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ARTICLE

The effect of protein and lipid level on the specific dynamic action and post-prandial nitrogen excretion in subadult of white shrimp *Litopenaeus vannamei*

El efecto del nivel de proteína y lípidos sobre la acción dinámica específica y la excreción postprandial en sub-adultos del camarón blanco *Litopenaeus vannamei*

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Resumen.- El objetivo del estudio fue evaluar el efecto de 4 niveles de proteína (20, 30, 40 y 50%) y lípidos (2, 4, 8 y 16%) sobre la magnitud y duración de la acción dinámica específica (ADE) y la excreción nitrogenada en subadultos del camarón blanco *Litopenaeus vannamei* usando una cámara metabólica controlada por computadora (respirómetro de flujo continuo). Se determinó la tasa de consumo de oxígeno a intervalos de 1 hora hasta que la tasa de consumo de oxígeno postprandial regresó al nivel de pre-alimentación. Los camarones alimentados con todas las dietas tienen una tasa de respiración significativamente alta después de la alimentación debida a la ADE. El consumo de oxígeno, el coeficiente de ADE y la magnitud de ADE aumentó notablemente con el incremento del contenido de proteína en la dieta. Los camarones alimentados con el 20% de proteína en la dieta tuvieron el nivel más bajo de respiración de pre y post-alimentación y la más baja ADE. Un cambio significativo en el coeficiente de ADE relativo a la energía digestible no fue demostrado para cada uno de los niveles de lípidos. Adicionalmente, la excreción nitrogenada aumento con el incremento del nivel de proteína en la dieta, pero no con el incremento del nivel de lípidos. Al estimar la ADE de subadultos se encontró que la tasa metabólica estándar (SMR) fue menor que la reportada para juveniles y postlarvas de *L. vannamei.*

Palabras clave: Oxígeno, ADE, amonio, camarón, proteínas, lípidos

Abstract.- The study aimed to evaluate the effect of 4 levels of dietary protein (20, 30, 40 and 50%) and lipids (2, 4, 8 and 16%) on the magnitude and duration of specific dynamic action (SDA) and postprandial nitrogen excretion in the subadult white shrimp *Litopenaeus vannamei* using computer-controlled metabolic chambers (continuous-flow respirometer). We determined the oxygen consumption rate at 1 h intervals until the postprandial oxygen consumption rate returned to the pre-feeding level. Shrimp fed all the diets had significantly higher respiration rates after feeding due to the SDA. Oxygen consumption, the SDA coefficient and the SDA magnitude increased notably with increasing dietary protein content. Shrimp fed the 20% protein diet had the lowest levels of pre- and post-feeding respiration and the smallest SDA. A significant change in the SDA coefficient relative to each lipid level was not demonstrable. Additionally, nitrogenous excretion increased with an increase of dietary protein but not with an increase of lipid level. By estimating the SDA of subadults, the response to standard metabolic rate (SMR) was lower than that reported for juveniles and postlarva white shrimp.

Key words: Oxygen, SDA, ammonia, shrimp, protein, lipid

INTRODUCTION

The specific dynamic action (SDA) reflects the metabolism of protein, lipids and carbohydrates, and the deamination and synthesis of proteins are the greatest contributing factors (Beamish & Trippel 1990). In crustaceans, the SDA has been described in a number of species including isopods, amphipods and decapods (Whiteley *et al.* 2001, McGaw & Curtis 2013), and it manifests as an increase in oxygen uptake which usually reaches peak values within 4 h of feeding and may remain elevated for over 48 h (McGaw & Reiber 2000, Mente *et al.* 2003). In decapod, postprandial rate of oxygen consumption (MO_2) can increase 2-4 fold over resting metabolic rate (Penney *et al.* 2016) and can remain raised for between 12 and 72 h

(McGaw & Curtis 2013). Protein utilization can be determined by the rate of oxygen consumption and ammonium excretion (Frisk *et al.* 2013), which has been shown to vary with species, dietary composition, ration level, food and animal size (McGaw & Curtis 2013). In relation to the level of lipids (8.5 to 10.5 g kg^{-1}) in the diet, Toledo *et al.* (2016) have found that there are no significant differences in the growth performance of shrimp.

During fasting, the ammonium excretion rates are low and are usually associated with food retention and growth (Lyytikäinen & Jobling 1998). There are some studies on the influence of dietary and non-dietary factors on the SDA in *L. vannamei*, such as protein content and ammonia excretion (Rosas *et al.* 1996, 2001a), growth rate (Nuno 1996) and water salinity (Du-Preez *et al.* 1992, Rosas *et al.* 2001b), but the results were fragmentary.

