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Oxygen consumption and ammonia excretion of *Penaeus setiferus*, *P. schmitti*, *P. duorarum* and *P. notialis* postlarvae fed purified test diets: effect of protein level on substrate metabolism

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Abstract

Taking into account that the relationship between metabolic substrate and the characteristics of the diet is essential for understanding the mechanisms associated with the use of the components of a certain food substance, the aim of this study was to examine the metabolic substrate used by *Penaeus setiferus*, *P. schmitti*, *P. duorarum* and *P. notialis* postlarvae PL 35-40 (35-40 days after metamorphosis) fed purified diets with different levels of proteins (40, 50, 60 and 65 %). Oxygen consumption and ammonia excretion were measured in animals in intermoult which had been acclimatized to each diet over a minimum of 5 days. Measurements were taken on fasted (24 h) and fed animals. Oxygen consumption of fasting animals increased with a rise in the proteins of the diet in the four species. In the fed animals, this tendency was observed in *P. setiferus* and *P. notialis*, while in *P. schmitti* and *P. duorarum* oxygen consumption was a weak relationship, decreasing or increasing with a diet with 50 % protein. Both in fasting and feeding animals, the ammonia excretion increased with the increase in proteins in the diet. The O : N atomic ratios of the four species decreased (between 30 and 3), according to increase in dietetic protein : energy ratio, (10.4 to 17.0 mg protein/kcal). The highest value of O : N ratio was observed in *P. setiferus* (40 % of protein requirement, O : N = 24 to 34) and lowest in *P. schmitti* (60 % of protein requirement, O : N = 4 to 11), with intermediate values in *P. duorarum* and *P. notialis* (50 % protein requirement, O : N = 8 to 14 and 13 to 19, respectively). These results support previous research that showed that the omnivorous-herbivorous species, like *P. setiferus*, use protein-lipids normally as energy source in contrast to omnivorous-carnivorous species such as *P. duorarum* which use protein preferentially as a source of energy.

Keywords: Oxygen consumption, nitrogen excretion, metabolism, *Penaeus* sp., protein level.

Consumo de oxigeno y excreción de amonio de las postlarvas de Penaeus setiferus, P. schmitti, P. duorarum, *y* P. notialis *alimentadas con dietas purificadas: effecto del nivel de proteínas en el sustrato metabólico.*

Resumen

Teniendo en consideración que la relación entre el sustrato metabólico y las características de la dieta son esenciales para entender los mecanismos asociados con el uso de los componentes del alimento, el objetivo de este estudio fué el de examinar el sustrato metabólico usado por las postlarvas PL 35-40 (35-40 días después de la metamorfosis) de *Penaeus setiferus*, *P. schmitti*, *P. duorarum* y *P. notialis* alimentadas con dietas purificadas elaboradas con diferentes niveles de proteínas (40, 50, 60 y 65 %). El consumo de

oxígeno y la excreción de amonio fueron medidos en animales en intermuda los cuales fueron aclimatados a cada dieta por un mínimo de 5 días. Las mediciones metabólicas fueron realizadas en animales con 24 h de ayuno y después de haber sido alimentados. El consumo de oxígeno de ayuno de los animales de las cuatro especies aumentó en relación al incremento de las proteínas en la dieta. En animales alimentados, esta tendencia fué observada en *P. setiferus* y en *P. notialis*, mientras que en *P. schmitti* y en *P. duorarum* el consumo de oxígeno no presentó relación con el aumento en las proteínas de la dieta. Tanto en los animales en ayuno como alimentados de las cuatro especies, la excreción de amonio aumentó con el incremento de las proteínas de la dieta. La razón O:N de las cuatro especies disminuyó (entre 30 y 3), de acuerdo con el incremento de la razón proteína:energía de la dieta (10.4 a 17 mg proteina/kcal). Los mayores valores de O:N fueron observados en *P. setiferus* (40% de requerimiento proteico O:N=24 a 34) y los menores valores en *P. schmitti* (60% de requerimiento proteico y O:N=4 a 11), con valores intermedios en *P. duorarum* y *P. notialis* (50% de requerimiento proteico y O:N=8 a 14 y 13 a 19, respectivamente). Estos resultados sostienen hipótesis previas que demuestran que especies omnivoras-herbivoras, tales como *P. setiferus* usan las proteínas y los lípidos normalmente como sustrato energético en contraste con las especies omnivoras-carnivoras que, como *P. duorarum*, usan preferencialmente las proteínas como fuente de energía.

Palabras clave : Consumo de oxígeno, excreción nitrogenada, metabolismo, *Penaeus* sp., nivel proteico.

