REPORT OF THE EIFAC, IUNS AND ICES WORKING GROUP ON STANDARDIZATION OF METHODOLOGY IN FISH NUTRITION RESEARCH

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REPORT OF THE EIFAC, IUNS AND ICES WORKING GROUP ON STANDARDIZATION OF METHODOLOGY IN FISH NUTRITION RESEARCH

(Hamburg, Federal Republic of Germany, 21-23 March 1979)

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PREPARATION OF THIS DOCUMENT

The present document was prepared by the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research which was convened in Hamburg, Federal Republic of Germany, by Prof.Dr. K. Tiews from 21 to 23 March 1979, at the recommendation of the Tenth Session of the European Inland Fisheries Advisory Commission (EIFAC). This report is an elaboration of the guidelines of the ICES Cooperative Research Report No. 65 (1977) for the standardization of experimental designs and evaluations. It is published in this series with the agreement of ICES.

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1. INTRODUCTION

1.1 Origin and Objectives of the Working Group

The Symposium on finfish nutrition and feed technology organized by the European Inland Fisheries Advisory Commission (EIFAC) in conjunction with the Tenth Session of EIFAC (Hamburg, 20-27 June 1978) with the support of the International Council for the Exploration of the Sea and the collaboration of the General Fisheries Council for the Mediterranean (GFCM) and the International Union of Nutritional Sciences (IUNS) recommended (EIFAC recommendation 78/4) that priority should be given to the establishment of a Working Group of nutrition specialists sponsored by EIFAC, ICES and IUNS to:

(i) recommend standard technology to make research results more comparable and to develop formulae to report results in the field of fish nutrition;

(ii) advise EIFAC of the benefits of an International Network of Feed Information Centres (INFIC) and the establishment of an International Fish Feedstuffs Nutrient Data Bank.

1.2 Organization of the Working Group

The Working Group was convened by Prof. Dr. K. Tiews (Federal Republic of Germany) in Hamburg from 21 to 23 March 1979. Dr. J.D. Castell (Canada) was appointed Rapporteur. The participants were:

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1.3 Standardization

The Working Group agreed on the desirability of harmonizing the reporting of results, experimental design, diet formulation, species selection, etc.

Much of the existing literature on fish feeding and nutrition is less useful than it might be because it lacks details of diet composition, methods of preparation, etc. It was recommended that specific details be reported so that research results may be meaningfully compared. This is one of the first steps necessary in standardizing methodology.

1.4 Appendixes

One of the needs recognized was for a common vocabulary to be used in fish nutrition. It was recommended that the report of the Working Group should have an Appendix I with definitions of terms related to fish nutrition work. This appendix should include a review and updating of definitions in EIFAC Technical Report No. 12 (Salmon and Trout Feeds and Feeding), terms defined in the Symposium Review Paper No. 1 (Standard methods and terminology in finfish nutrition, by F. Utne, now published in Finfish Nutrition and Feed Technology, Halver/Tiews, 1979, Vol. II, pp. 432-44), plus any additional terms which were found valuable by the Working Group.

It was further recommended that Appendix II give the exact formula and description for preparation of one or more standard reference diets. One example to be used was diet H-440 given in the Symposium Review Paper No. 8, Vitamin requirements of finfish by J.E. Halver now published in Finfish Nutrition and Feed Technology, Halver/Tiews, 1979, Vol. I, pp. 45-58).

Other appendixes were recommended as follows:

Appendix III Proposed List of Data on Feed Ingredient Composition of Interest to Fish Feed Producers

Appendix IV Europe, Raw Materials to be Characterized as Potential Ingredients in Fresh or Salt Water Practical Diets

Appendix V World Mariculture Society Nutrition Task Force Recommended for Nutrition Papers Published

Appendix VI References of Interest to Fish Nutrition Researchers, Fish Culturists and Fish Feed Manufacturers

2. GENERAL CONSIDERATIONS AND RECOMMENDATIONS

2.1 Diets

2.1.1 Identification of diet and dietary ingredients

The full recognized name and International Feed Number of all ingredients should be given for all prepared foods. Where possible, source and exact species from which ingredients were prepared should be given together with details of preparation and extraction methods. The chemical formulae and quality of mineral components and forms and quality of vitamins should be reported. If a commercially prepared diet or ingredient (raw material) is used, the full name of the diet and manufacturer, with the manufacturer's code and lot number, should be given.