The energy expended in mechanical and biochemical processes, expressed as the SDA, and post-prandial nitrogen excretion (PPNE) have been related to the growth rate of shrimp. In shrimp, it has been found in both post-larvae and juveniles that a lower SDA and PPNE are related to better shrimp growth (Rosas *et al.* 1996, Taboada *et al.* 1998). In other aquatic organisms, it has been found that excessive protein in the diet causes high proportions of protein and energy to be used for excretion (Wang *et al.* 2016). A significant amount of energy consumed by shrimp is lost as nitrogenous excretory products. The ammonia excretion of penaeids has been studied for *L. vannamei* (Racotta & Hernández-Herrera 2000, Lin & Chen 2001, Li *et al.* 2007, 2016). The protein content of a meal can influence the SDA, and because protein is the most expensive dietary component in aquaculture, there is a great interest in the relationship between the SDA and the protein content of the diet. Cost-effective shrimp production requires optimal dietary protein-to-lipid ratios to minimize amino acid catabolism and maximize anabolism (Cho 1992). Furthermore, protein requirements change with shrimp size and its stage of growth (Lim & Sessa 1995), with young shrimp requiring more protein compared with larger shrimp on maintenance or production diets (Hilton & Slinger 1981). Consequently, despite the extensive research on optimal dietary protein-to-lipid ratios in various cultured shrimp species (Carter & Mente 2014), conflicting results still exist.

The objectives of this study were to evaluate the effect of different levels of protein and lipids in the diet on feeding metabolism in shrimp, to test whether the postprandial metabolic cost increases with increased protein and lipid levels in the diet of *L. vannamei* subadults.

Materials and methods

Shrimp collection and acclimatization

Intermolt subadult white *L. vannamei* (weight 11.0 ± 2.6 g) shrimp were obtained from a commercial farm in Sinaloa state, Mexico (23°10'43.37''N; 106°20'34.46''W). The animals were acclimated to laboratory conditions for at least 7 days. The shrimp were maintained in 8 cylindrical plastic tanks (600 L) at salinity of 35 ± 1.5 ups, with a 12 L:12 D photoperiod, within the optimal temperature range for growth (28 ± 0.5°C) for white shrimp (Ponce-Palafox *et al.* 1997) and within an aerated recirculation system. The stock shrimp were fed twice daily (2% biomass/day) with a diet containing approximately 35% protein and 6% lipid (commercial shrimp pellets). The animals were fasted for 3 d prior to supplying them with the protein and lipid diets. This period allowed all food to be evacuated from the digestive system but avoided the large-scale physiological changes associated with starvation (Wallace 1973).

Diet formulation and preparation

For the protein and lipid level experiments, two series of experimental dry diets (Table 1) were isoenergetic, containing 20, 30, 40 and 50% protein (15.60 to 16.50 MJ of estimated DE kg^{-1}) and 2, 4, 8 and 16% lipid (15.30 to 16.56 MJ of estimated DE kg^{-1}).

The diets were prepared at the Coastal Bioengineering Lab, National School of Fisheries Engineering, Autonomous University of Nayarit in San Blas, Nay, Mexico by first mixing all the finely ground dry ingredients together for at least 30 min in a Hobart Commercial Mixer (Hobart Manufacturing Company, Troy, OH, USA). The dry mashes were then steam pelleted using a California model CL 2 Laboratory pellet mill. Thereafter, the pellets were dried immediately in a custom-made vertical cooler. Subsequently, the pelleted diets were kept in air-tight containers at 4°C until required.

Proximate analysis was performed using standard methods (AOAC 2000). Prior to experimentation in the metabolic chambers and during the trials, shrimp were fed for 10 days in the stock tanks with the corresponding diet.

Oxygen consumption

Thirty-five intermolt subadult shrimps were utilized from each treatment to analyze individual oxygen consumption in a continuous-flow respirometer. Oxygen consumption (MO_2, mg O_2 kg^{-1} h^{-1}) was determined according to the method described by Li *et al.* (2007). Every hour during a 24 h period, the O_2 concentration (mg L^{-1}) in the metabolic chamber and control water was measured.

$$MO_2 = ((O_0 - O_t + O_c) \cdot V) \cdot (WT)^{-1} \quad (Ec.1)$$

where O_0 is the initial oxygen (mg L^{-1}); O_t is the final oxygen concentration in the shrimp container (mg L^{-1}); O_c is the final oxygen in the control (mg L^{-1}); V is container volume (L); W is weight of the shrimps (g) and T is the duration (h).

Six metabolic chambers (18.5 x 6.5 x 15.5 cm), each with a capacity of 1,864 mL, were supplied from a flow-through respirometry system modified of Chakraborty *et al.* (1992). The water was matched to that of the stock holding conditions. The water quality in the recirculation system was controlled using aeration, solid filtration and charcoal filtration. The metabolic chambers were partitioned internally using a stiff mesh sheet so that no shrimp could transfer between the upper and lower sections. The capacity of the upper active chamber was 900 mL. Water flow for each chamber was calculated from the volume of water that passed through the chamber (the median water flow was 3.45 ± 0.27 L h^{-1}). A small feeding port in the lid allowed the introduction of the diet. The lower chamber could be evacuated via a valve which allowed feces and water to be collected for determination of ammonia excretion content. The metabolic chambers were managed according to the protocols of Chakraborty *et al.* (1992).

