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Research Article

Influence of dietary starch and cellulose levels on the metabolic profile and apparent digestibility in penaeoid shrimp

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ABSTRACT. The present study compared the effect of different starch/cellulose ratios (30/0, 20/10, 10/20, 0/30) on the metabolic response and apparent digestibility in two species of penaeoids: *Artemesia longinaris* and *Pleoticus muelleri*. Adult animals were used in order to obtain sufficient quantities of haemolymph and faecal material for analysis. No significant differences were found in levels of plasma metabolites in *P. muelleri*, but in *A. longinaris*, a significant increase was observed in glucose, total protein, and cholesterol in correlation with increased dietary starch. The apparent digestibility coefficients decreased from 83.7% to 51.2% (*A. longinaris*) and from 71.9% to 7.6% (*P. muelleri*) as the dietary starch levels increased. The ratio of amylase activity to protease activity (A/P ratio) declined in *A. longinaris* when the percentage of dietary starch increased. In contrast, the A/P ratio for *P. muelleri* increased with higher starch concentrations. These results demonstrated a close relationship between the feeding habits and digestive physiology of the two species studied; they also suggest a more herbivorous behavior for *A. longinaris* and more omnivorous habits for *P. muelleri*.

Keywords: *Artemesia longinaris*, *Pleoticus muelleri*, apparent digestibility, carbohydrate metabolism, cellulose, starch, haemolymph, Argentina.

Influencia del nivel de almidón y celulosa en la dieta sobre el perfil metabólico y digestibilidad aparente en camarones penaeoideos

RESUMEN. En el presente estudio se comparó el efecto de diferentes concentraciones de almidón/celulosa (30/0; 20/10; 10/20; 0/30) sobre la respuesta metabólica y la digestibilidad aparente en dos especies de peneidos, *Artemesia longinaris* y *Pleoticus muelleri*. Se utilizaron animales adultos a fin de obtener cantidades suficientes de hemolinfa y heces para los análisis. No hubo diferencias significativas en los niveles de metabolitos plasmáticos en *P. muelleri*, en cambio en *A. longinaris* se observó un incremento significativo de la glucosa, proteínas totales y colesterol en relación con el aumento del almidón en la dieta. Los coeficientes de digestibilidad aparente disminuyeron de 83,7% a 51,2% (*A. longinaris*) y de 71,9% a 7,6% (*P. muelleri*) a medida que los porcentajes de almidón en la dieta aumentaron. El cociente entre la actividad de amilasa y proteasa (A/P) se redujo en *A. longinaris* con los mayores porcentajes de almidón dietario; por el contrario, el cociente A/P en *P. muelleri* aumentó cuando la concentración fue más alta. Estos resultados demostraron que existe una estrecha relación entre los hábitos alimentarios y la fisiología digestiva de las dos especies estudiadas; sugiriendo un comportamiento más herbívoro para *A. longinaris* y más omnívoro para *P. muelleri*.

Palabras clave: *Artemesia longinaris*, *Pleoticus muelleri*, digestibilidad aparente, metabolismo de carbohidratos, celulosa, almidón, hemolinfa, Argentina.

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INTRODUCTION

The major achievements in crustacean nutrition include the identification of a protein sparing effect of dietary carbohydrates and lipids, leading to considerably lower protein requirements than those originally suggested. The proteins are the higher reserve substrate in shrimp, which can be converted to carbohydrates following the gluconeogenic pathway (Campbell, 1991). The carbohydrates are not essential for crustaceans; shrimp appear to be able to utilize complex carbohydrates better than simple ones such as glucose, which is quickly absorbed and released into the haemolymph, resulting in a physiologically abnormal elevation of plasma glucose levels (New, 1976, 1990; Shiau & Peng, 1992). Starch is nowadays the typical carbohydrate in formulated feeds for crustaceans; it is well hydrolyzed by shrimp such as *Fenneropenaeus indicus* and *Litopenaeus vannamei* but poorly hydrolyzed by lobsters (Verri *et al.*, 2001).

The rate of nutrient absorption depends on the rate at which nutrients come into contact with the absorptive epithelium. Dietary fibers, such as cellulose, are associated with the delay in stomach emptying and contribute to the efficient utilization of dietary protein (Gomez Díaz & Nakagawa, 1990). Determination of digestibility can be used to select ingredients that optimize the nutritional value and reduce costs of formulated feeds. Among the methods employed in feed digestibility studies in crustaceans, the use of chromic oxide as an inert indicator is recommended with procedural steps to insure accuracy (Fenucci *et al.*, 1980, 2009; Lee & Lawrence, 1997; Divakaran *et al.*, 2002).

The carbohydrates are incorporated in aquaculture feeds to reduce costs and for their binding properties during feed manufacturing. Waste management has become a prime concern for shrimp farming in many countries. It is generally accepted that a better understanding of feed utilization by the shrimp is essential to reduce environmental pollution through both ammonia excretion and feces egestion; carbohydrate utilization can be achieved and consequently lead to a decrease in the amount of nitrogen waste.

Haemolymph is the prime component involved in the defense mechanism of crustaceans; several metabolic variables of haemolymph, such as proteins, glucose, and cholesterol have been proposed to monitor the effect of environmental conditions on wild and cultured shrimp (Hall & Van Ham, 1998; Sánchez *et al.*, 2001; Pascual *et al.*, 2003). Fluctuations in biochemical variables are also associated with the physiological response to stress, but these variables levels can only be properly interpreted if the nutritional state of the shrimp is carefully controlled.

