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Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei*: effects of dietary protein source

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Summary

The effect of dietary protein on protein synthesis and growth of juvenile shrimps *Litopenaeus vannamei* was investigated using three different diets with equivalent protein content. Protein synthesis was investigated by a flooding dose of tritiated phenylalanine. Survival, specific growth and protein synthesis rates were higher, and protein degradation was lower, in shrimps fed a fish/squid/shrimp meal diet, or a 50% laboratory diet/50% soybean meal variant diet, than in those fed a casein-based diet. The efficiency of retention of synthesized protein as growth was 94% for shrimps fed the fish meal diet, suggesting a very low protein turnover rate; by contrast, the retention of synthesized protein was only 80% for shrimps fed the casein diet. The amino acid profile of the casein diet was poorly correlated with that of the shrimps. 4 h after a single meal the protein synthesis rates increased following an increase in RNA activity. A model was developed for amino acid flux, suggesting that high growth rates involve a reduction in the turnover of proteins, while amino acid loss appears to be high.

Key words: shrimp, *Litopenaeus vannamei*, growth, protein synthesis, diet, amino acid flux, casein, protein turnover.

Introduction

Optimal dietary protein levels in penaeid shrimps, measured as growth response, vary from 50–55% in *Penaeus japonicus*, to 40–46% in *Penaeus monodon* and over 30–60% in *Litopenaeus vannamei* (Teshima and Kanazawa, 1984; Cousin et al., 1993). Commercial shrimp feeds contain 30–50% crude protein, composed mostly of marine animal protein products such as fish, shrimp and squid meal. These feed materials have high nutritive value and palatability but are expensive and not readily available (Lim and Persyn, 1989). There is much interest in finding alternative, less expensive, protein sources to replace part of the fish meal content of diets. The importance of soybean meal as a source of protein has long been recognized (Kanazawa, 1992). Its utilisation in shrimp diets has increased due to its 'nutritional quality, lower cost and consistent availability' (Akiyama, 1988). Casein, however, although generally used as a standard protein source for the nutrient requirement studies of fish and other vertebrates, is poorly utilised by several shrimp species (Deshimaru, 1982).

Attempts have been made to optimise the utilisation of these proteins by determining the optimal dietary amino acid profile (Cowey and Forster, 1971). This optimal dietary amino acid profile will depend on the amino acid requirement of an animal for protein synthesis and the use of individual amino acids as energy substrates or for other purposes (Ronnestad and Fyhn, 1993). Deshimaru and Shigeno (1972) suggested that the amino acid composition of the food should be very similar to that of the animal's proteins. Ogata et al. (1985) measured the total essential free amino acid concentrations of the European eel *Anguilla anguilla* and noted that it was correlated with dietary protein content. To minimize the effect of different sample pretreatment, the A/E ratio (the concentration of each essential amino acid as a percentage of the concentration of total essential amino acids, including tyrosine) was calculated in some studies. Arai (1981) based diets for coho salmon fry upon A/E ratios found in the whole-animal tissue of this species. Penaflorida (1989) used the profile of essential amino acids of whole shrimp to calculate an essential amino acid index (the *n*th root of the product of the ratios of each essential amino acid in the feed to that of a reference protein). Changes in the levels of free amino acids in tissue after a meal have been used as a criterion for determining amino acid requirements, based on the hypothesis that the concentration of an individual free amino acid will remain low until its requirement has been met (Wilson, 1994).

Protein turnover can be divided into its constituent processes, protein synthesis, protein growth and protein degradation (reviewed by Houlihan, 1991). At any particular time, protein growth (k_g, protein growth as a percentage of the total protein

mass) is the net balance between protein synthesis (k_s) and protein degradation (k_d), i.e. $k_g=k_s–k_d$ (Millward et al., 1975, 1976). Preliminary results from invertebrates suggest that high specific growth rates may be achieved by relatively low rates of protein turnover (equivalent to protein degradation in growing animals). In order to improve our understanding of protein metabolism in crustaceans, the amino acid flux model may be useful (Houlihan et al., 1995a,b). At the heart of this model is the relationship between dietary amino acid intake, the free amino acid pool and the protein pool, linked *via* protein synthesis, protein degradation and amino acid metabolism (Houlihan et al., 1995a,b). The model allows ratios between its components to be calculated.

The aims of this study were to determine the effects of replacing fish meal protein with soybean or casein protein in practical diets for shrimp and to determine the effects of the different diets on the rates of protein metabolism in shrimps. Models of amino acid flux and protein turnover with the above different diets were constructed using the protein synthesis data. Four experiments were performed. Experiment 1 was mainly a methodological trial to verify the validity of the method used by Garlick et al. (1980) for measuring protein synthesis in shrimps. Experiment 2 examined the effect of dietary protein source on growth and protein turnover in shrimps. Experiment 3 examined whole-animal protein synthesis after a meal and RNA:protein concentrations, to investigate whether starvation and refeeding altered synthesis and RNA concentrations. Experiment 4 measured free amino acid levels in tissues of *L. vannamei* at various times following feeding. Free amino acid concentrations in *L. vannamei* tail muscle and whole animals were investigated with respect to dietary amino acids.

Materials and methods

Experimental animals, tanks and seawater system

The three growth experiments were carried out at the Laboratory of Aquaculture and Artemia Reference Centre (Gent, Belgium). *Litopenaeus vannamei* Boone, postlarval stage 5–6, were obtained from a commercial hatchery in Florida, USA and gradually acclimated for 4 weeks to the experimental conditions. During this period they were fed *ad libitum*, initially with *Artemia nauplii* (EG/Decar, type, INVE Aquaculture NV, Baasrode, Belgium) (Sorgeloos et al., 1986) and then with a standard diet (containing 50% protein). Postlarvae were acclimatised to the test diets for 7 days prior to the start of the experiment.

The experiments were set up as a randomized complete block design, with five blocks containing one replicate of three diets (Coutteau et al., 1995). Each block consisted of an independent recirculating system with six, 30 l dark grey rectangular tanks (rearing units), a mechanical filter, a biological filter, a carbon filter and a water reservoir (100 dm^3) equipped with heaters and aeration. Approximately 10% of the water in each tank was replaced every day with fresh seawater.

At the start of the experiment a control group of 50 shrimps were weighed, killed and kept frozen at –80°C for estimation of initial protein content. A total of 120 healthy juveniles were selected randomly, blotted dry in tissue paper, weighed (224±0.01 mg wet mass, 51.58 mg dry mass) to the nearest mg and transferred to the experimental tanks (rearing units). Each experimental tank contained eight randomly chosen juveniles. The experimental tank conditions were kept constant: temperature 27±1°C, photoperiod 12 h:12 h light:dark, salinity 30‰; [NH_4^+] and [NO_2^-] never exceeded 0.5 and 0.4 p.p.m, respectively. The water circulation provided adequate levels of dissolved oxygen. The experimental conditions remained stable throughout the experiment and were within the limits normally considered acceptable for the growth and survival of penaeid shrimps (Wickins, 1976). Shrimps were hand-fed 12% of their body mass twice a day at 9:00 h and 17:00 h for a period of 28 days. Feeding rate was adjusted every week. Faeces and any uneaten feed were siphoned daily prior to the first feeding. After the 28th day, experiments 1–3 were conducted (see Experimental design).

For measurement of the free amino acid (FAA) concentrations at various times following feeding (Experiment 4), four rearing units were used (in two separate blocks, block 6 and 7) each containing ten shrimps. The shrimps were starved for 1 week, then those in two tanks were fed diet 1 and in the other two tanks diet 3, for 14 days. The shrimps were offered food *ad libitum* once per day.

Experimental diets

The diets were prepared at the Artemia Reference Centre, according to a method and formulation modified by Teshima et al. (1982). A mixture of fish meal, squid and shrimp powder (INVE Aquaculture, Belgium) was used to make the protein source for diet 1 (45% protein), whilst for diet 2, half the fish/squid/shrimp meal was replaced by soybean meal (45.2% protein) (INVE Aquaculture, Belgium) (Table 1). Diet 3 was a casein-based microbound diet (powdered diet with carrageenan as a binder) (44.5% protein). The three experimental diets were designed to be isonitrogenous and isoenergetic. Diets were analysed for crude protein using the Kjeldahl procedure (Williams, 1984). Total lipids were extracted according to the method of Folch et al. (1957), modified for freshwater invertebrates by Herbes and Allen (1983). Moisture and ash content were estimated by difference in mass after 24 h in a drying oven at 60°C (moisture) and 6 h in a muffling furnace at 600°C (ash). Total carbohydrates were obtained by adding the percentage values determined for moisture, crude protein, lipid content and ash and subtracting the total from 100 (Tacon, 1990). The carbohydrates, lipids and protein were multiplied by their respective fuel value (kal g^{-1}) and the sum obtained defined the energy value of the feed (Halver, 1989). The solubility of the diets was estimated from the loss of total dry matter after rotating 0.25 g of the diet for 5 min and 60 min in 50 ml of distilled water, centrifugation for 35 min at 3100 ***g*** and discarding the supernatants. The water stability of diets 1, 2 and 3 varied from 69%, 74% and 69% after 5 min to 63%, 69% and 63%, respectively, after 60 min of emersion in deionized water (Camara, 1994).