Table 1. Formulation and proximate composition of experimental diets / Formulación y composición proximal de las dietas experimentales

Ingredient	Protein (%)				Lipids (%)			
	20	30	40	50	2	4	8	16
Fish meal[1]	19	25.2	32	35	28	28	28	28
Soybean meal[2]	13	15	18	19	14	14	14	14
Shrimp meal[3]	6	6	6	6	3	3	3	3
Corn meal[4]	4	2	2	1	2	2	2	2
Fish oil[5]	5	5	5	5	0.5	1	4	6
Soybean oil[6]	2	2	2	2	2	2	2	2
Starch[7]	45.3	39.1	29.3	26.3	44.8	44.3	41.3	39.3
Vitamins[8]	2	2	2	2	2	2	2	2
Minerals[9]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
C vitamin[10]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Binder[11]	2	2	2	2	2	2	2	2
Crude protein (g kg^{-1})	202.2	309.1	398.4	499.8	295.1	297.5	301.2	307.9
Crude lipid (g kg^{-1})	69.1	67.3	66.7	65.1	21.8	39.8	81.6	159.1
Ash (g kg^{-1})	56.3	65.3	75.1	87.8	75.7	57.4	65.2	50.5
DE (MJ kg^{-1})	15.60	16.04	15.70	16.50	15.32	15.30	16.12	16.56
DP*	284.0	299.4	317.7	352.0	270.6	271.5	280.1	286.3
DP:DE*	18.21	18.66	20.23	21.33	17.66	17.74	17.38	17.29

[1]Fish meal (Peruvian): crude protein 640 g kg^{-1}, crude lipid 87 g kg^{-1} (dry weight basis)

[2]Soybean meal (defatted): crude protein 465 g kg^{-1}, crude lipid 13 g kg^{-1}

[3]Shrimp meal crude protein 415 g kg^{-1}, crude lipid 185 g kg^{-1} (dry weight basis)

[4]Corn meal crude protein 456 g kg^{-1}, crude lipid 65 g kg^{-1} (dry weight basis)

[5]Kindly provided by Guangdong Yuehai Feed Group Co. Ltd (Zhanjiang, Guangdong, China)

[6]Kindly provided by Guangdong Yuehai Feed Group Co. Ltd (Zhanjiang, Guangdong, China)

[7]Starch

[8]Vitamin g kg^{-1} premix: premix: thiamin HCl 1.5, riboflavin 9.0, pyrodoxine HCl 3.0, DL Ca-Pantothenate 15.0, nicotinic acid 15.0, biotin 0.15, folic acid 0.54, Vitamin B12 0.006, choline chloride 300.0, inositol 15.0, menadione 6.0, Vitamin A acetate (20 000 IU g)1) 15.0, Vitamin D3 (400 000 IU g)1) 0.006, DL-alphatocopherol acetate (250 IU g)1) 24.0, L-ascorbyl-2-polyphosphate(25%) Active C, 0.067, Alpha-cellulose 595.731

[9]Mineral g kg^{-1} premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428

[10]C vitamin, [11]Binder

*Digestible protein (DP) and digestible energy (DE) were calculated using published from digestible coefficients (Cousin 1995)

Experimental protocol

Stock shrimp were starved for 24 h before being transferred to the respirometers. The shrimp were blotted with absorbent paper to remove excess moisture before being individually weighed and placed into the metabolic chambers. Once installed, the water flow was reinstated and data collection was commenced, with the respiratory rate being logged every hour. The metabolic chambers were left without handling for 24 h to allow the shrimp to recover from handling stress and to adapt to the chambers. Of the 6 chambers available, one was always used as a control and contained no shrimp.

Prior to all experiments, data were collected for a period of 24 h, during which the shrimp were unstressed and unfed. This allowed the determination of the minimum resting respiratory rate for each shrimp, which was subsequently used in calculating the parameters of the SDA.

The resting metabolic rate (post-absorptive, minimal activity) was recorded for a 3 h control period. The shrimp were then fed a meal, and all had finished feeding by the time the first postprandial oxygen consumption reading was completed. Oxygen consumption was recorded until it returned to pre-feeding levels. For each experiment, the following parameters were calculated: a) the time to reach peak oxygen consumption following feeding, b) the scope of the SDA, c) the duration of the SDA response, and d) the SDA of each animal in kJ (as a function of individual mass).

For each diet, feeding commenced on the first morning and was always carried out between 10:00 and 11:00 a.m. The water flow to the chambers containing shrimp was turned off, and the bung in the feed port was gently removed. Individually weighted pellets of approximately 40 mg were dropped one at a time into the chamber until either the whole meal was consumed or a simple small pellet remained uneaten. The flow was reinstated and the fecal traps were immediately flushed to remove uneaten food and feces. Some shrimp did not feed well in the metabolic chambers, and data from these were discarded. In all, a total of 30 shrimp gave satisfactory SDA responses, in addition to five sham results.

Nitrogen excretion

Nitrogen excretion of unfed and fed animals was measured to assess postprandial nitrogen excretion (PPNE). Water samples were taken every 1 h from each chamber. Metabolism chamber 6 held no shrimp and was used as the control chamber to measure the background ammonia in the system water. Nitrogen excretion in the form of ammonia was measured as the total ammonia in the water using the indophenol and azo-dye colorimetric methods (Rodier 1989). During fasting, a 5 ml water sample was retrieved from the respirometer immediately before the waiting period started, again immediately before the measuring period ended, at MMR. This procedure was repeated 2, 10, 20, and 35 h post feeding and when the oxygen consumption rate had returned to SMR.

