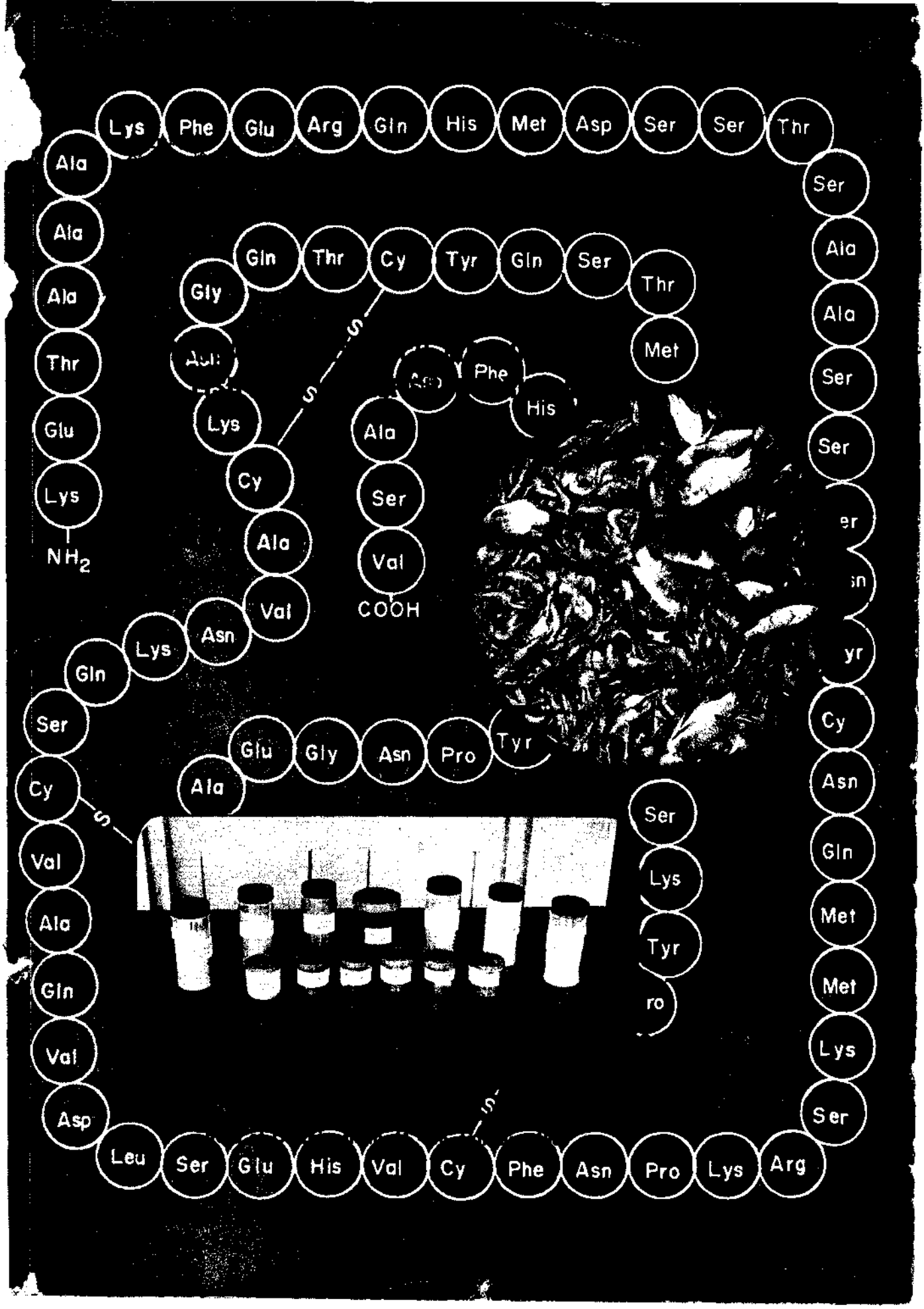
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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