Table 1. *Composition of the three experimental diets*

Ingredient	Composition (% wet mass) Diet 1	Diet 2	Diet 3	Source
Fish meal	24.91	12.46	–	INVE Aquaculture NV, Belgium
Shrimp meal	12.07	6.04	–	INVE Aquaculture NV, Belgium
Squid meal	22.28	11.14	–	INVE Aquaculture NV, Belgium
Soya	–	23.65	–	Supro 500E isolated soy protein, Protein Technologies International
Casein	–	–	17	Sigma C-8654
Sodium casein	–	–	27	Sigma C-7078
Arginine	–	–	1	Sigma A-5131
Sucrose	5	5	5	Sigma S-9378
Cellulose	0.445	6.415	8.705	Sigma C-8002
Wheat starch	10	10	10	Sigma S-5127
Vitamin mix	1.1	1.1	1.1	Kanazawa et al. (1977)
Mineral mix	4	4	10	Kanazawa et al. (1977)
Vitamin C	1	1	1	Stay-C (0.15% act), Roche
Vitamin E	0.02	0.02	0.02	Sigma T-3001
Choline chloride	0.15	0.15	0.15	Sigma C-1879
Attractant mix	3	3	3	Chen (1993)
Cholesterol	0.5	0.5	0.5	Sigma C-8503
Sodium alginate	2	2	–	Sigma A-7128
Sodium hexametaphosphate	1	1	–	Fluka 71600
Calcium citrate	1	1	–	Aldrich 35, 973-4
k-Carrageenan	–	–	4	Sigma C-1013
Olive oil	3	3	3	Vande Mortale olive oil
Soybean oil	3.5	3.5	3.5	Belgium soybean oil
Fish oil	5	5	5	INVE Aquaculture NV, Belgium
Butylated hydroxytoluene	0.005	0.005	0.005	Federa NV, Belgium
Butylated hydroxyanisole	0.005	0.005	0.005	Federa NV, Belgium
Ethoxyquin	0.015	0.015	0.015	Sigma E-8260

Experimental design

Validation of the protein synthesis measurement (Experiment 1)

At the end of the experiment, individual shrimps (1–2 g wet mass) were randomly selected and fractional rates of tail-muscle protein synthesis were measured using the flooding-dose method. After an incorporation period of 10, 30, 60 and 120 min, eight shrimps were killed at each time point and samples were taken and analysed as described below.

Protein turnover (Experiment 2)

At the end of the experiment, fractional rates of whole-animal protein synthesis were measured in eight shrimps (final masses 1–2 g, Fig. 2) randomly selected from each of the three diets. These shrimps were fasted for 1 day before the measurements. After an incorporation period of 1 h (see results for Experiment 1), samples of tail muscle and the remaining whole animal were taken and analyzed as described below.

Protein synthesis levels after a meal (Experiment 3)

On day 30, immediately before feeding (t=0) and 1, 2 and 4 h after feeding, fractional rates of whole-animal protein synthesis were measured in five shrimps (at each time point) fed diet 1 using a flooding dose of ^{3}H-phenylalanine. These shrimps were fasted for 24 h before the measurements. Five shrimps were denied food for 6 days (starved group). At the end of the incorporation time, all the shrimps were killed, frozen in liquid nitrogen and stored in –80°C until analysis as described below.

Amino acid levels after a meal (Experiment 4)

After 7 days without food, a group of ten shrimps (1.2 g) were removed, killed and used as the prefeeding t=0 group. The remaining 30 shrimps were fed normally and groups of five shrimps fed diet 1 and another five fed diet 3, selected at random, were removed at 4, 9 and 24 h after feeding. Each shrimp was individually netted, removed and killed, and samples of tail muscle were quickly dissected out. Tail-muscle tissue and the whole animal samples were frozen in liquid nitrogen and stored at –80°C until analysis.

Measurements of growth rates, protein synthesis rates and RNA concentrations

Whole-animal specific growth rates (SGR), expressed as a percentage increase in body mass per day, were calculated for days 0–28 using the growth rate equation of Ricker (1979):

$$\mathrm{SGR} = 100[\log_e(W_f/W_i)]/t\,, \quad (1)$$

where W_i and W_f are the initial and final wet mass (g),

respectively, of the experimental shrimps and t is the length of the experimental period (in days). Whole-animal fractional rates of protein synthesis were measured using the flooding-dose method of Garlick et al. (1980). Protein synthesis rates were measured in eight shrimps (intermoult stage; Smith and Dall, 1985), randomly selected from each of the three diet groups. Injections were made *via* the first pair of pleopods into the haemolyph, by passing the needle through the anterior dorsal section of the first abdominal segment, without anaesthesia. The injected solution contained 135 mmol l^{-1} L-phenylalanine and L-[2,6-^{3}H]phenylalanine (Amersham International; 3.7 MBq ml^{-1}, 100 μCi ml^{-1}). Shrimps were injected at a dose of 10 μl g^{-1} wet mass, administered using a 0.5 ml micro-fine (0.33 mm × 12.7 mm) syringe. The precise time of injection was noted, and each shrimp was returned to aerated seawater (26°C) for 1 h (see results for Experiment 1). Recovery of the shrimps from the injection procedure was 100%. After the incorporation period, the shrimps were removed and killed, and tail-muscle samples were dissected within 2 min of death. The tissue and whole-animal samples were immediately frozen in liquid nitrogen and stored at –80°C until analysis. Duplicate whole-animal samples (100 mg) were taken from each shrimp to measure: (i) the free-pool phenylalanine specific activity of the tail muscle and the whole animal, (ii) the tail-muscle protein-bound phenylalanine specific activity and (iii) protein and RNA content, as described in Houlihan et al. (1995a,b).

Samples (100 mg) of the whole bodies were weighed and homogenised in 0.2 mol l^{-1} perchloric acid (PCA), and centrifuged, releasing intracellular 'free' (i.e. unbound) amino acids into the PCA-soluble fraction. Following separation of this fraction by centrifugation, the supernatants were retained for phenylalanine analysis as described by Houlihan et al. (1995a,b). Protein content was determined (Lowry et al., 1951) as modified by Schacterle and Pollock (1973) after solubilisation in 0.3 mol l^{-1} NaOH. Whole-animal RNA content was measured, following extraction, using the orcinol method (Mejbaum, 1939), comparing the samples against known RNA standard (Type IV, calf liver, Sigma) concentrations determined spectrophotometrically (absorbance at 665 nm, using quartz cuvettes and a Perkin Elmer LamdaUV/vis spectrophotometer) and expressed as RNA:protein concentration (μg RNA mg^{-1} protein). This was used as an indication of the animal's capacity for protein synthesis. The concentrations of phenylalanine in intracellular free-pools, protein pellets and the solution injected into the animals were measured, using a fluorometric assay following enzymatic conversion of the phenylalanine to β-phenylethylamine (PEA) (Houlihan et al., 1995a). Specific activities of the PEA from the intracellular free-pools, protein pellets and injected solution were measured using a scintillation counter. Fractional rates of protein synthesis (k_s), the percentage of the protein mass synthesised per day (% day^{-1}), were estimated using the flooding-dose method equation (Garlick et al., 1980; Houlihan et al., 1988):

$$k_s = (S_b/S_a) \times (1/t) \times 1440 \times 100, \quad (2)$$

where S_b is the specific activity of protein-bound phenylalanine, S_a is the specific activity of free-pool phenylalanine, t is the time (min) from start of incubation and 1440 is the number of minutes in 1 day. Whole-animal protein growth rates (k_g, % day^{-1}) were calculated using Equation 1 by substituting for W_1 the mean estimated initial total protein content, measured in the 20 animals killed initially, and for W_2 the final total protein content measured in the flooding-dose injected animals. Protein degradation (k_d, % day^{-1}) can be calculated by subtracting protein growth from protein synthesis. RNA activity (k_{RNA}, g protein synthesised g^{-1} RNA day^{-1}) was calculated as the ratio of k_s and RNA:protein concentration (Millward et al., 1973).

Amino acid profiles

The amino acid composition of the whole bodies was measured using the method outlined in Lyndon et al. (1993). Briefly, 200 mg samples, taken from the tail muscle and whole bodies, were homogenised in 4 ml absolute ethanol plus 100 μl aqueous norleucine (2.5 μmol ml^{-1}). The homogenate was centrifuged to pellet the precipitated proteins. Duplicate samples of the supernatant were then taken to measure the free-pool amino acid composition. A subsample of 100 μl was dried down under vacuum and reconstituted in 100 μl 0.1 mol l^{-1} HCl and filtered, and a 5 μl sample was taken for amino acid analysis, using an Applied Biosystems 420A amino acid analyser. By this method, cysteine was only 20–30% recoverable and was therefore not quantified. A validation experiment was conducted to check the solubility of the amino acids in 95% ethanol. Known amounts of hydroxyproline, asparagine, glutamine, taurine, tryptophan and norleucine were added to the supernatant described, at a concentration range (over 1 nmol) known to be within the linear range of the amino acid analyser. Recovery was 100%. The amounts of protein-bound amino acids (PAA) of the tissues were determined after hydrolysis in 6 mol l^{-1} HCl (Finn et al., 1995) on samples that had been extracted for FAA as described above. Tryptophan is destroyed by acid hydrolysis, so separate samples of the tissues were prepared by alkaline hydrolysis for analysis of the tryptophan content. Briefly, 50–75 mg samples, taken from the tail muscle and whole bodies, were hydrolysed for 22 h (4.2 mol l^{-1} NaOH, 110°C) and filtered. 2.5 ml of filtrate sample with 1 ml internal standard solution were dried at 40°C. The residue was further dissolved in 2.5 ml acetic acid and 10 μl of each of the samples were analysed in a Kontron 450 data system analyser. The same procedure was followed for analysing the diets.