The present study was designed to compare the dietary effect of different ratio starch/cellulose on selected physiological, metabolic, and hematological responses and the apparent digestibility in two species of penaoids (*Artemesia longinaris* and *Pleoticus muelleri*). Both species present seasonal and yearly fluctuations in catches; it is therefore important to establish the feasibility of culturing them on commercial basis to keep a continue supply of these species to the market. The Aquaculture group from the University of Mar del Plata has been working with both species, but mainly with *P. muelleri,* on different aspects of the biology, nutrition, maturation, massive larval culture, and growth out in ponds. Some studies have demonstrated good survival and growth under culture conditions (Fenucci *et al.*, 1983, 1990; Petriella *et al.*, 1984; Díaz *et al.*, 1996), and determined the nutritional requirements (Fernández-Gimenez & Fenucci, 2002; Romanos-Mangialardo & Fenucci, 2002) as well as gonadal maturation in captivity (Díaz *et al.*, 1997, 2001; Díaz & Fenucci, 2004), and characterized the digestive proteinases in relation to the molting cycle (Fernández-Gimenez *et al.*, 2002).

MATERIALS AND METHODS

Feed and feeding trials

Artemesia longinaris and *Pleoticus muelleri* were obtained from a commercial fisherman in the coastal waters of Mar del Plata, Argentina (38°S). Large adult animals were used in these experiments to obtain sufficient quantities of faecal material for the analysis of feed digestibility. All individuals were kept in 150 L glass aquaria with continuous aeration during four weeks. Filtered seawater (to 5μm) was exchanged at a rate of 50% per day. Shrimp were exposed to constant conditions of photoperiod (11 h light-13 h dark), temperature (18°C), pH 7, and salinity (31 ppt). The ammonium concentration never exceeded 0.2 mg L^{-1}. All groups were fed *ad libitum* once a day (09:30 h). Formulated feeds were tested in triplicate groups for both species, each *A. longinaris* group consisted of 6 shrimp (2.7 ± 0.9 g mean weight) and *P. muelleri* groups had 4 shrimp (9.7 ± 2.0 g mean weight), randomly chosen.

The treatments consisted of four dry formulated feed prepared to contain different ratios starch/cellulose (30/0; 20/10; 10/20; 0/30), with 0.5% chromic oxide as an inert indicator (to calculate the apparent protein digestibility coefficients). Formu-

lations were made according to the chemical composition results of the by-products meal in order to obtain isoproteic and isolipidic diets. The chemical composition of the formulated feeds was confirmed through proximate analysis (Table 1) according to AOAC (1997). All ingredients, from a local feed manufacturer, were mixed and cold pelleted (< 50°C) by extrusion (Fenucci & Zein-Eldin, 1976) and were oven-dried for 24 h at 50°C.

Apparent digestibility

After a 7-day period of adjustment to the new conditions and diets was beginning of fecal collection. To determine the apparent digestibility for crude protein, before each feeding, feces were collected during two weeks by siphoning and rinsed with distilled water to eliminate the excess of salts. The fecal material from each tank was pooled and frozen at -20ºC for analysis.

Proximate analyses of faecal samples were carried out using AOAC methods (1997). The chromic oxide levels were measured with a spectrophotometer (540 nm) (Shimadzu UV-2102 PC, UV-visible Scanning Spectrophotometer). The apparent digestibility coefficient (ADC) was estimated according to Fenucci *et al.* (1980): ADC (% protein digestibility) = 100 - (I_a/I_b . II_b/II_a . 100) where: I_a = %Cr_2O_3 feed; I_b = % Cr_2O_3 feces; II_a = % protein food; II_b = % protein feces.

Sampling and analyses of metabolic variables

At the end of the experiments (four weeks), shrimp were anaesthetized in ice water for approximately 5 min, then the haemolymph was extracted and midgut gland was removed. All shrimp used in the analysis were in the intermolt stage (Petriella, 1984; Díaz & Petriella, 1990). Samples collected from eight individuals (n = 8) of each treatment group were analyzed separately.

Approximately 200-300 µL of haemolymph were extracted from the arthrodial membrane of the fifth pereiopod of each organism; using a 1 mL syringe rinsed with a 10% cooled anticoagulant solution of sodium citrate. The haemolymph was centrifuged at 800 *g* for 5 min, and plasma transferred into a new tube and stored at -20°C for further analysis.

Midgut glands were carefully dissected and immediately frozen at -20ºC and homogenized in chilled distilled water and centrifuged for 30 min (10,000 *g* at 4ºC), the upper aqueous phase was stored at -20°C.

Glucose, total protein and cholesterol concentrations in plasma and supernatants from homogenate were measured using commercial kits for medical diagnosis (Wiener Laboratories SAIC, Argentina), according to the manufacturer's protocols and quantified in a Metrolab 1600DR (Wienerlab Instrument). The extracts were analyzed in triplicate.

Enzyme assays

The amount of soluble protein in the homogenized midgut glands used for enzyme analysis was determined by the Bradford method (1976). Albumin from chicken egg white (Sigma) was used as the standard.

Total proteinase determination was performed with 1% azocasein in 50 mM Tris-HCl, pH 7.5 as substrate. Triplicates of 5 µL of enzyme extracts were mixed with 0.5 mL of buffer and 0.5 mL of substrate solution. The reaction mixtures were incubated for 10 min at 25°C. Proteolysis was stopped by adding 0.5 ml of 20% trichloroacetic acid TCA, and the mixture was centrifuged in Eppendorf tubes for 5 min at 14 000 *g*. The supernatants were separated from the undigested substrate and the absorbance at 366 nm was recorded for the released dye (García-Carreño, 1992).

Total α-amylase activity was determined using commercial kits for medical diagnosis (Wiener Laboratories SAIC, Argentina), according to the manufacturer's protocols and quantified in a Metrolab 1600DR (Wienerlab Instrument).

