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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
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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.

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CHAPTER 12

PREPARATION OF ARTIFICIAL DIETS FOR NUTRITIONAL STUDIES*

1 TEST DIET FOR FISHES

The H 440 standard reference diet of Halver (1969) has* proven satisfactory for use with a variety of species of fishes. If this exact formula does not prove satisfactory for growth and survival of the test fish, slight modifications of clearly explained ingredient changes still permit meaningful comparisons of the test fish results with other species.

Diets may be prepared as moist, semi-moist or dry diet, or as a powder, rolled pellets, extruded pellets, or compressed pellets.

1.1 Standard Reference Diet H-440

| <u>Complete test diet</u> | (g) | <u>Vitamin Mixture</u> | (g) |
|---------------------------|-----|--------------------------|--------|
| Vitamin-free casein | 38 | L-cellulose | 8.000 |
| White dextrin | 28 | Choline chloride | 0.500 |
| Gelatin | 12 | Inositol | 0.200 |
| Corn oil | 6 | L-Ascorbic acid | 0.100 |
| Cod liver oil | 3 | Nicotinic acid | 0.075 |
| Vitamin mixture | 9 | Ca-pantothenate | 0.050 |
| Mineral Mix | 4 | Riboflavin | 0.020 |
| | | Thiamine HCl | 0.005 |
| Total | 100 | Pyridoxine HCl | 0.005 |
| Water | 200 | Menadione (K) | 0.004 |
| | | Folic acid | 0.0015 |
| Total diet as feed | 300 | Vitamin B ₁₂ | 0.0011 |
| | | Biotin | 0.0005 |
| | | L-tocopherol-acetate (E) | 0.040 |

* Prepared by Akio Kanazawa, Professor of Nutritional Chemistry, University of Kagoshima, Japan, R. Paul Raj and Syed Ahamed Ali, Central Marine Fisheries Research Institute, Cochin-18.

| <u>Mineral Mix</u> | (g) | <u>USP XII No.2</u> | (g) |
|----------------------|-------|----------------------------------|--------------|
| USP XII No.2 | 99.50 | Calcium biphosphate | 13.58 |
| $AlCl_3 \cdot 6H_2O$ | 0.015 | Calcium lactate | 32.70 |
| $Zn SO_4 \cdot H_2O$ | 0.300 | Ferric citrate | 2.97 |
| CuCl | 0.010 | Magnesium sulphate | 13.20 |
| $MnSO_4 \cdot H_2O$ | 0.080 | Potassium phosphate (dibasic) | 23.98 |
| KI | 0.015 | Sodium biphosphate | 8.72 |
| $CoCl_2 \cdot 6H_2O$ | 0.100 | Sodium chloride | 4.35 |
| | | | <u>99.50</u> |

1.2 Preparation of Halver's diet

Gelatin

Take in the container, add cold water and heat with stirring in a water bath at 80°C to dissolve gelatin. remove from heat

add with stirring - dextrin (first) Casein (second) minerals, oils and vitamins as temperature decrease mix well until the temperature decreased to 40°C

Warm diet

transfer into another container put into the refrigerator at 5°C

Artificial diet

Mix L-tocopherol with oils

Mix 1 part of vitamins with 8 parts of L-cellulose and

Add vitamin B₁₂ in water during final mixing.

2 TEST DIET FOR THE PRAWN, PENAEUS JAPONICUS

| <u>Ingredients</u> | <u>g</u> |
|-----------------------|----------|
| Casein (vitamin-free) | 50.0 |
| Glucose | 5.5 |
| Sucrose | 10.0 |

| <u>Ingredients</u> | <u>g</u> |
|--------------------|----------|
| a-Starch | 4.0 |
| Glucosamine HCl | 0.8 |
| Sodium citrate | 0.3 |
| Sodium succinate | 0.3 |
| Cholesterol | 0.5 |
| Pollack liver oil | 12.0 |
| Minerals*1 | 3.5 |
| Vitamins*2 | 3.2 |
| Cellulose powder | 1.9 |
| + Water 130-135 ml | 100.02 |

*1, *2 Minerals and vitamins for the test diet of prawn, P. japonicus

| <u>Vitamins</u> | <u>mg</u> | <u>Vitamins</u> | <u>mg</u> |
|--------------------------------|-----------|------------------------------------|--------------|
| Thiamine HCl (B ₁) | 4.9 | Menadione | 4.0 |
| Riboflavin (B ₂) | 8.0 | β -Carotene | 9.6 |
| P. Aminobenzoic acid | 10.0 | α -Tocopherol (vitamin E) | 20.0 |
| Biotin | 0.4 | Calcipherol | 1.2 |
| Inositol | 400.0 | Cyanocobalamine (B ₁₂) | 0.08 |
| Niacin | 40.0 | Na Ascorbate (vitamin C) | 2000.00 |
| Ca Panthothenate | 60.0 | Folic acid | 0.30 |
| Pyridoxine HCl | 12.0 | Choline HCl | 600.00 |
| | | <u>Total</u> | <u>32000</u> |

| <u>Minerals</u> | <u>g</u> |
|---|--------------|
| K ₂ HPO ₄ | 2.0 |
| Ca ₃ (PO ₄) ₂ | 2.72 |
| NaH ₂ PO ₄ ·2H ₂ O | 0.79 |
| MgSO ₄ ·7H ₂ O | 3.02 |
| MnSO ₄ ·5H ₂ O | 0.004 |
| FeSO ₄ ·7H ₂ O | 0.015 |
| <u>Total</u> | <u>8.549</u> |

2.1 Preparation of Test Diets for the Prawn

- i) Vitamins are dissolved in either water or ethanol and stored in a refrigerator at 5°C.
- ii) Minerals are powdered into a fine particle with a mortar and mixed well.
- iii) The pH of diet is adjusted with 0.5N-NaOH (in most case, 65-70 ml. of 0.5N-NaOH solution) to pH 6.8-7.0 (optimum pH for the prawn diet).

Detailed procedures:

All the ingredients are weighed and powdered into a fine particle. Casein, glucose, sucrose, α -starch, glucosamine HCl, Na citrate, Na succinate, cholesterol, and agar are combined and mixed thoroughly. To this, cellulose powder, minerals, vitamins, and pollack liver oil are added in turn and mixed well. Finally, distilled water (130-135 ml) is added to the mixture of dry ingredients and mixed again, the pH of diet being adjusted to pH 6.8-7.0 with 0.5N-NaOH (or 1N-HCl). The mixture so obtained is heated in autoclave (without addition of pressure) at 100°C for 10 min. The container is taken out from the autoclave, and the ingredients are mixed well and again heated at 100°C for 10 min. by the same manner. The heated mixture of the ingredients is packed into the plastic tubes (Cleharon tubes) by the similar manner to that of casing of sausage-making. The cased diet is again heated at 100°C for 10 min. and then cooled.

3 REFERENCES

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