Calculations

Amino acid results were expressed as μmoles of amino acid per gram of diet (μmol g^{-1}) and as grams per 100 g determined amino acid for protein. The essential amino acid (A/E) ratio (Arai, 1981) of each essential amino acid (EAA) was calculated as the percentage of the total EAA. The essential amino acid index (EAAI) of the two diets was determined from the formula:

$$\mathrm{EAAI} = \sqrt[n]{aa_1/AA_1 \times aa_2/AA_2 \ldots\ldots\ldots \times aa_{11}/AA_{11}}, \quad (3)$$

where aa_1 is the A/E ratio in the feed [(EAA/total EAA+tyrosine)×100], AA_1 is the A/E ratio in the shrimps [(EAA/total EAA+tyrosine)×100]. The EAAI is patterned after the formula for *Penaeus monodon* (Penaflorida, 1989) using juvenile *P. monodon* as the reference protein. In this study, whole *L. vannamei* juveniles were used as the reference protein, following the hypothesis that an efficient diet should have a similar amino acid profile to that of the experimental shrimp (D'Abramo et al., 1997).

Amino acid flux

In order to present a metabolic model for protein and amino acid metabolism for the two diets, the model of Millward and Rivers (1988) was used. The models used here describe the amino acid flux for a 1.25 g and a 1.12 g *L. vannamei* offered diets 1 and 3, at 28°C, at a consumption rate 4% of the body mass (Gopal and Raj, 1990). The assimilation efficiencies were assumed to be 90% for marine meal protein and 91% for casein (Fenucci et al., 1982; Dall, 1992; D'Abramo et al., 1997). Nitrogenous excretion was taken as 0.9 mg N g^{-1} day^{-1} (Wickins, 1985). The nitrogen protein content was calculated from the N content using a conversion factor of 5.85 (Gnaiger and Bitterlich, 1984) and a protein equivalent of amino acid nitrogen was calculated assuming that there are 9 mmol amino acids per g protein (Houlihan et al., 1995c). Faecal amino acid losses were calculated as consumption minus absorption. The total free amino acid (FAA) and protein concentrations were taken from direct measurements of whole-animal tissues for both diets. Data of the fractional rates of protein synthesis, protein growth and protein breakdown were used to calculate the respective flux components.

Statistical analysis

All values are means ± S.E.M. and differences present at 5% level (P<0.05) were considered significant. Data were compared by Student's *t*-test, analysis of variance (ANOVA) followed, where applicable, by Tukey's or Scheffe's multiple-comparison tests (Zar, 1996). The F_{max} test for homogeneity of variances was used to determine whether the assumption of equal variance was met. Pearson correlation coefficients between dietary and tissue amino acid composition were calculated according to Zar (1996).

Results

Experiment 1

Experiment 1 examined the time course of incorporation of [^{3}H]phenylalanine and showed that the flooding-dose technique is suitable for the study of protein turnover in shrimps. The incorporation times for individual shrimps were 30–120 min. The mean phenylalanine (Phe) specific radioactivity (S_a, d.p.m. $nmol^{-1}$ Phe) in the tail-muscle free pool was elevated within 10 min of injection and did not decline significantly with time (Fig. 1A). The mean free-pool specific activity at each time interval was significantly lower (ANOVA, P<0.05) than the specific activity of the injection solution. The free-pool S_a in the tail muscle reached a mean value of 52% of the S_a of the injection solution (2312±89.62 d.p.m. $nmol^{-1}$ Phe). Following injection, the free phenylalanine concentrations in the tail-muscle free pool (nmol Phe g^{-1} wet mass) were elevated over the range of incorporation times (ANOVA, P<0.05) (Fig. 1B). Taking a tail-muscle free phenylalanine concentration of 400 nmol Phe g^{-1} wet mass in uninjected shrimp (see below), the results indicate a fourfold increase in free phenylalanine levels following injection. Labelling of the tail-muscle protein-bound phenylalanine (S_b, d.p.m. $nmol^{-1}$ Phe) was linear over the incorporation time t (min) (Fig. 1C) and was described by S_b=0.122+0.005t (r^2=0.933, N=8, P<0.005). From the intercept of the regression line, it is possible to estimate how soon after injection the radiolabel began to be incorporated into body protein. In Fig. 1C, the intercept is not significantly different from zero (t=1.12, P<0.27), indicating that labelling in the tail muscle began very soon after injection, and incorporation of the radiolabel into muscle protein doubled with time over the incorporation period. These findings also indicate a uniform rate of labelling. The fractional rates of protein synthesis measured 10, 30, 60 and 120 min post-injection were not significantly different for the tail muscle (mean 1.1±0.08% day^{-1}; ANOVA, P<0.13).

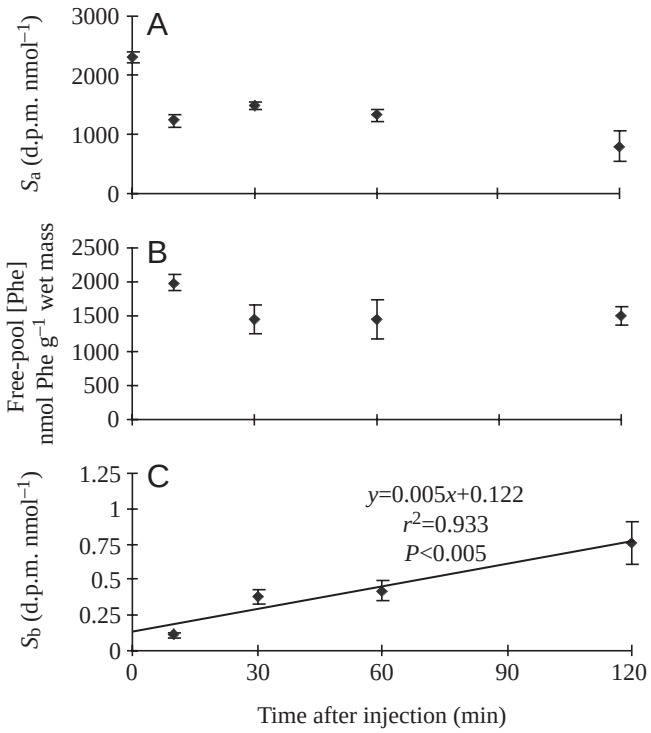

Fig. 1. (A) Mean tail-muscle free-pool phenylalanine (Phe) specific activity (S_a, d.p.m. $nmol^{-1}$); the value at 0 min is the specific activity of the injection solution. (B) Mean tail-muscle free-pool phenylalanine concentration (nmol Phe g^{-1} wet mass). (C) Mean tail-muscle protein-bound phenylalanine specific radioactivity (S_b, d.p.m. $nmol^{-1}$) for shrimps from Experiment 1 at various times after injection. Values are means ± S.E.M. at each time interval (N=8).

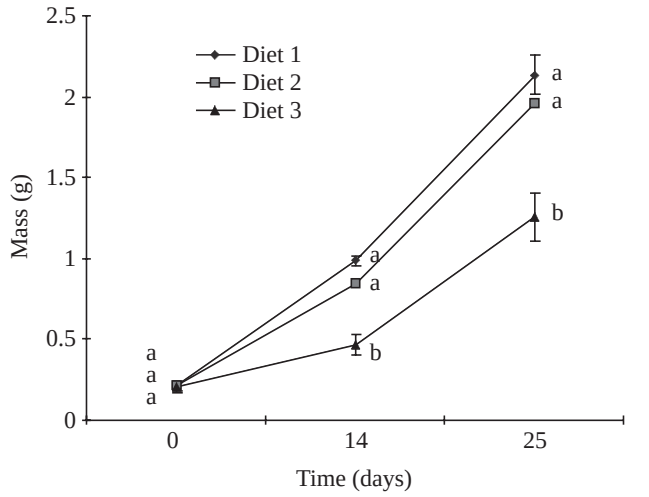

Fig. 2. Mass gain (mean ± S.E.M.) of shrimps *L. vannamei* fed the experimental diets 1–3 in the tanks. Values annotated with the same superscript are not significantly different at the $P<0.05$ level.