2.1.2 Preparation of experimental diets

Mixing may present special problems. The choice of components may assist in achieving homogeneous mixtures. Preparation of premixtures of microcomponents will facilitate more homogeneous distribution. The addition of preservatives, stabilizers or other special function ingredients (such as flavour attractants) is often necessary. Details of all preparation methods and ingredients or additives must be clearly stated.

The physical form of presentation will depend on the preference of the experimental animal and methods of feeding. The various physical constraints in feeds were noted for crustaceans, eels, marine flatfish, molluscs, salmonids and other finfish. Diets may be presented as: flakes, microgranules, microencapsulated particles, pellets (dry or moist) or as a mash (wet feed). Details about particle size and, where needed, directions for use should be given.

2.1.3 Analysis of diets

Physical and chemical analysis of diets should be done with internationally accepted official methods. Specific references for the methods used should be given along with details of any modification necessary for analysis of the feed. Information on the following is important in the evaluation of any diet and should be provided:

moisture
crude protein (N x 6.25)
crude fat (ether extract)
ash
crude fibre
nitrogen-free extracts (N.F.E.)

This proximate analysis should be corrected to total 100 percent. Information on the following is also desirable, for example,

vitamins
minerals
fatty acids
antioxidants used
binders
method of preparation
particle size

Physiological values, such as digestibility, metabolizable energy, NPU, etc. (see Appendix I), are also valuable for interpreting results.

2.1.4 Standard reference diet(s) (SRD)

The principle of establishing a standard reference diet was endorsed. The use of SRD in all fish nutrition research would permit direct comparison of results among all laboratories.

In selecting a reproducible SRD, the following factors must be considered:

nutrient balance
nutrient positive control
reproducibility between lots and between laboratories
market-availability of components
standard processing
lot identification of diet and ingredients
form of nutrients
availability and utilization of nutrients
stability or shelf life

It was recommended that each fish nutrition experiment have a standard reference diet, a control diet (which may be positive and/or negative control) and treatment diets.

2.2 Experimental Conditions

Information on the following experimental conditions will greatly facilitate comparisons between different research results:

1. Temperature profile or standard environmental temperature (SET);
2. Dissolved gases, in particular O_2, CO_2, N_2;
3. Ammonia dissociation;
4. Nitrite, nitrate (especially in recirculated systems) and other ions of interest;
5. Total dissolved solids (TDS);
6. Salinity;
7. pH;
8. Turbidity;
9. Description of experimental rearing units giving dimensions and any unusual characteristics;
10. Lighting type and photoperiod;
11. Location;
12. Sanitation and water treatment (UV, O_3, filtration, etc.);
13. Velocity, flow or exchange rate of water;
14. Size, age, sex, state of maturation, stocking density, previous rearing conditions, feed, source and strain;
15. Other stress factors.

It was further recommended that these conditions be reported in units recognized by the International Committee for Standardization of Weights and Measures.

2.3 Methods of Measurements

2.3.1 Growth

Growth is measured as the difference (gain or loss) in initial and final body biomass or body composition or as the partial difference in each sub-period. The inherent errors in wet weight determination must be recognized. The specific procedure used for weighing must be stated. Other methods for reporting growth may be substituted for weight, increase in body nitrogen content or body length. The condition factor, a combination of weight and length, is sometimes reported for estimating health status of fish (see Appendix I).

2.3.2 Feed conversion

Several methods of presentation are possible; definitions of such expressions as feed conversion, feed efficiency, net protein retention and net energy retention, will be found in Appendix I of this report.

2.3.3 Body composition

It was recommended that the proximate analysis and energy content of an adequate number of whole fish or specific tissues, such as adipose tissue, muscle and viscera, be recorded before and after the feeding trial in order to determine the increments in fish body constituents and to relate these increments to the intake of the different feedstuff components. The sample number for this analysis may be quite small because of the lower variation in the standard deviation of the means of body composition parameters compared with the variation in the means and length and body weight.

2.3.4 Health status

If mortalities are higher than should normally be expected, specific comments are required. At the termination of the experiment, examination of the gross external and internal appearance, and similarly, histological examination of body tissues should be reported where necessary. Bacterial, viral, fungal or other disease or parasitic organisms should be considered possible explanations for mortality or poor health of experimental fish. Whenever possible, fish diseases should be diagnosed and reported.