Total cellulase activity was determined according to Martínez *et al.* (1999) modified method. A solution of crystalline cellulose was prepared in substrate buffer sodium acetate 0.05 M; pH 5. A 24 mL volume of this substrate solution was then mixed with 1 mL of midgut gland extract, incubated with gentle agitation, for 2 h at 30°C. The reaction mixture was centrifuged, 2 ml of supernatant was separated and added 0.1 mL starch solution (100 mg mL^{-1}) and 2 mL of 3.5-dinitrosalicilic acid. Then, the extract was incubated for 5 min at 100°C and the absorbance was recorded at 490 nm. The extracts were analyzed in triplicate.

Statistical Analysis

One-way ANOVA and Duncan's multiple comparisons of means were preformed to compare the data obtained. Homogeneity of variances was verified with Cochran's test. An arcsine transformation was applied before processing percentages data. Protein digestibility among different treatments was evaluated through regression analyses. A correlation coefficient was used to describe the fit of the data on the regression line. ANCOVA was used to test differences among regression lines. To find out the relationships among different ratio starch/cellulose in the diet and metabolic variables, Pearson's rank correlation

Table 1. Ingredient composition of formulated feeds.
Tabla 1. Composición de las dietas formuladas.

Ingredient	Formulated feed (g 100 g^{-1} dry feed)			
	D1	D2	D3	D4
Fish meal[a]	48	48	48	48
Soybean meal[b]	15.5	15.5	15.5	15.5
Manioc starch[c]	30	20	10	0
Cellulose microcrystalline[d]	0	10	20	30
Fish oil	2	2	2	2
Lecithin	0.5	0.5	0.5	0.5
Cholesterol	0.5	0.5	0.5	0.5
Vitamin supplement[e]	0.5	0.5	0.5	0.5
Minerals[f]	0.5	0.5	0.5	0.5
Fish soluble	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5
Na alginate	1	1	1	1
Proximate composition (% dry matter)				
Dry matter	99.3	97.6	98.2	99.2
Crude protein	37.9	38.2	38.2	37.8
Total lipid	12.4	13.2	12.0	12.7
Ash	11.0	11.6	10.8	10.2

[a]Agustinier S.A. Mar del Plata, Argentina. [b]Melrico S.A. Argentina.
[c]Molinos Chacabuco, Argentina. [d] Merck, Germany.
[e]Vitamin premix (mg kg^{-1} of premix): cholecalciferol 35; thiamin 163; rivoflavin 156; pyridoxine 213; calcium pantothenate 250; biotin 250; niacin 500; folic acid 25; B_{12} HCL 20; ascorbic acid Rovimix STAY C 781; menadione 34; inositol 300; choline chloride 200; a-tocopherol acetate 1750; vitamin A acetate 180 (Roche, Argentina).
[f]Mineral premix: calcium 1,000 mg; magnesium 500 mg; potassium 99 mg; zinc 30 mg; 10 mg; iron 10 mg; copper 2 mg; iodine 150 μg; selenium 200 μg; chromium 200 μg; molybdenum 500 μg (Twin Laboratories, Inc. USA).

coefficient was done. A probability level of 0.05 was used to assess significance in all measured parameters. (Sokal & Rohlf, 1995).

RESULTS

Metabolic variables

Mean values of metabolic variables for each species are shown in Figure 1. There were no significant differences in levels of plasma metabolites in *P. muelleri* when shrimp were fed with different ratio starch/cellulose. Significant variations were observed in the metabolic variables of *A. longinaris*, a significant increase in glucose, total protein, and cholesterol was noted in correlation with the increase in starch.

The analysis of the midgut gland from shrimp fed with different levels of starch/cellulose showed that concentration of metabolites was different in both species. The highest level of cholesterol was observed in *A. longinaris* fed with D1 (30/0, starch/cellulose), whereas concentrations of glucose and total protein were not significantly different. In contrast, in *P. muelleri* glucose was significantly higher in shrimp fed with D1 than in those fed with diet containing lower starch levels.

Enzyme assays

Soluble protein content of the midgut gland was 15.3 ± 1.68 mg mL^{-1} in *A. longinaris* and 17.3 ± 2.30 mg mL^{-1} in *P. muelleri*. No significant differences were observed in proteinase and α-amylase activity in midgut gland extracts from the four dietary groups in both species (Table 2). No specific cellulase activity was observed in the midgut gland extracts.

The ratio of amylase activity to protease activity (A/P ratio) decreased in *A. longinaris* when the