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 9

DETERMINATION OF DIGESTIBILITY COEFFICIENT*

1 INTRODUCTION

Nutrients present in the feedstuffs are not completely available to the animal body. Large portions of the nutrients are excreted in the faeces because of being not digested in the alimentary tract. Therefore, the digestibility of the feedstuff is defined as the portion of a feed or nutrient of feed which is not recovered in faeces, i.e., the portion which has been absorbed by the animal. When the digestibility is expressed in percentage it is known as digestibility coefficient. Digestibility coefficients are calculated for dry matter, crude protein, crude fibre, ether extract and nitrogen-free extract. Digestibility of gross energy present in the food can also be determined. The digestibility coefficients normally determined are the apparent digestibility coefficients since the nutrients found in the faeces contain small proportion of nutrients from the previously utilised food in the form of mucosal debris, unspent enzymes etc.

2 DIRECT FAECES COLLECTION METHOD

2.1 Apparatus and reagents required

(a) Specially designed aquarium tanks for collection of faeces
(b) Aerators
(c) Feeding trays
(d) Polythene tubes
(e) Porcelain crucibles
(f) Hot air oven
(g) Centrifuge
(h) Single pan balance
(i) Equipment and reagents required for protein analysis
(j) Equipment and reagents required for fat analysis

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* Prepared by R. Paul Raj and D.C.V. Easterson, Central Marine Fisheries Research Institute, Cochin-18.

(k) Equipment and reagents for crude fibre analysis
(l) Equipment and reagents for determination of energy
(m) Experimental animals (fishes or crustaceans)

2.2 Procedure

(a) Take three specially designed aquarium tanks of identical size, with provision for continuous collection of faeces. Fill them with water and maintain the level, volume and temperature of water similar in all the aquaria. The water used in all the aquaria should be of the same source and salinity level. Aerate the aquaria well to maintain oxygen levels near saturation in all the tanks.

(b) Introduce 10 healthy experimental animals of the same species, age, size group, etc in each of the tanks.

(c) During the pre-experimental feeding, the animals are fed on the feed or feedstuff in question for a period of atleast 6 days to remove the effects of previous feeds. (The number of pre-experimental feeding days can be adjusted depending on the gastric evacuation time).

(d) At the end of six days the feeding trial can be started. Feed the three groups of experimental animals with a known quantity of feed, weighed accurately to 0.1g in a feeding tray and allow the animals to feed for about 12 hours overnight. The feeding time can be increased or decreased based on the actual time taken for consumption by the experimental animals.

(e) Carefully remove the feeding trays from the aquarium tanks and collect the left-over food by centrifuging or filtering. Dry the left-over food in an oven at 105°C.

(f) Collect the faeces at 2 hourly intervals using a polythene tube by siphoning out the faecal pellets if there is no specially designed aquaria for collecting faeces. Centrifuge or filter the faeces and dry in an oven at 105°C.

(g) Continue the feeding, and collection of left-over food and faeces for a period of 10 days.

(h) After drying, the representative samples are kept separately in polythene bags.

(i) At the end of the digestibility trial all the faeces samples of each aquarium tanks are composited and analysed for the dry matter, crude protein, crude fat, crude fibre, NFE and energy contents.

2.3 Calculation

Apparent digestibility coefficient of nutrients = Da = $\frac{I-F}{I}$

where I = nutrient intake and F = faecal nutrient

True digestibility coefficient = TD = $\frac{A}{I} = \frac{I-(F-Fk)}{I}$

where A = absorbed nutrient; I = nutrient intake; F = faecal nutrient and Fk = metabolic nutrient excreted with the faeces.

3 CHROMIC OXIDE INDICATOR METHOD

A breakthrough in digestibility studies of nutrients was the use of inert materials, and in particular the use of chromium oxide (Cr_2O_3).

