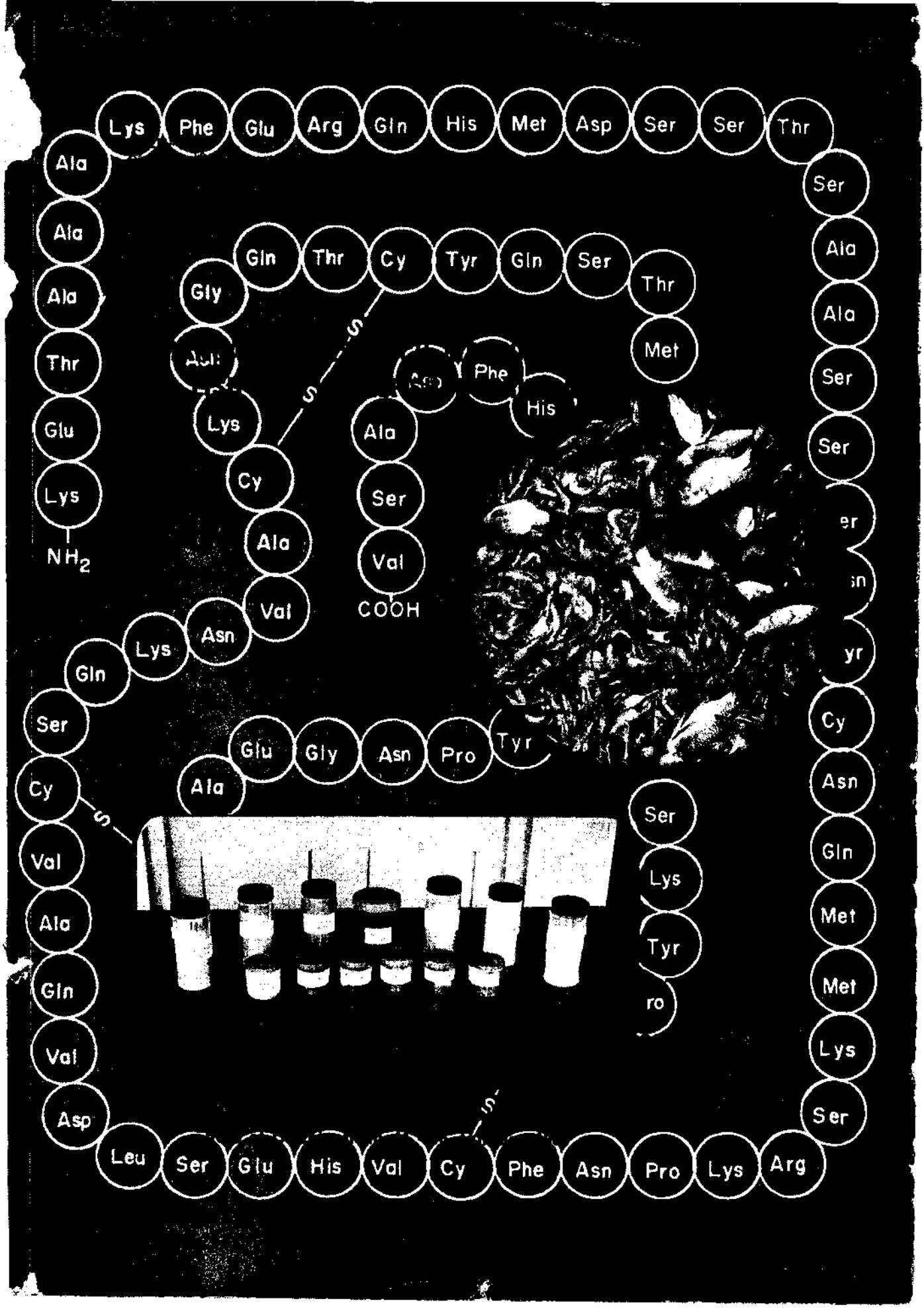
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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

Issued on the occasion of the Workshop on
METHODOLOGY FOR FISH AND SHELLFISH NUTRITION
organised by
The Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
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Published by: **E. G. SILAS**
Director
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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 8

METHODOLOGY OF NUTRITIONAL BIOENERGETICS - AN OUTLINE*

1 Principle

Nutritional Bioenergetics, the study of transformation and partitioning of food energy offers a conceptional framework to anabolism and catabolism. While the rate of transfer can be expressed as dB/dt, the whole process can be expressed in the form of a simplified equation, which is also known as energy budget.

$$C = F + U + M + \Delta B \qquad \ldots\ldots \quad (1)$$

where 'C' denotes the amount of food energy consumed also known as ration, 'F' that part lost as faeces, 'U' non-faecal nitrogenous loss, 'M' loss by way of metabolism and 'ΔB' change in materials of body, growth. (Fig. 1).

