

# HOW FOOD CONSUMPTION AND DIET QUALITY CAN INFLUENCE THE GROWTH OF TROPICAL SHRIMPS AND LOBSTERS

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## *Abstract*

This study investigates the protein metabolism in decapod crustaceans (shrimps and lobsters) and examines how food consumption and diet quality can affect their growth. Protein synthesis rates were quantified *in vivo* by measuring the incorporation of a single radiolabeled essential amino acid into whole animal using a single flooding dose injection to administer the radiolabel. The results of this study have shown that a 50% replacement of marine animal meal did not affect the growth rates or protein turnover in shrimps *L. vannamei*. The efficiency with which synthesised protein was retained as growth was 38% for the lobsters and 93% for the shrimps. The free amino acids pools in the whole body of the shrimps appear to remain constant after a meal. The amino acid flux of the shrimps suggests higher protein conversion efficiency (growth/intake) compared to the lobsters. This study contributes to the improvement of our knowledge on nutritional requirements of the above species.

Keywords: protein metabolism, amino acids, crustaceans

## 1 INTRODUCTION

The past 30 years have seen significant advances in the commercial aquacultural production of fin fish species such as salmonids, sea bass and sea bream. However, the crustacean aquaculture industry is presently in its infancy in Europe and therefore it would benefit from fundamental research that seeks to examine how the growth and food conversion efficiency of candidate species (shrimps, lobsters and crabs) can be maximised. The efficient culture of species can be compared to a length of chain. It is as strong as its weakest link. It is dependent on knowledge of nutritional requirements of the species in order to develop and deliver suitable diets to the growing animals to minimise food wastage, to promote efficient

food conversion (maximise protein deposition in the animal) and to maximise growth performance under culture conditions. Understanding the physiological basis of observed growth in terms of anabolic and catabolic processes will then enable informed decisions to be made on the modification of diets and feeding regimes. The particular cost-effective feed being formulated should contain many individual nutrients that are essential for optimal crustacean performance. The nutritive value of a dietary protein is governed by the extent to which its content of amino acids reflects the needs of the animal in question. The changes in the tissue free amino acid levels after a meal have been used as a criterion for determining AA requirements, based on the hypothesis that the concentration of an individual free AA will remain low until its requirement is met [1]. The influences of diet on growth rate of various stages of *L. vannamei* have been studied [2], however, very little is known about the protein metabolism of the shrimp and the lobster although some studies have been carried out on the crustaceans ([3], [4], [5]). This study examines the effect of dietary protein source on growth and protein turnover in juveniles shrimps and lobsters. It also provides information on feeding frequency for lobster culture.