INTRODUCTION

The estimated dietary protein requirements of most shrimp species vary between 25 and 60%, which suggests that amino acids are a substrate for the energy metabolism (García and Galindo, 1990; Gaxiola, 1994; Harris, 1959; Tacon, 1990). It has been reported that proteins (Andrews *et al.*, 1972; Alava and Lim, 1983) provide the amino acids for growth or other metabolic functions (intracellular osmotic regulation, intermediate metabolism, etc.). Nitrogen metabolism that supports the oxidation of amino acids may follow different pathways depending on the feeding experimental conditions (Mayzaud and Conover, 1988). Ammonia is the primary excretory product of protein catabolism in crustaceans and accounts for over 70% of the nitrogen excreted (Quetin *et al.*, 1980). For that reason nitrogen excretion, measured as ammonia excretion, is a good indicator of oxidation of amino acids. When shrimp are fed with different protein levels, the evaluation of nitrogen excretion may be used as an indicator of the capabilities of a species for using protein as energy source (Regnault, 1981; Dall and Smith, 1986).

The oxygen:nitrogen ratios (O:N) have been used in estimating the nature of the substrate being oxidized in Crustacea (Harris, 1959; Conover and Corner, 1968; Dall and Smith, 1986; Regnault, 1979, 1981). Theoretical values of O:N have been indicated for the use of different substrates. For example, values of 3 to 16 have been suggested for proteins, while equal amounts of lipid and protein catabolism will correspond to values between 50 and 60. Values greater than 60 will correspond to lipids and carbohydrates (Mayzaud and Conover, 1988). Taking into account that the relationship between metabolic substrate and the characteristics of the diet is essential for an understanding of the mechanisms associated with the use of the components of a certain food substance, this study is focused on defining the metabolic substrate used by the postlarvae (PL 35-40) of *P. setiferus*, *P. schmitti*, *P. notialis* and *P. duorarum* fed purified diets with different levels of proteins.

MATERIAL AND METHODS

Animals and conditioning

The postlarvae of the four species were obtained from larvae bred under controlled laboratory conditions. Postlarvae of *P. setiferus* and *P. duorarum* were obtaining in the experimental hatchery laboratory located in the Centro Regional de Investigaciones Pesqueras of Lerma, Campeche, Mexico. Postlarvae of *P. schmitti* and *P. notialis* were obtained from the Hatchery Commercial Center of Tunas de Zaza, Cuba. In all cases the breeding temperature was $28 \pm 2°C$ and salinity was $36 \pm 2‰$ and were placed under a light/dark cycle of 14/10 hours. Once the organisms had reached PL_5 (5 days after metamorphosis) shrimp species were kept in plastic tanks ($23 \times 30 \times 45$ cm-$H \times W \times L$; 30 l) at densities of 1.6 animals/l. The animals remained in these tanks for 30 days, a temperature of $28 \pm 2°C$, a pH of 8.5 ± 0.1 and an oxygen concentration greater than 5 mg/l. During this time, a standard balanced diet (55% protein) (García and Galindo, 1990) was given twice a day *ad libitum*. To avoid accumulation of uneaten food and metabolic excreta, the water in the tanks was partially changed (50%) twice a day.

Two hundred postlarvae of each species (*P. setiferus* live weight 23.4 ± 2.3 mg/animal; *P. schmitti* live weight 28.2 ± 1.2 mg/animal; *P. duorarum* live weight 31.3 ± 2.3 mg/animal; *P. notialis* live weight 27.3 ± 3.3 mg/animal) were then separated into four groups and assigned a diet containing one of four different protein levels (40, 50, 60 and 65%, *table* 1). Casein, with 90% protein, was employed as the main source of protein in the purified diets. L-Arginine-HCl, was added to the casein to improve the amino acid balance, and to approximate it to the amino acid

Table 1. – Composition (%, dry weight) of diets containing different levels of protein fed to experimental groups.

Ingredients	A	B	C	D
Caseine	43.3	54.1	64.9	70.4
Arginine	1.1	1.4	1.7	1.8
Malto-dextrine	37.9	26.8	15.7	10.1
Cod liver oil	2.3	2.3	2.3	2.3
Sunflower seed oil	2.25	2.25	2.25	2.25
Soybean lecithin	1.0	1.0	1.0	1.0
Cholesterol	0.5	0.5	0.5	0.5
Ascorbic acid	0.5	0.5	0.5	0.5
Vitamins and minerals pre-mix**	2.5	2.5	2.5	2.5
Carboxymethyl cellulose	5.0	5.0	5.0	5.0
Proteins, %	40	50	60	65
* Digestible Energy kcal/g	3.83	3.83	3.83	3.83
P/E	10.40	13.05	15.70	16.97
Lipids, %	6.50	6.50	6.50	6.50
CHO: lipids	5.83	4.12	2.41	1.55

* Calculated from Nose (1979) with values of 4 kcal/g to proteins and carbohydrates and 9 kcal/g to lipids.

** From Purina de Mexico.

composition known for the penaeid abdominal muscle (Teshima *et al.*, 1986). The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding water until a stiff dough resulted. This was then passed through a meat grinder to form 5 mm diameter pellets. These pellets were dried at 60°C using an electrical stove. After drying, the diets were broken up and sieved into convenient pellet size and stored at −4°C.