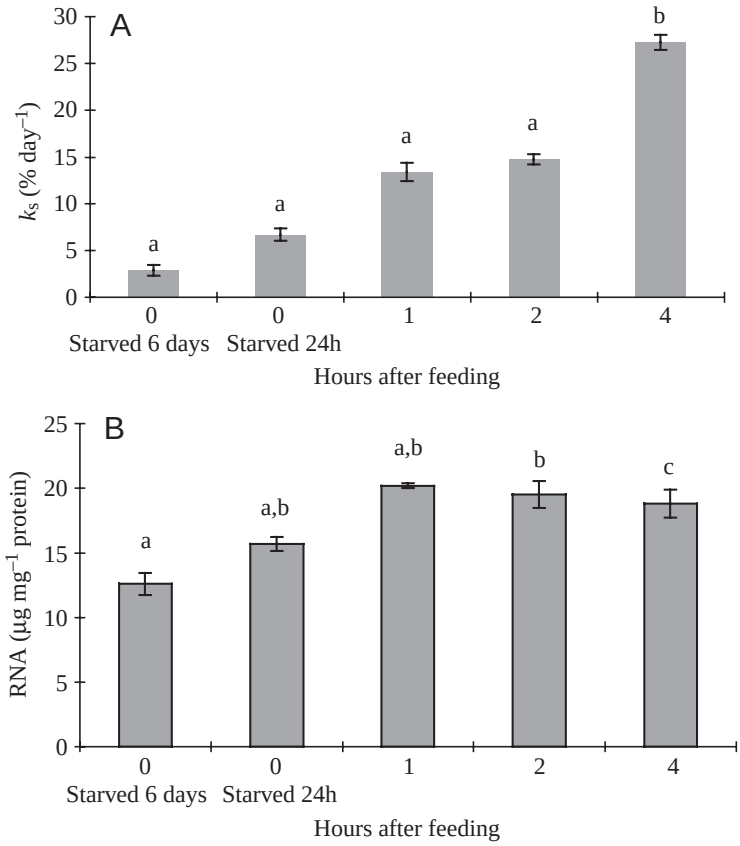

Fig. 3. (A) Fractional rates of whole-animal protein synthesis (k_s,% day^{-1}, mean ± S.E.M., N=5) of *L. vannamei* shrimps starved for 6 days or for 24 h, and at various times (h) after being offered a single meal of diet 1. (B) RNA:protein concentrations (µg RNA mg^{-1} protein; means ± S.E.M., N=5) of whole *L. vannamei* shrimp starved for 6 days or 24 h, and at various times (h) after being offered a single meal of diet 1. Values annotated with the same superscript are not significantly different at the $P<0.05$ level.

Experiment 2

At the start of the experiment there were no significant differences in means or range of initial body mass between shrimps fed the three diets (ANOVA, $P>0.05$). There were no significant differences between the final mean masses of shrimps fed diets 1 and 2 over the first 14 days and 24 days (ANOVA, $P>0.05$) (Fig. 2). However, the mean final masses of shrimps fed diet 3 on days 14 and 25 were significantly lower than the mean final masses of shrimps fed diets 1 and 2 (ANOVA, $P<0.05$). The growth performance of shrimps fed diet 1 (Table 2*)* was not significantly different from those fed diet 2, but was significantly different from those fed diet 3 (ANOVA, $P<0.05$). Survival rates of shrimps were 98% (diet 1) to 93% (diet 2) (Table 2). Shrimps fed diet 3 had a significantly lower survival rate than shrimps fed diets 1 or 2. The effect of diet quality on whole-animal protein turnover is summarized in Table 2. Whole-animal fractional rates of protein synthesis (k_s, % day^{-1}), were not significantly different between diets 1, 2 and 3, but tended to be lower for diet 3 (ANOVA, $P>0.05$). However, diet 3 had statistically significant lower fractional protein growth rate (k_g, % day^{-1}) and statistically significant higher fractional protein breakdown (k_d, % day^{-1}) compared to diets 1 and 2 (ANOVA, $P<0.005$).

The efficiency with which synthesised protein was retained as protein growth in the whole animal was similar for all the diets (80–94%; Table 2). A significant correlation was found between k_s and k_g for individual shrimps fed diet 1 (y=0.3379x+6.5468, r^2=0.65, N=8), indicating that shrimps with a higher protein synthesis were more efficient in retained growth protein ($P<0.05$).

Experiment 3

The mean whole-animal fractional protein synthesis rates over the first 4 h following feeding of diet 1 was 15% day^{-1}. 1 h after feeding the absolute protein synthesis rates of whole animals tended to increase, although this was not statistically significant (Fig. 3A), compared to those starved for 24 h and 6 days. Fractional protein synthesis rates 2 h after feeding were higher than in shrimps starved for 24 h and for 6 days, and significantly different from those in shrimps 4 h after feeding. In the 6-day and 24 h starved groups the low k_s was accompanied by high RNA:protein concentrations.

The RNA:protein concentration increased significantly 2 h and 4 h after feeding compared with the 6-day starvation group (Fig. 3B). RNA:protein concentration of the 6-day starved shrimps was not significantly different from those starved for 24 h. RNA activity (k_{RNA}) increased significantly 4 h after feeding (ANOVA; $P<0.05$). The relationship between k_s and RNA:protein after feeding was significant [y=–21.465+2.001×RNA:protein, N=5 (at each time point for each diet), r^2=0.48, $P<0.05$]. The slope of the line (not shown) corresponds to the amount of protein synthesised per unit RNA (k_s×10/RNA) (Millward et al., 1973). Thus

Table 2. *Specific growth rates and survival over the 25-day period for groups of shrimps* L. vannamei *fed diets 1, 2 or 3*

	Diet 1	Diet 2	Diet 3
SGR (% day^{-1})	9.53±0.29^{a} (8.81–10.27)^{a}	9.11±0.29^{a} (8.20–9.86)^{a}	7.58±0.48^{b} (6.67–8.79)^{a}
Survival (%)	97.5^{a}	92.5^{a}	62.5^{b}
k_s (% day^{-1})	9.75±1.5^{a} (4.78–12.55)^{a}	9.60±1.54^{a} (4.05–11.03)^{a}	9.05±2.14^{a} (4.67–11.54)^{a}
k_g (% day^{-1})	9.20±1.03^{a} (7.26–10.69)^{a}	8.98±0.49^{a} (6.47–11.23)^{a}	7.28±1.06^{b} (5.41–8.66)^{a}
k_d (% day^{-1})	0.54±0.23^{a} (–5.22–10.65)^{a}	0.62±0.43^{a} (–4.45–9.56)^{a}	1.76±0.44^{a} (6.22–14.56)^{a}
k_g/k_s (%)	94.33±0.07^{a} (0.56–1.08)^{a}	93.53±0.15^{a} (0.45–1.37)^{a}	80.49±0.09^{a} (0.34–1.14)^{a}

The specific growth rates (SGR) were calculated using the mean wet mass on days 0 and 24 for the shrimps.

Fractional rates: k_s, whole-animal protein synthesis; k_g, growth; k_d, degradation; k_g/k_s, retention efficiency.

Values are means ± S.E.M. N=8, range in parentheses.

Pairs of data with the same superscript (across rows) are not significantly different from each other (P<0.05; Tukey's or Scheffe's multiple-comparison test for means, variance-ratio test of ranges).

Table 3. *Post-prandial changes in the free amino acid concentrations of the tail-muscle and whole-animal free pools of juvenile shrimp fed two experimental diets*

	Time after feeding (h)			
FAA (μmol g^{-1} wet mass)	0	4	9	24
Diet 1				
Tail muscle				
EAA	2.414±1.5^{a}	10.43±3.9^{a}	6.88±2.5^{a}	6.18±2.8^{a}
NEAA	17.98±7.0^{a}	34.13±10^{a}	19.19±8.4^{a}	19.24±9.3^{a}
TAA	10.73±4.4^{a}	22.28±8.7^{a}	13.04±4.5^{a}	12.71±4.9^{a}
Whole animal				
EAA	2.25±0.8^{a}	5.20±1.7^{a}	5.16±1.6^{a}	4.53±1.4^{a}
NEAA	10.03±4.0^{a}	14.30±6.6^{a}	14.35±6.6^{a}	11.11±5^{a}
TAA	6.14±2.2^{a}	9.75±3.5^{a}	9.76±3.4^{a}	7.82±2.6^{a}
Diet 3				
Tail muscle				
EAA	2.41±1.5^{a}	5.59±2.1^{a}	5.64±2.1^{a}	3.80±2.1^{a}
NEAA	17.98±7.0^{a}	21.03±8.0^{a}	21.19±7.6^{a}	18.96±8.5^{a}
TAA	10.73±4.4^{a}	13.31±4.6^{a}	13.41±4.25^{a}	11.98±4.8^{a}
Whole animal				
EAA	2.25±0.8^{a}	4.53±1.2^{a,b}	8.55±1.6^{b}	2.64±1.0^{a}
NEAA	10.03±4.0^{a}	14.06±5.2^{a}	17.15±4.6^{a}	10.87±4.3^{a}
TAA	6.14±2.2^{a}	9.30±2.8^{a}	11.83±2.6^{a}	6.75±2.3^{a}

FAA, free amino acids.

Values are means ± S.E.M. (N=5) of the total levels of free essential amino acids (EAA), free non-essential amino acids (NEAA) and the total free-pool (TAA) concentrations.

Pairs of data with the same superscript (across rows) are not significantly different from each other (P<0.05) (ANOVA, followed by Tukey's multiple-comparison test for means).

the increase in the slope after feeding indicates that there is an increase in k_s/RNA. A meal brings a significant increase in RNA activity.

Experiment 4

The total FAA (EAA + NEAA) concentrations in the tail muscle and whole-animal tissue of the unfed group were compared with those in the three groups (4, 9 and 24 h after feeding) of shrimps fed diets 1 and 3 (Table 3). Generally, the mean concentrations of total and non-essential whole-animal FAA for shrimps fed diets 1 and 3 were not significantly different compared with the unfed shrimps. However, with diet 3, 9 h after feeding there was a statistically significant increase in the concentration of the essential FAA in the whole animal (ANOVA; P<0.05). There were no statistically significant differences between the mean concentrations of total, essential and non-essential tail muscle FAA for the two dietary treatments following feeding. A two-way analysis of variance showed that diet has a major effect on the total essential amino acid concentration in the tail muscle (P<0.03). The total EAA and NEAA concentrations in the tail muscle and whole-animal tissues of the unfed group and those 24 h after feeding were not significantly different from each other in any of the tissues.