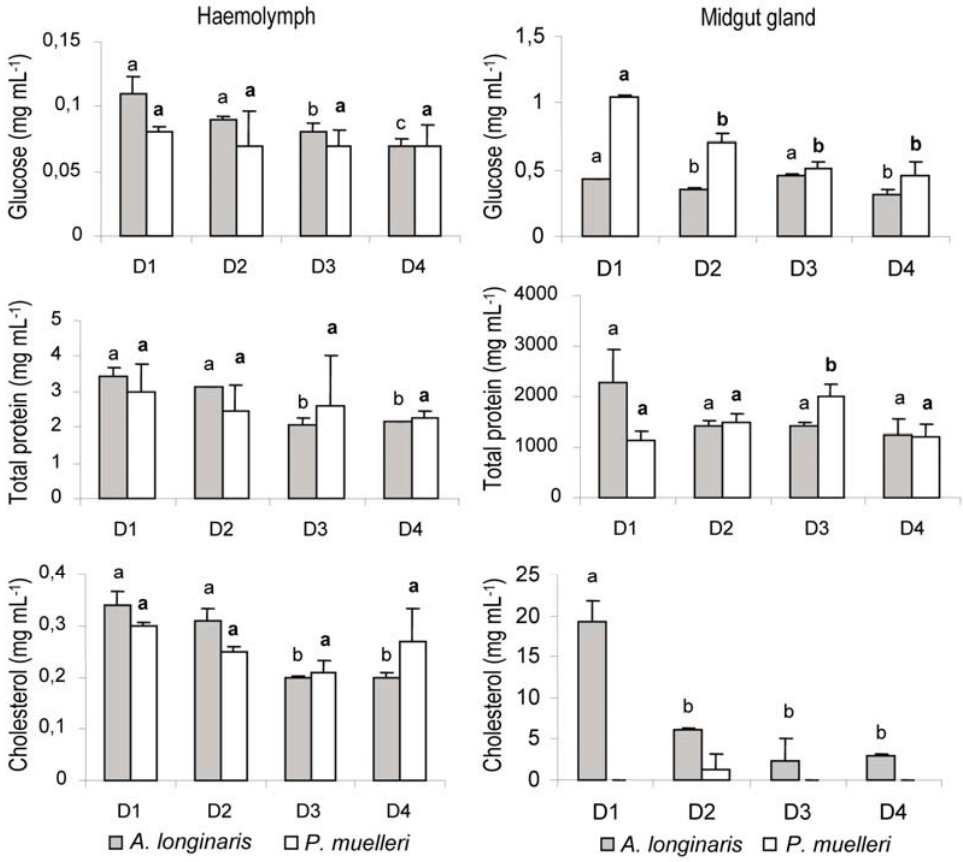

Figure 1. Metabolic variables in haemolymph and midgut gland of *A. longinaris* and *P. muelleri* fed with different ratio starch/cellulose in the diet. Error bars indicate standard deviation. Different letters indicate statistical differences ($P < 0.05$).

Figura 1. Variables metabólicas en hemolinfa y hepatopáncreas de *A. longinaris* y *P. muelleri* alimentados con diferentes niveles de almidón/celulosa en la dieta. La barra indica la desviación estándar. Letras distintas indican diferencias estadísticas significativas ($P < 0,05$).

percentage of dietary starch increased. In contrast, the A/P ratio for *P. muelleri* increased when starch concentration was high (Fig. 2).

Apparent Digestibility

The apparent protein digestibility coefficients decreased from 83.7% to 51.2% in *A. longinaris*) and from 71.9% to 7.6% in *P. muelleri* with the increase in dietary cellulose levels, and was significantly different among treatments. *In vivo* apparent protein digestibility was related to dietary cellulose contents as shown by the regression analysis ($y = 0.095x^2 - 4,2647x + 88,671$; $R^2 = 0.7198$ for *A. longinaris*, and $y = 97,476\ e^{-0,0829x}$, $R^2 = 0.724$ for *P. muelleri*) (Fig. 3).

Pearson's Correlation

Pearson correlation coefficients showed that all variables exhibited a greater degree of correlation with the cellulose rate, except glucose in haemolymph for *A. longinaris* (Table 3) and glucose in midgut gland for *P. muelleri* (Table 4). Metabolic variables were correlated with each other.

DISCUSSION

Dietary nutritional requirements of two species of penaeoids were investigated on the basis of their haemolymph metabolic contents and the apparent digestibility analysis to determine which the dietary components that were better assimilated are. The penaeoids shrimp do not present a dietary glucose requirement since this compound can come from the gluconeogenesis from the amino acids. Nevertheless, these organisms own a complete enzymatic structure for the digestion of proteins and polysaccharides, such

Table 2. Specific enzyme levels (± SEM) recorded from midgut gland at four levels of dietary starch/cellulose in *A. longinaris* and *P. muelleri*.

Tabla 2. Niveles enzimáticos (± ESM) específicos registrados en hepatopáncreas de *A. longinaris* y *P. muelleri* alimentados con cuatro niveles dietarios de almidón/celulosa.

Formulated feed	Protease		Amylase	
	A. longinaris	*P. muelleri*	*A. longinaris*	*P. muelleri*
D1	0.11 ± 0.007^{a}	0.25 ± 0.117^{a}	2.06 ± 0.007^{a}	3.78 ± 0.410^{a}
D2	0.11 ± 0.056^{a}	0.24 ± 0.009^{a}	2.30 ± 0.007^{a}	2.99 ± 0.183^{a}
D3	0.09 ± 0.011^{a}	0.32 ± 0.019^{a}	2.56 ± 0.084^{a}	2.20 ± 0.128^{a}
D4	0.05 ± 0.024^{b}	0.47 ± 0.066^{a}	2.56 ± 0.275^{a}	3.69 ± 0.134^{a}

Specific protease activity expressed as Abs/mg protein/min. Specific amylase activity expressed as mg starch hydrolyzed/mg protein/min. Different superscripts are significantly different, $P < 0.05$.

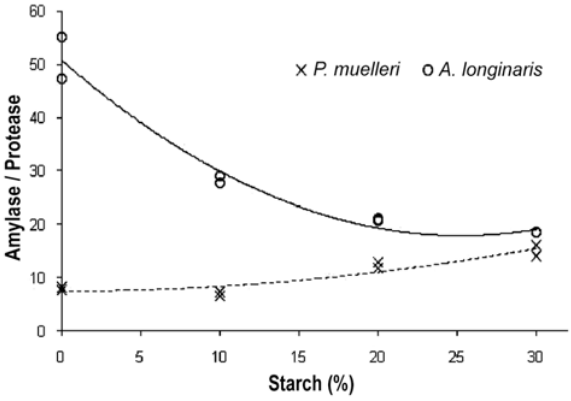

Figure 2. The effect of dietary starch levels on ratio of amylase specific activity to protease specific activity on polynomial regression analysis in *A. longinaris* and *P. muelleri* ($y = 0.051x^2 - 2.602x + 50.80$, $R^2 = 0.969$ for *A. longinaris*, and $y = 0.009x^2 + 0.001x + 7.387$, $R^2 = 0.867$ for *P. muelleri*).