For each experiment, the following parameters were calculated: total ammonia nitrogen excretion (TAN) (µg-atóm N kg^{-1} h^{-1}), analyzed standard TAN excretion rate (STR), maximum fasting TAN excretion rate (MTR), postprandial peak, factorial postprandial scope (postprandial peak divided by STR), time to peak (TTP) (h) and duration (h) (Frisk *et al.* 2013).

Data analysis

We quantified standard metabolic rate (SMR), maximum metabolic rate (MMR), the SDA of each animal in kJ (as a function of individual mass), the scope of the SDA - maximal oxygen consumption (peak MO_2) divided by the basal pre-feeding rates (RMR), postprandial metabolic peak (the maximum peak oxygen consumption induced during feeding), factorial scope of that peak (postprandial peak divided by SMR), time to peak (TTP), duration of the SDA response - until oxygen dropped back to pre-feeding levels, the SDA calculated as the integrated oxygen cost in excess of SMR for the duration of the SDA, and the SDA coefficients were calculated by dividing the SDA by the energy of the diet (kJ) (Grigoriou & Richardson 2008, Frisk *et al.* 2013). Oxygen consumption rates were converted to units of energy using a standard oxycalorific conversion: 1 ml O_2 = 4.8 cal or 20.1 J (Elliott & Davison 1975, Wells & Clarke 1996).

Statistical analysis

As previously described by Ross & McKinney (1998), there is variability in the raw data. The underlying trend can, however, be revealed by processing the raw data using a smoothing routine. The data from these experiments were reprocessed using a 42 twice-running median technique (Velleman & Hoaglin 1981). Integrals of area under curve (AUC) and curve fittings were performed using Table Curve 2D version 5.5 software (Systat Software Inc., Chicago, Illinois, USA). Oxygen consumption data for the shrimp in different treatments were evaluated with analyses of variance (ANOVA) and covariance (ANCOVA). Percentages were arcsine transformed (Morman 2014). If significant differences were detected ($P < 0.05$) among the means, Tukey's test was applied.

Results

MO_2

Increasing the protein level from 20 to 50% increased the standard metabolic rate (SMR) from 217.9 to 258.2 mg O_2 kg^{-1} h^{-1}, while the maximum metabolic rate (MMR) increased approximately 50% (from 308.2 to 586.8 mg O_2 kg^{-1} h^{-1}, Table 2). Although the protein level affected the kinetics of the SDA, subadult white shrimp displayed a typical response at all protein levels: MO_2 increased rapidly followed by a slow decline. The change in the oxygen consumption (MO_2) is shown for each dietary protein level in Figure 1. The postprandial SMR increased by 1.41 to 2.72 times that of the pre-prandial SMR of shrimp. The postprandial peak of MO_2 was significantly higher at the 50% protein level than at 40, 30, and 20% (Table 2) protein. As a result of the higher SMR at 50%, the factorial postprandial scope at 40 and 50% tended to be larger than at 30 and 20%. The TTP was significantly different between 20% and the other treatments, with the value at 20% being the shortest (2.2 h). The duration of the SDA was significantly lower in 20 and 30% protein (13 and 15 h, respectively) than in 40 and 50% (20 and 21 h, respectively). Differences in the total metabolic expenditure on SDA were observed between 20% and other treatments; there were significant differences between the SDA coefficients of 20 to 30% and 40 to 50%. The SDA magnitude increased significantly with dietary protein levels. The values for the SDA magnitude ranged from 911.1 mg kg^{-1} with 20% dietary protein to 1,788.1 mg kg^{-1} with 50% protein content. The SDA coefficient was significantly correlated with the protein content of the diet and increased from 20 to 30% and 40 to 50% dietary protein. The mean SDA coefficients with 20, 30, 40 and 50% dietary protein were 2.7, 5.3, 11.2 and 11.5, respectively.

The effects of different dietary lipid levels on the SDA are summarized in Table 3. The metabolic rate of unfed *L. vannamei* was between 210 and 266 mg kg h^{-1} at different dietary lipid levels. The metabolic rate of fed animals increased to between 1.6 and 2.3 times that of the unfed animals. The SDA coefficient was between 5.7 and 7.9%, although the differences were not statistically significant.

Nitrogen excretion

The STR was smaller at 20% than at the 30 to 50% protein level, but no significant difference was observed between 30, 40 and 50% (Table 2). The MTR followed the same pattern as the STR. The postprandial course of TAN excretion was essentially in line with the course of MO_2, with an initial steep increase followed by a slow decline. The postprandial peak of TAN excretion at 20% was significantly lower than others

