Specific Amino Acid–Free Semi–Purified Diets for *Penaeus monodon* Juveniles

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Abstract

To determine the effects of deficiency of individual amino acids believed to be essential to *Penaeus monodon* juveniles, a 28–day feeding experiment was carried out with *P. monodon* postlarvae.

P. monodon postlarvae weighing around 120 mg each were randomly distributed in individual perforated one liter jars, 10 jars in 60–liter, fiberglass tanks, in a flow through seawater system, which passed through a 0.35 micron filter before use. Animals were reared in these jars for 28 days on semi–purified moist diets. Salinity and temperature ranged from 27 to 32 ppt and 26 to 28.9°C, respectively.

Percentage weight gains and survival rates were not significantly different among test diets. However, some amino acids seemed to be more critical than others in the diets. Animals given phenylalanine–free, leucine–free, and methionine–free diets gained more than 300% compared to those fed the complete amino acid diets. A low weight gain of 228.6% was observed in animals fed arginine–free diet. Animals fed the histidine–free, lysine–free, threonine–free and valine–free diets also gained less than 200%. Survival rates were 60% for those given threonine–free and isoleucine–free diets, 70% for histidine–free and complete amino acid diets, whereas those fed the phenylalanine–free, leucine–free and methionine–free diets had survival of 80 to 85%. These results suggest the possibility that some amino acids were taken from sources outside the diets and could have come from whatever bacterial growth there was in the rearing jars.

Key Words:Amino acid, Essential amino acid, Prawn, *Penaeus monodon*

In developing practical diets for *Penaeus monodon* juveniles, not only the quantity but also the quality of protein in diets is very important because amino acids are the building blocks for protein. DESHIMARU and KUROKI (1975) showed that certain amino

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acids were needed by *P. japonicus*. KANAZAWA and TESHIMA (1981) by a radioisotopic method using [^{3}H] acetate reported that little or no radioactivity was incorporated in arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and isoleucine indicating that these amino acids are not synthesized *de novo* and therefore are probably essential for prawn growth. SHEWBART *et al.* (1973) have shown that *P. aztecus* need 10 amino acids in their diets. COLOSO and CRUZ (1980) determined the amino acids required by the prawns by radioactive assay and showed that *P. monodon* juveniles cannot synthesize arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Likewise, in *Macrobrachium ohione*, MIYAJIMA and BRODERICK (1977) showed that the animal incorporated little or no ^{14}C in the same amino acids mentioned by COLOSO and CRUZ (1980). Similar patterns have been found in *P. serratus* (COWEY and FORSTER, 1971). Although essential amino acid patterns have been defined, specific amino acid requirements of *P. monodon* have not been studied.

It is important in the formulation of practical diets that essential amino acid pattern of the diets is optimum for the prawn to provide for an effective diet. Thus, this study was carried out to determine the amino acids needed by the prawn by biological method.

Methodology

P. monodon postlarvae from one spawner, weighing around 120 mg each that had been fed mussel for 2 weeks were randomly distributed in perforated one liter jars, 10 jars in 60–liter fiberglass tanks, in a flow through seawater system, passed through 0.35 micron filter before use. The animals were reared in these jars for 28 days on semi-purified moist diets, the basic composition of which is shown in Table 1. Table 2 gives the amino acid composition of the dry diet and Table 3 the vitamin and mineral mixtures.

Essential amino acids were individually weighed so that each diet deficient in one of the essential amino acids (Diets 1 to 10) while Diet 11 contained a simulation of the amino acid profile of *P. monodon* juveniles. Carboxymethyl cellulose (CMC) was added to the beaker containing all the amino acids and 5 ml distilled water was added to the mixture to bind the amino acids with cellulose. The mixture was kept over night at -70°C to -80°C and subsequently freeze–dried for 25 hours. The freeze–dried amino acid mixture was added to the other dry ingredients, including the dry vitamin mixture. Cellulose replaced the weight of essential amino acid excluded from the diet. α–Tocopherol was added to the pre–weighed pollack liver oil, soybean oil and soybean lecithin mixtures and then added to the dry mixture.