Figura 2. Regresión polinomial del efecto de los niveles de almidón en la dieta sobre el cociente entre la actividad específica de amilasa y la de proteasa en *A. longinaris* y *P. muelleri* ($y = 0{,}051x^2 - 2{,}602x + 50{,}80$; $R^2 = 0{,}969$ para *A. longinaris*; $y = 0{,}009x^2 + 0{,}001x + 7{,}387$; $R^2 = 0{,}867$ para *P. muelleri*).

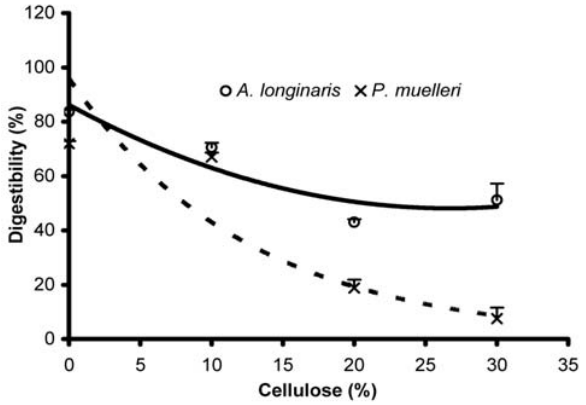

Figure 3. Apparent protein digestibility at four levels of dietary starch/cellulose in *A. longinaris* and *P. muelleri*. Values are means of three observations. Error bars indicate standard deviation.

Figura 3. Digestibilidad aparente de proteínas con cuatro niveles de almidón/celulosa en la dieta de *A. longinaris* y *P. muelleri*. Los valores representan la media de tres observaciones. La barra indica la desviación estándar.

as starch, glycogen, laminarin, and chitin that are natural components of their diet. (Tacon, 1990). The starch is a straight-chain consisting of glucose molecules linked together by α (1-4) bonds, conformed by two units' amylose and amylopectin. The starches rich in amylose are poorly digestible because α-amylase cannot hydrolyze the amylase, in contrast, the starch rich in amylopectin is relatively well digested (Gaxiola *et al.*, 2006). The cellulose is also a polymer of glucose but it differs in its glycoside bonds, which are β (1-4). The inclusion of starch in the shrimp's diet represents the greater source of energy. The incorporation of cellulose was used to compensate the amount of carbohydrates added to the experimental diet or to increase stomach emptying (Shiau, 1997).

The type of food is a dominant factor affecting shrimp haemolymph metabolites (Pascual *et al.*, 2003). Baseline levels of the haemolymph metabolites were obtained by Rosas *et al.* (2002) in *Litopenaeus vannamei* to be used as reference parameters. Glucose is the major component of circulating carbohydrates in crustaceans, but its concentration varies markedly

Table 3. Correlation matrix of metabolic variables and cellulose in feed for *Artemesia longinaris.*

Tabla 3. Matriz de correlación de las variables metabólicas y el nivel de celulosa en la dieta para *Artemesia longinaris*.

	C	GH	PH	ChH	GMG	PMG	ChMG	APD
C	1							
GH	-0.983	1						
PH	-0.919*	0.901*	1					
ChH	-0.941*	0.928*	0.998	1				
GMG	-0.432*	0.445*	0.042*	0.106*	1			
PMG	-0.865*	0.941*	0.749*	0.785*	0.524*	1		
ChMG	-0.866*	0.940*	0.842*	0.865*	0.313*	0.973	1	
APD	-0.874*	0.889*	0.983	0.980	-0.014*	0.786*	0.893*	1

*$P < 0.05$. C-Cellulose feed; GH -Glucose Haemolymph; PH- Total Protein Haemolymph; ChH - Cholesterol Haemolymph; GMG -Glucose Midgut Gland; PMG -Total Protein Midgut Gland; ChMG - Cholesterol Midgut Gland; APD - Apparent Protein Digestibility

Table 4. Correlation matrix of metabolic variables and cellulose in feed for *Pleoticus muelleri.*

Tabla 4. Matriz de correlación de las variables metabólicas y el nivel de celulosa en la dieta para *Pleoticus muelleri*.

	C	GH	PH	ChH	GMG	PMG	ChMG	APD
C	1							
GH	-0.775*	1						
PH	-0.866*	0.871*	1					
ChH	-0.445*	0.751*	0.346*	1				
GMG	-0.954	0.913*	0.868*	0.674*	1			
PMG	0.245*	-0.536*	-0.753*	-0.960	-0.478*	1		
ChMG	-0.252*	-0.340*	-0.232*	-0.138*	0.063*	0.025*	1	
APD	-0.947*	0.620*	0.661*	0.471*	0.886*	-0.346*	0.515*	1

*$P < 0.05$. C-Cellulose feed; GH -Glucose Haemolymph; PH - Total Protein Haemolymph; ChH -Cholesterol Haemolymph; GMG -Glucose Midgut Gland; PMG -Total Protein Midgut Gland; ChMG - Cholesterol Midgut Gland; APD -Apparent Protein Digestibility

among the species (Chang & O'Connor, 1983). Increases in haemolymph glucose are also associated with the physiological response to stress in shrimp, but levels can only be properly interpreted if the nutritional state of the shrimp is carefully controlled (Hall & Van Ham, 1998). Furthermore, the glucose in haemolymph is an indicator of the carbohydrates metabolism and the level of this nutrient in the diet. In the present study, the glucose levels were similar to those reported in juvenile *L. setiferus*, *L. vannamei* and *L. stylirostris* (Rosas *et al.*, 2000). In *A. longinaris*, the glucose in haemolymph was no correlated with cellulose level in the diet (Table 3); shrimp could activate its compensation mechanism that allowed the recovery of haemolymph metabolites to maintain the homeostasis. This mechanism could involve the use of reserves stored in digestive gland, such as demonstrated in other species (Pascual *et al.*, 2003).

