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Changes in Hemolymph Aminoacid Profiles during Starvation in the Penaeid Prawn, *Penaeus monodon*

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A 5-day starvation of penaeid prawn *Penaeus monodon* resulted in a significant decrease in the concentrations of selected FAA — the maximum decrease occurred in glutamic acid, proline, methionine, serine, aspartic acid, isoleucine, alanine, glycine, leucine, phenylalanine, valine, tryptophan and lysine. Trace amounts of arginine, cystine and histidine were also recorded. The concentrations of total AA's (bound and free) for each AA measured were not significantly affected due to starvation - induced stress. From the results obtained it is suggested that AA's rapidly removed from hemolymph during short-term starvation were probably most critical in meeting the demands for tissue metabolic profiles or physiological and biochemical functions inducing osmotic homeostasis and energy derivation.

Key Words : Aminoacid, Penaeid prawn, *Penaeus monodon*

Introduction

In recent times studies on shrimp nutrition have become important with the advent of commercial shrimp culture gaining momemtum. Crustaceans encounter starvation during periods of food scarcity and undergo periods of voluntary fasting during the molt cycle and seasonal dormancy. Most of the techniques involved for the determination of essential AA requirements for crustaceans are based on radioactive labelling studies (Watanabe 1975) and also observing differential rates of growth among crustaceans fed with selected AA deficient diets or commercialy available diets selectively supplemented with AA's (Farmanfarmaian & Lauterio 1980). In cruataceans individual AA's contribute to metabolic nitrogen equilibrium and considered to be the index of AA requirements. The dietary and metabolic requirements for individual AA's are not known for penaeid prawns, despite the variety of techniques and experimental approaches that have been attempted. In the present investigation an attempt was being made to estimate metabolic requirements for selected AA's in the penaeid prawn, *Penaeus monodon*, and changes in the profiles of

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FAA in hemolymph were measured after subjecting to short term stress of starvation. This particular aspect was chosen since it reflects the natural AA fluxes between hemolymph and tissues during the period of stress. Such accentuation in AA fluxes allows detection of AA's necessary for critical metabolic processes.

Materials and Methods

Penaeus monodon were collected from the estuaries around Thummalapenta seacoast, Kavali, A.P, India. Intermolt prawns of 10±1g were selected and acclimatised to laboratory conditions for a period of one week at a constant salinity of 15±1 ppt, *p*H 7.1±0.2 and temperature of 23±2°C. They were fed with 55% protein diet twice a day during accimatization. The medium was changed at regular intervals and continuous aeration was provided to prevent hypoxia. Prawns were divided into two batches one batch subjected to starvation for 5 days (A 5-day starvation period was selected since it resulted in catabolic metabolic processess prior to the degradation of structural proteins in marine invertebrates, Riley 1980), while the other served as controls (fed with formulated diet twice a day). Control and experimental, intermolt animals (as defined by Skinner 1985) were bled by pericardial puncture after 3 hr postprandial period. Only intermolt animals were used in the present study to prevent any discrepancies associated with ecdysis. Samples from three individual animals were pooled and used for analysis. The hemolymph was centrifused at 5000 g for 5 min at 4°C and the samples were stored frozen. Samples were prepared for analysis by hydrolysing 0.1 ml of hemolymph with 1 ml of 6N HCl. The ampule was evacuated with nitrogen, sealed and heated to 110°C. The hydrolysates were diluted with distilled water and freeze-dried. The residue was dissolved in 1 ml of 0.1M sodium citrate buffer (*p*H 2.5) and made upto a volume of 5 ml. The same hydrolysis procedure was used for 50 mg of formula feed that was grounded in a micromill and fat extracted. Fat extraction was performed using methanol : chloroform (1:1). Whole animal (controls) samples were analysed in a LKB Biochrom Automatic aminoacid analyser (Moore et al. 1958). FAA's were extracted by modifying the procedure used by Van Marraewijk and Ravenstein (1974).