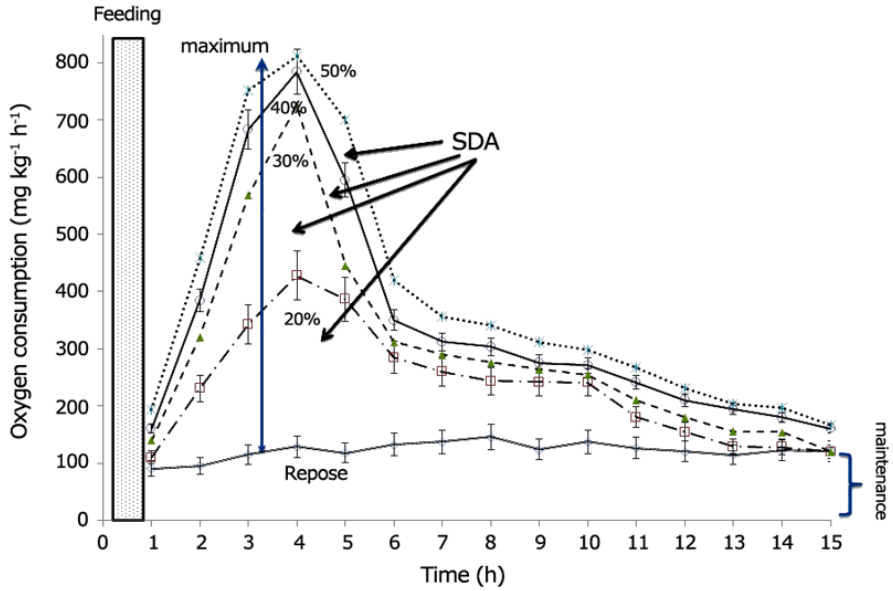

Figure 1. Post-prandial change in oxygen consumption rate in *L. vannamei* fed diets containing different levels of protein / Cambio post-prandial de la tasa de consumo de oxígeno en *L. vannamei* alimentado con dietas que contienen diferente nivel de proteína

Table 2. Measurements of postprandial rate of oxygen consumption ($\dot{M}O_2$) and ammonia excretion (TAN) in *L. vannamei* fed diets differing in protein contents / Medidas del consumo de oxígeno ($\dot{M}O_2$) y excreción de amonio (TAN) en *L. vannamei* mantenido con dietas con diferente nivel de proteína

Item	Protein (%)			
	20	30	40	50
$\dot{M}O_2$				
SMR (mg O_2 $kg^{-1}h^{-1}$)	217.9±6.5^a	234.5±6.3^b	242.1±8.7^b	258.2±7.7^c
MMR (mg O_2 $kg^{-1}h^{-1}$)	308.2±12.3^a	543.2±18.2^b	572.3±14.3^b	586.8±12.7^b
ΔSMR (%)	141.4±1.5^b	222.2±3.1^a	226.9±3.5^a	227.3±3.4^a
Metabolic scope	2.0±0.6^b	2.97±0.52^a	3.11±0.75^a	3.14±0.82^a
Postprandial peak	428.2±15.7^a	727.4±20.4^b	784.3±17.2^b	811.8±14.9^c
ΔSMR maximum (%)	195.4±3.9^a	297.5±4.8^b	311.1±3.7^b	314.4±3.9^b
Postprandial scope	1.33±0.12^a	1.34±0.10^a	1.37±0.10^a	1.38±0.11^a
Time to peak (TTP, h)	2.2±0.1^a	3.0±0.2^b	3.0±0.1^b	3.0±0.2^b
Duration (h)	13.0±1.5^b	15.0±1.5^b	20.0±1.5^a	21.0±1.5^a
Magnitude (mg kg^{-1})	911.1±14.8^a	1421.4±19.4^b	1623.2±21.0^c	1788.1±33.1^d
SDA (kJ)	0.17±0.008^a	0.23±0.005^b	0.26±0.003^b	0.28±0.005^b
SDA Coefficient	2.7±1.2^a	5.3±1.9^b	11.2±1.3^c	11.5±1.4^c
TAN				
STR (μg-atóm N $kg^{-1}h^{-1}$)	3.64±1.12^a	6.92±1.43^b	7.04±2.25^b	7.00±2.08^b
MTR (μg-atóm N $kg^{-1}h^{-1}$)	5.95±3.01^a	18.07+4.87^b	18.85±9.92^b	18.95±9.37^b
ΔSTR (%)	163.46±5.0^a	261.13±8.0^b	267.76±7.5^b	270.7 0±5.3^b
Scope	2.92±0.9^a	3.71±0.6^a	5.15±0.7^b	6.85±0.9^b
Postprandial peak (μg-atóm NH_4 $kg^{-1}h^{-1}$)	10.63±4.92^a	26.18±27.72^b	35.70±9.12^{b,c}	41.73±12.92^c
ΔSTR maximum (%)	291.68±8.3^a	378.32±11.2^b	507.1±12.1^c	684.80±11.9^d
Postprandial scope	1.78±0.4^a	1.39±0.5^a	1.96±0.3^b	2.21±0.3^c
Time to peak (TTP, h)	1.5±0.4^a	1.5±0.3^a	2.0±0.4^b	2.0±0.2^b
Duration (h)	12±1.5^b	13±1.5^b	18±1.5^a	19±1.5^a
Magnitude (mg kg^{-1})	0.32±0.05^a	1.06±0.45^b	1.19±0.76^b	1.89±0.94^b
SDA (kJ)	7.95±1.1^a	29.64±4.8^b	26.29±3.9^b	46.99±6.8^c
SDA Coefficient	0.20+0.02^a	0.37+0.06^b	0.38+0.04^b	0.60+0.08^c
MTR/SDA (%)	74.8	60.9	71.7	40.3

$\dot{M}O_2$: oxygen consumption; TAN: ammonia-N excretion. Different superscripts in each row indicate significant differences among treatments ($P < 0.05$)

treatments. However, when comparing postprandial scopes, no differences were apparent between 20 and 30%. At 20 and 30%, the TTP of TAN excretion was significantly shorter than at the 40 and 50% protein level. The TTP differed between MO_2 and TAN excretion, where TAN excretion peaked first. Similarly, the duration of elevated TAN excretion was shorter than the duration of SDA at all treatments.