## 2 MATERIAL AND METHODS

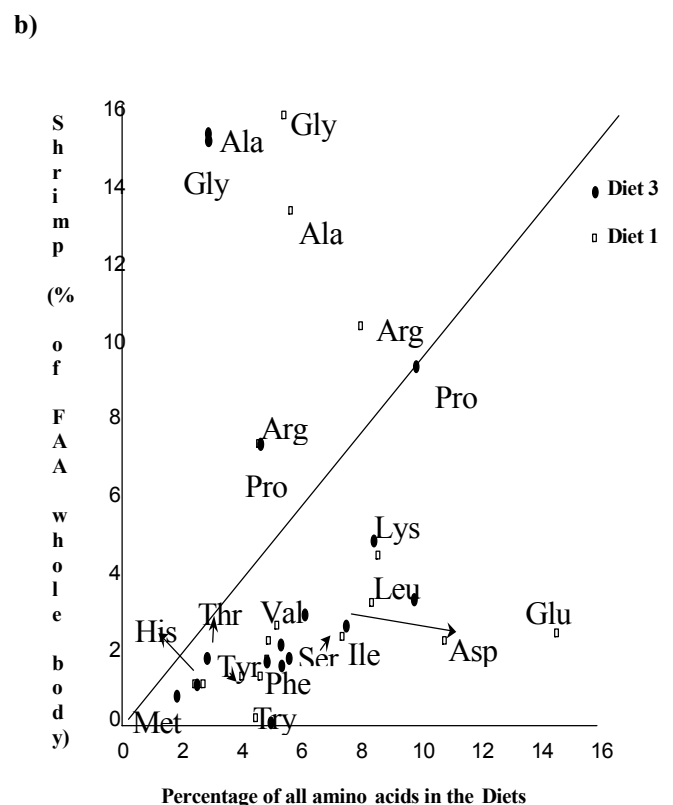
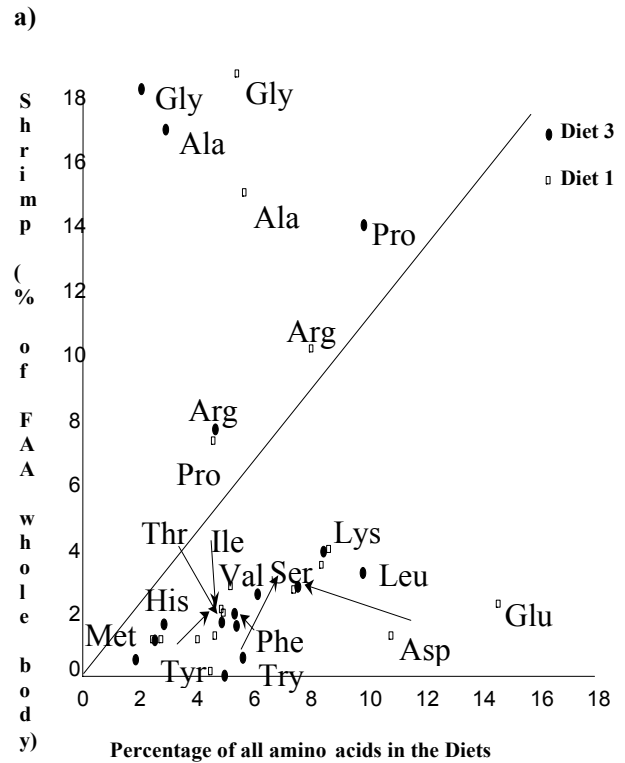
Shrimps (*L. vannamei*) were fed either a control diet, where fish/squid/shrimp meal was the sole source of dietary protein (Diet 1), or a replacement diet, where 50% of the above fish/squid/shrimp meal was replaced by soybean meal (Diet 2) or a casein-based microbound diet (Diet 3). Temperature was kept at 27±1°C. Each diet was evaluated in five replicates (forty shrimps per diet). The effect of dietary protein on protein synthesis and growth of juvenile shrimps *L. vannamei* was investigated. Fourth (IV) stage lobsters *H. gammarus* were fed individually a marine animal meal (60% herring meal mixed with 40% mussels tissue) for 56 days. The animals were divided into five groups of 25 individuals each. They were fed daily a ration corresponding to 5%, 10% and 20% of their body weight respectively and fed every 4 days a ration

corresponding to 20% of their body weight. Temperature was kept at  $19 \pm 1^\circ\text{C}$ . The effect of feeding frequency on growth and protein metabolism in the European lobster, *H. gammarus*, was investigated. Protein synthesis has been investigated by a flooding dose of tritiated phenylalanine. The flooding dose technique, together with measurements of protein growth, was successfully applied in both studies to measure protein turnover in shrimps, and lobsters. The criteria of the technique [6] were fully satisfied. The amino acid composition of the whole body of the shrimp was measured using the method given by Lyndon *et al.* [7]. In order to gain some insight into the likely amino acid concentrations occurring in shrimps, the effects of dietary amino acids on free amino acid (FAA) profile in whole-body were examined. Free amino acids pattern in the body may also be strongly affected by the time after feeding. Therefore, post-feeding change in FAA pattern should be sufficiently studied in order to apply the FAA profile for assessments of the protein quality of a particular diet. The amino acid model of Millward and Rivers, [8] was adapted for lobsters and shrimps in order to present the amino acid metabolism. Data were analysed using the Student's t-test when comparisons between two groups or two measurements within a group (i.e. initial and final weights) were made, and by one way ANOVA tests when more than two groups were compared. Where the ANOVA indicated a significant effect, Scheffe's or Tukey's multiple comparison test were used as appropriate depending on sample sizes. In the statistical analysis differences present at 5% level were considered significant.

