylirostris* juveniles fed with different levels of dietary carbohydrates. Mean±S.E. Different letters or symbols (*) indicate statistical differences ($P < 0.05$).](image)
observed for animals fed diets containing each of the other carbohydrate levels ($P < 0.05$) (Fig. 5b).

4. Discussion

The concept that CBH can substitute for the proteins as an energy source in shrimp food, thereby reducing the costs associated with feed production, is already documented (Cruz-Suarez et al., 1994). However, many authors have demonstrated that marine shrimp have severe restrictions for the utilization of dietary CBH (Bages and Sloane, 1981; Alava and Pascual, 1987; Shiau and Peng, 1992).

A significant induction of $\alpha$-amylase and $\alpha$-glucosidase activities related to an increase in dietary CBH levels was found for juvenile $L.\ stylirostris$. There is evidence for an adaptation of these digestive enzymes to the dietary level of wheat flour. Similar results have been reported for the amylasic activity in $Homarus\ americanus$ (Hoyle,
1973) and *Palaemon serratus* (van Wormhoudt et al., 1980). In *L. vannamei* a regulation at the level of transcription for amylase was reported when animals were fed diets containing a constant level of starch (Le Moullac et al., 1996). For *L. stylirostris* the regulation of enzymatic activity needs to be studied.

The α-glucosidase participates in the final intracellular digestion of the dietary carbohydrates in the B cells (blister-like cells) of the midgut gland of shrimps (Le Chevalier and Van Wormhoudt, 1998). The main function of shrimp α-glucosidase is to complete the breakdown of maltose and oligosaccharides liberated by amylase hydrolysis (α- [1–4]-malto-oligosaccharide residues obtained after the action of α-amylase). Glucose is the final product of this process and it is evident that the glucose content of the extract of the midgut gland (137±5 mg/ml), and the activity of dietary α-glucosidase increased according to CBH increment. This enzyme has a specific activity more pronounced for linear chains of glucose, which can explain the similar digestibilities observed for starches in *L. vannamei* and *L. stylirostris* (Cousin, 1995). Cousin et al. (1996) showed that native starch or precooked starch were similarly digested by juvenile shrimp and without any difference in contribution to growth.

Intermediary metabolic enzymes may provide additional information about how shrimp can utilize glucose. Activities measured confirmed (i) the low entry of glucose into the citric acid cycle; (ii) the influence of dietary CBH on the rate of conversion of glucose to glycogen; and (iii) the role of PEPCK and gluconeogenesis at low dietary CBH levels. Hexokinase of crab was quickly saturated because of its low $K_m$ value, and no specific phosphatase was present (Loret, 1990), but this response remains to be evaluated in shrimps fed diets containing different levels of CBH. The interest lies in identifying the routes of glucose metabolism via phosphorylation, via pentose phosphates or via the synthesis of acetylglucosamine and the activities of enzymes related to such pathways.

Glucose and glycogen levels of *L. stylirostris* reached different levels according to the type of diet. In both cases levels were higher after feeding, showing that CBH metabolism is affected by dietary CBH levels. Higher levels occurred between 1 and 3 h after feeding, depending on the CBH level. In all cases, initial CBH values were reached between 6 and 7 h after feeding (Fig. 2). Similar results have been reported by Rosas et al. (1995b) for *L. setiferus* fed with squid, for *L. vannamei* and *M. japonicus* fed diets containing different sources of CBH (Abdel-Rahman et al., 1979; Cousin, 1995) and *M. japonicus* fed corn starch (Shiau and Peng, 1992). Here, the onset of the glucose hemolymph level was affected by the dietary CBH level. Shrimp fed with low CBH level (1 and 10% CBH) needed 3 h to reach the glucose peak in comparison to 2 h for shrimp fed a diet containing 20% CBH and only 1 h in shrimp fed a diet containing 30% CBH (Fig. 2). Abdel-Rahman et al. (1979) reported that serum glucose levels in *M. japonicus* increased rapidly after administration of glucose, resulting in a physiologically abnormal elevation of serum glucose concentration. The abnormal level impaired utilization of CBH as an energy source and inhibited the absorption of amino acids in the intestine.

Once the food is digested in the gut, chyme and fine particles are digested in the lumen and absorbed by diffusion to the inner portions of the digestive gland tubules, thus initiating the accumulation of glycogen (Al-Mohanna and Nott, 1987). The
glycogen accumulation after feeding was affected by the dietary CBH, showing a saturation curve with the low values observed in shrimp fed with low CBH diets and a higher values observed in animals fed between 21 and 33% CBH (Fig. 3). A similar saturation curve was observed in α-amylase activity indicating a limited capacity of L. stylirostris to store and process dietary CBH, which is around 20% of dietary CBH. A similar saturation curve of post-prandial glycogen concentration has been obtained in our laboratory, with the maximum glycogen concentration occurring in L. setiferus and L. vannamei juveniles fed diets containing 23% CBH (Rosas et al., unpublished). Taking into account these results the highest possible level of dietary CBH level should be considered the level at which the maximum capacity to store and process dietary CBH occurs.