Commonly accepted or clinical physical or biochemical applications uniquely developed or adapted from standard clinical methods used for other animals must be clearly described.

2.3.5 Product criteria

The role of nutrition factors on final consumer quality and commercial value of the fish should be recognized in designing nutrition experiments. The quality factors measured will be dependent upon the ultimate use of the reared fish; flavour, texture, colour and general appearance are important for fish used for human consumption while survival and percent returns of released fish are assessments of fish for stocking purposes. In evaluating these quality factors, there are standard methods which are available and should be used (see Appendix VI).

2.4 Methods of Evaluation of Results

All nutritional variables are dependent rather than independent variables. Each nutrient plays some role in the evaluation of other nutrients.

2.4.1 Bias

All efforts must be made to minimize bias; for example, randomization of feeding order and placement of animals in the experiment is one way of reducing bias. It was noted that specific text books exist giving detailed methods for experimental design (see Appendix VI).

2.4.2 Significant numbers

Results are often reported with several digits after the decimal point when the results may be really only accurate to two figures.

2.4.3 Statistics

Classical methods were emphasized as the key factor in considering statistical methods for analysis of fish nutrition results in light of the interdependence of experimental variables noted above.

In cases where the total number of replicates as a basis for statistical evaluation is rather small, range tests [list, e.g., U-Test (Wilcoxon, Mann and Whitney), H-Test (Kruskal and Wallis)] should be preferred.

2.4.4 Conclusions

The significance of conclusions must be limited by considerations of the specific population sampled, size of the fish and experimental conditions. The limitations in interpreting results based on a very select sample taken from an entire population were noted. It was also noted that when taking a representative sample from that whole population is impossible, generalizations from a limited sample are the best first approximation for the population.

2.5 Experimental Design

2.5.1 Hypothesis

In designing an experiment, only one hypothesis should be evaluated at a time.

2.5.2 Replication

The design will be determined by the question asked, but it should allow statistical evaluation of the results. The number of replicates cannot be categorically stated but depends on the variability of the test animals and the desired accuracy of the experimental results. Replication is essential for any statistical evaluation of results.

2.5.3 Diets

The basal control diet should supply all nutrients required by the test fish and allow reasonable growth and survival for the experimental period. During the experiment, there should be no change in the basal diet except for design nutrient treatments. The energy requirements may be met by feeding either isoenergetic diets or isoenergetic rations. The composition and chemical evaluation of the standard and experimental diets should be recorded.

2.5.4 Experimental parameters

2.5.4.1 Test animals should be deliberately selected for maximum homogeneity and then randomly distributed among treatment groups in appropriate numbers relevant to the experimental hypothesis to fulfil biological and statistical requirements of the experiment.

2.5.4.2 Differences in numbers of experimental animals at the start of an experiment and at the end should be noted, accounted for, and incorporated into evaluations. This should include sampling losses, mortalities, escapes, cannibalism or any other unexpected losses or gains.

2.5.4.3 The stocking density (expressed both as weight/volume and number of fish/volume) should be consistent with experimental objectives.

2.5.4.4 The feeding method and schedule should be clearly stated.

2.5.4.5 The frequency and methods of handling must be recorded.

2.5.4.6 Description of the environmental conditions and any changes experienced during the course of the experiment should be recorded.

2.5.4.7 The intrusion of unwelcome species which might interfere with the experiment must be noted.

2.5.4.8 The working hypothesis should be clearly stated.

2.5.4.9 A minimum replication of treatments should be incorporated into the experimental design for maximum significance of difference in response between treatments. Individual lot treatments should be randomly positioned in the laboratory to eliminate positional bias.

2.5.4.10 Relevant boundaries and limits should be stated and considered in the experimental design.

2.5.4.11 Consideration of both total biomass and experimental biomass must be given within the context of the experimental system.

2.5.4.12 Total biomass = experimental fish + food organisms + others. Experiments may be designed for constant or expanding biomass. Specific details of representative random sampling programmes for each of the above alternatives must be given. Preferably not less than five random samples per treatment should be collected at each period.

