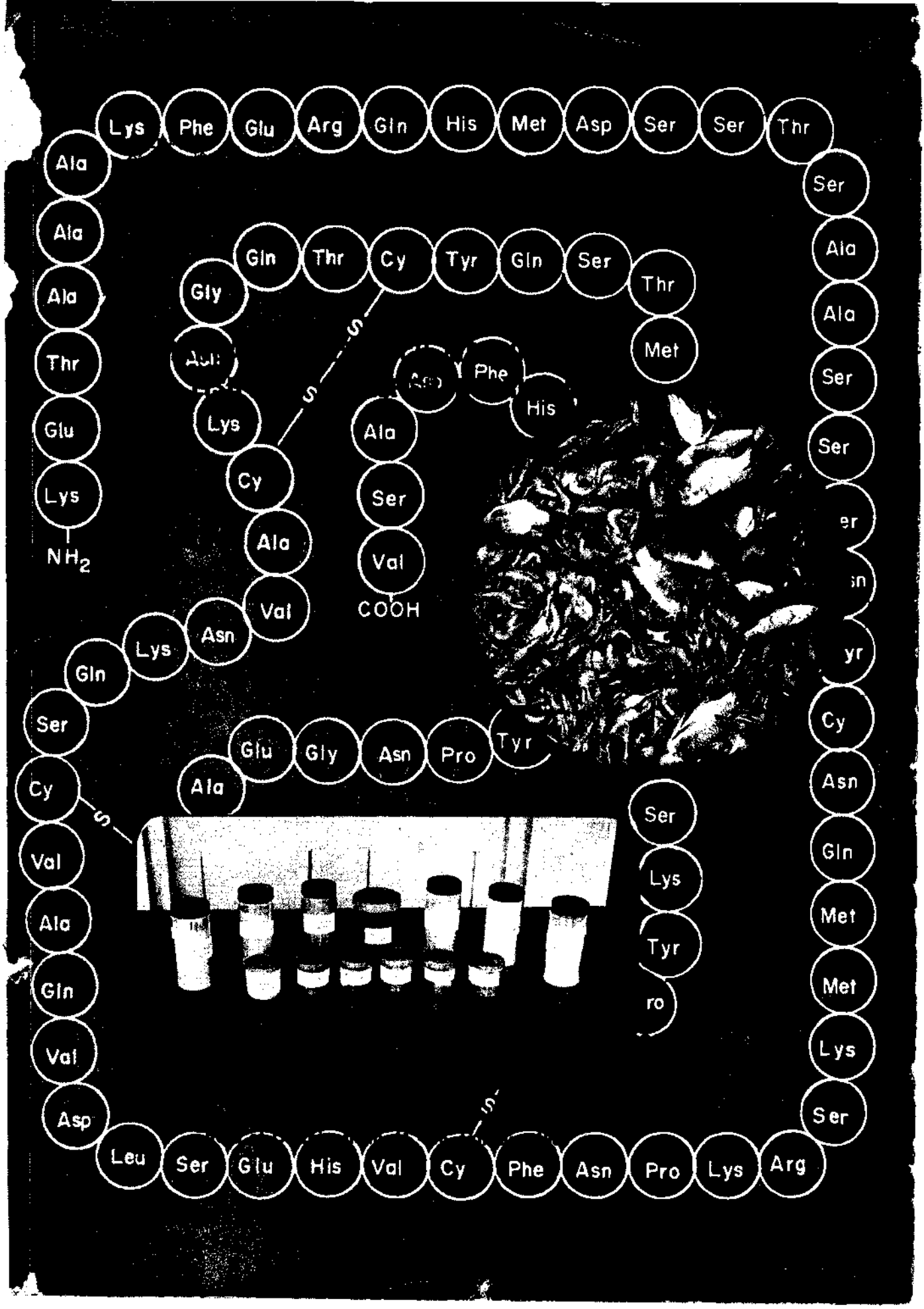
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Ala Ala Ala Thr Glu Lys NH2
Ser Ala Ala Ser Ser er in yr Cy Asn Gln Met Met Lys Ser
Gln Thr Cy Tyr Gln Ser Thr Met
Gly
Lys Cy Ala Val Asn Lys Gln Ser Cy Val Ala Gln Val Asp Leu Ser Glu His Val Cy Phe Asn Pro Lys Arg
Ala Ser Val COOH
Phe His
Glu Gly Asn Pro Tyr
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Ser Lys Tyr ro