Each group was given the test diet twice a day *ad libitum* for a period of not less than 5 days. During this time, water quality parameters similar to those during acclimatization were maintained.

Oxygen consumption

Oxygen consumption was measured individually in a closed system of respirometer chambers of 50 ml. These chambers were placed in a temperature-controlled water bath and kept at 28 ± 0.2°C and filled with sterilized (UV) natural sea water (36 ‰). Before the experiments were begun, 20 postlarvae fasted for 24 hours were acclimatized to the chambers for 60 min to reduce the effects of handling. Preliminary results showed that this period of fasting and acclimatization produced a low and uniform metabolism which reflects metabolism during fasting.

After acclimatization water in the chambers was replaced by water previously filtered, sterilized (UV) and aerated. An initial sample was carefully taken before the chambers were closed for 2.5 h. The oxygen consumption of the animals that were fasting was calculated from the difference between the concentration of oxygen before and after the closing of the chambers. The concentration of oxygen was measured with a digital oxygen meter and an YSI 50 B polarographic sensor (± 0.05 mg/l) that had previously been calibrated with O_2 saturated water. These results were corrected using the data obtained from two control chambers with no shrimp.

The same shrimp in the chambers were fed the test diet at the rate of 3 mg/animal (mean of 10 % of body weight) after the fasting metabolism was ascertained. The chambers were carefully opened and the sea water partially and slowly changed. Afterwards, the food was added to each chamber. The same amount of food was placed in two control chambers to eliminate a source of error in the form of food decomposition and microbial action due to uneated food. In almost all cases the oxygen consumption of control chambers was negligible. To measure the oxygen consumption in fed animals, the process described above was followed. Preliminary experiments demonstrated that the shrimp reached the peak of oxygen consumption during the first 3 hours after feeding. In almost all cases shrimps ingested all the food presented within 30 minutes. The respirometric chambers were covered to eliminate the possible interference provoked by personnel in the laboratory.

Nitrogen excretion

In order to determine the nitrogen excretion, water samples were taken at the same time the oxygen of the chambers was measured. As in the case of oxygen consumption, the concentration of ammonia was checked before and after closing the chambers. The concentration of ammonia was measured with an ammonia electrode connected to an ORION 720A ion multianalyzer and calibrated according to the recommendations of Dall and Smith (1986).

Statistical analysis

Both metabolic rates were measured between 10.00 and 16.00 h. A paired t-test was used to compare metabolic rate and nitrogen excretion before and after feeding the same animals with each diet. The Kruskal-Wallis non-parametric test was used to define the significant differences between the oxygen consumption and the nitrogen excretion of each species (Zar, 1974). Level of significance was determined at $p < 0.05$.

RESULTS

Oxygen consumption (values are expressed in μg-at O_2 g^{-1} dry weight h^{-1})

Oxygen consumption in fasting *P. setiferus* (*fig.* 1*a*) ranged between 61 and 238 μg-at O_2 g^{-1} dry weight h^{-1}. Diets containing 40 and 50 % protein produced values significantly lower than those from shrimp fed diets containing 60 and 65 %. Similarly, oxygen

consumption of fed animals was lowest with 40 and 50% of protein (93.8 and 128.2 respectively) and greatest with 60 and 65% (513 and 435 respectively). Oxygen consumption increased progressively between fasted and fed shrimp as protein levels in the diet increased.

Oxygen consumption in *P. schmitti* (*fig.* 1*b*) showed generally higher levels than those registered for *P. setiferus*. Oxygen consumption for fasted shrimp previously fed a diet containing 40% protein (134.5) was significantly lower than that for fasted shrimp previously fed a diet containing 50, 60 and 65% protein (170.3, 177.4 and 198.1 respectively). The lowest value for oxygen consumption among fed postlarval *P. schmitti* were those fed the diet containing 50% protein (265) followed for those fed 40% protein (354.5) and 60 and 65% (392 and 402). Oxygen consumption of fed animals under the respective diets was between 150 and 260% greater than oxygen consumption of fasted animals.

Oxygen consumption during fasting in *P. duorarum* (*fig.* 1*c*) was irregular with respect to the protein content of the diet. The greatest oxygen consumption values were registered with shrimp fed 40 and 65% protein (305 and 300 respectively) and the lowest with those fed 50% protein (214) and 60% protein (264). The lowest values for fed animals were obtained with 40% (416) and 60% protein (439) and the greatest with 50% (558) and 65% protein (553) ($p<0.05$). The increase between fed and fasted animals was between 140 to 260%; the lowest level of increase with 40% protein and the greatest with 50% protein.