2.5.4.13 Animal numbers will depend on homogeneity, size, somatic index and limitations of the system and should include at least the minimum number of animals for statistical analysis. The maximum number will depend upon the carrying capacity of the system.

3. RECOMMENDATIONS TO EIFAC AND ICES REGARDING INTERNATIONAL NETWORK OF FEED INFORMATION CENTRES (INFIC) AND FISH NUTRITION DATA BANK

3.1 Fish nutrition researchers of all member nations should obtain information and input forms from the nearest INFIC centre and submit all pertinent published results of analyses of fish feed ingredients to that centre. The benefit to be gained would be a more complete data bank, which would be of interest to all those involved in fish feeding and fish diet formulation.

3.2 The format of submitted data should be amended to store and make available up-to-date specific data of interest to fish feed formulaters and producers (see Appendix III and Appendix IV).

3.3 A Fish Nutrition Requirement Data Bank should be established. One site might be the National Academy of Sciences Committee on Animal Nutrition in the U.S.A. All relevant data from accepted published reports should be submitted to:

Dr. Philip Ross
Executive Secretary
Board on Agriculture and Renewable Resources
National Academy of Science
101 Constitution Avenue
Washington, D.C. 20240

APPENDIX I

Description of Terms Related to Fish Nutrition

This appendix contains a description of some key words and phrases often used in the fields of feeding or nutrition of fish. In all cases, these should be accepted only as descriptions of terms. The methods used to determine any of these factors should be in accordance with an internationally or officially recognized standard method such as those given in the Official and Tentative Methods of the Association of Official Analytical Chemists (AOAC) quoting the specific method number or reference for the specific type of sample being analysed and following exactly that procedure, including the recommended sample preparation procedures.

Chemical Analysis Terms

1. Proximate or Weende-analysis: composition of ingredients or complete feeds according to the Weende system. The following items are determined: crude protein, crude fat (ether extract), crude fibre, ash and moisture. The nitrogen-free extract (NFE), an estimate of soluble carbohydrate, is then determined by difference. The total of all items must add up to 100.

2. Moisture content: derived by drying a sample to constant weight (not longer than 24 h) at 104°C.

3. Crude protein: nitrogen content (usually by Kjeldahl) x 6.25.

4. Crude fibre: materials insoluble in boiling weak acids and alkalis corrected for ash content of the residue.

5. Crude fat: derived by extracting a finely ground sample of feed with ether continuously for some hours in a suitable apparatus.

6. Ash: that portion of a sample remaining after burning (at up to 500°C) until the residue is free of organic matter.

Feed Conversion or Utilization Terms

7. Apparent digestibility coefficient by faecal method

$$D_a = \frac{I - F}{I}$$

where I is measured feed intake and F is the total faecal output without correction for metabolic faecal losses.

Apparent digestibility by indicator method. Estimates are made by including an inert indicator at a known level in the food and then measuring the nutrient level in food and faeces relative to that inert indicator

$$D_a(\%) = 100 - 100 \times \frac{\% \text{ indicator in feed} \times \% \text{ nutrient in faeces}}{\% \text{ indicator in faeces} \times \% \text{ nutrient in feed}}$$

8. True digestion coefficient

$$TDC = \frac{I - (F - F_m)}{I} = \frac{\text{Food absorbed}}{\text{Food consumed}}$$

where F_m is the metabolic faecal nutrient excreted.

9. Feed conversion: the dry weight of feed per unit wet weight gain (feed/gain).

10. Feed efficiency (the inverse of feed conversion): wet weight gain per unit dry weight of feed (gain/feed).

11. Gross energy of feed: the amount of energy (kcal) obtained by total oxidation of the feed in a bomb calorimeter.

12. Apparent digestible energy of feed: the gross energy of feed minus gross energy of the total faeces produced per unit weight of consumed food

$$DE = \frac{R_E - F_E}{R_E}$$

R_E = ration energy

F_E = faecal energy

13. Metabolizable energy of feed is the gross energy of feed minus gross energy of the total faeces minus gross urinary energy minus gross branchial waste energy per unit feed intake.