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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

Issued on the occasion of the Workshop on
METHODOLOGY FOR FISH AND SHELLFISH NUTRITION
organised by
The Centre of Advanced Studies in Mariculture,
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Published by: **E. G. SILAS**
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COCHIN

PREFACE

The Centre of Advanced Studies in Mariculture established at the Central Marine Fisheries Research Institute has been conducting Workshops in Research Methodologies on specialised disciplines with a view to enhance the competence of the scientific workers specialising in researches connected with mariculture. The main emphasis in mariculture research has been directed towards the development of economically viable culture techniques for culturable species of fish and shellfish, with a view to augmenting the fish and shellfish production of the country. In order to develop low-cost technologies the essential operational inputs have to be rationally utilized.

It has been well established that feeding constitutes the major cost of production, often exceeding 50 per cent of the operating costs in intensive aquaculture operations. Two main factors affecting the cost of feeding are composition of the diet and efficiency of feed conversion. In order to develop least-cost formula diets of high conversion efficiency, knowledge of the nutritional requirements of the different species during the different phases of the life cycle and the nutritive value of the complex feed ingredients available in the country to the candidate species is a prerequisite.

The existing information on the nutritional requirements of cultivated species of fish and shellfish in India, is meagre and recently research has been intensified in this area. If researches on this field could be carried out using standardised experimental procedures, the data obtained on the nutritional requirements of the different species could be stored in a fish and shellfish nutrition data bank, from where data could be disseminated to the users such as feed manufacturers, farmers, extension workers and research workers as and when required. It is also necessary that the data collected on the chemical composition of the feed ingredients and their nutritive value for the species should be based on standard chemical methods and experimental procedures so that the data could be stored in

the data bank which eventually could become a National Fish Feed Information Centre.

To undertake studies on the above lines, especially by the technicians and research workers entering afresh into the field, the need of practical guides describing the research techniques and methods, planning of investigations, collection of data and their interpretation need not be emphasized. Keeping this in view, the present manual on Research Methods in Fish and Shellfish Nutrition is issued by the Centre of Advanced Studies in Mariculture on the occasion of the Workshop on Methodology of Fish and Shellfish Nutrition.

Dr. Akio Kanazawa, Professor of Nutritional Chemistry, University of Kagoshima, Japan and Consultant in Fish and Shellfish Nutrition at the CAS in Mariculture, has been kind enough to cooperate with the Scientists of CAS in Mariculture of the Central Marine Fisheries Research Institute in the preparation of this manual. There are chapters in this manual covering various methods on composition analysis of feeds, including growth inhibitors and toxins; determination of digestibility coefficient; protein evaluation; bioenergetics; determination of essential amino acid requirements using radioisotope method; research test diets for fishes and prawns; feed formulation methods; experimental design, etc. Methods of preparation of microparticulate diets, phytoplankton and zooplankton culture methods, etc. are also included to facilitate larval nutrition studies. Many of the methods given in the manual have been standardized for fish and shellfish nutrition studies in India and abroad. The users can also gain maximum benefit by suitable modifications of other methods which are given as guidelines.

I would like to thank all the scientific and technical staff especially Shri S. Ahamed Ali, Dr. K. Alagarswami, Shri D.C.V. Easterson, Shri C.P. Gopinathan, Shri T. Jacob, Shri M.S. Muthu, Dr. R. Paul Raj, Dr. A.G. Ponniah and

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CHAPTER 11

DETERMINATION OF ESSENTIAL AMINO ACID REQUIREMENT OF PRAWN*

(Radioisotopic tracer method)

1 INTRODUCTION

The requirement of essential amino acids (EAA) are generally estimated on the basis of the following parameters: (1) The weight gain in the feeding experiments using the test diets (amino acid-diets) whose protein sources were replaced entirely or partly by the mixture of amino acids; or (2) The daily increase of each EAA in the fish bodies when they were fed on the diets containing proteins with high biological values.