From the entire set of metabolites studied in *A. longinaris* plasma, glucose, total protein, and cholesterol were the most affected by the increase of starch in diet. But in *P. muelleri* there were no significant variations under the same conditions.

In *P. muelleri,* cholesterol in midgut gland showed slight fluctuations, however in *A. longinaris*, significant differences were observed among dietary treatments (Fig. 1). The shrimp's midgut gland is considered the main storage organ, mainly accumulating lipids, and to a lesser degree, glycogen. It has been proposed in crustaceans, that cholesterol is conserved due to their role as structural component of cell membranes, and is also an essential nutrient for crustaceans since they are incapable of *de novo* synthesis of the steroid ring (Rabid *et al.*, 1999). The dependence of haemolymph cholesterol on dietary lipids levels is related to the ability of shrimp to store and synthesize lipids. Mourente & Rodríguez (1991) and Teshima (1998) showed that because of the

limited space in shrimp's digestive gland, lipids must be processed rapidly and delivered into the haemolymph, where they are transported to the different tissues to be metabolized. With regards to *P. muelleri,* this species will be using cholesterol of the diet quicker than *A. longinaris*. Probably for that reason it is observed higher levels of cholesterol in *A. longinaris* haemolymph and midgut gland and in *P. muelleri* only in the haemolymph. As all supplied diets had the same cholesterol level, it was evident that *P. muelleri* presented a greater requirement of this nutrient, which was demonstrated by a smaller level of reserve. *P. muelleri* would be more carnivorous and require cholesterol from animal sources. This is in agreement with the results of digestibility, which decreased with increasing cellulose inclusion

Borrer & Lawrence (1989) observed that the level of dietary cellulose affected apparent protein digestibility in *Farfantepenaeus aztecus*, by lowering digestibility values as cellulose levels increased. A similar result was observed in *Macrobrachium rosenbergii* (González-Peña *et al*., 2002). On the other hand, in *L. vannamei* the protein digestibility did not change with the different levels of cellulose (Borrer & Lawrence, 1989; Guo *et al*., 2006). It was evident that *A. longinaris* fed with diets poor in cellulose content (D1 and D2) assimilated better the nutrients than *P. muelleri* and this fact was reflected by an increase of circulating metabolites and the apparent digestibility (Fig. 3). In both species, the digestibility values decreased from D1 to D4, nevertheless the values of apparent digestibility in *A. longinaris* were higher than in *P muelleri.* This fact would be related to the rate of nutrient absorption that depends on the time at which nutrients come into contact with the absorptive epithelium. According to other authors, the relative influence of dietary fiber on the movement of nutrients along the gastrointestinal tract affects nutrient absorption (Shiau, 1997). So, it is possible that the dietary fiber with high viscosity reduces the interaction between the enzymes and substrates, reducing absorption rates. The binding capability of the dietary fiber is another possible factor that can reduce the availability of nutrients (González-Peña *et al.*, 2002).

The proteins are essential nutrients for the penaeoids shrimps because they are basic for growth, the regulation of the internal osmotic pressure, the gluconeogenesis and the immune system (Rosas *et al.*, 2002). Therefore variations of this nutrient in haemolymph can be used as good indicator of general physiological state. The increment in haemolymph proteins observed in *A. longinaris* feed D1 and D2 (high levels starch) could be related with amino acids synthesis from the transamination pathway, and its posterior storing (Rosas *et al.*, 2001). The values of proteins in haemolymph and digestibility for the different treatments in *P. muelleri* did not adjust to a same pattern. Whereas digestibility was greater in D1 and D2, there were no differences in the circulating proteins. From the analysis of the proteins data it can be postulated that *P. muelleri* employs these absorbed proteins for muscular development or growth.

Penaeid shrimp adapt quite well to changes in diet composition by the induction of digestive enzymes synthesized and secreted in midgut gland. These enzymes hydrolyze a variety of substrates and various factors are involved in their regulation, including diet (Gamboa-Delgado *et al.*, 2003). Therefore, the digestive enzyme profile can be used to illustrate the capacity of shrimp to exploit diet in order to meet nutritional requirements. Carnivorous species generally have a wide range of proteolytic enzyme at high concentrations, which is consistent with their ability to hydrolyze high levels of dietary protein (Johnston & Yellowlees, 1998). However, omnivores and herbivores have a wide range and higher concentration of carbohydrases, consistent with their ability to hydrolyze plant and animal dietary carbohydrate (Wigglesworth & Griffith, 1994). The decline in the ratio of amylase to protease observed in *A. longinaris* in our study, confirms that carbohydrate digestion and its utilization change with the increase in dietary starch levels. In contrast, in *P. muelleri* the A/P ratio was correlated with dietary starch level. This shift in relative importance of carbohydrate and protein highlights the strategy outlined above, whereby carbohydrate energy reserves are used initially, while protein becomes important once carbohydrate reserves are depleted.

In the current study, there was no cellulase activity in the midgut gland of both species. It is now clear that cellulases are produced endogenously in a number of invertebrate taxa that includes crustaceans; genes for cellulose enzymes (β-glucosidase and endo-β-1,4-glucanase) are present in the genome of crayfish (*Cherax* sp.) (Linton *et al*., 2006). However, in penaeoid species cellulose digestion has not been unequivocally demonstrated (Shiau, 1997). More suitable substrates and assays for the determination of cellulase activity are required to clarify matters.