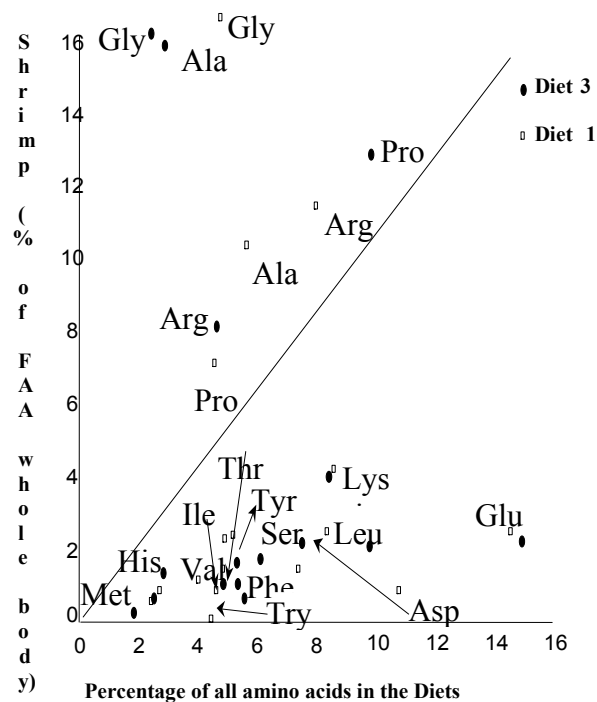
### 3 RESULTS

The results showed that the mean initial and final wet weight for shrimps from all groups were significantly different (Diet 1: t-test = 12.9,  $df=4$ ,  $p<0.05$ ; diet 2: t-test = 15.13,  $df=4$ ,  $p<0.05$ ; diet 3 t-test = 6.73,  $df=3$ ,  $p<0.05$ ). At the end of the experiment the protein synthesis rates were not significantly different for all groups of shrimps (ANOVA,  $F=0.04$ ,  $df=24$ ,  $p>0.05$ ). However, protein growth rates were not significantly different for shrimps, which received either a fish/squid/shrimp meal diet or a 50% replacement by soybean meal variant diet whereas casein diet caused a decrease in growth and an increase in the mortality (ANOVA,  $F=6.16$ ,  $df=25$ ,  $p<0.05$ ). The amino acid profiles of the whole-body protein of *L. vannamei* compared with casein diet showed a deficiency of asparagine, alanine, threonine and arginine concentrations. This is in line with the results of the low growth rates. Figure 1 shows that most of the free amino acids in whole-body of the shrimps turn over at high rates after feeding. The fact that free amino acids are precursors for protein synthesis is confirmed by the results, which also show an increase in the rate of protein synthesis after refeeding. The free amino acids

pools in the whole body tissue appear to remain constant after a meal (fig. 1). However, figure 1 also shows that alanine, proline, arginine, and glycine were abundant in the free amino acids of the whole-body but were poorly represented in the diets after feeding.



c)



**Figure 1:** Proportions (%) of amino acids in the diets (Diet 1 and Diet 3) and the appearance of free amino acids in whole body *L. vannamei* shrimps a) 9h, b) 4h and c) 24h after feeding. The line is the best fit line.