Diets containing different lipid levels had an increase in nitrogen excretion rates of between 1.61 to 2.08 times that of the unfed animals (Table 3). The energy lost varied from 33.25 J day^{-1} with 16% lipid diet to 22.67 J day^{-1} with 2% lipid diet, suggesting a decrease in energy loss with increasing dietary lipid. The PPNE decreased with dietary lipid level, although the trend was statistically significant at 16%.

Discussion

Oxygen consumption was measured in fasted and fed subadult shrimp. Those fed the 20% protein diet had lower levels of prefeeding oxygen consumption than shrimp fed either of the 30 to 50% diets (Table 2). The shrimp fed the 20% diet were obtaining energy from a substrate that requires less oxygen to metabolize. The mean SMR from subadults fed a diet containing 30 to 50% protein (234.5 to 258.2 mg O_2 kg^{-1} h^{-1}, respectively) was lower than the range reported for SMR (350.0 to 600 mg O_2 k g^{-1} h^{-1}, respectively) in juvenile *L. vannamei* (Rosas *et al.* 2001a). The SMR of subadult shrimp were 1.5 to 2.3 less than that of juveniles with diets of 30 to 50% protein, respectively. The peak postprandial MO_2 for subadult shrimp was 428.2 to 811.8 mg O_2 kg^{-1} h^{-1} for 20 to 50% dietary protein, respectively, which represents postprandial increases of 195 to 314% above

Table 3. Measurements of postprandial rate of oxygen consumption ($\dot{M}O_2$) and ammonia excretion (TAN) variables in *L. vannamei* fed diets differing in lipid contents / Medidas del consumo de oxígeno ($\dot{M}O_2$) y excreción de amonio (TAN) en *L. vannamei* mantenido con dietas con diferente nivel de lípidos

Item	Lipid (%)			
	2	4	8	16
$\dot{M}O_2$				
SMR (mg O_2 $kg^{-1}h^{-1}$)	265.6±8.3^a	258.8±6.0^a	235.7±5.8^b	229.9±3.9^b
MMR (mg O_2 $kg^{-1}h^{-1}$)	428.31±11.7^a	437.66±13.6^a	456.60±12.5^b	478.50±17.9^b
ΔSMR (%)	161.3±6.1^a	168.9±4.9^a	193.8±8.1^b	210.9±9.5^c
Metabolic scope	2.46±0.8^a	2.22±0.9^a	3.32±0.7^b	3.03±0.6^b
Postprandial peak	654.2±13.4^b	575.1±16.7^a	695.9±18.8^b	687.6±12.9^b
ΔSMR maximum (%)	246.3±5.4^a	274.1±7.1^a	269.0±8.3^a	236.9±8.7^a
Postprandial scope	1.53±0.2^a	1.52±0.6^a	1.52±0.5^a	1.49±0.8^a
Time to peak (TTP, h)	3.0±0.5^a	3.0±0.5^a	3.0±0.5^a	3.0±0.5^a
Duration (h)	10±1.0^a	10±1.0^a	9±1.0^a	11±1.0^a
Magnitude (mg kg^{-1})	1526.0±31.8^a	1576.9±22.6^a	1585.2±23.2^a	1534.1±35.2^a
SDA (kJ)	0.23±0.01^a	0.24±0.02^a	0.28±0.04^a	0.29±0.03^a
SDA Coefficient	6.1±2.8^a	7.9±1.0^b	7.7±4.1^b	5.7±3.7^a
TAN				
STR (µg-atómN $kg^{-1}h^{-1}$)	9.41±4.52^a	9.69±5.14^a	8.59±4.62^a	5.00±3.08^b
MTR (µg-atómN $kg^{-1}h^{-1}$)	13.14±3.31^a	17.86±5.92^a	13.67±9.12^a	10.87±4.52^b
ΔSTR (%)	139.26±3.5^b	141.54±4.6^b	162.15±5.6^c	129.78±3.7^a
Scope	2.17±0.3^a	2.24±0.2^a	2.33±0.5^a	2.13±0.7^a
Postprandial peak (µg-atómN $kg^{-1}h^{-1}$)	42.06±11.53^b	44.06±5.09^b	34.00±7.61^a	27.71±9.29^a
ΔSTR maximum (%)	216.72±10.1^a	223.81±8.6^a	232.96±7.9^a	213.12±8.2^a
Postprandial scope	1.82±0.5^a	1.58±0.3^a	1.43±0.2^a	1.64±0.2^a
Time to peak (TTP, h)	2.0±0.5^a	3.0±0.5^b	3.0±0.5^b	3.0±0.5^b
Duration (h)	9±1.0^a	9±1.0^a	8±2.0^a	10±1.0^a
Magnitude (mg kg^{-1})	0.91±0.23^b	1.12±0.21^c	1.26±0.38^c	0.53±0.09^a
SDA (kJ)	22.67±1.7^a	27.83±1.9^a	31.32±2.1^a	33.25±0.9^a
SDA Coefficient	0.32+0.07^a	0.33+0.05^a	0.34+0.09^a	0.39+0.01^a
MTR/STR (%)	100	100	75.7	72.5