In the case of the prawn, however, the amino acid-diets are known to attain only poor growth as also observed in the carp. This makes it difficult for us to estimate the EAA requirements of prawn by the feeding trials. Therefore, to define the amino acids essential for the growth of the prawn, radio-isotopic methods are used.

2 PROCEDURE

2.1 Injection of 14^C Acetate to the prawn

Two speciments of the prawn, about 6.0g in total body weight, are each injected with 15 uCi of 14^C sodium acetate dissolved in 1.0 µl of aqueous solution containing carrier sodium acetate (0.7%). The radioactive acetate is injected into the muscle between the carapace and the second abdominal segment, and then the prawns are maintained on the fresh meat of short-necked clam in the aquarium (30 l) at 25°C.

2.2 Fractionation of the prawn

Six days after injection of 14^C acetate, the whole body of prawns is fractionated into 4 fractions by the method of

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* Prepared by Akio.Kanazawa, Professor of Nutritional Chemistry, University of Kagoshima, Japan.

SCHNEIDER; that is, the prawns are minced, homogenized in an ice-cold trichloroacetic acid (TCA), and separated into the TCA-soluble compound fraction and the residue. The residue is then extracted with organic solvents such as ethanol-water (4:1), ethanol, and ethanol-ether (3:1) to give the lipid fraction. The defatted residue is further separated into the protein and nucleic acid fractions by using a hot TCA solution.

2.3 Separation and Quantification of individual amino acids

The protein fraction is oxidized with performic acid as described by HIRS and then hydrolized in a sealed test tube with 5.7N hydrochloric acid by the method of MOORE and STEIN. The performic acid oxidation converts cysteine and cystine to cysteic acid and methionine to the corresponding sulphone. The amino acids of protein hydrolysate are separated and quantified by the method of MOORE et al. using column chromatography on Amberlite IR-120 (type II, Rohm and Hass co.) with sodium citrate buffers. Tryptophan is also quantified and separated in the same manner after hydrolyzing the protein fraction in 5N sodium hydroxide solution as mentioned by OELSHLEGEL et al.

2.4 Measurements of Radioactivity

The radioactivity of lipid, TCA-soluble compound, and nucleic acid fractions from the prawns and individual amino acids from the protein fraction is measured wth a Beckman Liquid Scintillation Counter LS-230 using a dioxane solution of ppo (0.6 %) and naphthalene (11.2%) as a scintillation cocktail. The radioactivity of protein fraction is determined in the similar manner after hydrolysis with hydrochloric acid.

Distribution of radioactivity in the fractions obtained from the prawns 6 days after injection of acetate (30 μCi)

Fraction	Total radioactivity recovered (μCi)
TCA-soluble compounds	0.39
Lipids	0.73
Nucleic acids	0.03
Proteins	0.15
Whole body of prawns	1.30
Sea Water	25.80
Total radioactivity recovered	27.10

Incorporation of radioactivity into the individual amino acids of protein fraction in the prawns 6 days after injection of acetate

Amino acids	Specific activity cpm/μmol)	Amino acids	Specific activity (cpm/μmol)
Aspartic acid	203	Valine	0.1
Serine	102	Methionine	2.5
Glutamic acid	196	Isoleucine	0.7
Proline	138	Leucine	0.1
Glycine	132	Phenylalanine	0.1
Alanine	187	Lysine	0.2
Cysteic acid	159	Histidine	0.7
Tyrosine	1.2	Arginine	1.2
Threonine	1.9	Tryptophan	0.4

Radioactivity is unambigously incorporated into aspartic acid, serine, glutamic acid, proline, glycine, alanine, and cysteic acid. These amino acids are suspected to be unnecessary for the prawn. On the other hand, little or no radioactivity is incorporated into valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, threonine, and tryptophan. These 10 amino acids are not synthesized de novo and are probably essential for the growth of the prawn. Tyrosine is thought to be formed from the ingested phenylalanine.

3 REFERENCE

1. Manual Book of Experiments, Laboratory of Fisheries Chemistry, University of Kagoshima, Japan. 1980-81.