$$ME = R_E - (F_E + U_E + B_E)$$

B_E = branchial waste energy

F_E = urinary energy

14. Net energy is metabolizable energy minus heat increment or energy retained per unit feeding.

15. Net energy for maintenance is that fraction of net energy expended to keep the animal in energy equilibrium.

16. Net energy for production is that fraction of net energy expended for growth and metabolic production.

17. Biological value of protein

$$B_V = \frac{N_i - (N_f - N_m) - (N_u - N_{en}) - (N_b - N_{eb})}{N_i - (N_f - N_m)}$$

where N_i = nitrogen intake

N_f = faecal nitrogen

N_m = metabolic faecal nitrogen

N_u = urinary nitrogen

N_{en} = endogenous urinary nitrogen

N_b = branchial nitrogen

N_{eb} = endogenous branchial nitrogen

18. True net protein utilization (NPU)

$$NPU = \frac{N_i - (N_f - N_m) - (N_u - N_{en}) - (N_b - N_{eb})}{N_i} = \frac{N_{ct} - N_{co}}{N_i}$$

where N_{ct} = carcass nitrogen of test group

N_{co} = carcass nitrogen of group receiving a nitrogen-free diet.

19. Apparent net protein utilization (productive protein value)

$$\text{app NPU} = \frac{N_i - N_f - N_u - N_b}{N_i} = \frac{N \text{ retained}}{N \text{ consumed}}$$

where N_b is branchial nitrogen.

20. Protein efficiency ratio

$$\text{PER} = \frac{\text{Weight gain}}{\text{Protein intake}}$$

21. Chemical score: the ratio of the most limiting indispensable amino acid in a test protein to the percent weight of that amino acid in standard reference whole egg protein.

22. Indispensable amino acid index: the n^{th} root of the product of the ratios of indispensable amino acids in test protein to content of each of those amino acids in whole egg protein.

$$\text{EAA} = \sqrt[n]{\frac{aa_1}{AA_1} \cdots \frac{aa_n}{AA_n}}$$

where aa_1 is amino acid in test protein and AA_1 is amino acid in whole egg protein.

Diet Description Terms

23. Standard reference diet (SRD): a precisely defined and reproducible test diet satisfying the nutritional needs of fish for use in feeding studies to facilitate comparisons between various experiments, species, locations, researchers and other factors and conditions.

24. Reference diet (RD): a diet with which one can compare response to experimental design and dietary treatments.

25. Control diet: may be either a negative or positive reference diet used to compare dietary treatment responses. It can be SRD or RD.

Animal Parameters

26. Mortality: number of recorded deaths per unit time or percent of total number of animals which died per unit time.

27. Growth: weight gain per unit time.

28. Morbidity: number of recorded deaths due to disease per unit of time or percent of total number of animals which were ill per unit of time.

29. Relative growth: growth as a percentage of initial body weight

$$\text{RG} = \frac{W_t - W_o}{W_o} \times 100$$

where W_t is body weight at time t and W_o is initial body weight.

30. Specific growth rate:

$$W_t = W_o \left(1 + \frac{\alpha}{100}\right)^t$$

where W_t = weight at time t

W_o = weight at time o

t = time

α = specific growth rate

31. Survival of stocked fish estimated by percentage of tag returns

$$\frac{\text{tags returned}}{\text{tags released}} \times 100$$

In reporting tag (or marked) returns, it is important to specify the type of tag or mark used, location of release and location and method of recapture.

32. Condition factor:

$$k = \frac{100 \times \text{weight (g)}}{\left[\text{length (cm)}\right]^3}$$

APPENDIX II

Exact Formula and Description for the Preparation of Standard Reference Diets

The H-440 standard reference diet (Table 1) is an example of an SRD which has proven satisfactory for use with salmonids, char, catfish, carp, sea bream, sea bass, perch, redfish, pompano, red snapper, black cod and black bass. 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. An example is the addition of 0.5 to 1.0 percent cholesterol to satisfy the essential sterol requirements of a crustacean species (Table 1).

Lot numbers of purified diet ingredients should be listed.