The lack of differences in pre-prandial glucose and glycogen levels between treatments, indicates that shrimp can produce and store CBH from dietary carbohydrates and the gluconeogenesis pathway. The present results suggest that shrimp can use the gluconeogenesis pathway according to CBH availability, with a higher gluconeogenic activity in shrimp fed diets containing comparatively low amounts of CBH, and low or no gluconeogenic activity in shrimp fed diets containing more than 20% CBH. An intermediate gluconeogenic activity could be expected in shrimp fed with intermediate levels of CBH. Realizing that the protein is the source for gluconeogenic formation of CBH (Campbell, 1991), the shrimp protein metabolism should be affected by diets containing different CBH levels following gluconeogenesis.

The osmotic capability (OC) has already been evaluated in studies with Homarus americanus (Young-Lai et al., 1991), M. japonicus (Charmantier-Daures et al., 1988) and L. vannamei (Charmantier et al., 1994) to monitor the physiological condition of crustaceans exposed to different experimental conditions (dissolved ammonia, salinity and dissolved oxygen). In the present study the OC was used to determine if there are any relationships among levels of dietary CBH levels, glucose hemolymph level and protein–carbohydrate metabolism. The differences observed between shrimp fed with different CBH levels could be related to the effect of dietary glucose level on CBH-protein requirements associated with salinity tolerance. The highest level of post-prandial plasmatic glucose of shrimp fed a diet containing 10% CBH, indicates a higher production of glucose derived from reserves of glycogen in muscle and midgut gland. If carbohydrates from the gluconeogenesis pathway are related to dietary protein content (Claybrook, 1983; Gómez et al., 1988) levels of hemolymph glucose are thought to have been derived from proteins, reducing the amounts of amino acids for osmoregulation. The present results show a dietary level of 10% CBH was insufficient to meet energy needs which could be satisfied by dietary protein. The low OC observed in shrimp fed a diet containing 10% CBH coincided with a relatively low PPNE which reflects a low concentration of amino acids circulating in hemolymph (Rosas et al., 1998). In contrast shrimp fed a diet containing a low level of CBH (1%) had enough protein (48%) to maintain an adequate level of glucids and free amino acids (FAA) for osmoregulation. The high OC observed in shrimp fed with that diet coincided with a high PPNE, which reflects a high amino acid catabolism. In L. stylirostris, the pool of amino acids could act as a buffer between the catabolism of body proteins and the requirements for energy for intermediate metabolism and osmotic pressure which is
apparently related to the CBH and protein requirements (McFarland and Lee, 1963; Claybrook, 1983; Mayzaud and Conover, 1988).

Depending on physiological condition the amino acids may be of alimentary or muscular origin (Regnault, 1986; Rosas et al., 1995a) and are reflected in the pre- and post-prandial ammonia excretion. Low values were found in fasting shrimp and fed shrimp produced high values (Fig. 4).

In this study, fasting (12 h) caused a 50% reduction in the protein metabolism of *L. stylirostris* juveniles, showing a high sensitivity to food deprivation. This type of response may be related to the limited capacity of shrimp to store reserve substances, which has been observed previously in *L. setiferus*, *F. duorarum*, *L. schmitti* and *F. notialis* (Rosas et al., 1995a,b, 1999). In fed shrimp an increase in post-prandial nitrogen excretion reflects an increase in the amino acids of alimentary origin, which in turn relate to the role of dietary protein in energy metabolism. In the case of fed *L. stylirostris* juveniles, the regulating capacity of the ammonia excretion depends on the digested food protein which is inversely related to the level of dietary CBH (Fig. 4). This type of dependence has been observed in other shrimp species reflecting the high plasticity of shrimp species to use protein as a source of metabolic energy (Rosas et al., 1996).

The level of ammonia excretion demonstrates that protein catabolism in this shrimp species is comparatively high (Fig. 4) (Claybrook, 1983; Pierce and Crawford, 1996). Ammonia excretion is not regulated by shrimp but directly related to the level of dietary protein, as shown in this and other studies with shrimp (Gauquelin, 1996) and for lobster (Capuzzo, 1980). The ammonia produced reflects the catabolism for essential amino acids for energy (Barclay et al., 1983). Catabolism of essential amino acids (EAA) yields a higher metabolizable energy than that derived from glucose dextrins. Realizing that crustaceans are ammoniotelic and use less energy than birds and mammals during mechanical and biochemical transformations of food (called apparent heat increment or specific dynamic action) (Cowey and Forster, 1971), we can expect shrimp to be better adapted to use proteins as a source of energy than carbohydrates, which have an apparent limit of 20% in diet in *L. stylirostris*.

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