Oxygen consumption during fasting in *P. notialis* (*fig.* 1*d*) showed a maximum oxygen consumption rate is shrimps previously fed a diet containing 60% of protein with values of 398, ($p<0.05$). Fasted shrimp previously fed 65% protein exhibited a value similar to that obtained with fasted shrimp previously fed a diet containing 40% protein (208). Oxygen consumption in fed animals paralled for fasting shrimp. Values for 60% protein (613) were significantly greater than those obtained with shrimp fed with 40, 50 and 65% of protein (average value of 435). The increase in oxygen consumption between the fed/fasting animals varied between 154 and 203%.

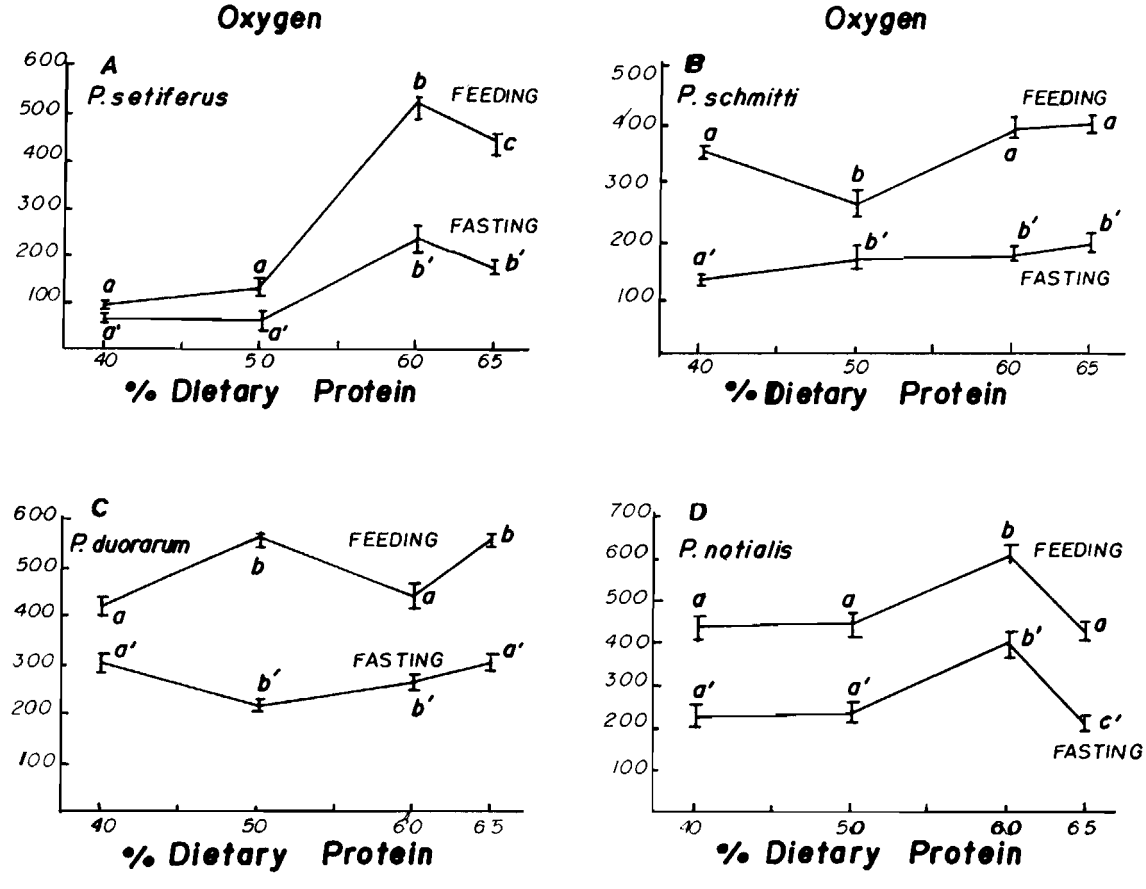

Figure 1. – Oxygen consumption (μg-atoms oxygen g^{-1} dw h^{-1}) of feeding and fasting shrimps. Each point represents mean + S.E. of 8 *P. setiferus* postlarvae, 18 *P. schmitti* postlarvae, 10 *P. duorarum* postlarvae and 19 *P. notialis* postlarvae. Different letters mean significant differences between means: a to c for feeding animals, a′ to c′ fasting animals.

Nitrogen excretion (values are expressed in μg-at N-NH_3 g^{-1} dry weight h^{-1})

The nitrogen excretion of *P. setiferus* (*fig.* 2*a*) during fasting increased with respect to the dietary protein and was lowest for 40 and 50% protein (2.1 and 2.5 μg-at N-NH_3 g^{-1} dw h^{-1}) and greatest for 60 and 65% protein (16.9 and 24). The same tendency appeared in the fed animals with 40 and 50% protein (2.6 and 3.8) being lower than those for animals fed the 60% (32.5) and 65% protein diets (54) ($p<0.05$). The amount of ammonia excreted by the fed animals was between 120 and 225% higher than that registered in fasting animals. Significant differences were not found between fasting and fed animals for 40 and 50% protein diets.