The changes in individual essential free amino acid concentrations after feeding are shown in Fig. 4. There was considerable variation in the profile of individual amino acids in the tail-muscle free pool and whole-animal over 24 h after feeding. Following feeding, there were significant increases in the concentrations of arginine, histidine, isoleucine, leucine, threonine and valine in whole animals fed diet 1 (Fig. 4B). In contrast, in whole animals fed diet 3 there were significant increases only in isoleucine, leucine and valine concentrations (Fig. 4C,D). Following feeding, the concentrations of valine, isoleucine, leucine and threonine increased significantly in the tail muscle for both dietary treatments. Arginine was the most abundant amino acid in the tail muscle and in whole animals. In both diet groups the most abundant EAAs in the tail-muscle free pool tended to be arginine, lysine, leucine and valine. In each tissue, tryptophan concentration was the lowest among the EAA and its level remained stable after feeding.

The concentrations of non-essential FAA in the white muscle and whole animal remained stable following feeding, and individual FAA exhibited few significant changes. Glycine, alanine, proline were the most abundant non-essential FAAs. In the tail muscle, asparagine, glycine, ornithine, hydroxyproline and tyrosine concentrations were significantly higher at 4 h after feeding. Although the total non-essential FAA concentrations in the whole animal did not change significantly following feeding diet 1, the concentration of six non-essential FAAs showed significant changes (P<0.05, Table 4). The concentrations of five NEFAAs changed significantly in shrimps after feeding diet 3 (P<0.05, Table 4). Taurine, tyrosine and alanine concentrations increased in tail

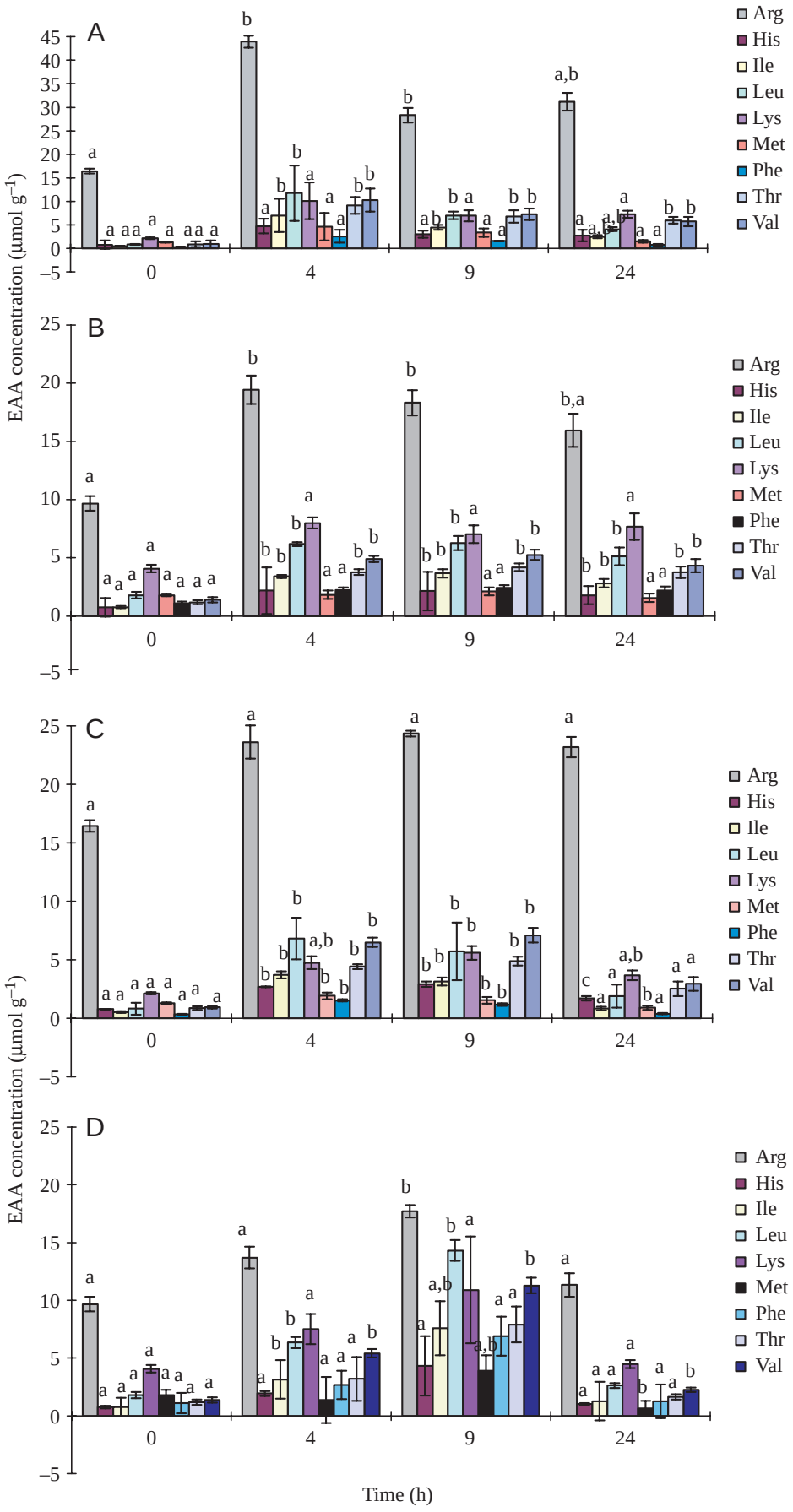

Fig. 4. (A,B) Essential free amino acid (EAA) concentrations (μmol g^{-1} wet mass) in shrimps fed diet 1, in (A) tail-muscle and (B) whole-animal tissue before and at various times (h) after feeding. (C,D) EAA concentrations in shrimps fed diet 3 in (C) tail-muscle and (D) whole-animal tissue before and at various times (h) after feeding. Values are means ± S.E.M. at each time interval (N=5). Values with the same letter (across columns) are not significantly different at the P<0.05 level. The tryptophan concentration was 0.1 μmol g^{-1} wet mass and is not included in the figure.

muscle and reached a plateau after feeding, whilst concentrations of serine and asparagine peaked before decreasing again. Glycine was the most abundant free amino acid in animals fed with either diet, followed by alanine, proline and arginine. Taurine in whole animals decreased significantly after feeding with both diets. Generally, non-essential amino acids were found in the free form in larger amounts than essential amino acids.

Amino acid profile of whole-animal protein

The amino acid profiles of the whole-animal protein from animals fed the two diets did not differ significantly with respect to their essential amino acid concentrations. However, when the amino acid compositions of the diets were compared with the animals' protein, significant differences were found for most of the non-essential amino acids in shrimps fed diet 3 (alanine, asparagine, glycine, glutamine, serine and proline; ANOVA; P<0.05) compared with the amino acid profiles in those fed diet 1. The essential protein amino acid composition of whole-animal and tail-muscle protein of shrimps fed diet 1 exhibited a higher correlation (whole animals, r=0.88; tail muscle, r=0.97, P<0.01) than of those fed diet 3 (whole animals, r=0.83; tail muscle, r=0.86, P<0.05). Fig. 5 shows the relationship between the amino acid patterns of diets 1 and 3 and those in protein of

Table 4. *The free non-essential amino acid concentration (μmol g^{-1} wet mass) in* L. vannamei *(whole animals) fed on diets 1 and 3*

		Time after feeding (h)					
		Diet 1			Diet 3		
Amino acids	0	4	9	24	4	9	24
Alanine	21.44±1.6^{a,b}	25.14±0.9^{b}	27.48±2.0^{b}	16.05±1.1^{a}	32.08±1.5^{a}	34.56±8.6^{a}	20.73±1.2^{a}
Asparagine	1.64±0.1^{a}	3.40±0.2^{c,b}	3.58±0.1^{c}	2.44±0.2^{a,b}	3.54±0.3^{b}	7.35±4.4^{b}	1.97±0.1^{a}
Aspartic acid	3.85±0.4^{a}	3.57±0.6^{a}	2.53±0.4^{a}	2.24±0.4^{a}	4.14±0.7^{a}	9.25±5.2^{b}	3.38±0.4^{a}
Glutamine	5.92±1.4^{a}	12.39±3.0^{a}	11.73±1.4^{a}	9.66±1.5^{a}	8.69±0.9^{a}	12.38±4.3^{a}	8.49±1.0^{a}
Glutamic acid	2.13±0.3^{a}	4.79±0.4^{b}	4.47±0.2^{b}	4.16±0.1^{b}	6.28±0.8^{b}	12.61±7.3^{b}	4.84±0.1^{b}
Glycine	41.44±4.3^{a}	70.90±5.8^{a}	70.15±8.7^{a}	54.45±4.4^{a}	53.52±3.1^{a}	49.11±7.5^{a}	45.03±5.2^{a}
Proline	14.80±2.3^{a}	11.36±2.5^{a}	11.46±1.3^{a}	11.10±2.7^{a}	20.19±2.4^{a}	25.84±6.2^{a}	15.75±5.5^{a}
Serine	0.91±0.08^{a}	4.25±0.1^{c,b}	5.13±0.5^{c}	3.12±0.4^{a,b}	3.25±0.4^{b}	8.74±5.6^{c}	1.30±0.3^{a}
Taurine	7.36±0.3^{a}	5.00±0.5^{b}	4.67±0.2^{b}	5.20±0.4^{b}	5.36±0.4^{b}	5.65±0.7^{b}	5.25±0.4^{b}
Hydroxyproline	0.16±0.01^{a}	0.72±0.05^{a}	1.23±0.08^{a}	1.0±0.03^{a}	2.1±0.001^{a}	1.80±0.01^{a}	1.02±0.02^{a}
Tyrosine	0.84±0.1^{a}	2.16±0.09^{b}	2.32±0.1^{b}	2.67±0.3^{b}	3.53±0.2^{a}	5.97±0.5^{a}	1.93±0.2^{a}

Values are means ± S.E.M. (N=5).
Means with the same letter (across rows) are not significantly different (P<0.05).

whole animals fed these diets. The relationship for diet 1 is close to the ideal line. The deviations from diet 3 for asparagine, alanine, threonine and arginine suggest a deficiency of these amino acids in this diet. The assimilation rate of all amino acids between the two diets was assumed to be the same.

