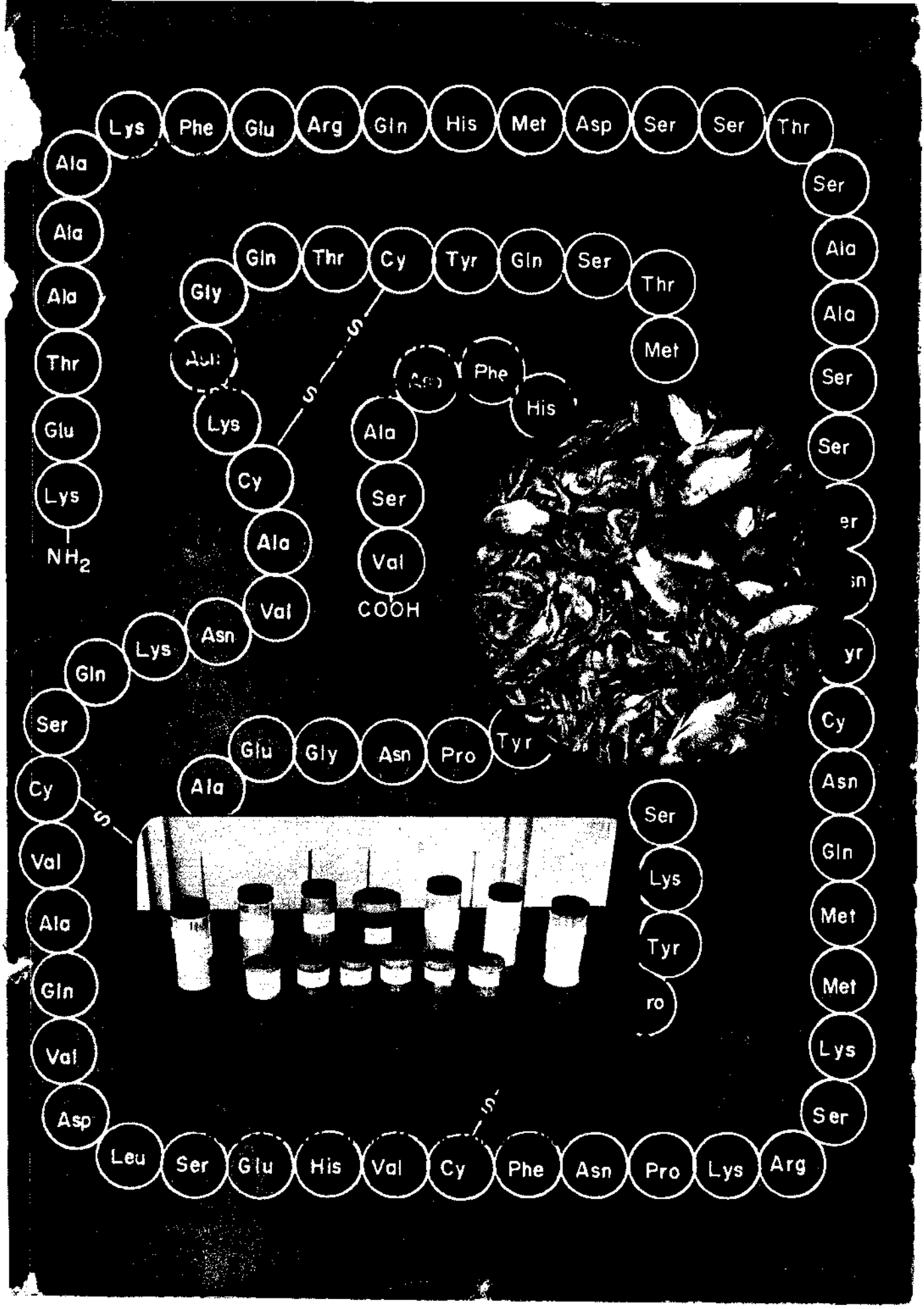
Lys Phe Glu Arg Gln His Met Asp Ser Ser Thr
Ala Ala Ala Thr Glu Lys NH2
Ser Ala Ala Ser Ser er in yr Cy Asn Gln Met Met Lys Ser
Gln Thr Cy Tyr Gln Ser Thr Met
Gly
Lys Cy Ala Val Asn Lys Gln Ser Cy Val Ala Gln Val Asp Leu Ser Glu His Val Cy Phe Asn Pro Lys Arg
Ala Ser Val COOH
Phe His
Glu Gly Asn Pro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

CMFRI SPECIAL PUBLICATION

Number 8

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,
Central Marine Fisheries Research Institute,
held at Cochin from 11 - 16 January 1982

Published by: **E. G. SILAS**
Director
Central Marine Fisheries Research Institute
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

Dr. P. Vedavyasa Rao who have rendered assistance during the preparation of this manual. Thanks are also due to Shri Johnson, Librarian and Shri Kambadkar, Technical Assistant, Central Marine Fisheries Research Institute, for the help rendered by them in printing this manual.

(E.G. Silas)
Director, CMFRI,
Sub-Project Coordinator,
Centre of Advanced Studies in Mariculture

CHAPTER 3

DETERMINATION OF MINERALS*

1 INTRODUCTION

Mineral elements have a great diversity of uses within the animal body. The prominence of each mineral element in body tissues is closely related to its functional role. Calcium and phosphorus are too major mineral elements that must be present in adequate amounts in the feeds supplied. The amount of sodium chloride and potassium in certain feeds should also be ascertained to screen out feeds for feeding fish and shellfish.