The nitrogen excretion of *P. schmitti* (*fig.* 2*b*) during fasting increased progressively with an increase in dietary protein level with significantly lower values for 40 and 50% protein (3.2 and 5.7) than those for 60% (17.3) and 65% protein (28.9). A progressive increase in the ammonia excretion was noted in the postlarvae of *P. schmitti* fed 40, 50 and 60% protein (21.7, 31.2 and 90.8 respectively) with a decline in ammonia excretion for animals fed 65% protein (63.46). The nitrogen metabolism related to food intake was between 266 and 670% greater for fed animals than for fasting animals.

The nitrogen excretion of *P. duorarum* (*fig.* 2*c*) during fasting significantly increased from animals previously fed 40% protein (23.59 μg-at N-NH_3 g^{-1} dw h^{-1}) to those fed 60% protein (41.3) and to 65% of protein (66.1). Fasted animals previously fed the 50% protein diet had the lowest amount of nitrogen excretion (17) which did not differ significantly from that obtained with 40%. The fed animals showed an increase in nitrogen excretion associated with the increase in proteins in the diet. Thus, levels of 38 (40% protein), 77.2 (50% protein), 98.2 (60% protein) and 237.6 (65% protein) were obtained. These values were between 161 and 454% greater than those obtained when fasting shrimps.

The nitrogen excretion of *P. notialis* (*fig.* 2*d*) during fasting increased progressively from 40 to 60% protein with a decrease for 65% protein. The values obtained were 12.7 (40% protein), 18.8 (50% protein), 45.3 (60% protein) and 20.1 (65% protein). This same tendency was seen in the fed animals with a

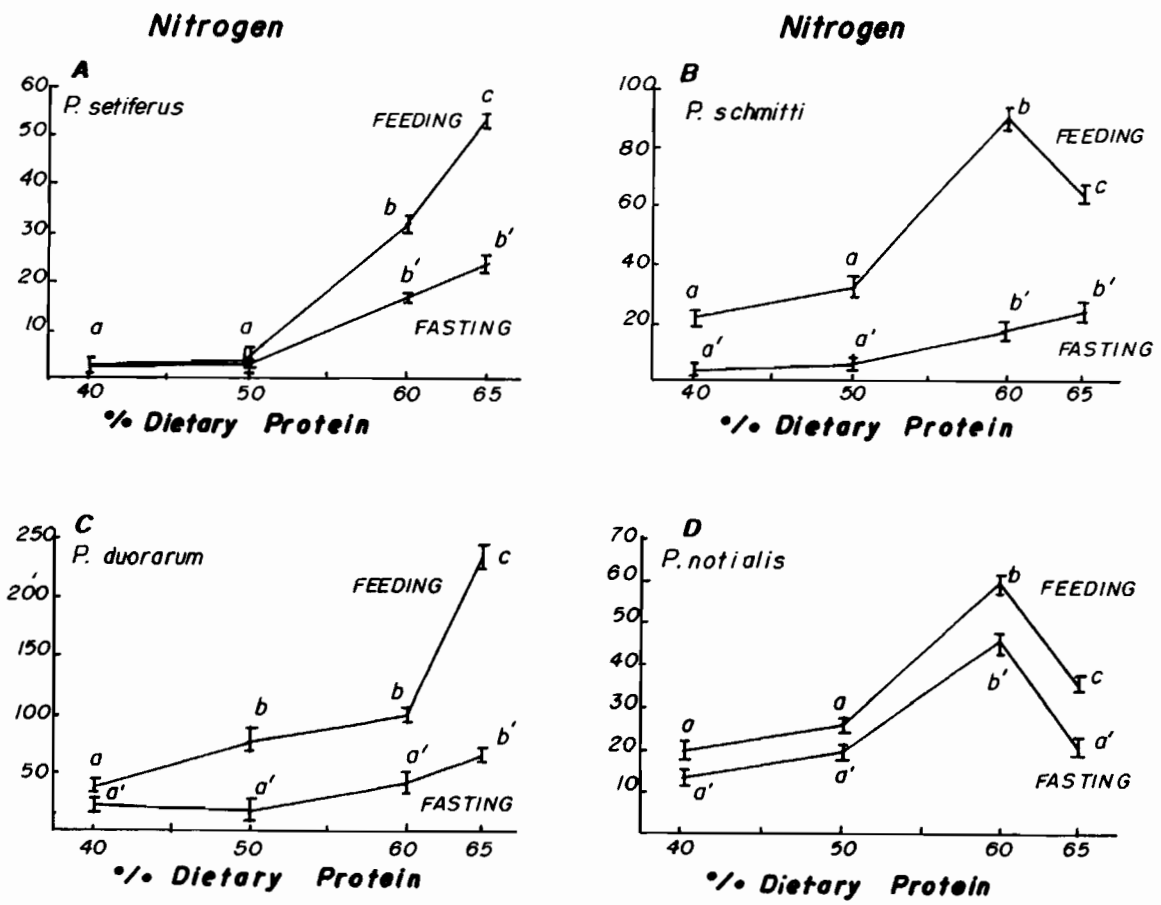

Figure 2. – The ammonia excretion (μg-atoms nitrogen g^{-1} dw h^{-1}) of feeding and fasting shrimps. Each point represents mean ± S.E. Number of animals as in *figure* 1. Different letters mean significant differences between means: a to c for feeding animals, a′ to c′ fasting animals.

