International Council for the
Exploration of the Sea

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Report of Study Group on Standardization of Methodology in Fish Nutrition Research Hamburg, March 21 - 23, 1978

EIFAC IUNS ICES

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The meeting was convened at 09:30 h by Prof. Dr. K. Tiews

The proposed agenda was accepted.

Dr. J. Castell was appointed Rapporteur.

1. INTRODUCTION

1.1 Recommendation of the Symposium on Finfish Nutrition and Feed Technology, Hamburg June 1978.

This working group was formed as a result of the recommendation of the Symposium held in Hamburg June 1978, that a group of international nutritionists gather to consider and modify the recommended standard methods for nutrition research as outlined in ICES Cooperative Research Report No. 65.

1.2 Objectives

The objectives of this study group were:
1. to recommend standard technology to make research results more comparable and to develop formulae by which to report results in the field of fish nutrition.

2. to advise EIFAC of the possible benefits of an International Network of Feed Information Centers (INFIC) and the establishment of an International Fish Nutrient Requirement Data Bank.

1.3 Standardization

The committee agreed on the desirability for 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 Appendices

1.4.1 Definitions:

One of the needs recognized was for a common vocabulary to be used in fish nutrition. It was recommended that the report of the study 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, terms defined in EIFAC/78 Symp/RI plus any additional terms which were found valuable by the study group.

1.4.2 Standard Reference Diets

The exact formula and description for preparation of one or more standard reference diets should be included as Appendix II. One example that could be used was diet H440 in EIFAC/78/Symp/R8.

1.4.3 List of Data on Feed Ingredient Composition of Interest to Fish Feed Producers.

Appendix III

1.4.4 List of Feed Ingredients of Interest to the Fish Feed Producer.

Appendix IV

1.4.5 List of Recommendations of Nutrition Task Force to World Mariculture Society, Hawaii January 1979

Appendix V

1.4.6 List of Literature References for Standard Methods

Appendix VI

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 flavor attractents) 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, micro-encapsulated 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, preferably 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 fiber
- nitrogen-free extracts (N.F.E.)

This proximate analysis should be corrected to give a total of 100%. 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 1) are also valuable for interpreting results.

1.2.4 Standard Reference Diet(s) (SRD)

The principle of establishing a standard reference diet was endorsed. The use of a SRD in all fish nutrition research would permit direct comparison of results between 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 Experiental 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 1

2.3.2 Feed Conversion

Several methods of presentation are possible, definitions of such expressions as feed conversion, feed efficiency, net protein retention, 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 from adequate numbers 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 of 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 quaity 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 the value 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 digets after the decimal point when the results maybe 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 U-Test (Wilcoxon, Mann and Whitney), H-Test (Kruskal and Wallis) should be preferred.

2.4.4 Conclusions

Significance of conclusions must be limited by considerations of the specific population sampled, size of fish and experimental conditions. The limitations in interpreting results based on a very select sample in terms of an entire population were noted. It was also noted that when 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 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 wt/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 interfer with the experiment must be noted.

2.5.4.8 The working hypothesis should be clearly stated.

2.5.4.9 Minimum replication of treatments for maximum significance of difference in response between treatments should be incorporated into the experimental design. 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.

Total biomass - expt. fish food organisms others.
Experiments may be designed for constant or expanding biomass.
Specific details of representative random sampling programs for each

of the above alternatives must be given. Preferably not less than 5 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.

3.0 RECOMMENDATIONS TO EIFAC AND ICES REGARDING INTERNATIONAL NETWORK OF FEED INFORMATION CENTERS (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 center and submit all pertinent published results of analysis of fish feed ingredients to that center. The benefit to be gained would be a more complete data bank 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 formulators and producers. (See Appendix III and Appendix IV).

3.3 A Fish Nutrition Requirement Data Bank should be established. One site may be the National Academy of Sciences Committee on Animal Nutrition in the USA. 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 Ave.
Washington, D. C. 20240

Appendix 1

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 analyzed and following exactly that procedure including the rcommended 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 fiber, ash and moisture. The nitrogen free extract (NFE), (an estimate of soluble carbohyrate) is then determined by difference. Total of all items must add up to 100.

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

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

4. Crude fiber: 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 (up to 500°C) until the residue is free of organic matter.