To minimize the effects of different sample pretreatment and hydrolysing agents, the A/E ratio was calculated (Table 5). The A/E ratio for arginine in diet 3 appears to be unsatisfactory. The A/E ratio of *L. vannamei* fed diet 1 showed a decrease in methionine compared with *P. monodon* and *P. japonicus* (Table 5). The A/E ratio (Table 6) of the various animal protein sources generally showed higher arginine, lysine and methionine A/E ratios than those derived from plant sources. The common limiting amino acid for prawn diets utilising either animal or plant protein sources is arginine (Table 6). Only shrimp, squid meals and diet 1 contained arginine levels close to the ideal, confirming the observation that these are the best protein sources for *L. vannamei*.

Using the EAAI (Table 6), based on the method of Penaflorida (1989), a protein material was assumed to be good quality with an EAAI of 0.90 or greater, to be useful when it is approximately 0.80, and to be inadequate when it is below 0.70. Shrimp meal, squid meal, white fish meal, diet 1 and tuna meal were the best protein sources, with an EAAI of 0.98, 0.96, 0.96, 0.95 and 0.92, respectively. Soybean meal was also a good quality source, while casein and diet 3 are useful sources and the sweet potato meal is inadequate.

Amino acid flux

The daily amino acid fluxes of *L. vannamei* fed either diet 1 or diet 3 are shown in Fig. 6. The two groups were assumed to have consumed 0.22 mmol amino acid equivalents for diet 1 or 0.21 mmol amino acid equivalents for diet 3. The amount of amino acid that could be partitioned into protein synthesis were 59% and 57% of the consumed amino acids for diets 1 and 3, respectively, which represents 52% and 75%, respectively, of the FAA pool. The amount of recycled amino acids derived

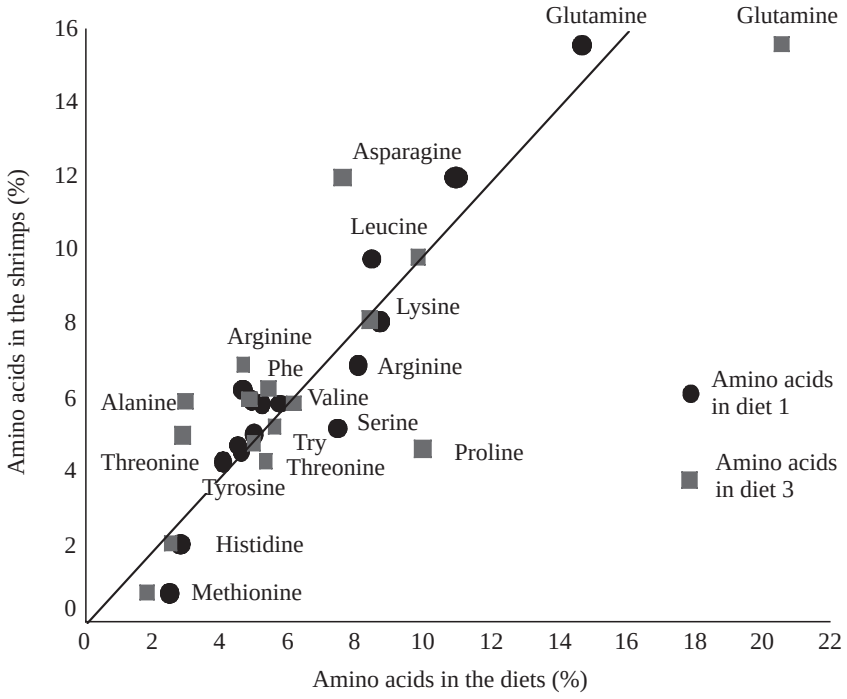

Fig. 5. Amino acid profiles of the shrimp *L. vannamei* fed the experimental diets 1 and 3. Each amino acid is expressed as the percentage of the total protein in the diet and in the shrimp. The 45° line represents the ideal line.

Table 5. *Essential amino acid (A/E) ratio[1] of diet 1, diet 3 and in the whole animal of* L. vannamei, P. monodon, P. japonicus *and* M. rosenbergii *at different stages of growth*

Amino acid	Diet 1	Diet 3	*L. vannamei* Juveniles[2] (2 g)	*P. monodon* Juveniles[3] (postlarvae 50)	*P. japonicus* Juveniles[6] (9 g)	*M. rosenbergii* Juveniles, tail muscle[7] (1.4 g)
Arg	14.75	8.83	13.70	15.25	15.2	20.6
His	5.04	4.78	3.80	4.74	4.5	4.53
Ile	8.96	9.25	10.78	8.49	8.6	7.22
Leu	15.41	18.6	16.29	14.60	15.0	14.76
Lys	15.86	16.0	14.36	14.46	15.8	17.32
Met	4.51	3.5	1.44	7.40[4]	7.5[4]	6.48
Phe	8.56	10.19	11.26	15.54[5]	16.8[5]	7.39
Thr	9.05	5.40	9.06	7.55	8.2	7.55
Trp	9.25	9.45	2.05	2.12	–	–
Val	9.58	11.65	10.58	9.85	8.3	7.32

Data on *Penaeus japonicus*, *Penaeus monodon* and *Macrobranchium rosenbergii* are included for comparison.
[1]A/E ratio = (essential amino acid/total essential amino acids)×100.
[2]Present study, shrimps fed diet 1.
[3]Data from Penaflorida (1989).
[4]Methionine plus cysteine.
[5]Phenylalanine plus tyrosine.
[6]Data from Deshimaru and Shigeno (1972).
[7]Data from Farmanfarmaian and Lauterio (1980).

Table 6. *Ratio of essential amino acids in feedstuffs to that of whole animals* L. vannamei *(aa/AA)[1] and essential amino acid index (EAAI)[2]*

Source	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	EAAI
Diet 1[3]	1.0	1.0	0.8	1.0	1.0	1.0	0.8	1.0	1.0	0.9	0.95
Diet 3[3]	0.6	1.0	0.8	1.0	1.0	1.0	0.9	0.5	1.0	1.0	0.85
White fish meal[4]	0.8	0.9	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.96
Tuna meal[4]	0.7	1.0	1.0	0.9	0.9	0.8	0.8	1.0	0.7	1.0	0.92
Shrimp meal[4]	1.0	0.9	1.0	1.0	0.9	0.8	1.0	1.0	1.0	0.9	0.98
Squid meal[4]	1.0	0.9	1.0	1.0	1.0	1.0	0.8	1.0	0.9	0.8	0.96
Casein[4]	0.3	1.0	1.0	1.0	1.0	0.7	1.0	1.0	0.4	1.0	0.81
Soybean meal[4]	0.8	1.0	1.0	1.0	0.8	0.7	1.0	1.0	0.4	0.9	0.87
Sweet potato meal[4]	0.5	1.0	0.9	1.0	0.6	0.5	1.0	1.0	0.01	1.0	0.53

[1]aa/AA = A/E ratio in feed/A/E ratio in shrimps (see Equation 1 for A/E ratio). aa/AA are set at 0.01 minimum and 1 maximum (Penaflorida, 1989).
[2]$EAAI = \sqrt[n]{aa_1/AA_1 \times aa_2/AA_2 \times \ldots\ldots \times aa_n/AA_n}$, where aa_1 is the A/E ratio in the feed; AA_1 is the A/E ratio in the shrimps; n is the number of essential amino acids.
[3]In this study.
[4]Taken from Penaflorida (1989).

from protein degradation of the protein pool was estimated to be 2.12% and 7%, respectively, for the two diets. The nitrogenous products excreted from the free amino acid pool were calculated to be 32% for the group fed diet 1 compared with 63% for those fed diet 3. It was estimated from the measured growth rates that the percentage of growth relative to consumption was 55% for diet 1 and 43% for diet 3. The FAA pools were calculated as 53% and 38%, respectively, of the protein pool.

Discussion

Validation of the methodology

Although some studies have been carried out on crustaceans (El Haj and Houlihan, 1987; Houlihan et al., 1990; Hewitt, 1992; El Haj et al., 1996), our knowledge of protein synthesis and growth in these animals is still limited. In the present study, the three criteria used to assess whether the rates of protein synthesis can be accurately measured *in vivo* by the high-dose phenylalanine method of Garlick et al. (1980) were validated.