progressive increase from 19.4 for 40% protein and 25.5 for 50% protein, to 60.3 for 60% protein and 35.1 for 65% protein. Increased excretion of nitrogen varied due to feeding between 133 and 175%, with the significant differences for 60 and 65% of protein.

Oxygen:Nitrogen ratio

A reduction of the O:N ratio in fasting shrimps was recorded for the four species with respect to an increase in the level of protein of the diet (*fig.* 3).

The O:N of fasting *P. setiferus* varied between 32.2 and 7.1 for 40 and 65% protein, respectively. No significant difference was found between the shrimp fed with 40 and 50% protein (36 and 34.1, respectively; *fig.* 3*a*). In the case of *P. schmitti* (*fig.* 3*b*), O:N values for fasting animals were 41 to 9.2 for 40 to 65% protein. The fed animals showed O:N values lower than those registered during fasting, with levels of 17.66 to 7.43 between 40 and 65% protein. The O:N during fasting varied between 13.2 with 40% protein and 4.6 with 65% protein for *P. duorarum* (*fig.* 3*c*). No significant difference was found between those fasting animals previously fed with 40 and 50% protein. Significantly lower values were observed in fed animals than in fasting ones and varied between 12.3 and 2.3 (40 to 65% protein, respectively). O:N ratios at all protein levels were statistically different from each other. In *P. notialis* (*fig.* 3*d*), O:N values during fasting showed levels between 24.4 and 10.7 with 40 and 65% protein, respectively. No difference was found between 60 and 65% protein. Similar variations were observed in the fed organisms with values slightly greater than those registered in fasting shrimps.

DISCUSSION

The O:N ratio of the four species showed that these shrimp are capable of utilizing different metabolic substrates according to the type of food they are offered. In the four species, O:N resulting from animals fed diets with 40% protein (between 12 and 40) is higher than from diets with 65% (between 2 and 12) both in fasting or feeding animals. The O:N ratio has been calculated during fasting in an attempt to separate the metabolism for maintenance from feeding