Feed conversion or utilization terms

7. Apparent digestibility coefficient by fecal method

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

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

Digestibility by indicator method. Estimates by including an inert indicator at a known level in the food and then measuring the nutrient level in food and feces relevant to that inert indicator:

$$D(\%) = 100 - 100 \times \frac{\% \text{ Indicator in feed} \times \% \text{ nutrient in feces}}{\% \text{ Indicator in feces} \times \% \text{ nutrient in feed}}$$

8. True digestion coefficient

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

where F_n is the metabolic fecal 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 feces produced per unit weight of consumed food.

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

R_E = ration energy
F_E = fecal energy

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

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

B_E - branchial waste energy
U_E - urinary energy

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

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

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

17. Biological value of protein:

$$TB_V = \frac{N_i - (N_f - N_m) - (N_u - N_{EN})}{N_i - (N_f - N_m)}$$

where N_i = nitrogen intake
N_f = fecal nitrogen
N_m = metabolic fecal nitrogen
N_u = urinary nitrogen
N_{EN} = endogenous urinary nitrogen

18. True net protein utilization (NPU)

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

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.

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

where N_b is bronchal nitrogen

20. Protein efficiency ratio:

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

21. Chemical score: the ration of the most limiting indispersible amino acid in test protein to 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 over content of that amino acid in whole egg protein.

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

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 of 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) 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. 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. Morbidity: number of recorded diseased deaths per unit of time or percent of total number of animals which were ill per unit of time.

28. Growth: weight gain per unit time.

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

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

Where W_t is body weight at time t
W_o is initial body weight

30. Specific growth rate:

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

Where W_t - weight at time t
W_o - weight at time o
t - time
- specific growth rate

31. Survival of stocked fish estimated by percentage tag returns

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

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.

33. Contition factor: $k - \frac{100 \times \text{weight (g)}}{\text{length (cm)}^3}$

Appendix II

The H-440 standard reference diet (Table 1) is given as an example of a 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% 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; and as a powder, rolled pellets, extruded pellets, or compressed pellets.

Table 1. Standard Reference Diet H-440[a]

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

Mineral Mix (gm)	
USP XII No. 2	100.00
$AlCl_3 \cdot 6H_2O$	0.015
$ZnSO_4 \cdot H_2O$	0.300
CuCl	0.010
$MnSO_4 \cdot H_2O$	0.080
KI	0.015
$CoCl_2 \cdot 6H_2O$	0.100

USP XII No. 2 (gm)	
Calcium biphosphate	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulfate	13.20
Potassium phosphate (dibasic)	23.98
Sodium biphosphate	8.72
Sodium chloride	4.35
	100.00

[a] Diet preparation: Dissolve gelatin 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 molecullarly 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%
- Crude protein
- Crude fat (ether extract)
- Nitrogen free extract NFE
- Crude fiber
- Ash
- Moisture

Apparent digestibility coefficients:

for crude protein

crude fat

NFE

crude fiber

kcal per gram digestable

for crude protein

crude fat

NFE

crude fiber

Metabolizable energy:

Calculated and estimated by biological assay.

Total amino acids of protein:

ten indespensable 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
Prox. analysis method treatment with antioxidant or formalin

1. Norwegian "Herring" mixed 70, 72, 74% CP stablized.
2. Danish tobis meal 71%/mixed fish 70% sabilized.
3. Skandinavian fish meal unspec. 70% not stabilized.
   All meals mainly or wholly steam dried.
4. Islandic cod/capelin meal 68% stabilized? dried?
5. Peruvian anchovy fish. 64% stabilized steam - flame dried.
   South American fish stabil/unstabil. 66% un% crude
7. South African mixed fish. 68% dried? stabilized?
8. Local fish meal. Specified. White fish mealn 78% crude
9. Other fish meals.
10. Fish hydrolysates low ash. 71, 88 etc. crude protein %
11. Special qualities fish meal: Norseamink. 72% crude protein.
12. Fish protein conc. defatted. Norsamin micro protein.
13. Dried fish solubles spec.

Meat and bone meals
Beef, pork and poultry meals

1. -6. High and low fat type tankage in several analysi types.
   60-7. 60-14. 20-30 ash.
   55 and 65 protein id. Heattreatment.
   % Carpenter av. lysin against total.
6. -12. 50 and 45 types m b. High/low fat.
13. High quality meat meal non sterilized.
    70%. 12-15%. fat. 75/id
14. Beef greaves meal. 65-70 CP 20-14 fat. 60-65 CP 20-
15. Beef greaves meal. 70-75 CP 20-14 fat
16. Poultry by product meal 64-68 CP.
17. Whale meat meal 85% CP.
18. Meat hydrolysate meal 85% CP. Meat prot. conc.
19. Miscelaneous products local-specified.
20. Bonemeal local spec. (30% CP) 50% ash.