In addition, the amino acid flux diagram presented in this study shows that the arrival of the ingested amino acids would have a relatively small effect on the free amino acid pool composition of the shrimps consuming the fish/squid/shrimp meal (Diet 1) (figure 2) whereas the opposite occurs in casein diet 3. The amino acid flux diagram also shows that 60% of the absorbed amino acids were incorporated into protein for Diet 1 and only 47% for Diet 3. It also indicates that protein synthesis acts as a major factor in the removal of amino acids from free pools.

Feeding every four days gave the lowest growth. Feeding a daily ration equivalent to 10% of their body weight (lobsters) gave better growth than feeding daily rations of 5% and 20%. Protein synthesis rates were not significant different for the three daily food rations but protein growth rates were significantly lower (ANOVA,  $F=13.3$ ,  $df=17$ ,  $p<0.05$ ) and protein degradation rates highest in the 5% body weight per day ration group. The efficiency with which synthesised protein was retained as growth was found to be 38% in the 10% ratio group (Table 1). Protein synthesis rates of lobsters were found to be lower than those for shrimps *L. vannamei* (Table 1).

**Table 1 :** Summary of fractional rates of whole body protein synthesis ( $k_s$ , %/day) and the efficiency of retention of synthesised protein ( $k_r/k_s$ , %) in vertebrates and invertebrates. The water temperature ( $T^\circ\text{C}$ ) and salinity ( $S\text{‰}$ ) is indicated for each experiment.

Species	Weight	$T^\circ\text{C}$	$S\text{‰}$	$k_s$
Sea Bass	2.3	18	34	2.80-9.40
Sea Bass	8g	18	34	1.7
Sea Bass	8g	18	34	1.6
Tilapia	10g	26	0	2.3
Grass carp	17g	22	0	2.2
Gold fish	5g	10	0	3.6
Octopus	150	22	33	6.3
White shrimp	2.1	27	30	9.75
White shrimp	1.2	27	30	9
Juveniles lobsters eating:				
a) 10% daily	stage IV	19	33	8.9
b) 20% every two days	"	19	33	7.1

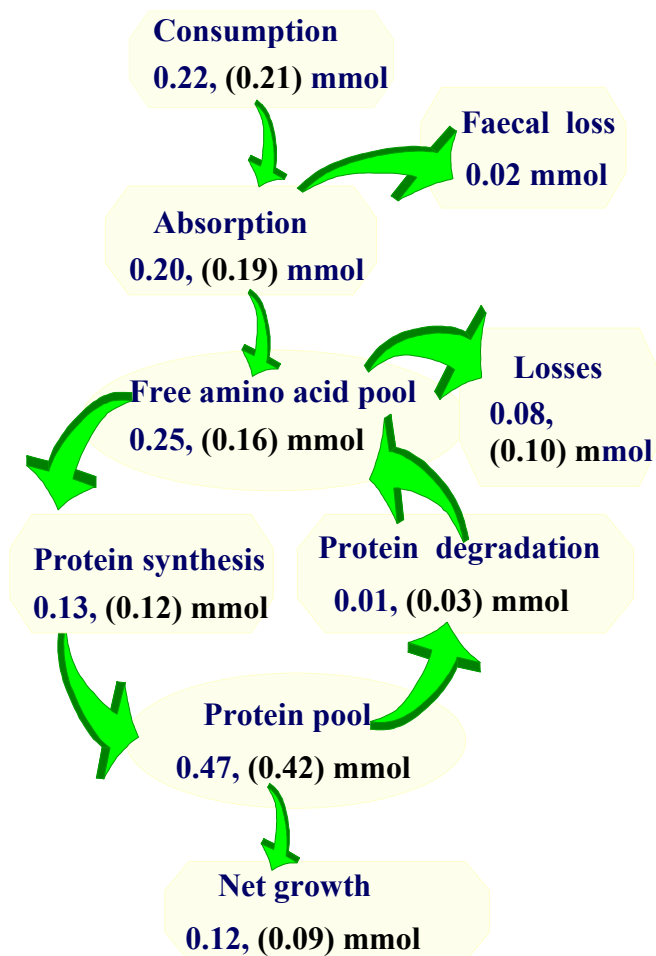
Species	$k_r/k_s$	Diet	References
Sea Bass	30-60	different diets	Langar <i>et al.</i> , [16].
Sea Bass	-	Fish meal	McCarthy, [17].
Sea Bass	-	50%fishmeal / 50%greaves meal	"
Tilapia	59	-	Houlihan <i>et al.</i> , [18].
Grass carp	54.2	Pellets	Carter <i>et al.</i> , [19a,b]
Gold fish	2.2		Heba, [20]
Octopus	95	crabs	Houlihan <i>et al.</i> , [21]
White shrimp	94	Diet 1	Mente <i>et al.</i> , [22]
White shrimp	80	Diet 3	"
Juveniles lobsters eating:			
a) 10% daily	38	marine animal meal	Mente <i>et al.</i> , [15]
b) 20% every two days	29	"	"