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

Table 1

Standard Reference Diet H-440[a/]

Complete Test Diet	(g)	Vitamin Mixture	(g)
Vitamin-free casein	38	α-cellulose[c/]	8.000
White dextrin	28	Choline chloride	0.500
Gelatin	12	Inositol	0.200
Corn oil[b/]	6	L-Ascorbic acid	0.100
Cod liver oil[b/]	3	Nicotinic acid	0.075
Vitamin mixture	9	Ca-pantothenate	0.050
Mineral mix	4	Riboflavin	0.020
Total	100	Thiamin-HCl	0.005
		Pyridoxine-HCl	0.005
Water	200	Menadione (K)	0.004
		Folic acid	0.0015
Total diet as feed	300	Vitamin B_{12}[d/]	0.0011
		Biotin	0.0005
		α-Tocopherol acetate (E)[e/]	0.040

Mineral Mix	(g)	USP XII No. 2	(g)
USP XII No. 2	100.000	Calcium biphosphate	13.58
$AlCl_3 \cdot 6H_2O$	0.015	Calcium lactate	32.70
$ZnSO_4 \cdot H_2O$	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
			99.50

a/ Diet preparation: Dissolve gelatine in cold water. Heat with stirring on water bath to 80°C. Remove from heat. Add with stirring - dextrin, casein, minerals, oils and vitamins as temperature decreases. Mix well to 40°C. Pour into containers; move to refrigerator to harden. Remove from trays and store in sealed containers in refrigerator until used. Consistency of diet adjusted by amount of water in final mix and length and strength of beating.

b/ For fat soluble vitamin test diet, delete oils, add 9 parts molecularly distilled fish oil plus vitamins A and D_3.

c/ Delete 2 parts α-cellulose and add 2 parts CMC for preliminary feeding.

d/ Add vitamin B_{12} in water during final mixing.

e/ Dissolve α-tocopherol in oil mix.

APPENDIX III

Proposed List of Data on Feed Ingredient Composition of Interest to Fish Feed Producers

Proximate analysis, corrected to 100 percent:

Crude protein
Crude fat (ether extract)
Nitrogen-free extract NFE
Crude fibre
Ash
Moisture

Apparent digestibility coefficients for:

Crude protein
Crude fat
NFE
Crude fibre

kcal per gramme digestible for:

Crude protein
Crude fat
NFE
Crude fibre

Metabolizable energy:

Calculated and estimated by biological assay

Total amino acids of protein:

Ten indispensable amino acids
Plus cystine and tyrosine

Digestible amino acids:

Fatty acid types as g/kg of total fat:

Saturated
Mono enoic
Poly enoic
$\Sigma\omega$ 6
$\Sigma\omega$ 3

Minerals:

Ca, P, NaCl, Na, K, Mg, Cl, CO_3, available phosphorus
Zn, Cu, Co, Se, Fe, Mn, I

Vitamins:

As listed in NRC reports

Sieve analysis to estimate particle size:

Smaller than 0.05, 0.1, 0.2, 0.5, 1 and greater than 1 mm

Volume weight:

g/ml

APPENDIX IV

Europe, Raw Materials to be Characterized as Potential Ingredients in Fresh or Salt Water Practical Diets

Fish meals

Specify by:
Proximate analysis (and its method)
Origin (region)
Source (fish species)
Technical treatment (dried by? stabilized by ?)
Other treatment (hydrolysed ? defatted ?)

Fish solubles

(Specifications: see Fish meals)

Meat and bone meal

Specify by:
Proximate analysis (and its method)
Lysine content (total and available)
Type (meat and bone, tankage, bone, misc.)
Source (animal species)
Technical treatment (dried by ? stabilized by ?)
Other treatment (sterilized ? hydrolysed ?)

Blood meal

Specify by:
Proximate analysis (and its method)
Lysine content (total and available)
Source (whole blood, blood plasma, blood cells)
Special guarantees (sterilized, pathogen free)

Other animal by-products

Specify by:
Proximate analysis (and its method)
Source (animal species, tissue type)
Treatment (dried by ? hydrolysed ?)

Corn

Specify as:
Yellow maize
White maize
Maize gluten feed (crude protein content ?)
Maize gluten meal (crude protein content ?)
Expanded maize
Hominy feed
Other maize by-products

Wheat

Specify as:
Wheat, soft (protein content ?)
Wheat, durum (protein content ?)
Wheat shorts (classified by crude fiber content)
Wheat middlings (crude fiber content ?)
Wheat bran (crude fiber content ?)
Expanded wheat (specification ?)
Wheat flour, first and second clears
Spaghetti offal
Custard powder (starch content ?)