Blood Meals
Spray Dried, Low Heat, High Heat Sterilized, Roller Dried

1. Blood meal drum dried 85% CP. Available/tot lysine
2. Blood meal drum dried 85% CP. Available/tot lysine
3. Blood meal spray dried LH 88%.
4. Blood meal spray dried HH 88%. Path. free
5. Blood meal 85% local quality spec. Available/tot
6. Blood plasma meal (centrif.)

Other Animal by Products

1. Shrimp meal white.
2. Shrimp meal red.
3. Shrimp offals (hullmeal) 45% white.
4. Shrimp offals (hullmeal) 45% red.
5. Hydrolyzed feather meal 80% CP.
6. Hydrolyzed feathe meal 80% CP.
7. Hydrolyzed feather meal 80% CP.
8. Liver meal 65 & spec.
9. Other products, krill meal etc.

A choice of 10 most currently available ingredients should be tested in vivo to derive a calculation method. Digestability factors, energy factors, aa's calculation. ME estimation.

Plant Materials

1. Maize gluten feed 23%.) yellow pigment
2. Maize gluten meal 42%) undesirable!
3. Maize gluten meal 60%)
4. White maize. Particle sizes
5. Yellow maize. Particle sizes
6. Expanded maize. Paricle sizes
7. Hominy feed. Particle sizes
8. Other maize by-produces. Particle sizes.

Wheat

1.-4. Wheat shorts. 2, 5, 7, 9% crude fiber.
6.-9. Wheat middlings- bran 7, 11, 15% crude fiber.
10. Wheat durum high prot. 17/18%
11. Wheat soft low prot. 10-11%

Wheat

12. Expanded wheat specif.
13. Spaghetti offal.
14. Wheat flow 1 st., 2 nd clears.
15. Custard powder. 85 NFE (starch).

Other Cereal and Miscell.

1. Steamed rolled oat groats.
2. Steam rolled barley.
3. Maize starch
4. Potato flakes
5. Steamed maniok
6. Row maniok (tapioca whole roots)
7. Maize dextrin water soluble
8. Biscuit meals specified
9. Glucose monohyd. (dext.)
10. Denat sugar (spec.)
11. Mollasses cane or beet

Milk Product

1. Whey powder sweet/acid spray
2 Delactosed whey powder. Spray dried. 24-30% CP.
3. Skimmed milk powder spray.
4. Acid casein -spray dried/roller
   -drum dried/roller
5. Ca or Na neutralized casein spray dr.
6. Others like lactalumin 60% etc.

Fillers

1. Rice polishings white, brown
2. Rice hulls
3. Grape hulls dried.

Soy Products

1. Soy bean extract. 50% standard microfine, 2.5% C Fi.
2. Soy bean extract. 44% standard 7.0% Fi.
3. Soy bean partly extr. Soy-assim. 4-6% oil.
4. Whole beans expanded. Hisoy 40-20%.
5. Soy bean extr. 42%/47% C Fi. 9/4%.

Miscellaneous

6. Brewers dr. yeast. 45% CP
7. Torula yeast.
8. Local yeast.
9. Single cell proteins sources
10. Other plant prod. like cotton seed meal etc.

Oils

1. Linseed oil
2. Fish oi. Specified!
4. Maize oil
5. Cod liver oil (AD_3)
6. Lard (melting point)
7. Soy lecithin
8. Norsalmoil, capelin oil etc.

Dist. Byproduct

1. Dist. dried solubles whiskey (scotaferm)
2. Dist. dried solubles corn maize
3. Dist. dried solubles molasses
4. Dist. dried solubles whey. etc.

Aminoacids, Press Aids, etc.

1.-3. L-Arginine, DL Meth. L Lysine %!
4. Lignin sulfonate.
5. Clays
6. Propoionic acid, NH_4 propionate, etc.

Minerals

1. Limestone flour. $CaC0_3$ (specify)
2. Mono Ca phos. Dicalphosphate (spc.!)
3. Na phosphate
4. Phosphjoric acid. 75%
5. Salt (spec.)
6. Ca-lactate etc.

Acids Org.