## 4 DISCUSSION

Both studies confirm that the methodology, previously used to measure protein synthesis in a wide variety of mammals, fish (reviewed by Houlihan *et al.*, [9] and amphibians [10] can be successfully applied to measure protein synthesis in crustaceans. Protein synthesis rates have been measured using the flooding method in the same species as in this study (crabs: [3] and in lobsters [5]) although the size of the experimental animals were not similar.

Protein metabolism is fundamental to the growth of all animals and therefore an understanding of such process is essential. Protein synthesis is essential to provide a supply of structural proteins to promote growth of the animal. Knowledge of the dynamics of muscle protein deposition and the relationship between diet quality, protein synthesis and muscle protein

deposition is required. Protein turnover is considered to be a highly dynamic process which results in a continuous flux of amino acids into the protein pool via anabolic processes (i.e. protein synthesis,  $k_s$ ) and out of the protein pool via catabolic processes (i.e. protein degradation,  $k_d$ ). The efficiency of retention of



**Figure 2:** Amino acid flux for a 1.25g shrimp [22] fed Diet 1 and for a 1.12g shrimp fed Diet 3 (the numbers in parenthesis) based on the model of Millward and Rivers [8]. The apparent absorption efficiency was assumed to be 90% for marine meal protein and 91% for casein [23]. One gram of protein is equal to 9 mmole of free amino acids [9].

synthesised protein (protein growth rate x 100/ rate of protein synthesis) is used as a key indicator of strategies of protein metabolism (Table 1). Dietary amino acids imbalance lead to a reduction in growth rate and a decrease in the efficiency of retention of the synthesised protein (Table 1, Diet 3). High efficiencies of retention of synthesised proteins indicate reduced protein degradation rates and hence low turnover rates

in growing animals. Indeed, this study, shows that in fast growing shrimps the efficiency of whole-body protein retention was 94%. In tropical shrimps *L. vannamei* there is an increase in protein synthesis retention efficiency with increasing growth rates. It seems that tropical shrimps sacrifice protein turnover in order to maximise retention efficiencies of synthesised protein. The present results confirm the results reported by [4] who examined the shrimp *Penaeus esculentus* and suggested low protein turnover rates (i.e., degradation) in invertebrates. This study offers further insight by modelling protein metabolism and by presenting amino acids flux diagrams.

The amino acid flux model for the shrimps (fig 2) showed that the animals regulate free amino acid concentrations and it appears that protein synthesis acts as a mechanism for regulation of free amino acid concentrations. Free amino acid pools in white muscle are relatively unaffected by feeding [11]. In this study the total free amino acid pool concentrations showed stability with time after feeding (fig.1). The concentration of free glycine makes up about one-third of the total free amino acids on crustaceans [12] and it is the major constituent of the FAA pool together with proline, arginine alanine and serine [13]. The lack of any correlation between the concentrations of free amino acid in whole-body and dietary amino acid composition does not preclude the possibility that amino acid requirements of crustaceans could be estimated by analysis of levels in the hemolymph [14]. The amino acid flux of the lobsters also suggests low protein conversion efficiency [15] compared to 55% found in shrimps and 63% found in larval herring. The results suggests that lobsters are slow, periodic feeders and that growth can be readily increased by manipulation of particular environmental factors such as feeding frequency.

