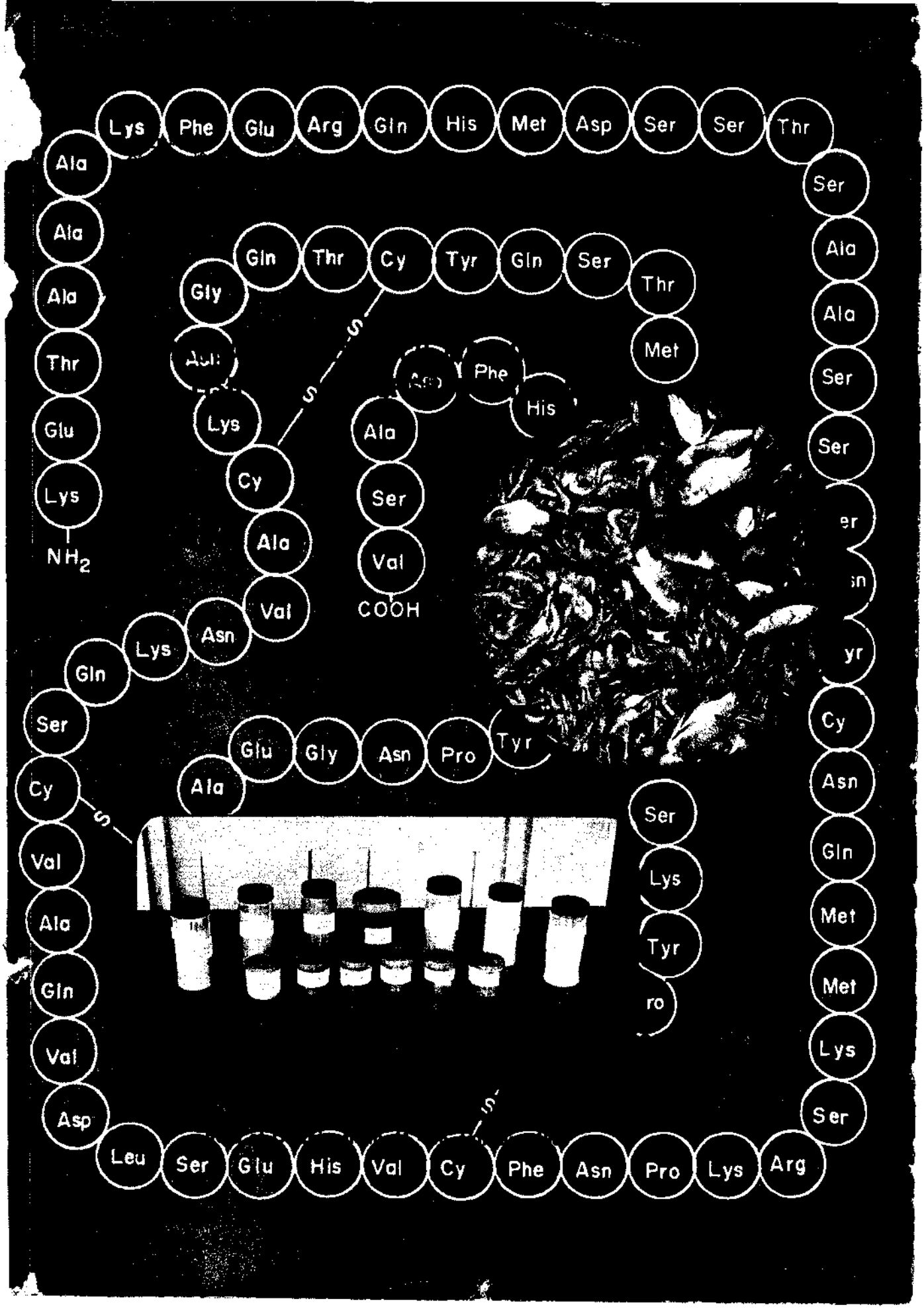
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MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

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**
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

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CHAPTER 2

DETERMINATION OF GROSS ENERGY OF FEEDS*

1 Principle

The amount of heat, measured in calories, that is released when a substance is completely oxidized in a bomb calorimeter containing 25 to 30 atmospheres of oxygen, is called the gross energy (GE) of the substance. A sample of the material to be tested is weighed into a combustion capsule. The combustion capsule is placed in an oxygen bomb containing 25 to 30 atmospheres of oxygen. The oxygen bomb is covered with 2000 g of water in an adiabatic calorimeter. After the bomb and calorimeter have been adjusted to the same temperature, the sample is ignited with a fuse wire. The temperature rise is measured under adiabatic conditions. From the hydrothermal equivalent of the calorimeter the temperature rise minus some small corrections for fuse wire oxidation and acid production, the caloric content of the sample is calculated.

2 Apparatus

(a) Parr oxygen bomb calorimeter and accessories or equivalent

(b) The calorimeter may be equipped with an automatic temperature controller. If the temperature controller is on the calorimeter, it will take less labour to run the analysis, but the controller is not necessary to obtain accurate results.

(c) Solution or trip balance with capacity to 3000 g and accurate to 0.1 g.

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* Prepared by R. Paul Raj and A.G. Ponniah, Centre of Advanced Studies in Mariculture, Central Marine Fisheries Research Institute, Cochin-18.

3 Reagents

(a) Standard sodium carbonate solution, equivalent to 1 cal/ml (3.658g Na_2 Co_3 per litre)
(b) Methyl orange indicator
(c) Benzoic acid combustion tablets or primary standard grade crystals

4 Determining the hydrothermal equivalent of the bomb

(a) Determine the hydrothermal equivalent of the bomb, bucket and water by determining the temperature rise using the same procedure as outlined above, but with a sample of known caloric content (benzoic acid combustion tablet). Make at least four determinations and use the average value. Once this value is determined, it should not change unless some parts of the bomb are replaced.