2 DETERMINATION OF CALCIUM

2.1 Apparatus

(a) Porcelain dishes
(b) Volumetric flasks, 250 ml
(c) Beakers, 250 ml
(d) Quantitative filter paper and funnels, and
(e) Burette

2.2 Reagents

(a) Hydrochloric acid (1-3 v/v)
(b) Nitric acid (70%)
(c) Ammonium hydroxide (1-1 v/v)
(d) Methyl red indicator (dissolve 1g in 200 ml alcohol)
(e) Ammonium oxalate (4.2% solution)
(f) Sulphuric acid (98%), and
(g) Standard potassium permanganate solution (0.05N)

2.3 Procedure

Weigh 2.5 g of finely ground material into a porcelain dish and ash as above (alternatively use residue from ash

---

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

determination). Add 40 ml hydrochloric acid and a few drops of nitric acid to the residue, boil, cool, and transfer to a 250 ml volumetric flask. Dilute to volume and mix.

Pipette a suitable aliquot of the solution (100 ml for cereal feeds, 25 ml for mineral feeds) into a beaker, dilute to 100 ml and add 2 drops of methyl red. Add ammonium hydroxide one drop at a time until a brownish orange colour is obtained, then add two drops of hydrochloric acid to give a pink colour. Dilute with 50 ml water, boil, and add while stirring 10 ml of hot 4.2 percent ammonium oxalate solution. Adjust the pH with acid to bring back pink colour if necessary. Allow precipitate to settle out, and filter, washing precipitate with ammonium hydroxide solution (1+50 v/v). Place the filter paper with precipitate back in beaker and add a mixture of 125 ml water and 5 ml sulphuric acid. Heat to 70°C and titrate against the standard permanganate solution.

2.4 Calculation

Calcium (%)

$$= \frac{\text{ml permanganate solution}}{\text{wt. sample}} \times \frac{\text{aliquot used (ml)}}{250} \times 0.1$$

3 DETERMINATION OF PHOSPHORUS

3.1 Apparatus

(a) Spectrophotometer to read at 400 mμ and
(b) Graduated flasks, 100 ml.

3.2 Reagents

(a) Molybdovanadate reagent

Dissolve 40 g ammonium molybdate $4H_2O$ in 400 ml hot water and cool. Dissolve 2g ammonium metavanadate in 250 ml hot water, cool, and add 450 ml 70 percent perchloric acid. Gradually add the molybdate solution to the vanadate solution with stirring and dilute to 2 litres.

(b) Phosphorus standards

Prepare stock solution by dissolving 8.788g potassium dihydrogen orthophosphate in water and making up to 1 litre. Prepare the working solution by diluting the stock 1 in 20 (working concentrate 0.1 mgP/ml).

3.3 Procedure

Pipette an aliquot of the sample solution prepared as for the calcium determination into a 100 ml flask and add 20 ml of the molybdovanadate reagent. Make up the volume, mix, and let stand for 10 min. Transfer aliquote of the working standard containing 0.5, 0.8, 1.0 and 1.5 mg phosphorus to 100 ml flasks and treat as above. Read sample at 400 mμ setting the 0.5 mg standard at 100 percent transmission. Determine mg phosphorus in each sample aliquot from a standard curve.

4 DETERMINATION OF POTASSIUM

4.1 Apparatus

(a) Silica crucibles
(b) Flame photometer and
(c) Muffle furnace

4.2 Reagents

(a) Hydrochloric acid (concentrated)
(b) Potassium standard

To prepare stock solution (500 ppm K), dissolve 0.477g potassium chloride (Analar) and make up to 500 ml with distilled water. To prepare working standard (10 ppm), dilute 1:50.

4.3 Procedure

Dry 2g of sample in a silica crucible at 100°C to expel moisture. Add a few drops of pure olive oil and heat over flame until swelling stops. Ash at 500°C in muffle furnace for 24 h, cool, and add 2 ml concentrated hydrochloric acid to dissolve the residue. Make up to 100 ml. Take 1 ml of this solution and make a further dilution to 100 ml.

Set the flame photometer to give a reading of 100 with the 10 ppm standard, and then read sample solution. If the sample reading does not fall between 50 and 100 make a fresh dilution to give an appropriate reading.

5 DETERMINATION OF SODIUM CHLORIDE

5.1 Apparatus

(a) Conical flasks
(b) Pipettes
(c) Burettes

5.2 Reagents

(a) Standard 0.1 N silver nitrate solution
(b) Standard 0.1 N ammonium thiocyanate solution
(c) Ferric indicator - saturated aqueous solution of ferric aluminium
(d) Potassium permanganate solution - 6% w/v
(e) Urea solution - 5% w/v and
(f) Acetone (A.R. grade)

5.3 Procedure

Weigh 2g sample into a 250 ml conical flask. Moisten sample with 20 ml water and then add, by pipette, 15 ml 0.1 N silver nitrate solution and mix well. Add 20 ml concentrated nitric acid and 10 ml potassium permanganate solution and mix. Heat mixture continuously until liquid clears and nitrous fumes are evolved; then cool. Add 10 ml acetone and 5 ml ferric

indicator, and back titrate the excess silver nitrate with the 0.1 N thiocyanate solution to the red brown end point.

5.4 Calculation

Calculate results as sodium chloride,

$$\% \text{ NaCl} = \frac{(15.00 - \text{ml } 0.1 \text{ N } NH_4CNS \times 0.585)}{\text{g sample taken}}$$

6 REFERENCES

1. AOAC - 1965 — Official Methods of Analysis of the Association of Official Agricultural Chemists. 1965, 10th Edition Published by the Association of Official Agricultural Chemists, Washington, D.C.

2. Chow, K.W. 1980 — Quality control in fish feed manufacturing. In Fish feed Technology, Aquaculture Development and Coordination Programme, FAO ADCP/Rep/80/11 p. 369-385.

3. Harris, L.E. 1970 — Nutrition research techniques for domestic and wild animals. Volume 1. Utah State University, Logan, Utah.