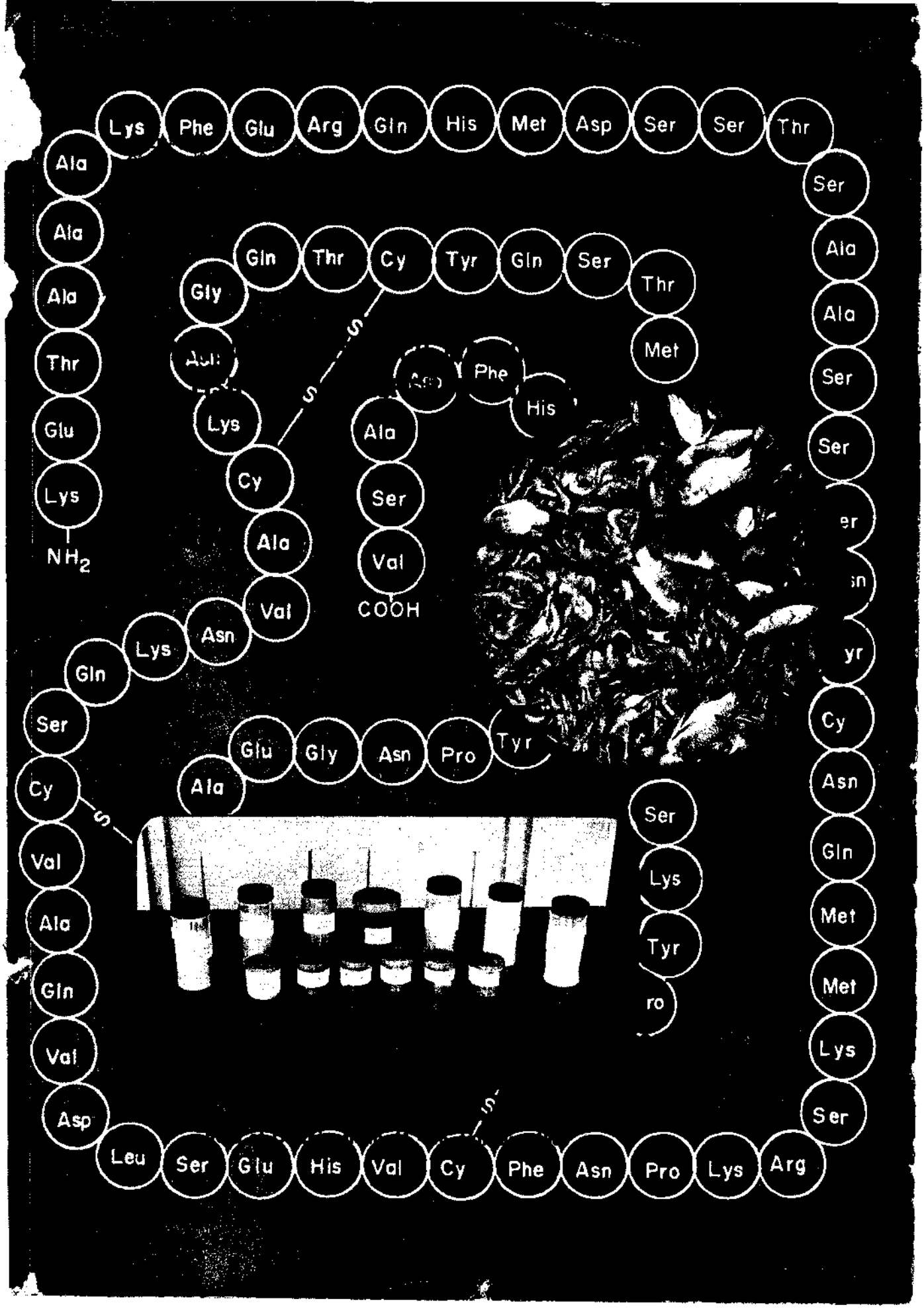
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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

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 10

SYSTEMS OF EXPRESSING THE PROTEIN VALUES OF THE FEED AND METHODS OF THEIR ESTIMATION*

1 INTRODUCTION

In chemical composition studies, proteins in the feeds are estimated by determining the nitrogen content and multiplying by a factor 6.25 and the resultant value is the crude protein. However, considerable variation in the efficiency of conversion of different dietary sources of proteins have been reported in the literature using isonitrogenous diets, and these variations have been attributed to the quality difference among proteins particularly in their amino acids profile. The following terms are widely used in the biological evaluation of proteins in fishes and crustaceans.