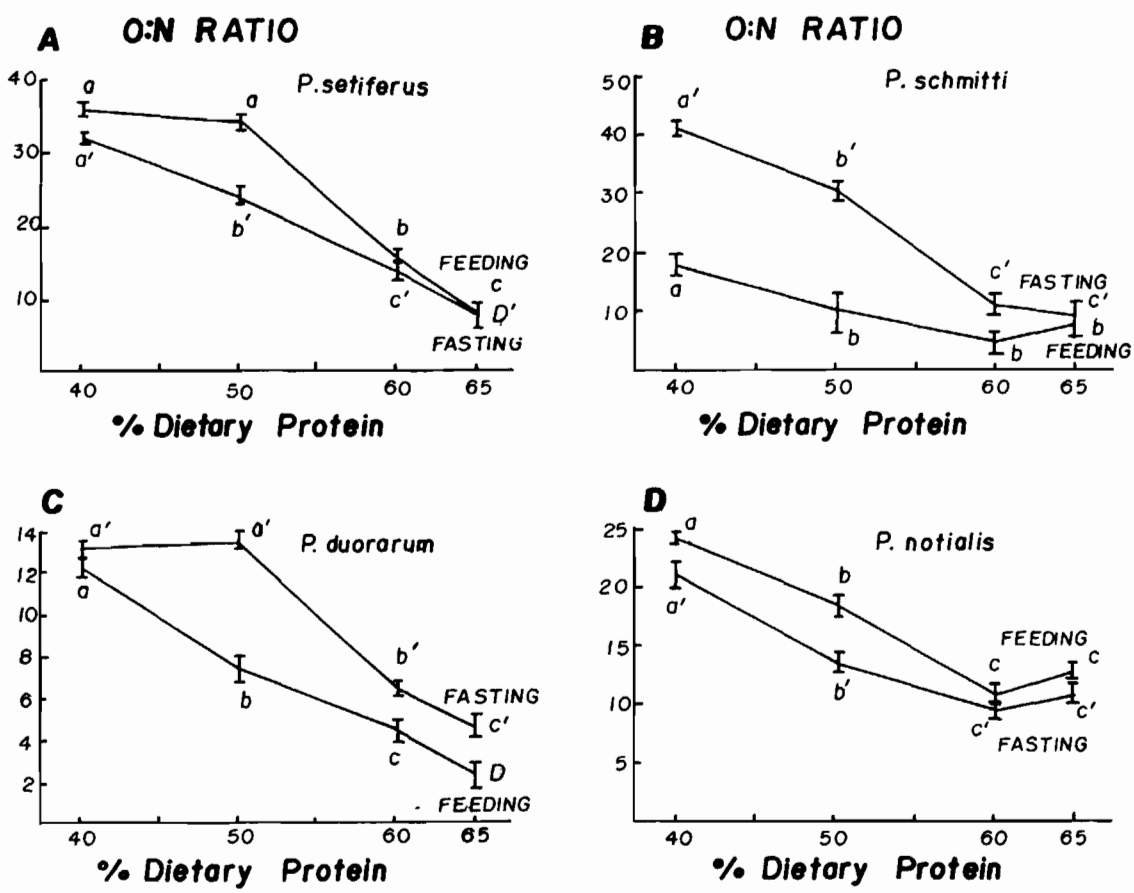

Figure 3. – Oxygen:Nitrogen (atomic ratios) of feeding and fasting shrimps. Each point represents mean ± S.E. of 8 *P. setiferus* postlarvae, 18 *P. schmitti* postlarvae, 10 *P. duorarum* postlarvae and 19 *P. notialis* postlarvae.

metabolism and to define the relationship between the energy reserves for immediate use and feeding.

Metabolic rate was lower in fasted animals. Similarly oxygen consumption and nitrogen excretion were significantly lower than values recorded in fed animals. The recorded increase in nitrogen excretion related to changes in the diet protein contents, in addition to the peak values in oxygen consumption observed in fasted *P. setiferus* and *P. notialis*, may reflect a difference in its metabolic response due to feeding before the experiment. Despite the difficulty in determining anabolism, it can be indirectly estimated and distinguished from catabolism, through observations of the metabolic activity in fasting animals. Since catabolism depends on both the nutrition state and the trophic history it can be used as a valuable indicator of the diet contribution to the storage reserve system (Conover and Mayzaud, 1976). Nitrogen metabolism that supports the oxidation system may follow different pathways depending on the feeding experimental conditions (feeding vs fasting) as has been shown by Mayzaud and Conover (1988) in *Calanus finmarchicus*. Under fasting conditions the amino acid pool functions as a buffer between body protein degradation and the intermediate metabolism requirement, which is a reflection of the ability of the organism to withstand such a condition. The amino acid pool is determined by the nutritional history of the individual (Bidigare, 1983), therefore any changes in the ammonia excretion and in the respiratory metabolism can be attributed to this factor. In fasted animals changes recorded in the ammonia excretion may be a reflection of the protein content of the supplied diet.

Mayzaud and Conover (1988) have shown, through theoretical estimations, that protein catabolism in crustaceans exhibits O:N ratios ranging from 3 to 16. Equal quantities of protein and lipids from catabolism present ranges from 50 to 60. In the present study these intervals were used to interpret the biochemical fractions from the shrimp catabolism. Thus when fasting, *P. setiferus* and *P. schmitti* utilized lipids and protein (40 and 50% protein), and protein (60 and 65% protein), *P. duorarum* oxidized proteins and *P. notialis* oxidized lipid and protein (40% proteins) and mainly proteins (50, 60 and 65% protein).

Barclay *et al.* (1983) indicated muscle as the storage site of metabolic reserves, which at short notice could supply the lipid and protein necessary for the supply of energy. Furthermore, various authors have demonstrated that while protein are the most important reserve substrate during prolonged fast in crustaceans, lipids and carbohydrates are utilized in normal conditions of feeding or during short fasts (Dall and Smith, 1986; Winget *et al.*, 1977; Dean and Vernberg, 1965; Chang and O'Connor, 1983; Chan *et al.*, 1988; Rosas *et al.*, 1992). Thus, a minimum period of 5 days is sufficient to evaluate the effects of a certain type of food on shrimp postlarvae and the metabolic substrate accumulated by the organisms under a particular diet. The high metabolic rate that is characteristic of this stage of development makes possible the rapid metabolization of the components of the food tested in this study.

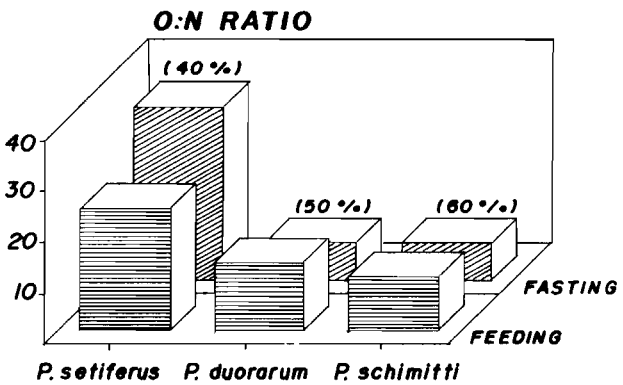

Figure 4. – Oxygen : Nitrogen ratio of feeding and fasting *P. setiferus*, *P. duoraum* and *P. schmitti* postlarvae in relation to protein requirement (in parentheses).

