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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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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. Alagarwami, Shri D.C.V. Easterson, Shri C.P. Gopinathan, Shri T. Jacob, Shri M.S. Nuthu, Dr. R. Paul Raj, Dr. A.G. Ponniah and

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CHAPTER 13

FEED FORMULATION METHODS*

1 INTRODUCTION

The requirements for protein and energy levels of the candidate species should be balanced in the formula diets. In addition, the specific amino acid levels, vitamin level, mineral level and roughage should also be balanced. Each feed ingredient in any feed formulation should serve some specific purpose, and have least cost. Usually in animal diets the protein level is adjusted first and the energy level is adjusted by addition of high energy supplements.

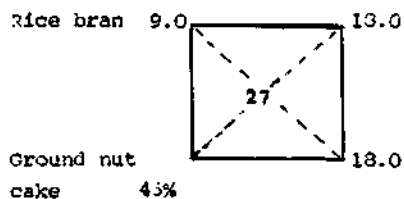
2 SQUARE METHOD

2.1 Balancing crude protein level

2.1.1. Using two ingredients:

Using rice bran (crude protein 9%) and ground nut cake (C.P. 45%) a fish diet with 27% crude protein can be prepared as follows:

A square is constructed and the two ingredients are put on the two left corners along with the protein content of each. The desired protein level of the feed is placed in the middle of the square. Next the protein level of



the feed is subtracted from that of the ingredients and the answer is placed in the opposite corner. The positive or negative sign is ignored.

* Prepared by Syed Anamed Ali, Central Marine Fisheries Research Institute, Cochin-18.

Now add the figures on the right side corner of the square $18 + 18 = 36$

$$\text{Then the \% of ricebran} = \frac{18}{36} \times 100 = 50\%$$

$$\% \text{ of ground nut cake} = \frac{18}{36} \times 100 = 50\%$$

To make 100 kg of feed, 50 kg of ricebran and 50 kg of groundnut cake have to be utilised.

2.1.2 Using more than two ingredients:

Using prawn waste (C.P. 35%), fish meal (C.P. 60%), wheat bran (C.P. 15%) and tapioca (C.P. 2%) a prawn diet with 32% crude protein can be prepared as follows:

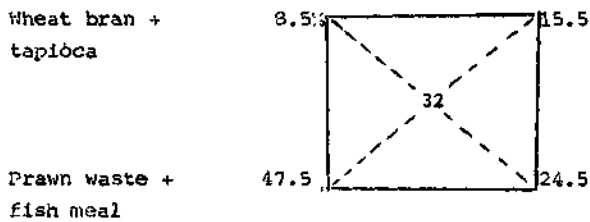
Group the ingredients with less than 20% crude protein and average their protein content

Wheat bran	15%
Tapioca	2
	<hr style="width: 100%;"/>
	17
Average	= 8.5%

Group the ingredients with more ^{than} 20% crude protein and average their protein content

Fish meal	60%
Prawn waste	35%
	<hr style="width: 100%;"/>
	95
Average	= 47.5%

Now the averaged protein contents are put on the two left corners of the square, and the rest is same.



Now add the figures on the right hand side corners of the square.

$$15.5 + 24.5 = 40.0$$

$$\text{Wheat bran + tapioca} = \frac{15.5}{40} \times 100 = 38.75$$

$$\text{Prawn waste + fish meal} = \frac{24.5}{40} \times 100 = 61.25$$

$$\text{Wheat bran} = 38.75/2 = 19.375\%$$

$$\text{Tapioca} = 38.75/2 = 19.375\%$$

$$\text{Prawn waste} = 61.25/2 = 30.625\%$$

$$\text{Fish meal} = 61.25/2 = 30.625\%$$

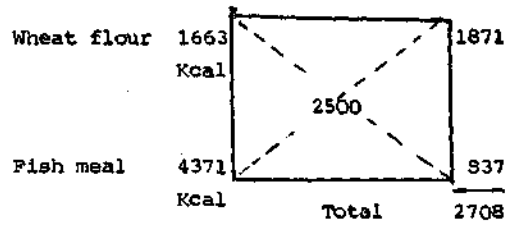
The composition of the feed is

Prawn waste	30.625%
Fish meal	30.625%
Wheat bran	19.375%
Tapioca	19.375%
Total	100.00

2.2 Balancing energy levels

The square method can also be used to calculate the proportion of feed ingredients to mix together to achieve a desired dietary energy level.

Using wheat flour 1663 Kcal Me/kg and fish meal 4371 Kcal Me/kg, a feed containing 2500 Kcal Me/kg can be prepared as follows:



$$\text{Wheat flour} = \frac{1871}{2708} \times 100 = 69.1\%$$

$$\text{Fish meal} = \frac{837}{2708} \times 100 = 30.9\%$$

To make 100 kg of the feed with 2500 Kcal of ME/kg we have to mix 69.1 kg of wheat flour and 30.9 kg of fish meal.

The square method cannot be used to balance for both crude protein level and energy level simultaneously.

3 REFERENCE

1. Hardy, R. 1980. Fish feed formulation. In Fish feed Technology, Aquaculture Development and Coordination Programme, FAO ADCP/Rep/80/11. p. 233-239