The mixture containing all the other feed ingredients was added to 5g κ–carrageenan in 175 ml distilled water heated at 85°C in a water bath with constant stirring until a homogeneous gel was formed. The diets were cooled and kept in a freezer. Feed enough for 2 or 3 days was kept in tared container and kept in the refrigerator. Feed

Table 1. Percentage diet composition

Ingredient	g / 100g dry diet
Casein (Vitamin free)	10
Amino acids	42.47
Glucose	5.5
Sucrose	10
α-Starch	4
Glucosamine HCl	0.8
Na-citrate	0.3
Na-succinate	0.3
Cholesterol	0.5
Pollak liver oil	5
Soybean oil	5
Soybean lecithin	3
Mineral mixture	8
Vitamin mixture	3
Taurine	0.5
Betaine	0.5
Glutathione	0.1
Cellulose	1.03
Total	100
k-carrageenan	10
CMC	1

Table 2. Amino acid composition of complete diet (Diet No.11)

Amino acid	g / 100g dry diet
Non-essential	
L-Sodium aspartate	7.20
L-Serine	1.10
L-Glutamic acid	7.56
L-Proline	2.18
Glycine	3.72
DL-Alanine	2.12
L-Cystine	0.18
L-Tyrosine	0.98
Essential	
L-Threonine	1.17
L-Valine	1.21
L-Methionine	1.08
L-Isoleucine	1.27
L-Leucine	2.43
L-Phenylalanine	1.37
L-Histidine	0.63
L-Lysine monohydrochloride	1.73
L-Tryptophan	0.78
L-Arginine monohydrochloride	5.76
Total	42.47

Table 3. Compositions of mineral and vitamin mixtures

Mineral mixture	g / 100 g dry diet
K_2HPO_4	1.872
$Ca_3(PO_4)_2$	2.545
$MgSO_4 \cdot 7H_2O$	2.846
$NaH_2PO_4 \cdot 2H_2O$	0.739
Vitamin mixture	**mg / 100g dry diet**
ρ-Aminobenzoic acid	10.0
Biotin	0.4
Inositol	400.0
Niacin	40.0
Pantothenic acid Ca Salt	60.0
Pyridoxine HCl	12.0
Riboflavin	8.0
Thiamine HCl	4.0
Cyanocobalamine	0.08
Ascorbic acid	2000.0
Folic acid	0.8
Choline chloride	600.0
Menadione	4.0
β-Carotene	9.6
α-Tocopherol	20.0
Calciferol	1.2

was offered twice daily at 8:30 a.m. and 4:00 p.m.

Salinity and temperature ranged from 27 to 32 ppt and 26°C to 28.9°C, respectively. Feces were siphoned out every Monday, Wednesday and Friday and the tanks were scribbed on Mondays and water totally drained out. The essential amino scids in the animals before and after the rearing period individually quantified by the method described by KANAZAWA and TESHIMA (1981). (Table 4). A one way analysis of variance was carried out to determine statistical significance among treatment means (STEEL and TORRIE, 1960).

Results and Discussion

Percentage mean weight gains and survival were not signigicantly different from each other (Table 5). However, some trends indicate that some amino acids seemed to

Table 4. Amino acid residue composition (g / 100g material) in whole of *P. monodon* juveniles

Amino acid	Initial	Diet No. 1 (THR)	No. 2 (VAL)	No. 3 (MET)	No. 4 (ILE)	No. 5 (LEU)	No. 6 (PHE)	No. 7 (HIS)	No. 8 (LYS)	No. 9 (TRP)	NO. 10 (ARG)	NO. 11 (Complete)
Essential												
MET	3.06	3.14	2.84	2.97	2.95	2.80	2.29	3.22	3.10	3.10	3.35	3.22
THR	1.79	1.83	1.89	1.94	1.88	1.75	1.70	1.73	1.63	1.83	1.92	1.84
VAL	1.82	2.00	2.07	2.06	2.16	2.04	2.06	2.14	1.83	2.13	2.20	2.24
ILE	1.62	1.77	1.83	1.85	2.01	1.96	1.90	1.96	1.78	1.95	2.00	2.03
LEU	3.10	3.26	3.22	3.51	3.39	3.32	3.19	3.11	3.10	3.25	3.30	3.25
PHE	2.20	2.41	2.42	2.53	2.24	2.36	2.27	2.18	2.19	2.25	2.29	2.27
HIS	1.02	1.09	1.13	1.14	1.21	1.14	1.08	.109	0.96	1.10	1.15	1.14
LYS	3.05	3.04	3.22	3.49	3.37	3.04	3.03	3.12	2.86	3.39	3.13	3.08
TRP	0.61	0.42	0.46	0.45	0.41	0.56	0.38	0.36	0.48	0.52	0.38	0.38
ARG	3.71	3.83	4.04	4.06	4.07	3.80	3.60	3.70	3.42	3.78	3.74	3.92
Non-essential												
ASP	4.25	4.46	4.66	4.91	4.85	4.76	4.40	4.55	4.60	4.72	4.79	4.86
SER	1.70	1.71	1.76	1.86	1.82	1.67	1.56	1.57	1.48	1.70	1.73	1.66
GLU	6.59	6.66	6.98	7.39	7.46	7.08	6.13	6.94	6.38	7.30	7.49	7.31
PRO	2.09	2.53	2.76	3.19	2.60	2.96	3.04	2.72	2.95	2.93	3.07	3.07
GLY	3.51	3.21	3.54	3.54	3.52	3.53	3.14	3.79	3.17	3.83	3.88	3.64
ALA	2.32	2.52	2.57	2.72	2.54	2.47	2.63	2.82	2.48	3.00	3.09	2.95
CYS	0.56	0.76	0.70	1.00	0.59	0.59	0.42	0.51	0.59	0.67	0.71	0.90
TYR	1.92	2.06	2.09	2.22	1.92	2.04	1.89	1.76	1.88	1.85	0.76	1.80
TAU	0.64	0.52	0.56	0.58	0.56	0.55	0.51	0.52	0.43	0.49	0.51	0.51
Total amino acid residue	45.56	47.22	48.84	51.41	49.55	48.42	45.22	47.79	44.77	49.79	50.49	49.89