Other cereals and by-products

Specify as:
Oat (steamed, rolled groats ?)
Barley (steam rolled ?)
Potato flakes
Manioc (steamed ? flour or whole roots ?)
Molasses (cane or beet ?)
Biscuit meal
Starch (kind of cereal, treatment)
Dextrin (kind of cereal, water soluble ?)
Glucose monohydrate, dextrose
Sugar (denatured ? specification)

Milk products

Specify by:
Proximate analysis
Lysine content (total and available)
Kind of drying
Treatment (delactosed, neutralized by)

Specify as:
Skimmed milk powder
Buttermilk powder
Whey powder (sweet or acid)
Casein
Whey protein concentrate

Fillers

Specify as:
Rice polishings (white or brown)
Rice hulls
Grape hulls, dried

Soy products

Specify as:
Soy bean oil meal (44 % crude protein)
Soy bean oil meal dehulled (50 % crude protein)
Soy bean partly extracted (ether extract ?)
Whole beans (expanded, e.g. HISOY 40 % CP, 40 % CF)
Soy protein concentrate (crude protein ?)

Miscellaneous

Specify as:
Brewers dried yeast (crude protein ?)
Torula yeast
Single cell protein (specification)
Other plant products (e.g. cotton seed meal, etc.)
Distillers dried solubles (fermentation source)

Oils and fats

Specify by:
Melting point
Free fatty acids, peroxide number)
Source (e.g. animal, tissue)
Treatment (hydrogenated, special fraction ?)

Specify as:
Soy oil
Soy lecithin
Linseed oil
Maize oil
Fish oil
Code liver oil (vitamin content ?)
Lard
Tallow

Minerals

Specify by:
Salt form
Mineral content
Source

A selection of the currently most available and used ingredients should be tested in vivo in order to develop calculation methods to determine digestibility coefficients, metabolizable energy and availability of amino acids.

Also include any other raw materials that might be of interest as fish feed ingredients. The characteristics, chemical and physical, should be described in detail, as it is relevant to its physiological impact on digestibility, metabolizable energy, etc.

Also particle size is of interest.

APPENDIX V

World Mariculture Society Nutrition Task Force
Recommended for Nutrition Papers Published

I. Diets

1. Full name of diet manufacturer, manufacturer's code, lot number, etc.
2. Complete composition of diet expressed as percent, g/kg, or mg/kg, dry weight of diet
3. Ingredients: full recognized name or international feed number
4. Moisture content of diet as feed
5. Purified chemicals: recognized chemical name or formula
6. Micronutrient premix: give recognized name or formula
7. Method of preparation: binding, flaking, drying, etc.
8. Indicate results of any chemical analysis or calculated content based on published values in accordance with a specific study

II. Feeding Procedures

1. Frequency of feeding
2. How many animal units (replicates) fed each diet
3. Amount of feed per unit expressed in weight/day or week
4. Determination of actual food consumption

III. Experimental Animals

1. Species - scientific name and common name
2. Source
3. Age and sex, if appropriate
4. Initial weight and, if appropriate, length, carapace length (crustaceans), etc.
5. Number of individuals per replicate
6. Previous dietary regimen
7. If appropriate, the dietary regimen of parent stock

IV. Methods of Handling, Management and Collection of Data

1. Description of experimental rearing units, surface area, volume (dimensions), and unusual characteristics
2. Length of experiment: days, weeks, etc.
3. Important environmental conditions, photoperiod, etc., which might affect nutritional experiment
4. Water Quality
   (a) Temperature ^{o}C
   (b) Concentration of nutrients in water, e.g., Ca, Mg, etc.
   (c) Dissolved oxygen
   (d) Indicate source of water supply to each unit, noting any difference between units, recirculation, etc.
   (e) Water treatment can be in terms of published reference work or give actual details: UV, filtration, etc.
5. Description of methods used to make measurements with reference, e.g., carapace length
6. Complete description of statistical methods, analysis of variance, regression analysis, etc., with citation or reference for method

V. Results

1. Survival, giving details of any differential survival and overall survival
2. Mean final weight or gain
3. Mean cumulative amount of food fed per unit
4. Results of any special measurement. Symptoms of deficiency, any conditions related to feeds observed
5. Results of statistical analysis of data, including a measure of experimental variability
6. Any special observations pertaining to effects of treatment: fish going off feed, lobsters throwing food out of tank, etc.

APPENDIX VI

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