(b) Dry the benzoic acid at 105°C overnight, cool in dessicator and weigh by difference from a covered weighing bottle one tablet or approximately a 1g sample of dry calorific standard grade benzoic acid crystals. Determine the temperature rise from the benzoic acid in the bomb as with other samples.

Hydrothermal equivalent per degree (Cal)

$$\frac{\text{Wt. of benzoic acid} \times \text{Calories per gram benzoic acid} + \text{Length of fuse wire burned} \times \text{Cal/cm fuse wire} \times \text{ml } Na_2 Co_3}{\text{(final temp. - initial temp.)}}$$

4.1 Example:

A 1.0622g sample of benzoic acid had a heat of combustion of 6319 cal per g. The corrected initial temperature of the bomb was 20.280°C and the final corrected temperature was 23.045°C. There were 4.8 cm of fuse wire burned with a caloric value of 2.3 cal per cm. There were 7.5 ml of Na_2Co_3 titrated (equivalent to 7.5 cal).

Hydrothermal equivalent calories per degree

$$= \frac{(1.0622 \times 6319) + (4.8 \times 2,3) + 7.5}{(23.045 - 20.280)} = 2434 \text{ small calories}$$

5 Procedure

(a) Weigh by difference approximately 1.0g of sample and place in a clean, empty combustion capsule. Samples may be pelleted, but this is usually not necessary.

(b) Attach a 10 cm length of fuse wire between the electrodes of the bomb (oxygen)and set the combustion capsule with sample in place in the loop electrode. Adjust the fuse wire so that it touches the sample.

(c) Place about 1 ml of water in the bomb cylinder and swirl it around to wet the sides. This is not necessary if the bomb is still wet from a preceeding determination.

(d) Assemble the bomb, tighten the screw cap, close the pressure release valve and fill with oxygen to 25 atmospheres gauge pressure. Place the bucket (oval) in the calorimeter, set the bomb in the bucket, and attach the clip terminal.

(e) Weigh 2000g distilled water on the solution or trip balance (use a 2000 ml volumetric flask to hold the water) and carefully pour into the calorimeter bucket. The water temperature must be within the range of the calorimeter thermometers.

(f) Close the cover, lower the thermometer and start the water circulating motor. Remove the cap from the jacket cover and fill the cover with water until it runs out of the drain hose.

(g) Adjust the temperature of the water in the outer jacket to approximately equal that of the calorimeter by adding hot or cold water, and allow one minute to

attain equilibrium. Then carefully adjust the temperature to be exactly equal and check the calorimeter temperature at one minute intervals for three minutes.

(h) Read and record the initial temperature to the nearest 0.0002° and ignite the sample. Turn in hot or cold water to keep the jacket temperature equivalent to the calorimeter temperatures during the period of rise.

(i) Compare and adjust the temperature of the outer jacket to the inner bucket of the calorimeter temperature frequently and carefully to insure adiabatic condition or that the temperatures are equal. Read and record the final temperature after the same temperature is observed in three successive one minute intervals.

(j) Raise the thermometers. Open the calorimeter, take the bomb from the calorimeter bucket, release the residual pressure of the bomb and open. Carefully remove the remaining pieces of fuse wire from the electrodes; straighten and measure the combined total length in centimeters. The calories of wire burned can be determined with the measuring scale that is supplied with the wire.

(k) Rinse all inner bomb surfaces with a stream of neutral distilled water and collect all washings in a clean beaker. Titrate the washings with the standard sodium carbonate solution using methyl orange indicator to determine the amount of acid formed from the incidental oxidation of nitrogen and sulphur compounds. A correction is made to take care of the heat liberated in the formation of the acid.

(l) Correct the initial and final temperatures from the calibration curve supplied with the thermometer.

6 Calculations

6.1 GE (cal/g) on as fed basis

$$= \frac{\left[\begin{matrix}\text{final temp.} - \\ \text{initial temp.}\end{matrix}\right] \begin{matrix}\text{Hydrothermal} \\ \text{equivalent of} \\ \text{bomb}\end{matrix} - \left[\begin{matrix}\text{Length of} \\ \text{fuse wire burned}\end{matrix}\right] \times \left[\begin{matrix}\text{Cal} \\ \text{per} \\ \text{cm}\end{matrix}\right] - \left[\begin{matrix}\text{ml} \\ Na_2 \\ Co_3\end{matrix}\right]}{\text{(weight of sample)}}$$

6.1.1 Example

A 1.0214 g sample of feed (as fed basis) was used. The initial temperature was 23.13°C and the final temperature was 25.25°C. The hydrothermal equivalent of the bomb is 2412 cal per degree C. There was 7.0 cm of fuse wire burned with a correction of 2.3 cal per cm of wire and 6.0 ml of Na_2 Co_3 titrated (equivalent of 6.0 cal).

$$\text{GE (cal/g)} = \frac{(25.25 - 23.13)\ 2412 - (7.0 \times 2.3) - 6.0}{\text{1.0214 g sample on as fed basis}}$$

$$= 3804 \text{ cal/g or } 3804 \text{ K cal/kg}$$

6.2 Adjusting to dry basis:

$$\text{GE (Kcal/kg)} = \frac{\text{GE(Kcal/kg) on as fed sample}}{\text{dry matter \% of as fed sample}} \times 100$$

7 References

1. Harris, L.E. 1970 — Nutrition Research Techniques Volume I Utah State University, Logan, Utah.

2. Parr Instrument Company 1966 — Oxygen bomb calorimetry and combustion methods. Technical Manual No.130. Parr Instrument Company, Moline, Illinois.