2 EXPERIMENTAL PROCEDURES

Experiments are conducted in specially designed aquaria with provision for collection of the different nitrogen fractions. The experimental set-up devised by Ogino, Kakino and Chen (1973) and shown in Fig.2 of Chapter 9 is an example. Experimental animals of the same species, size group and age are acclimatized in the aquaria for a stipulated period. During the acclimatization period feeding is done with the experimental protein diet. The oxygen, temperature, salinity levels are maintained similar in all the aquaria. The experimental protein diets are fed to the animals once or twice a day in fixed timings. Data on the various nitrogen fractions are collected by sampling food, water, faeces and also carcass analysis. Periodically weight of the animals are recorded to adjust and maintain the feeding level. At the end, the total protein consumed, wt. gained, various forms of nitrogen losses, carcass nitrogen, etc., are analysed and composed.

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* Prepared by R. Paul Raj, Central Marine Fisheries Research Institute, Cochin-18.

3 PROTEIN EFFICIENCY RATIO (PER)

In this case the nutritive value of the dietary protein is determined by the rate at which the animal grows. It is defined as the weight gain per unit intake of protein and may be calculated from the following formula:

$$PER = \frac{\text{gain in body weight (g)}}{\text{protein intake (g)}}$$

PER will vary with the percentage protein in the diet, with a maximum efficiency at a certain level. The determination of PER demand feeding over a longer period, and in fishes and crustaceans it is strongly related to the water temperature.

4 TRUE NET PROTEIN UTILIZATION (NPU)

This term originally proposed by Bender and Miller (1953) is one of the most used terms in relation to protein evaluation. The original formula was modified for fishes (Castell and Tiews 1980).

$$NPU = \frac{Ni-(Nf-Nm) - (NU-Nen) - (Nb-Neb)}{Ni} = \frac{Nct - Nco}{Ni}$$

where Ni = nitrogen intake
Nf = faecal nitrogen
Nm = metabolic faecal nitrogen
NU = Urinary nitrogen
Nen = endogenous urinary nitrogen
Nb = branchial nitrogen
Neb = endogenous branchial nitrogen
Nct = carcass nitrogen of test group
Nco = carcass nitrogen of group receiving a nitrogen-free diet

The first fraction in this equation require determinations of faecal and urinary nitrogen in groups on test diet as well as on non-protein diet. One of the major problems encoun-

tered is the solubility of the faecal nitrogen and collection of this part of the nitrogen is very difficult with the experimental devises developed so far.

The second fraction, however, can be more easily applied to fishes. Body nitrogen can be determined by analysis of each single fish in the case of fingerlings, whereas, careful homogenization would give values based on analysis of samples from larger fish. The method however, require adjustment of test and control diet to equal caloric contents.

5 APPARENT NET PROTEIN UTILIZATION
(PRODUCTIVE PROTEIN VALUE)

If no correction is made for endogenous nitrogen losses, the apparent net protein utilization would be determined. This function is identical with the term productive protein value (PPV).

$$\text{app NPU} = \frac{Ni-Nf-Nu-Nb}{Ni} = \frac{\text{N retained}}{\text{N consumed}}$$

where Nb is branchial nitrogen

The advantage of the method is that it does not require a control group, and the nitrogen retention can be conveniently and very precisely determined. The method is well suited for assay of different proteins, as the endogenous nitrogen may be considered equal in all groups.

6 BIOLOGICAL VALUE OF PROTEIN (BV)

It is defined as the percentage of the absorbed protein which is utilized by the body. In this case losses in digestion and metabolism are taken into consideration.

$$BV = \frac{Ni-(Nf-Nm) - (Nu-Nen) - (Nb-Neb)}{Ni - (Nf-Nm)}$$

From net protein utilization and protein digestibility, we can derive this value

$$BV = \frac{\text{Net protein utilization}}{\text{True digestibility of protein}}$$

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