The quantification and identification of different AA's was done using standard AA mixture containing glutamic acid (GLU), proline (PRO), leucine (LEU), methionine (MET), lysine (LYS), phenylalanine (PHE), alanine (ALA), aspartic acid (ASP), serine (SER), valine (VAL), isoleucine (ISE), tryptophan (TRP), glycine (GLY), threonine (THR), cysteine (CYS), tyrosine (TYR), histidine (HIS) and arginine (ARG). Calibration curves were obtained for each AA by plotting peak area against known AA's. AA's in the bound form were determined by subtracting the free AA concentration from the total AA concentration.

Results and Discussion

The FAA profiles significantly decreased during the starvation period (figure 1), however the concentrations of respective total AA's (bound and free) were not significantly affected during starvation period (figure 2). In addition to the relatively constant total AA concentration found in the hemolymph of both fed and starved prawns, the relative proportions of AA's were found to be similar

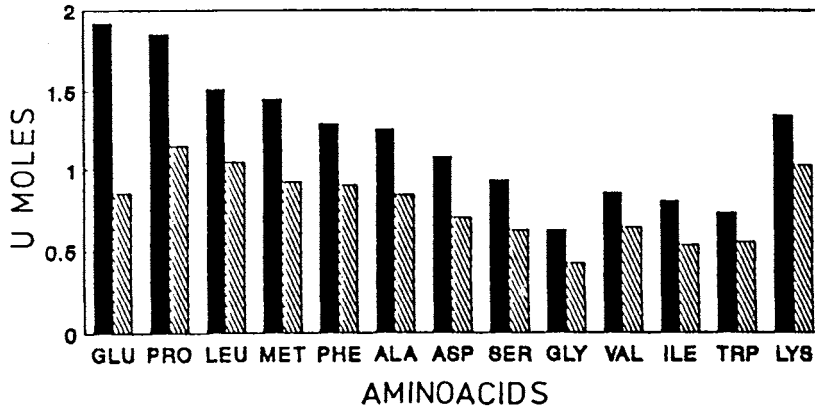

Figure 1 Comparison of free aminoacid concentrations in the hemolymph of fed and starved prawn, *P. monodon*

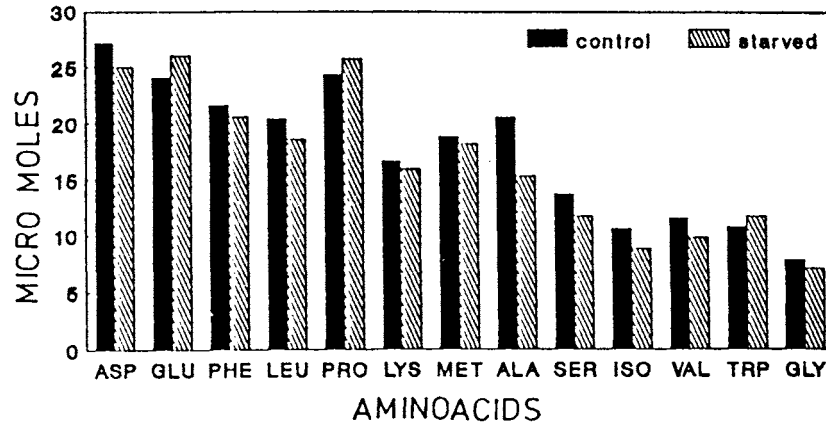

Figure 2 Comparison of total aminoacid concentrations in the hemolymph of fed and starved prawn, *P. monodon*