Table 5. Mean weight, survival and weight gain of *P. monodon* juveniles fed essential amino acid deficient and complete diets*

Diet (Amino acid free)	Mean initial weight g	Mean final weight g	Mean weight gain %	Mean survival %
Isoleucine	0.09	0.38	332.2	60
Arginine	0.14	0.46	228.6	75
Histidine	0.14	0.50	257.1	70
Leucine	0.11	0.50	354.5	85
Valine	0.14	0.52	271.4	85
Methionine	0.09	0.40	344.4	80
Threonine	0.13	0.48	269.2	60
Lysine	0.12	0.44	266.7	70
Tryptophan	0.13	0.54	315.4	75
Phenylalanine	0.10	0.47	370.0	80
Complete amino acid	0.10	0.44	340.0	70

*Feeding trials were conducted by using 3 jars on each test diet. Data shown in Table are the means of 3 jars. Statistical analysis showed no significant difference ($p>0.05$) among dietary groups.

be more critical in the diets than others. Animals given phenylalanine–free, leucine–free, and methionine–free diets in 4 weeks gained more than those fed the complete amino acid diets. The highest of which were those given phenylalanine–free diet (370%). Those fed the diet containing all the amino acids also gained 340%. Animals fed isoleucine–free and tryptophan–free diets also gained more than 300% but grew poorly more than those receiving the complete amino acid diet. Juveniles that gained less than 300% were those that were given the arginine–, histidine–, lysine–, threonine–, and valine–free diets.

Survival rate was 60% for those given threonine–free and isoleucine–free diets, and 70% for histidine–free and complete amino acid diets. These results indicate the possibility that animals might get them from sources outside the diets such as bacterial growth which occurred in the plastic jars. The three diets, phenylalanine–, methionine–, and leucine-free diets that gave the highest weight gains, had also the highest survival rates of from 80 to 85%.

According to STAHL and AHEARN (1978) the dietary lysine, arginine, methionine and tryptophan were found dispensable for growth when each was omitted from the semipurified diets for *Macrobrachium rosenbergii* diets. The authors suspect that gut or tank bacteria may have supplied the prawns with some amino acids in amounts enough for growth.

MURAI *et al.* (1982a,b,c) determined the effect of coating crystalline amino acids with casein on carp and found the the coating improved the balance of essential amino acids in the tissues. Likewise, RUMSEY and KETOLA (1975) observed that supplementing casein or soybean meal with crystalline amino acids to simulate levels of essential amino acids on isolated fish protein and trout eggs significantly imporved growth of Atlantic salmon *Salmo salar* and rainbow trout (*S. gairdneri*), respectively. The utilization of various amino acids by carp (*Cyprinus carpio*) has been found by FISCHER and LIPKA (1983) to depend on the kind protein ingested and not on the percentage of different amino acids.

Results obtained by DESHIMARU (1981) showed that dietary free arginine assimilation into juvenile prawn's crude protein was very low compared to a proteinaceus form of arginine. Therefore, it is suggested that when deficient essential amino acid diets are supplemented with crystalline amino acids, such diets do not affect the growth of prawn. On the other hand, according to MASON and CASTELL (1980), the use of purified proteins such as casein or fish protein concentrate and addition of crystalline amino acids as a technique for quantifying amino acid requirements is a promising technique.

Hence, further work is required to determine the quantifying amino acid requirements of crustaceans.

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