Penaeid shrimp are known for using protein as a main source of energy (Regnault, 1979, 1981), and therefore the determination of their nutritional requirement may consider amino acid supply to be used for growth, and carbohydrate intake for chitin production and lipid synthesis (Tacon, 1990). Hence the fed shrimp O:N ratio should reflect not only the type of oxidized substrate, but also the metabolic relation between the animals and the biochemical composition of the ingested food (Mayzaud and Conover, 1988). Postlarvae of *P. setiferus* and *P. notialis* fed with diets with 40 and 50% protein and those of *P. schmitti* with 40% protein displayed O:N ratios greater than 16 indicating both protein and lipid catabolism. However, when the protein percentage in the experimental diet increases, the O:N ratio fluctuates from 3 to 16, which can be interpreted as protein degradation. Ikeda and Dixon (1984) and Hewitt (1992) have pointed out that this process may well be associated to an increased deamination related to a protein surplus, rather than to a greater use of the dietary protein fraction as the metabolic substrate. Depending on the existing balance between the diet characteristics and the protein requirements, the deamination products (alpha-ketoacids) may or not be part of energy producing pathways in the Krebs cycle (Mayzaud and Conover, 1988).

One of the objectives of shrimp producers is to distribute diets that are converted more efficiently into matter and energy for growth. This efficiency, according to Tacon (1990), depends as much on the quality of the components as on the requirements. In this sense, O:N values may hold a greater importance if they are compared with the protein requirements for growth of each species. The relationship between the O:N and the protein requirements for growth

registered for three of the four species of shrimp postlarvae studied here fasting and feeding is showed in *figure* 4 (García and Galindo, 1990; Gaxiola, 1991; Gaxiola *et al.*, 1994). There is an inverse relationship between the O:N and the requirement of proteins with greater values (24 to 34: lipids and proteins) in the species with the lower requirement (40% *P. setiferus*) and lower values (4.5 to 10) in the species with the higher requirement (60% *P. schmitti*). A value between 7.4 and 13.5 was calculated with the requirement of 50% protein for *P. duorarum* (Gaxiola, 1994). The lack of information on the protein requirement of *P. notialis* makes a comparison impossible.

Several authors (Guitart and Hondares, 1980; McTigue and Zimmerman, 1991) have pointed out that carnivorous species tend to utilize substrates with high levels of protein more efficiently than omnivorous species, and this is reflected in O:N values. Thus, carnivorous crustaceans had values of O:N reported of 13.6 to 16.4 for *P. esculentus* (Dall and Smith, 1986) and 12.9 to 23.3 for *Homarus americanus* (Capuzzo and Lancaster, 1979), while those of omnivorous crustaceans had values of 21.8 to 54.7 for *Macrobrachium rosenbergii* depending on diet (Lipid to CHO ratio; Clifford and Brick, 1983), 6.1 to 34.2 for *Palaemonetes varians* depending on the time of year (Snow and Williams, 1971) and 27 for the Palaemonids *Crangon crangon* (Regnault, 1981). The four species studied herein utilized differently the diet components. In the purified diets, it was observed that when protein and the ratio protein/energy (P/E) increased, the ratio CHO/lipids diminished. Assuming that the dietary components and the capacity of the shrimp to use each nutritional fraction determine the catalyzed fraction, then the O:N values can be related to the efficiency of each species to assimilate its diet. Lovett and Felder (1990) suggested that such a capacity is genetically determined, and that it is a product of an adaptive mechanism which allows the species to optimize the use of available food resources in the ecosystem. *P. setiferus* and *P. schmitti* have been considered as omnivorous-herbivorous species, whereas *P. duorarum* and *P. notialis* are known for their omnivorous-carnivorous trophic habits (Guitart and Hondares, 1980; McTigue and Zimmerman, 1991). Results revealed that the postlarvae of *P. setiferus*, *P. notialis* and *P. schmitti* used more protein-lipids when CHO/lipids is higher and *P. duorarum* postlarvae used protein when the CHO/lipids ratio was high. Enzymatic studies conducted by Lovett and Felder (1990) have shown that *P. setiferus* postlarvae had limited proteolytic activity, related to a greater amylase activity. These differences have been attributed to an omnivorous-herbivorous strategy of this species (Lovett and Felder, 1990). Thus the O:N ratio has been demonstrated to be useful as an index of the differences between trophic habits of four shrimp postlarvae studied here. In this case the O:N ratio resulted in a form to evaluate the nutritional state of shrimp postlarvae fed with different protein levels. The relationship between O:N ratio and other physiological measures (osmotic pressure, apparent heat increment, etc.) should be studied before establishing the definitive relationship between both nutritional and physiological indices that improve postlarval shrimp culture.

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