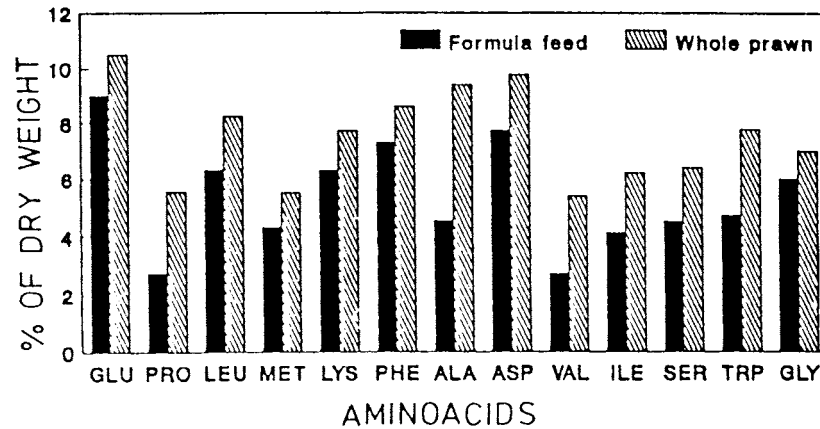

Figure 3 Comparison of TAA's in 55% protein diet and whole prawn, *P. monodon*

in the formula feed and whole prawn (figure 3).

Glutamic acid and proline may be preferential sources of metabolic ammmonia nitrogen principally as α-amino nitrogen since they exhibited the greatest decrease in individual FAA concentration after starvation. Many of the individual AA's in the relatively large FAA pool of aquatic invertebrates including crustaceans can be metabolically synthesized or obtained from protein catabolism (Claybrook 1983). However, one of the most readily available sources of those AA's utilized in osmotic regulation or having a high turnover rate in cellular metabolism is absorption from the blood plasma following absorption from the intestine. Glutamic acid is known to have a relatively high turnover rate in cellular metabolism and is an important osmoeffector in intracellular fluids of aquatic invertebrates. Glutamate also contributes to ammonia production. This statement gains support that ammonia content of tissues and hemolymph were significantly elevated (Srinivasulu Reddy et al. 1988). It is therefore, reasonable to hypothesize that metablolic demands for glutamic acid could exceed cellular capability of synthesis and necessitate some supplementation via absorption from the hemolymph. Such absorption would decrease hemolymph concentrations of glutamic acid during the period of starvation. Another possible reason will be the mobilization of glutamic acid into the tissues through transamination phenomenon for the synthesis of 2-oxoglutarate thereby contributing towards the synthesis of one of the intermediate products of Krebs cycle, succinate. Similarly, proline, a metabolic precursor for glutamate synthesis, could be in demand from hemolymph during the period of starvation. Since no AA assimilation from the intestine to the hemolymph can occur during starvation, a period of starvation may merely accentuate the normal supplementation of AA's to the cellular metabolism from the hemolymph. Similarly, leucine, methionine, lysine, phenylalanine, alanine, aspartic acid, serine, valine, isoleucine, tryptophan and glycine also significantly decreased during starvation. Although total concentration of glutamic acid was relatively high in the formulated feed and also in the whole prawn, dietary supplementation with glutamic acid and AA's with a high turn over-rate may reduce over all protein uptake by the organism. Since proline has a high turn over rate and also is considered to be the precursor for glutamic acid, it is also reasonable to assume that it should be considered as a dietary supplement. Furthermore, the differences between pyruvate and α-ketoglutarate derived AA's are indicative of metabolic requirements and a preferential route of oxidative degradaton. The α-ketoglutarate derived AA's such as proline and glutamine, and those AA's usually considered essential (methionine, leucine, phenylalamine etc) significantly decreased in the concentration of FAA's in starved prawns along with pyruvate derived AA's, such as glycine, alanine, serine and others.