$\dot{M}O_2$: oxygen consumption; TAN: ammonia-N excretion. Different superscripts in each row indicate significant differences among treatments ($P < 0.05$)

the SMR. This is smaller than other species (up to 514% increase) such as *Penaeus monodon* (Du-Preez *et al.* 1992). Pascual *et al.* (2004) determined the post-prandial high oxygen consumption in juvenile shrimp fed 15% protein. Once subadult shrimp were fed, oxygen consumption increased, reaching a peak 2.2 to 3.0 h after feeding for the 20 to 50% protein level; in juveniles, the peak occurred at 1 and 2 h (Pascual *et al.* 2004) after feeding with 15 to 40% dietary protein. Generally, the time to peak reached in subadult shrimp was higher than in juveniles at one hour. Despite the fact that the quantity and quality of food supplied to juvenile shrimp in the studies of Rosas *et al.* (2001a) and Pascual *et al.* (2004) is different and higher feed rate, there is a tendency of the parameters studied to be lowest in subadult

The method of estimating SDA varies among studies and depends on the respirometry system and the SMR calculation method (Eliason *et al.* 2007). Accurate estimates of SDA require a separation of metabolism associated with activity and stress from that associated with feed intake (Brett & Groves 1979). This may also result in an overestimation of the SDA in the case of shrimp.

Findings obtained with several species of shrimp (Rosas *et al.* 1996) and *L. vannamei* (Rosas *et al.* 2001a) at postlarve and juvenile early stages showed that proteins ingested through the diet have an effect on the SDA, showing that diets with high protein levels could result in a higher metabolic cost (Taboada *et al.* 1998), most likely because more protein is being synthesised from the meal at the cellular level. There are no studies that assessed the effect of diet on both the SDA and postprandial ammonia using the same group of subadult shrimp. However, in this study, we found that more advanced life stages of shrimp, the subadults, exhibit the same pattern of excretion of nitrogen. This is because nitrogen metabolism in white shrimp is a key factor in the SDA because the deamination and synthesis

of protein are probably the greatest contributors (Rosas *et al.* 1996). In subadult shrimp fed with a high protein level (50%), the SDA was 165% higher than that obtained in shrimps fed with a diet containing a low protein level (20%) ($P < 0.05$). In subadult shrimp, the SDA was 1.6 times lower (this work) than in juveniles (Rosas *et al.* 2001a).

Postprandial nitrogen excretion is a measure of excreted ammonia of alimentary origin in shrimp; it can be associated with the SDA through the STR/SDA ratio. The STR in the SDA (STR/SDA) is lower in subadult shrimp (40.3%) fed diets containing high protein (50%) in comparison with shrimp fed diets containing lower protein levels (60.9 to 74.8%). These results confirm that the metabolism of *L. vannamei* subadults, similar to juvenile shrimp (Rosas *et al.* 2001a), is controlled by dietary protein levels. The prefeeding ammonia excretion rate was significantly lower for shrimp fed the 20% diet (Table 2), so the lower level of deamination of amino acids may well be responsible for some of the differences in prefeeding respiration rates.

The maximum oxygen consumption rate (MMR) and maximum fasting TAN excretion rate (MTR) changed similarly with the level of dietary protein, although this was only significant between 20% and 30 to 50%. This is in accordance with findings in *L. vannamei*, where instantaneous protein use changed during rest and exercise (Duan *et al.* 2014, Zhang *et al.* 2006).

An increase in the duration of the SDA response has been noted in carp (Chakraborty *et al.* 1992). This effect is minimal in *L. vannamei*, suggesting a much more rapid digestive metabolism. The total magnitude of the response increased (Table 2), although the standard deviation of the data also increased. The coefficients measured in this study were generally higher than those recorded in shrimp from the Gulf of Mexico (Rosas *et al.* 1996). The metabolic scope in this study was within the range of 2 to 5, as reported in other aquatic organisms (McCue 2006, Luo & Xie 2008). The postprandial peak and duration of the SDA differed between the protein content of 20% and the 30-50% groups, by contrast shrimp digest and assimilate food remains without significant change with increasing levels of lipid in the diet.