Although the bibliography indicates that both species studied in this work present a similar nourishing regime (Boschi, 1989; Gavio & Boschi, 2004), studies in culture conditions showed that both species have a 92% apparent digestibility of proteins with diets having fish meal as the main ingredient; when this ingredient is replaced by soybean meal, the

digestibility is 83% for *A. longinaris* and 47.7% for *P. muelleri* (Fenucci *et al.*, 2009). This difference in digestibility can be related to the more carnivorous behavior of the latter species. The present study shows that apparent protein digestibility was influenced by the levels of dietary starch and cellulose in both species; increasing cellulose levels caused significant reduction in protein digestibility. The inclusion of starch as source of carbohydrates in the diet seems to be more suitable than cellulose.

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REFERENCES

AOAC. 1997. Official methods of analysis of AOAC International. In: P. Cuniff (ed.). 16th ed. AOAC International. Gaithersburg, Maryland, 1995 pp.

Bradford, M.M. 1976. A refined and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Boschi, E.E. 1989. Biología pesquera del langostino del litoral patagónico de Argentina (*Pleoticus muelleri*). Contrib. Inst. Nac. Invest. Des. Pesq., 646: 1-71.

Borrer, S. & A.L. Lawrence. 1989. Effects of lipids and cellulose on the digestibility of penaeid shrimp diets. J. World Aquac. Soc., 20: 18 (Abstract).

Campbell, J.W. 1991. Excretory nitrogen metabolism. Comparative Animal Physiology. 4th Willy-Liss, New York, pp. 227-324.

Chang, E. & J.D. O'Connor. 1983. Metabolism and transport of carbohydrates and lipids. The Biology of Crustacea. Academic Press, New York, 5: 263-289.

Díaz, A.C., A.M. Petriella & J.L. Fenucci. 1996. Ablación peduncular del langostino *Pleoticus muelleri* Bate (Crustacea, Decapoda, Penaeoidea): relación con el crecimiento. Actas del IX Congreso Latinoamericano de Acuicultura, Chile, pp. 136-139.

Díaz, A.C., A.M. Petriella & J.L. Fenucci. 1997. Efecto de la ablación peduncular en la maduración gonadal de *Pleoticus muelleri* Bate (Decapoda, Solenoceridae). I. Interacción entre la muda y desarrollo ovárico. Rev. Bras. Oceanogr., 45(1/2): 53-60.

Díaz, A.C. & J.L. Fenucci. 2004. Effect of artificial nutrition on the induction of precocious maturation in *Pleoticus muelleri* Bate (Crustacea, Penaeoidea). Aquac. Res., 35: 1166-1171.

Díaz, A.C. & A.M. Petriella. 1990. Moult staging in the shrimp, *Pleoticus muelleri* Bate. J. Aquac. Tropics, 5: 181-189.

Díaz, A.C., A.V. Fernández-Gimenez, N.S. Harán & J.L. Fenucci. 2001. Reproductive performance of male Argentine red shrimp *Pleoticus muelleri* Bate (Decapoda, Penaeoidea) in culture conditions. J. World Aquac. Soc., 32(2): 236-242.

Divakaran, S., L. Obaldo & I.P. Forster. 2002. Note on the methods for determination of chromic oxide in shrimp feeds. J. Agric. Food Chem., 50: 464-46.

Fenucci, J.L., M.I. Müller & J.H. Magnaterra. 1990. Factibilidad de cría del langostino (*Pleoticus muelleri*). Frente Marítimo, 7(B): 103-108.

Fenucci, J.L., Z. Zein-Eldin & A.L. Lawrence. 1980. The nutritional response of two penaeid species to various levels of squid meal in a prepared feed. Proc. World Aquac. Soc., 11: 403-409.

Fenucci, J.L., A.C. Díaz & A.V. Fernández-Gimenez. 2009. A review on the status of protein nutrition of argentine penaeoid shrimp: comparisons and contrasts within the Penaeidae. In: C.L. Browdy & D.E. Jory (eds.). The rising tide. Proceedings of the Special Session on Sustainable Shrimp Farming, World Aquaculture Society, pp. 164-176.

Fenucci, J.L., A.M. Petriella & M.I. Müller. 1983. Estudios sobre el crecimiento del camarón *Artemesia longinaris* Bate alimentado con dietas preparadas. Contrib. Inst. Nac. Invest. Des. Pesq., 424: 1-12.

Fenucci, J.L. & Z.P. Zein-Eldin. 1976. Evaluation of squid mantle meal as a protein source in penaeid nutrition. In: T.V.R. Pillay & W.A. Dill (eds.). FAO Technical Conference on Aquaculture Reports, Kyoto, Japan. Advances in Aquaculture. Fishing News Books Farnham, pp. 601-605.

Fernández-Gimenez, A.V. & J.L. Fenucci. 2002. Vitamin E requirement of the prawn *Artemesia longinaris* (Decapoda, Penaeidae). In: E. Escobar-Briones & F. Alvarez (eds.). Modern approaches to the study of Crustacea. Kluwer Academic, New York, pp. 85-90.

Gamboa-Delgado, J., C. Molina-Poveda & C. Cahu. 2003. Digestive enzyme activity and food ingesta in juvenile shrimp *Litopenaeus vannamei* (Boone, 1931) as a function of body weight. Aquac. Res., 15: 1403-1411.

Gavio, M.A. & E.E. Boschi. 2004. Biology of the shrimp *Artemesia longinaris* Bate, 1888 (Decapoda: Penaeidae) from Mar del Plata coast. Nauplius, 12(2): 83-94.

García-Carreño, F.L. 1992. The proteases of langostilla (*Pleuroncodes planipes*, Decapoda): their partial characterization, and the effect of feed on their composition. Comp. Biochem. Physiol., 103B(3): 575-578.

Gaxiola, G., C. Rosas, L. Arena & C. Gerard. 2006. Requerimientos de carbohidratos. In: C. Rosas, O. Carrillo, R. Wilson & E. Andreatta (eds.). Estado actual y perspectivas de la nutrición de los camarones peneidos cultivados en Iberoamérica. Publidisa Mexicana, Mexico, DF, pp. 143-153.