Chromium oxide mixed with prepared diets and measured in the faeces provides a general comparison of the overall digestibility of a feed expressed as:

Percent digestibility = 100 $\frac{\%Cr_2O_3 \text{ in faeces}}{}$

When the percentage of a nutrient in the feed and faeces is analysed as well as the corresponding percentage of the indicator substance the digestibility percentage can be calculated by the formula:

$$\text{Apparent digestibility coefficient} = 100 - \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in feed}}$$

Caution:

The chromic oxide for commercial use has to be purified for removing the toxic chromium compounds. The toxic chromium compounds can be removed by repeated washing in N HCl using glass fibre filter (Whatman GFC) and borosilicate glassware. After repeatedly treating with hydrochloric acid the powder is washed, thoroughly in distilled water, dried at 120°C for 24 hours and stored. Thus, treated Cr_2O_3 is mixed in a definite percentage to the feed.

4 DETERMINATION OF CHROMIC OXIDE IN FAECES

4.1 TITRIMETRIC METHOD

4.1.1 Principle

The well mixed faecal matter containing organic waste and chromic oxide marker is digested with nitric acid to remove organic matter. The resultant solution is suitable for estimation of minerals in addition to chromium. On the addition of perchloric acid the chromium oxide is oxidised to dichromate which is estimated by adding an excess of ferrous ammonium sulphate and titrating the mixture.

4.1.2 Reagents

(a) Hydrochloric acid
(b) Nitric acid, Analar
(c) Perchloric acid, 60%
(d) Sulphuric acid (6N-167 ml of Analar sulphuric acid diluted to 1 litre in distilled water)

(e) Potassium dichromate (0.1 N) standard:

Dissolve 4.90g of analar grade solid previously heated to 180°C, cooled and stored in a dessicator in 1 litre of 2 N sulphuric acid. Check the normality.

(f) Ferrous ammonium sulphate (0.1 N):

Dissolve about 40g ferrous ammonium sulphate ($FeSO_4(NH_4)_2.6H_2O$) in 1 litre of 2N sulphuric acid. Check the normality each day before use.

(g) Indicator solution:

Dissolve 0.1g of N-Phenylanthranilic acid in 2 ml of 5% sodium carbonate and dilute to 100 ml with distilled water.

4.1.3 Procedure

(a) Weigh about 50-100 mg of faeces containing 1-3 mg of Cr_2O_3 into a 100 ml conical flask or a kjeldahl flask.

(b) Add 5 ml of conc nitric acid

(c) After 5 minutes, boil the contents gently in a hot plate for about half an hour in a fume cup board (Alternatively samples can be digested in a Tecator Kjeltec system, if available). Additional acid may be added to prevent the content becoming dry.

(d) Cool and add 3 ml of 60% perchloric acid. Heat by keeping inside a fume cupboard with plastic screen until no more fumes evolves and all nitric acid has been removed.

(e) If chromium is present the solution becomes brilliant golden in colour. Cool and wash the digest into a 50 ml volumetric flask with distilled water.

(f) To this add 5 ml of 6N-H_2SO_4, 5 ml of 0.1 N ferrous ammonium sulphate and a drop of indicator. Titrate the mixture in a 10 ml burette with standard 0.1 N potassium dichromate until the green colour of the solution turns to slate-grey, which turns to cherry colour on standing.

4.1.4 Calculation

If 't' ml of 0.1 N potassium dichromate solution is required, then the dichromate in the digest has reached with 5 't' ml of 0.1 ferrous ammonium sulphate. 1 ml of 0.1 N dichromate is equivalent to 2.53 mg of Cr_2O_3. Thus 2.53 (5-'t') mg of Cr_2O_3 can be present in the digest.

4.2 SPECTROPHOTOMETRIC METHOD

4.2.1 Reagents

As in the titrimetric method

4.2.2 Equipment

Spectrophotometer

4.2.3 Procedure

Follow procedures 'a' to 'd' as given in titrimetric method.

(e) Cool and wash the digest into a 100 ml volumetric flask and make up the volume by adding distilled water.

(f) Mix well and after allowing a minimum of 5 minutes measure the optical density at 350 mμ in a spectrophotometer. The percent chromic oxide is read from a standard curve where 'Y' is the optical density at 350 mμ and 'X' the Cr_2O_3 content of the sample in mg/100 ml.

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