The magnitude of the SDA depends on the composition of the meal in the diet. The values were lower for lipidic diets when compared with proteic diets; these values are in agreement with the findings by McCue (2006), which showed a relatively lower value (1,211.1 mg kg^{-1} to 1,688.1 mg kg^{-1}) for fat and higher values (1,526.0 mg kg^{-1} to 1,585.2 mg kg^{-1}) for protein. The protein influences the magnitude of the SDA in the 20-30% range of dietary protein, levels in excess of 30-50% had no effect on SDA, which appears to be a threshold phenomenon (Clifford & Brick 1978), operating independently of the quantity of food intake. In our study, the SDA and magnitude did not differ between the shrimp fed the low-lipid diet and those fed the high-lipid diet, which suggests that the effect of food composition on the SDA in shrimp is species-dependent (Luo & Xie 2008). Studies in fish found that changing the meal lipid content had no impact on the SDA (Peres & Oliva-Teles 2001). A significant change in the SDA relative to digestible energy was not demonstrable with dietary protein for each lipid level.

The SDA coefficient is comprised of two components: an obligatory one, which consists mainly of the energetic cost of first digesting and then converting food to its primary storage forms, and a facultative one, which is an adaptive mechanism for dissipating extra calories as heat (Fu *et al.* 2007). It is interesting to note that no sparing of dietary protein could be observed with a higher lipid diet, as was reported by Ross *et al.* (1992) in *O. niloticus*. The continuous quantitative appraisal of nitrogen excretion to determine energy loss is necessary for a better understanding of endogenous nitrogen excretion and nitrogen excretion from different proteinaceous diets. In the present study, post-feeding subadult shrimp had an increased ammonia excretion pattern similar to the SDA response. Ammonia excretion increased with the protein content and, hence, the nitrogen content of the diet. A similar effect was reported by Rosas *et al.* (1996), where the amount of nitrogen excreted as ammonia by *P. notialis*, *P. duorarum*, *P. schmitti* and *P. setiferus* depended largely on the quantity of protein assimilated during feeding.

High dietary protein means high cost of feed and pollution from waste, which will not support sustainable development of shrimp culture (Wang *et al.* 2015), it has been demonstrated in laboratory and on farm that shrimp *L. vannamei* can grow sustainably in ponds with a level of digestible energy:crude protein ratio (DE: CP) from 11.9 to 28.57 kcal g^{-1} protein (Cousin *et al.* 1993, Lawrence *et al.* 1995[1], Kureshy & Davis 2002, Patnaik & Samocha 2009), mainly in subadult organisms. Due in part to the low cost of energy expenditure to eliminate excess nitrogen as shown in the present work

In the present study, it was found that variations in the level of the protein:energy ratio affect the SDA and post-prandial nitrogen excretion of subadults, as has been found in juvenile shrimp (Rosas *et al.* 2001a). Furthermore, metabolic N

[1]Lawrence AL, P Aranyakananda & FL Castille. 1995. Estimation of dietary protein and energy requirements for shrimp. Proceedings, American Oil Chemists Association (AOCS) Conference, San Antonio, Texas, Inform 6(4): 520-521.

excretion by subadult shrimp tends to be lower when dietary lipid and the energy:protein ratio are high. Subadult shrimp fed the 50% protein diet consumed food with the highest DP/DE (21.33) and had the highest SDA and digested the greatest percentage of dietary protein. In general, it has been hypothesized that, as the optimal protein level is approached, there is a significant decrease in the metabolic rate of the shrimp (Hewitt & Irving 1990). Therefore, with the 20% protein diet, subadult *L. vannamei* may be operating at or near their optimum dietary protein level or protein/energy ratio. An optimal ratio of lipid, carbohydrate and protein would imply that subadult shrimp fed the 20% diet would have a smaller requirement for protein metabolism to provide energy and hence a lower oxygen consumption and ammonia excretion rate. This could also explain why it has been found that white *L. vannamei* shrimp grew properly when fed with 20 to 36% dietary protein (Venero *et al*. 2008), depending on size. It has also been found that subadult *L. vannamei* have lower requirements than juveniles for protein (Kureshy & Davis 2002), which is in agreement with this work. *L. vannamei* protein requirements have been determined at 40% for postlarvae, 20-30% for juveniles and subadults (Pedrazzoli 1998 and this work) and a low level of 15% CP in the diet (Lawrence *et al*. 1995[1]). Smaller organisms require more protein compared with larger organisms (Hilton & Slinger 1981), and the maximum and maintenance rations both decrease as organisms increase in size, with the maximum ration decreasing at a faster rate (Lim & Sessa 1995). This is because small organisms have a higher scope for growth. Furthermore, the oxygen consumption and nitrogen excretion in subadults were lower than those reported in juvenile shrimp fed with 20 to 50% dietary protein.

In conclusion, the results in this study show a relationship between respiration rate and the level of dietary protein ingested. *L. vannamei* have developed various physiological mechanisms to cope with variations in food quality, and they are able to adjust these mechanisms to a variable diet quality by regulating their metabolic. The SDA following ingestion of a 20% protein diet was the lowest, and there was a marked increase in the SDA when feeding a 30 to 50% protein diet. Knowledge of the protein-sparing effects of non-protein nutrients such as lipid and carbohydrates could be effective in reducing feed costs and enhancing growth. This work has confirmed and quantified the significant effect of dietary protein on oxygen consumption, the SDA coefficient and SDA magnitude in subadult *L. vannamei*, while the effect of dietary lipid has been shown to be minimal. Thus, a significant change in the SDA relative to digestible energy was not demonstrable with dietary protein for each lipid level.

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