This study showed that an increased rate of growth at temperature 27°C is a result of increased rate of protein synthesis and reduced protein turnover in shrimps. In contrast, lobsters (*H. gammarus*) showed lower growth rates at 19°C temperature and an increase in protein degradation. Thus, the results may be interpreting as indicating that there are species differences in protein synthesis rates. However it has been demonstrated that the efficiencies of retention of synthesised proteins increase with temperature (reduced protein degradation with increased temperature in growing animals is contrasted with increased degradation in starved animals, reviewed by Houlihan *et al.*, [9]). The common octopus, *Octopus vulgaris* grows extremely rapidly and as Table 1 shows has similar protein retention efficiencies with the values obtained for shrimps in this study at nearly the same temperature (22°C). Thus, although the results of this study showed that there are specific differences in

protein turnover between different species the interpretation remains ambiguous due to the effect of environmental factors such as temperature, salinity, body size and age.

## 5 ACKNOWLEDGEMENTS

We are grateful to the Marie Curie grant-FAIR GT961292- (under the 4th Framework program of the European Union, "Human Capital and Mobility" and "Training and Mobility of Researchers") for financial support.

## 6 REFERENCES

- [12] Awapara, J. (1962). Free Amino Acids In Invertebrates: A Comparative Study Of Their Distribution. In: Amino Acid Pools (Ed Holden, J.T.). Elsevier, Amsterdam, Pp 158-175.
- [19a] Carter, C.G. Houlihan, D.F., Brechin, J.G. & Mccarthy, I.D. (1993a). The Relationships Between Protein Intake Accretion, Synthesis And Retention Efficiency In Grass Carp, *Ctenopharyngodon Idella* (Val.). Canadian Journal Of Zoology 72:609-617.
- [19b] Carter, C.G., Houlihan, D.F., Brechin, J. Mccarthy, I.D. & Davidson, J. (1993b). Protein Synthesis In Grass Carp, *Ctenopharyngodon Idella* (Val.), And Its Relation To Diet Quality. In: Fish Nutrition In Practice (Eds. Kaushik, S.J & Luquet, P.). Proceedings Of The Ivth International Symposium On Fish Nutrition And Feeding, Inra, Paris, Pp 673-680.
- [11] Carter, C.G., He, Z.Y., Houlihan, D.F., McCarthy, I.D. & Davidson, I. (1995). Effect of feeding on tissue free amino acid concentrations in rainbow trout (*Oncorhynchus mykiss Walbaum*). Fish Physiology and Biochemistry 14: 153-164.
- [2] Cruz-Suarez, L.E., Ricque, D., Martinez-Vega, J.A. & Wesche-Ebeling, P. (1993). Evaluation of two shrimp by-product meals as protein sources in diets for *Litopenaeus vannamei*. Aquaculture, 115: 53-62.
- [23] D'Abramo, L.R., Conklin, D.E. & Akiyama, D.M. (1997). Crustacean nutrition, vol. 6, World Aquaculture Society, Louisiana, USA, pp 587.
- [3] El Haj, & Houlihan, D.F., (1987). In Vitro And In Vivo Protein Synthesis Rates In A Crustacean Muscle During The Moulting Cycle. J. Exp. Biol. 127:413-426.
- [5] El Haj, A.J., Clarke, R.S., Harrison, P. & Chang, E.S. (1996). In Vivo Muscle Protein Synthesis Rates In The American Lobster *Homarus Americanus* During The Moulting Cycle And In Response To 20-Hydroxyecdysone. Journal Of Experimental Biology, 199: 579-585.