1. Citric acid
2. Lactic acid
3. Fumaric acid. etc.

A choice of most currently used other ingredients should be tested in vivo to elaborate calculation methods to estimate ME values.

Plus 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 digestability, metabolizable energy, etc.

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 we weight of diet.
3. Ingredients, full recognized name or international feed number.
4. Moisture content of diet as fed.
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 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 per 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, 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 °C.
   (b) Concentration of nutrients in water, eg. Ca., Mg., etc.
   (c) Dissolved oxygen.
   (d) Indicate source of water supply to each unit, noting any differences 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 to make measurements with references, eg. carapace length.

6. Complete description of statistica methods, analysis of variance, repression 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 cumulated amount 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

Biolography of references of interest to fish nutrition researchers, fish culturists and fish feed manufacturers.

1. Chemical methods: -

A. O. A. C. (Association of Official Analytical Chemists) Official Official Methods of Analysis. 12th Edition 1975. George Banta Co. Inc., Manasha, Wisconsin.

A. P. H. A. Standard Methods of the Examination of Water and Waste Water 14th ed. 1975. Rand, M. C., a. E Greenberg and M. J. Taras (eds). American Public Health Association, American Waterworks Association and Water Pollution Control Federation joint publishers Washington, D. C.

2. Experimental Designs:-

Cochrin, W. D. and G. M. Cox. 1957. Experimental Designs. John Wiley and Sons, Inc., New York, London, Sydney.

3. Statistics:-

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of of Statistics with Special Reference to the Biological Sciences. McGraw-Hill, New York, Toronto, London.

Snedecor, G. W. and Cochran, W. G.: Statistical Methods. Iowa State University Press, Ames, Iowa, 1967.

Sachs, L.: Angewandte Statistik. Springer-Verlag, Berlin, Heidelberg, New York, 4th edition, 1974. ISBN 3-540-06443-5, Congress Catalog Card No. 73-11595.

4. Physiology of Fish:-

Young, W. D. and D. S. Robson (1978). Estimation of Population Numbers and Mortality Rates. In: Methods for the assessment of fish population in freshwater. Ed. T. Baganal. pp. 137-164. IBP Handbook No. 3, Blackwell Scientific Publications, Oxford and Edinburgh.

5. Feed Technology and Nutrition:-

Albanese, A. A. (ed.). 1959. Protein and Amino Acid Nutrition. Academic Press. New York and London.

Albanese, A. A. (ed). 1963, 1965, 1967, 1970, 1972. Newer Methods of Nutritional Biochemistry with Applications and Interpretations. A five volume series. Academic Press Inc., New York and London.

Cowey, C. B. and J. R. Sargent. 1972. "Advances in Marine Biology". In Fish Nutrition. Editors-F. S. Russell and M. Young. Vol. 10 pp. 383-492.

Crampton, E. W. and L. E. Harris. 1969. Applied Animal Nutrition. 2nd ed. A Series of Books in Agricultural Science. ed. G. W. Salisbury and E. W.Crampton. W. H. Freeman and Co. San Francisco.

Halver, J. E. (ed.). 1972. Fish Nutrition. Academic Press. New York and London.

Hoar, W. S. and Randall, D. J., Fish Physiology, Academic Press, New York, San Francisco, London:
Vol. 1: Excretion, Ionic Regulation and Metabolism, 1969
Vol. 2: The Endocrine System, 1969
Vol. 3: Reproduction and Growth, Bioluminescence, Pigments and Poisons, 1969
Vol. 4: The Nervous System, Circulation and Respiration, 1970
Vol. 5: Sensory Systrems and Electric Organs, 1971
Vol. 6: Environmental Relations and Behaviour, 1971
Vol. 7: Locomotion, 1978
Vol. 8: Bioenergetic and Growth, 1979

NRC. 1973. Nutrient Requirements of Trout, Salmon and Catfish. Nutrient Requirements of Domestic Animals. The National Research Council. National Academy of Sciences, Washington, D. C.

NRC. 1977. Nutrient Requirements of Domestic Animals. The National Research Council, National Academy of Science, Washington, D. C.

Russell, F.S. and Young M. Advances in Marine Biology. Vol. 10. In paragraph 5, p. 26.

Sebrell, W. J. Jr. and Harris, Robert S. The Vitamins; chemistry, physiology, pathology and methods. Volumes 1-7, 1967, 68, 71, 72. Academic Press Inc., New York, London