Food Energy (C)

Faecal Loss (F)

Digestible (Assimilated) Energy

Calorigenic Effect of food (SDA)

Nonfaecal Loss (U)

Net Physiologically useful energy

Metabolism (M)

Growth (ΔB)

i. Tissue repair & addition

ii. Exudates (mucos etc.,)

iii. Exuvia

iv. Gonadial products

Fig. 1. A diagramatic sketch showing partitioning of food energy.

* Prepared by D.C.V. Easterson and A.G. Ponniah, Central Marine Fisheries Research Institute, Cochin-18.

Since the equation is a balanced one, if one of the parameters is not known, it can be calculated. Though calorie (the measure of energy), is used as the unit of expression, units like carbon, nitrogen can also be used, when required. One gram calorie (g cal) is defined as the amount of heat required to raise the temperature of one gram of distilled water by 1°C at 15°C (1 Kcal or Cal = 1000 g cal = 3.9681 Btu = 4185 Joules).

2 Experimental Procedures

Before setting up the experiment, knowledge about the food and feeding habit of the experimental animal is necessary. For which, the study of mouth parts, gut and faecal contents would be informative. Some animals are nocturnal feeders and to these feeding should be done during the night. Preliminary studies on satiation ration, satiation time and frequency of meal are also essential.

For experimentation, healthy animals of uniform size and sexual stage are chosen and acclimatized in the chosen diet for a week. Before the starting of experiment, the animals are not fed for a fixed time to allow them to evacuate their gut. Afterwards, length and wet weight are taken and introduced into the experimental tank containing good filtered and aerated seawater. Water level, salinity and temperature are to be maintained constant. Feeding should be done at the fixed time in fixed quantities. The aeration should not be too much so as to agitate and break the faecal matter. The bottom of the tank should be of a colour in which faecal pellets can be easily recognized. If transparent containers are used, they should be covered inorder to prevent the animals getting excited. At the start of the experiment, another batch of three animals similar to the experimental ones should be rinsed with distilled water, external moisture removed, weighed and oven dried at 60-70°C for the estimation of percentage water and total calorific contents.

It is better that exuvia and faecal matter are removed soon after voided. On drying, salt from the adhering water adds up to the weight and also interferes in the chemical analysis. To avoid this, faeces and exuvia are transferred to a very fine

bolting silk fixed on the mouth of a beaker and washed gently with distilled water, transferred to a pre-weighed petridish and kept in a refrigerator or oven dried. The pooled dry matter is weighed finally. Exuvia and faecal matter need to be kept separately. Record should be maintained on moulting. One type of experimental set up is shown in Fig. 2.

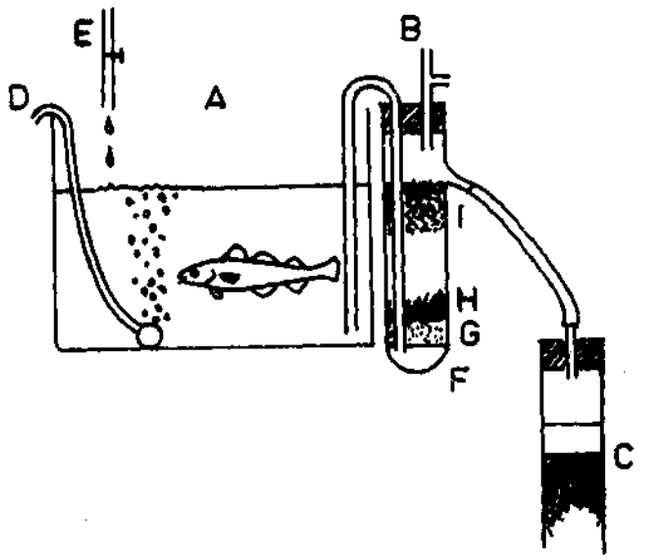

Fig. 2. The apparatus used for the separation of faecal N and urinary N.