- [10] Fraser, K.P.P. (1998). Growth, food consumption, protein metabolism and environmental adaptations in fish, amphibians and reptiles. PhD Thesis, University of Aberdeen, pp 196.
- [6] Garlick, P.J., McNurlan, A.M. & Preedy, V.R. (1980). A Rapid And Convenient Technique For Measuring The Rate Of Protein Synthesis In Tissues By Injection Of [3h]Phenylalanine. Journal Of Biochemistry, 192: 719-723.
- [20] Heba, H.M.A. (1992). Protein turnover in temperate and tropical fish. PhD thesis, University of Aberdeen., pp 151.
- [4] Hewitt, D.R. (1992). Dietary Protein Utilisation By The Brown Tiger Prawn, *Penaeus Esculentus*. Phd Thesis, Griffith University, Queensland, Australia.
- [21] Houlihan, D.F., McMilan, D.N., Agnisola, C., Trara Genoino, I. & Foti, L. (1990). Protein synthesis and growth in *Octopus vulgaris*. Marine Biology, 106: 251-259.
- [18] Houlihan, D.F., Pannevis, M.C. & Heba, H. (1993). Protein synthesis in juvenile tilapia, *Oreochromis mossambicus*. Journal of the World Aquaculture Society, 24:145-151.
- [9] Houlihan, D.F., Carter, C.G & McCarthy, I.D., (1995). Protein Synthesis In Fish. In: Biochemistry And Molecular Biology Of Fishes, Vol. 4, Chapter 8, Hochachka And Mommsen (Eds.), Elsevier Science B.V., Pp 191-220.
- [16] Langar, H. & Guillaume, J. (1994). Effect Of Feeding Pattern And Dietary Protein Source On Protein Synthesis In European Sea Bass (*Dicentrarchus Labrax*). Comp. Bioche. Physiol. A., 108: 461-466.
- [7] Lyndon, A.R., Davidson, I. & Houlihan, D.F. (1993). Changes in tissue and plasma free amino acid concentrations after feeding in Atlantic cod. Fish Phys. And Biochemistry, 10(5): 365-375.
- [17] McCarthy, I.D. (1993). Feeding Behaviour And Protein Turnover In Fish. Phd Thesis, University of Aberdeen, UK.
- [13] McCoid, V., Miget, R. & Finne, G. (1984). Effect Of Environmental Salinity On The Free Amino Acid Composition And Concentration In Penaeid Shrimp. J. Food Sci., 49: 327-330.
- [15] Mente, E., Houlihan, D.F., & Smith, K. (2001a). Growth, feeding frequency protein turnover and amino acid metabolism in European lobster *Homarus gammarus* L. Journal of Experimental Zoology, 289(7): 419-432.
- [22] Mente, E., Couteau, P., Houlihan D.F., Davidson, I. & Sorgeloos P. (2001b). Protein turnover, amino acid profile and amino acid flux in juvenile shrimp *Litopenaeus vannamei* Boone: Effects of dietary protein source. Journal of Experimental Biology. In press.
- [8] Millward, D.J. & Rivers, J. (1988). The nutritional role of indispensable amino acids and the metabolic basis for their requirements. European Journal of Clinical Nutrition, 42: 367-393.
- [14] Reed, L. & D'abramo, L.R. (1989). A Standard Reference Diet For Crustacean Nutrition Research. Iii. Effects On Weight Gain And Amino Acid Composition Of Whole Body And Tail Muscle Of Juvenile Prawns *Macrobrachium Rosenbergi*. Journal Of The World Aquaculture Society, 20: 107-113.
- [1] Wilson, R.P. (1994). Amino acid requirements of finfish. In: Amino acids in farm animal nutrition (ed. D'Mello, J.P.F.). CAB International, Wallingford, UK, pp 377-399.