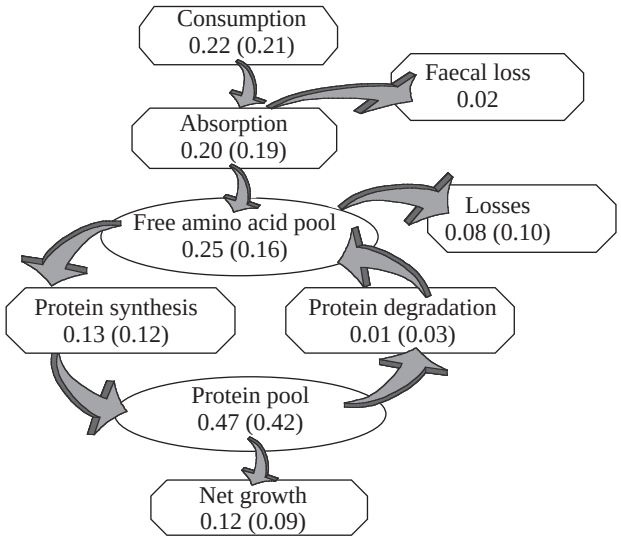

Fig. 6. Amino acid flux (mmol) for a 1.25 g shrimp fed diet 1. The numbers in parentheses are the amino acid flux (mmol) for a 1.12 g shrimp fed diet 3. (The various parameters used in the model are defined in the Materials and methods, amino acid flux.)

The phenylalanine specific activity of the tail-muscle free pool was not equal to that of the injected solution. However, the level remained elevated and stable over the incorporation period. Free phenylalanine levels showed a fourfold increase, and incorporation of radiolabel into protein was linear over the incorporation time. Data on the free amino acid content of *P. esculentus* muscle (Dall and Smith, 1987) indicate that the injected solution should increase the free phenylalanine pool by approximately sixfold, compared to the fourfold increase found in the present study. This difference may be due to the loss of some of the injected solution.

The effect of diet quality on growth and protein synthesis rates

Several studies have examined the relationship between growth and diet quality for *Penaeus* sp. Squid meal is known to be a good protein source for prawns (Kitabayashi et al., 1971; Deshimaru and Shigeno, 1972; Fenucci et al., 1980). Both fish meal and soybean meal are regarded as high quality protein and are highly digestible by shrimps; the apparent protein digestibility of soybean meal is 90% and of fishmeal, 80.7% (Akiyama, 1989). Akiyama et al. (1992) also reported that soybean meal has the best protein profile of all plant sources. Replacement of 50% of the fish meal and shrimp meal by soybean produced higher growth rates and better feed-conversion ratios in *P. californiensis* (Colvin and Brand, 1977). In *Macrobrachium rosenbergii* diets, soybean meal was successfully used to replace fish meal and shrimp meal (Balazs and Ross, 1976). Fenucci et al. (1980) replaced 50% of squid meal with a purified soy protein and obtained better growth, survival and feed conversion ratios in *P. setiferus* and *P. stylioritis*, while Akiyama (1988) indicated that soybean meal at a level of 20 to 50% can replace fish and shrimp head meals without affecting growth and survival of *P. schmitti*, *P. setiferus* and *L. vannamei*. In contrast, Forster and Beard (1973) observed a growth reduction of *Palaemon serratus* when all dietary fish meal was replaced by soybean meal.

The effect of diet quality on *in vivo* rates of protein synthesis has been little studied in crustaceans, although there are some studies in fish (McCarthy, 1993; Carter et al., 1993a,b). Despite the development of the flooding-dose method and the current interest in alternative protein sources for shrimp diets, there have been few studies on the effect of diet quality and protein turnover in juvenile shrimp. The rate of muscle (tail) protein synthesis measured at 27°C in this study (1.26% day^{-1}) was higher than that measured for *Carcinus maenas* carpopodite extensor muscle at a lower temperature (15°C, 1.15% day^{-1}) El Haj and Houlihan, 1987) and for *H. americanus* claw muscle (0.385 day^{-1}) (El Haj et al., 1996). Hewitt (1992) reports similar muscle protein synthesis rates (0.9–1.4% day^{-1}) in *P. esculentus* (5 g wet mass) to those obtained in the present study at 30°C.

Protein turnover

In the present study, whole-animal synthesis rates were 5–9.75% day^{-1}, depending on the diet and the growth conditions. Fractional protein-specific growth rate was higher in the shrimps fed the fish meal diet (diet 1) than those fed the casein protein diet (diet 3). Shrimps fed the fish meal diet and the 50% replacement diet with soybean meal showed enhanced retention of protein *via* a decline in protein degradation, while the casein diet decreased growth rate through an increase in protein degradation. The mortality rate was also high with the casein diet. Therefore casein is likely to be a poor protein source for crustaceans (Lim et al., 1979; Deshimaru, 1982). Experiment 4 investigated whether a dietary amino acid balance is responsible for the difference in protein turnover observed with the casein diet. The results show that the low poor performance of the casein diet may be due to its low content of arginine, an essential amino acid for protein synthesis.

In this study the efficiency of retention of synthesised protein [(whole-animal growth×100/whole-animal synthesis), k_g/k_s] was found to be as high as 94% (diets 1, 2) and 81.8% (diet 3), which is much higher than in fish (trout *Oncorhynchus mykiss*, 35–69%; plaice *Pleuronectes platessa*, 51%; salmon *Salmo salar*, 32%; cod *Gadus morhua*, 42%) (Houlihan et al., 1995b). *Octopus vulgaris* achieved high growth rates and very high retention efficiencies by increasing the level of protein synthesis in combination with very low rates of degradation (Houlihan et al., 1990). In *Mytilus edulis*, Hawkins (1985) found protein retention efficiencies as high as 92%. Thus, protein turnover rates in invertebrates may be much lower than in fish and mammals.

Fractional rates of whole-animal protein synthesis in these experiments were found to peak 4 h after a meal. A stimulation in k_s 3 h after a meal was reported for rainbow trout (McMillan and Houlihan, 1988). Protein synthesis rates also peaked 3 h after feeding in the shore crab *Carcinus maenas* (Houlihan et

al., 1990). In the present study, the fractional rates of protein synthesis of the 6-day starved group was not significantly different from those of after 24 h starvation. Protein synthesis rates are closely correlated with growth rates and RNA:protein concentration (Houlihan et al., 1988), and it is clear that the rate of protein synthesis relative to the RNA concentration can be radically elevated after a meal (Houlihan et al., 1990). The arrival of a meal brings a significant increase in the RNA activity (k_{RNA}). In this study the RNA activity followed the same pattern as the protein synthesis (elevated at 2 h and 4 h after a meal).

Free amino acids in shrimps

The concentration of free amino acids (FAA) in most crustaceans is higher than that in vertebrate tissues (Claybrook, 1983), possibly for osmoregulatory reasons (Awapara, 1962; Claybrook, 1983). The FAA profile for *L. vannamei* (intermoult stage) is similar to that of *P. kerathurus* (Torres, 1973). The FAA pattern in *L. vannamei* is also broadly similar to those in other crustaceans and its concentration is comparable with those in *Carcinus maenas* and *Palaemon xiphias* (D'aniello, 1980; Claybrook, 1983; Dall and Smith, 1987). In the present study, however, there were some notable differences between the FAA concentrations in whole animal and in tail-muscle tissue (Watts, 1968). The A/E ratio for arginine in diet 3 (Table 5) would appear to be unsatisfactory, implying that adequate arginine was not provided at the protein level in diet 3. In the present work, tryptophan was consistently present at the lowest concentration of all amino acids. Lyndon et al. (1992, 1993) found a significant increase in tryptophan levels in white muscle 12 h after a meal in cod, and correlated this increase with the protein synthesis rates at around this time. Tryptophan is a candidate amino acid that limits the rate of protein synthesis.

The present study demonstrated that although there was some variation in the free pool concentration of individual amino acids, the total level of essential and non-essential amino acids in the tail-muscle free pool remained stable over 24 h for both diet groups. This suggests that intracellular amino acid pools are not determined by passive movements of amino acids, but rather are regulated by active transmembrane transport. The consequence of this is that the tissue free pools are to some extent defended against sudden changes in concentration, although clearly feeding does cause an increase, although insignificant, in the tail-muscle free pools of both diets (4 h post-feeding). Previous work showed peak concentrations of plasma amino acids 12 h after feeding in pellet-fed rainbow trout (Walton and Wilson, 1986) and whole sandeel-fed Atlantic cod (Lyndon et al., 1993). We obtained similar results for whole-animal levels in shrimps fed diet 1; however, when fed diet 3, there was a significant increase 9 h after feeding, which is difficult to explain, but may be due to the combined effects of starvation and the inadequate nature of the diet. It has been suggested that the changes observed in refeeding studies would probably decrease with regular feeding, which would result in a more continuous supply of absorbed amino acids to the body tissue (Lyndon, 1990).