A: A tank for maintenance of fish (36 X 20 X 27 cm).
B: A test tube for collecting faeces (3.5 X 25 cm)
C: A column of resin for adsorbing dissolved urinary nitrogenous compounds (5.5 X 60 cm)
D: Air supply
E: Water supply
F: Chloroform
G: Cupric hydroxide
H: Feces collected
I: Glass wool
(Ogino, Kakino and Chen, 1973)

3 Food Consumption

3.1 Direct method:

Food offered - food left = food consumed (C)

3.2 Indirect method:

3.2.1 F + U + M + B = C. ..... (2)

3.2.2 Marker methods:-

(a) Use of Radioactive isotopes:

The food is labelled with one of the isotopes viz. ^{14}C, ^{32}P, ^{45}Ca, ^{35}S. In case of phytoplankton feeders, the feed alga is grown in medium containing isotopes; by this the isotope gets incorporated into the alga. In case of formula diets the isotope is mixed with the feed. The labelled food is followed in the animal and estimated either in the animal or calculated from the decrease in the medium. ^{32}P is a very convenient isotope having a very short half-life of 14.2 days and the radiation could be easily detected and measured in small animals as a total entity or in homogenized aliquots. But due to its short half-life, it can not be used for experiments of longer duration. ^{14}C has the advantage of a half-life of 5760 years. Since it is easily metabolised, the degree of labelling might differ in different food molecules and, as different food components are not uniformly assimilated, error is likely.

(b) Use of non-assimilable markers:

The most commonly used marker is Cr_2O_3 (chromium sesquioxide) which is well mixed at 5% level with the feed and its exact quantity in the feed and faeces is chemically estimated.

4 Assimilation

Assimilation (A) is calculated as follows:

4.1 Direct method:

$$A = C - F\ (A)$$

4.2 Indirect method:

$$A = C - \frac{\text{Concentration of indicator in food per unit weight}}{\text{Concentration of indicator in faeces per unit weight}}$$

5 Metabolism

5.1 Direct method:

By means of static or flow-through respirometers the metabolism (in terms of oxygen consumption) of animals fed with the tested diet is estimated. For this, the test animals should be acclimatized to the laboratory conditions, test diet and feeding schedule. If a diurnal rhythm in standard metabolic rate (M_s) is present, it should be taken into consideration in the schedule of feeding and oxygen consumption. After the excitment due to handling is over, hourly estimates of oxygen consumption is made for 24 hrs. This is totalled to arrive at the feeding metabolic rate (M) for a day for the tested diet. Energy equivalent of 4.63 Kcal/lit O_2 can be used to convert oxygen consumption data into calories.

5.2 Indirect method:

Metabolism (M) is calculated as the difference between sum of faecal and non-faecal loss, and growth and that of food consumed.

$$M = C - (F + U + \Delta B) \quad \ldots\ldots\ldots(3)$$

6 Specific dynamic action (SDA)

Specific dynamic action denotes the energy cost of biochemical transformation of ingested food into a metabolizable, excretable form. To estimate SDA, standard metabolism (M_s) and metabolism due to excitability and increased activity occurring in conjenction with food intake (M_e) are subtracted from total metabolism (M).

$$SDA = M - (M_s + M_e) \quad \ldots\ldots\ldots (4)$$

M - metabolic rate of fed animals;
M_s - metabolic rate of starved animals; and
M_e - the excited metabolic rate due to feeding procedure

(M and M_s are estimated in a respiration where activity is made constant by making the experimental animal to move against a steady current of water)

7 Growth

At the end of the experiment the animals are weighed, measured, and dried in the oven. It is better to determine the proximate composition, which would be of use in interpretation of the result. The weight is converted into the unit of experiment eg. Calories or Protein.

Analysis - formula:

(i) K_1 (Gross conversion efficiency) (%) $= \frac{\Delta B}{C} \cdot 100$

(ii) K_2 (Net conversion efficiency) (%) $= \frac{\Delta B}{A} \cdot 100$

(iii) Trophic coefficient $= \frac{C \text{ (in g dry wt)}}{P \text{ (in g dry wt)}}$

(iv) i. For an experiment of longer duration
Mean growth rate perday in % Body weight ($\overline{P}$) $= \frac{2\Delta B}{n(W_n + W_o)} \cdot 100$

ii. For short term experiments, especially in juveniles

$$\bar{P} = \left\{ 10^{\frac{1}{n}} (\log W_n - \log W_o) - 1 \right\} \times 100$$

8 Mean food consumption in percentage body weight per day (in ad libitum feeding studies) $\bar{C}$

$$\bar{C} = \frac{2C}{(W_n + W_o) \times n} \times 100$$

W_n = Wet weight on n day

W_o = Wet weight initial

9 Food conversion ratio (r)

Especially useful if there is any mortality to experimental animals

$$r = \frac{C}{(\bar{W}_n + D) - \bar{W}_o}$$

$\bar{W}_o$ = average initial weight (g)

$\bar{W}_n$ = average final weight (g)

D = weight of dead animals (g)

C = Total food consumed in g

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