Physiological adaptations of aquatic invertebrates may alter classic concepts of 'essential' and 'non-essential' AA's. While metabolic processes at the cellular level that dictate AA requirements will be somewhat similar for all the organisms, adaptations to a marine or estuarine environment or to growth by molting have necessitated specific AA requirements for aquatic arthropods (Dall 1975). Weber and Van Marraewijk reported that certain FAA were used as osmotic

effectors during adaptation to saline habitats. The relatively high concentrations of total glutamic acid and aspartic acids measured in both serum and also in the tissues of prawn, *P. monodon* clearly indicative of the ubiquitous role of these AA's in either structural protein component or physiological processes of marine invertebrates. Van Marraewijk and Ravestein (1974) reported that total glutamic and aspartic acids were the most concentrated of all total AA's measured in several tissues of crayfish, *Astacus leptodactylus*. Aspartic acid had a relatively low concentration in the hemolymph FAA pool and is usually a small component of the FAA pool of muscle tissues of crustaceans. Aspartic acid is therefore probably a structural component of crustacean protein rather than an active component in crustacean physiology. On the other hand, since glutamic acid is a relatvely large fraction of crustacean FAA pool (Van Marraewijk and Ravestein 1974), as well as in the total FAA fraction, it is probably required for physiological processes of aquatic crustaceans. It has been customary to estimate the individual AA requirements through the incorporation of isotopically labelled AA's from formula feeds into the tissues. Zandee (1966) demonstrated that the inability of the crayfish, *Astacus astacus* to synthesize the 10 AA's that are essential for domestic animals. Based on the isotope studies arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are considered as essential AA's for crustaceans (Claybrook 1983). Similary, Cowey and Forster (1971), Miyajima et al. (1975) and Shewbart et al. (1973) reported that the same list of 10 AA's mentioned were essential for *Palaemon serratus, Macrobrachium ohione and Penaeus aztecus*, respectively. Watanabe (1975) found similar results for *M. rosenbergii* except for lysine and obtained no results for threonine and tryptophan. Although some innate metabolic differences among several natantian crustacean genera and species may also have contributed to variations in essential AA's as reported by several investigators, differences in experimental design, methodology adopted and also the nature of experimental animal or selection of crustacean species probably contributed to most of the discrepancies cited above. The differences observed in a variety of AA's in many growth studies can be attributed to the fact that many factors other than the micro- or macro-nutrient being investigated synergistically affect the growth. Graham et al. (1981) reported that plasma AA concentrations have been used previously to quantitate essential AA concentrations and evaluate the quality of protein. This method is based on observing a reduction in concentration in plasma between a 3 hr postprandial sample and a fasting sample reflecting deficient concentrations of specific AA's. Fasting is considered to be a common phenomenon in the case of crustaceans. Dall (1974) observed a 48% reduction in hemolymph protein concentration of the rock lobster, Panulirus longipes after 4 weeks of starvation. Passano (1960) reported that prior to ecdysis, crustaceans will cease feeding until after the synthesis of new exoskeleton and followed by hardening of it, which may account for 10% of the molt cycle. Several authors showed that during molting, the AA concentrations and other components like water are drastically altered due to molting associated changes. In *C maenus* hemolymph, Busselen (1970) observed different glycoprotein levels during fasting

and concluded that hemolymph served as a "storage organ for proteins and amino acids". Duchateu et al. (1959) observed that proline and glutamic acid accounted for 50% of the post-molt decrease in *Carcinus maenus*. In *Geocarcinus lateralis*, proline, glycine and alanine in muscle, and water decreased during premolt, while lysine, methionine and tyrosine were significantly elevated (Yamaoka & Skinner 1976). But in the present investigation, molting associated changes were eliminated by way of choosing only inermolt individuals.

It may be concluded from the present investigation that a rapid mobilization of AA's from hemolymph to the corresponding tissues during starvation, is probably to meet the critical physiological, biochemical (or) metabolic functions of tissues. Although, it is rather difficult to estimate the individual AA's, which contribute to metabolism in the case of penaeid prawn, *P. monodon*, the results obtained from the current investigation compared to the previous determinations of nutritionally required AA's for marine crustaceans are a reflection of the experimental design employed to estimate the metabolic turnover rates. The discrepancies in AA requirments need to be verified through the turn over rates utilizing radioisotope techniques. At present these differences may be attributed to species differences.

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