Gomez Díaz, G. & H. Nakagawa. 1990. Effects of dietary carbohydrates on growth and body components of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquat. Living Resour., 3: 99-105.

González-Peña, M.C., A.J. Anderson, D.M. Smith & G.S. Moreira. 2002. Effect of dietary cellulose on digestion in the prawn *Macrobrachium rosenbergii*. Aquaculture, 211: 291-303.

Guo, R., Y.J. Liu, L.X. Tian & J.W. Huang. 2006. Effect of dietary cornstarch levels on growth performance, digestibility and microscopic structure in the white shrimp *Litopenaeus vannamei* reared in brackish water. Aquac. Nutrition, 12: 83-88.

Hall, M.R. & E.H. van Ham. 1998. The effects of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. J. World Aquac. Soc., 29: 290-299.

Johnston, D.J. & D. Yellowlees. 1998. Relationship between the dietary preferences and digestive enzyme complement of the slipper lobster *Thenus orientalis* (Decapoda: Scyllaridae). J. Crust. Biol., 18: 656-665.

Lee, P.G. & A.L. Lawrence. 1997. Digestibility. Adv. World Aquac., 6: 194-260.

Linton, S.M., P. Greenaway & D.W. Towle. 2006. Endogenous production of endo-β-1,4-glucanase by decapods crustaceans. J. Comp. Physiol., B 176: 339-348.

Martínez, P., J. Galindo & N. Aranda. 1999. Determinaction de la actividad proteolítica en rumen mediante un método colorimétrico. Rev. Cubana Cienc. Agric., 33: 83.

Mourente, G. & A. Rodríguez. 1991. Variation in the lipid contentof wild caught females of the marine shrimp *Penaeus keraturus* during sexual maturation. Mar. Biol., 110: 21-28.

New, M.B. 1976. A review of dietary studies with shrimp and prawn. Aquaculture, 9: 101-104.

New, M.B. 1990. Freshwater prawn culture: a review. Aquaculture, 88: 99-143.

Pascual, C., A. Sánchez, F. Vargas-Albores, G. LeMoullac & C. Rosas. 2003. Haemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: effect of an extreme temperature. Aquaculture, 218: 637-650.

Petriella, A.M. 1984. Estudio del ciclo de muda del camarón *Artemesia longinaris* Bate (Decapoda, Penaeidae). I. Setogénesis. Physis, Secc. A, 42(103): 93-100.

Petriella, A.M., M.I. Müller, J.L. Fenucci & M.B. Saez. 1984. Influence of dietary fatty acids and cholesterol on the growth and survival of the argentine prawn *Artemesia longinaris* Bate. Aquaculture, 37: 11-20.

Rabid, T., A. Tiezt, M. Khayat, E. Bohem, R. Michelis & E. Lubzens. 1999. Lipids accumulation in the ovaries of a marine shrimp *Penaeus simisulcatus* (De Haan). J. Exp. Biol., 202: 1819-1829.

Romanos-Mangialardo, R. & J.L. Fenucci. 2002. Effect of different dietary Arginine and Lysine leves for prawn *Artemesia longinaris* Bate (Crustacea, Decapoda). In: E. Escobar-Briones & F. Alvarez (eds.). Modern approaches to the study of crustacea. Kluwer, Academic, New York, pp. 79-84.

Rosas, C., G. Cuzon & G. Gaxiola. 2001. Metabolism and growth of juveniles of *Litopenaeus vannamei* effect of salinity and dietary carbohydrate levels. Aquaculture, 259: 1-22.

Rosas, C., G. Cuzon & G. Gaxiola. 2002. An energetic and conceptual model of the physiological role of dietary carbohydrates and salinity on *Litopenaeus vannamei* juveniles. J. Exp. Mar. Biol. Ecol., 268: 47-67.

Rosas, C., G., Cuzon, G. Gaxiola, C. Pascual, P. Brito, M.E. Chimal & A. van Wormhoudt. 2000. El metabolismo de los carbohidratos de *Litopenaeus setiferus*, *L. vannamei* y *L. stylirostris*. Avances en Nutrición Acuícola, 5: 340-359.

Sánchez, A., C. Pascual, F. Vargas-Albores, G. Le Moullac & C. Rosas. 2001. Haemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: the effect of acclimation. Aquaculture, 198: 13-28.

Shiau, S.Y. 1997. Carbohydrates and fiber. In: L.R. D'Abramo, D.E. Conklin & D.M. Akiyama (eds.). Crustacean nutrition. Advances in World Aquaculture. World Aquac. Soc., 6: 108-122.

Shiau, S.Y. & C.Y. Peng. 1992. Utilization of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon*, reared in seawater. Aquaculture, 101: 241-250.

Sokal, R. & J. Rohlf. 1995. Biometry, the principles and practice of statistics in biological research. W.H. Freeman, New York, 887 pp.

Tacon, A.G.J. 1990. Standard methods for the nutrition and feeding in farmed fish and shrimp. Argent Laboratories Press, Redmonth, Washington, 454 pp.

Teshima, S.I. 1998. Nutrition of *Penaeus japonicus*. Rev. Fish. Sci., 6: 97-111.

Verri, T., A. Mandal, L. Zili, D. Bossa, P.K. Mandal, V. Ingrosse, S. Zonno, S. Vilella, G.A. Ahearn & C. Storelli. 2001. D-Glucose transport in decapod crustacean hepatopancreas. Comp. Biochem. Physiol., 130A: 585-606.

Wigglesworth, J.M. & D.R. Griffith. 1994. Carbohydrate digestion in *Penaeus monodon*. Mar. Biol., 120: 571-578.

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