Ratios of amino acids

The ratios of bound to free amino acids vary for the individual amino acids, and are also specific for organs and, to some extent, for species (Simon, 1989). The amino acid pattern of the whole animal is mainly determined by the pattern of body proteins, and changes in the free amino acid pattern are of negligible influence. The results of the present study show that the concentrations of free amino acids after a single meal are lower in relative amount than those of protein-bound amino acids (Fig. 7), except for alanine, proline and glycine. Thus, free amino acids that are precursors for protein synthesis and substrates for oxidation appear to turn over at very high rates. In addition, the present study shows that there is a low protein turnover in shrimps and that high growth rates are achieved through efficient retention of synthesized proteins. Use of the whole-animal amino-acid profile of an animal to determine its dietary protein requirement was first considered by Philipps and Brockway (1956) in rainbow trout (*Salmo gairdneri*) and by Deshimaru and Shigeno (1972) in crustaceans (*P. japonicus).* A high correlation was assumed between the dietary profile of essential amino acids and that in the whole-animal protein. Penaflorida (1989) used the profile of essential amino acids of whole shrimp to calculate an essential amino acid index (EAAI). However, when formulating diets, the EAAI should be supported by feeding trials and digestibility tests to determine the incorporation of these protein sources. In the present study, diet 1 was the best protein source with an EAAI of 0.95, while diet 3 was useful with an EAAI of 0.85. Diet 3 had low growth rates and low fractional protein synthesis rates, which is consistent with the amino acid profile results.

Amino acid profile in the diets and in shrimps

In this study, for both diet 1 and 3 treatments, the percentages of essential amino acids and total amino acids were found to be greater than those reported by Farmanfarmaian and Lauterio (1980), who fed *M. rosenbergii* juveniles a commercial feed, and by Reed and D'Abramo (1989), who fed two standard reference diets. The amino acid profile of the whole *L. vannamei* was compared with those of the two diets. When compared to the whole-animal amino acid profile, diet 1 was low in isoleucine, phenylalanine and valine, while diet 3 was low in arginine, isoleucine, phenylalanine and threonine (Table 5). The contributions of asparagine and alanine were lower in diet 3 compared with diet 1 (Fig. 5**)**. The amino acid profile of diet 3 is rather imbalanced, which is consistent with the long-term growth results. No significant correlation was found between diet amino-acid profile and either whole-animal or tail-muscle free essential amino acids, up to 24 h post-feeding. Most of the amino acids were abundant in diets but were poorly represented in the whole animal after feeding. Thus, the FAA patterns in the whole animal and tail muscle seem to be relatively stable. Reed and D'Abramo

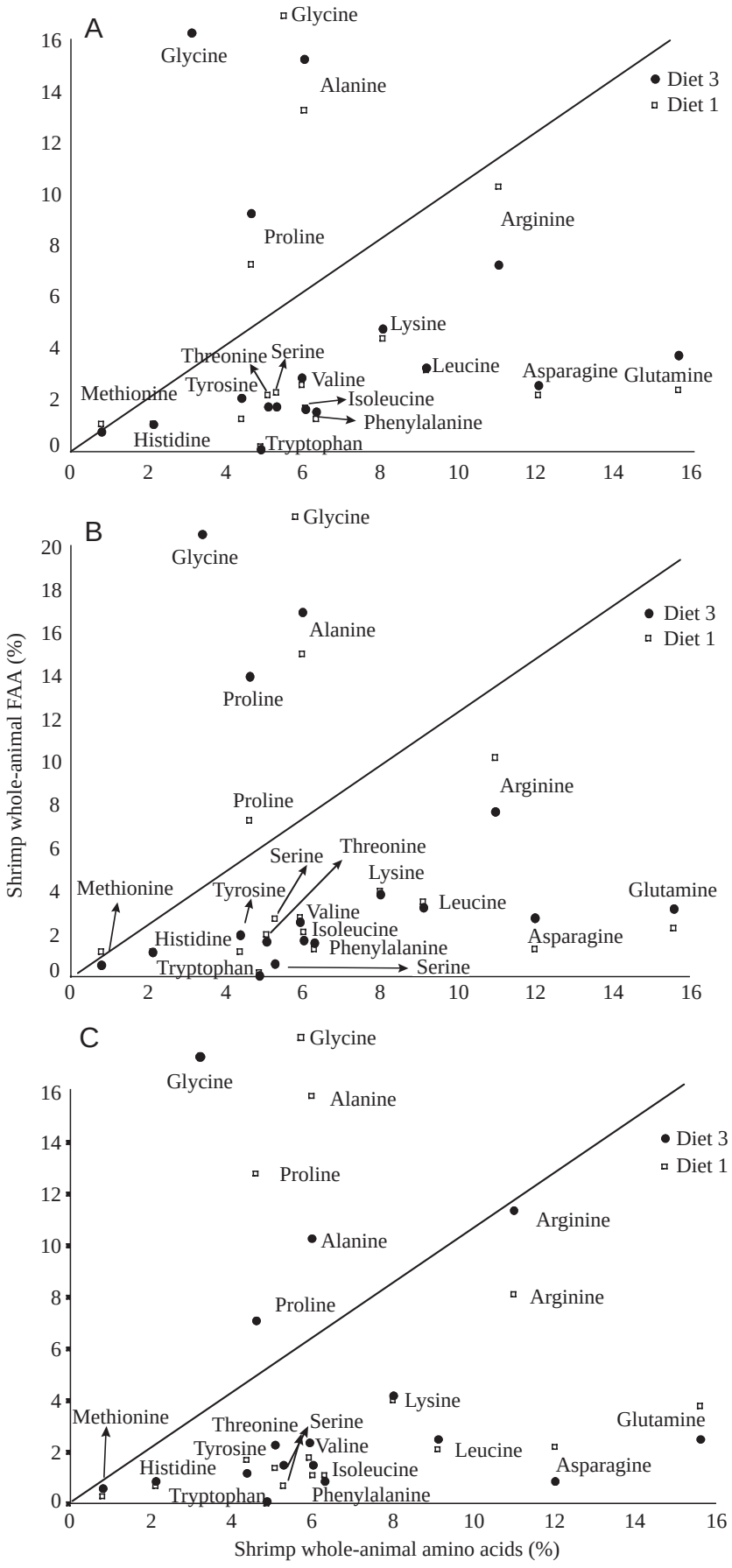

Fig. 7. Amino acid profile (whole-animal free amino acids/whole-animal protein-bound amino acids) of the shrimp *L. vannamei* at (A) 4 h, (B) 9 h and (C) 24 h after feeding diets 1 or 3. The 45° line represents the ideal line.

(1989) also found no correlation between dietary amino acid composition and the concentrations of FAA in the tissues (tail muscle and whole animal) of juvenile prawns *M. rosenbergii*. Other studies have found that the plasma FAA pattern shows the best correlation with the dietary amino acid pattern in fish 12 h after food intake (Nose, 1972; Ogata, 1986; Lyndon et al., 1993). There are no differences apparent when comparing the protein amino acid profiles of this study to those of other *Penaeid* sp. (Table 4). However, increasing arginine and lysine and decreasing isoleucine, leucine, valine and threonine levels with growth stage in *L. vannamei* and *P. japonicus* has been observed (Table 5). It is usually accepted that the amino acid profiles of the whole-animal protein differ little between and within species (Wilson and Poe, 1985; Wilson, 1994; Ramseyer and Garling, 1994).

Amino acid flux model

The amino acid flux models suggest a high food-conversion efficiency (growth/intake) compared to the range of values between 20% in juvenile and 48% in adult fish (Houlihan et al., 1995b), though it is not as high as the 63% found in larval herring (Houlihan et al., 1995b) and 93% found in turbot larvae (Conceição et al., 1997b). These high values apply to fast-growing larval fish, where high protein efficiencies are expected. Estimates of protein conversion efficiency in the present study should be treated with caution, since the estimated amino acid consumption is based on literature values. Shrimp have a nibbling feeding habit (Bordner and Conklin, 1981), and consumption measurements are not widely available in the literature due to the difficulties in measuring food and protein intake. Although there are a number of uncertainties, the amino acid model in this study does point to differences between the two experimental diets.

The amino acid flux diagram (Fig. 6) is comparable to the one published for larval herring (Houlihan et al., 1995b). The size of the FAA pool is 53% of the protein pool for diet 1 and 38% for diet 3, compared to 29% in herring larvae and 2.3% in rainbow trout (wet mass 250 g). This means that, on a daily basis, the

dietary amino acid intake is 88% (diet 1) of the whole animal's FAA pool. This might imply that the arrival of the ingested amino acids would have a relatively small effect on the FAA pool composition in diet 1, while in diet 3 the daily dietary amino acid intake represents almost the whole-animal FAA pool. In addition, protein synthesis will remove on a daily basis the equivalent of 52% (diet 1) or 75% (diet 3) of the FAA pool. We know that free pools remain relatively constant following a meal (present study; Houlihan et al., 1993). Protein degradation will return to the FAA pool 4% (diet 1) or 18.75% (diet 3) of its size. An amount equivalent to 60% of the absorbed amino acid was incorporated into protein for diet 1 and 47% for diet 3, respectively. The retention of synthesised protein was 92% (diet 1) and 75% (diet 3). Amino acid losses were 32% (diet 1) and 63% (diet 3) of the FAA. Thus, it appears that in shrimps there is a little scope for recycling of essential amino acids if these become limiting in the diet. The present study suggests that high growth rates seem to involve a reduction in the turnover of proteins while amino acid losses appear to be high. The high amino-acid oxidation may be related to the high FAA concentration. Lower protein turnover may conserve energy for growth (or other processes), as protein turnover is responsible for a large fraction of the energy budget (Conceição, 1997a); however, high protein turnover allows fast response of the organism to environmental/disease stress, through the synthesis of specific enzymes and other proteins (Conceição, 1997b).

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